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## Original Article

# Assessment of the key aroma compounds in rose-based products

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## ABSTRACT

In this study, headspace solid phase microextraction–gas chromatography–mass spectrometry and GC-olfactometry were used to analyze the key aroma compounds in three types of rose-based products, including low-temperature extracts (LTEs), high-temperature extracts (HTEs), and rose drinks (RDs). In combination with the Guadagni theory, it was confirmed that the key aroma components of LTE were  $\beta$ -phenyl ethyl alcohol, citronellol, geraniol, and eugenol. The main aroma compounds in HTE were  $\beta$ -phenyl ethyl alcohol, citronellol, geraniol, eugenol, linalool, and rose oxide. The four key aroma compounds in RDs were  $\beta$ -phenyl ethyl alcohol, eugenol, geraniol, and linalool.

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## 1. Introduction

Rose is a precious traditional Chinese medicinal material, which is also an important raw material in the perfume and food industries [9]. The demand for rose and its products has diversified in the international and domestic markets, resulting in it becoming a research focal point to determine the key compounds of the rose aroma found in rose-based products [4–6]. Currently, using headspace solid phase microextraction–gas chromatography–mass spectrometry (HS-SPME–GC-MS) technology, research into the aroma compounds of rose and its products is mainly focused on

quantifying the aroma constituents of the rose flower bud, petal, the rose flower recovered at different periods, and rose essential oil. In flower buds,  $\gamma$ -muurolene,  $\alpha$ -himachalene, and  $\alpha$ -pinene are the major constituents. Meanwhile,  $\beta$ -citronellol, citronellol acetate, phenethyl alcohol, geraniol are the major constituents in fresh flower at the early opening stage, and at the full opening stage,  $\beta$ -citronellol citronellol acetate, phenethyl alcohol, geranyl acetate, geraniol, phenethyl acetate, nerol, *n*-hexyl acetate and  $\alpha$ -myrcene, and alcohols are the major constituents [4,10,11]. In rose oil, there are 20 kinds of compounds whose content exceeds 1%, including 22.606% phenethyl alcohol, 12.015% citronellol, 6.772% geraniol, 6.194% eugenol, and 2.329% neroli alcohol

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[5,6,12]. As the thresholds of the compounds differ, the impact of the aroma at the highest content may not be the most significant; hence, determining the main aroma of rose-based products is crucial.

GC-olfactometry is an instrument used to detect odors online and simultaneously record the results. HS-SPME–GC-MS combined with GC-olfactometry technology has been widely used in determining the aromas of balsamic vinegar, distillate spirits, apple, cheese, and other foods [3,7,8,13]. However, analyzing the aroma of rose and its products using GC-MS combined with GC-olfactometry has not been previously reported.

HS-SPME–GC-MS combined with GC-olfactometry was used in this study to assess the odors of a serially diluted low-temperature extract (LTE), high-temperature extract (HTE), and rose drinks (RDs), to determine the main aroma substances of the three rose-based products. Meanwhile, according to the theory that a higher content and lower threshold confers a larger contribution to the sample, the key aromas of the samples were determined, providing a more reliable scientific basis for the quality control of rose-based products.

## 2. Methods

### 2.1. Materials

LTE refers to a distilled extract produced during the rose drying process, a colorless and clear liquid, produced on June 10, 2013. HTE is a distilled extract produced during the rose add-water distillation process, a colorless and clear liquid produced on May 3, 2013. RD refers to the rose drink named *jiuduomeigui*, a red and clear liquid, which is commercially available; its production process is not clear, but it was produced on June 21, 2013. These experimental materials were provided by NIS International Rose Industry Co. Ltd. (Zaoyang, Hubei Province, China).

All aroma standards were purchased from Sigma–Aldrich (St. Louis, MO, USA) and were of the highest purity available: rose oxide, linalool,  $\alpha$ -terpineol, citronellol, nerol, geraniol,  $\beta$ -phenyl ethyl alcohol, and eugenol. Sodium chloride AR was obtained from Beijing Chemical Plant (Beijing, China).

### 2.2. Instruments and equipment

GC-MS (Agilent Technologies, 7890A, 5975C), SPME handle (Supelco, USA), PDMS fibers (Supelco), and Olfactometry (ODP3; Gerstel, Germany) were used during the tests. Helium was used as the carrier gas, with a constant flow rate of 1.0 mL/min. The GC system was equipped with a split–splitless injection port at 230°C. The splitless mode was used to inject the fiber and used a 5-minute desorption time. An Elite Wax Etr column [30 m, 0.25 mm (ID), 0.25  $\mu$ mdf], supplied by Perkin-Elmer, was used to separate the volatiles. After injection, the column temperature was held at 50°C for 2 minutes and increased to 80°C at 10°C/min. Subsequently, the temperature was increased to 115°C at 10°C/min, followed by an increase to 240°C at 3°C/min, with a final holding time of 5 minutes. The temperature of the transfer line to the mass spectrometer was

set at 240°C. The mass spectrometer was operated using the electron impact ionization mode (70 eV) at 230°C.

### 2.3. Experimental

#### 2.3.1. Sample preparation

Depending on which of the rose-based products was to be tested, the samples were diluted to varying ratios. First, 2 mL of the LTE stock solution, as well as two-fold diluted, fold-fold diluted, and 10-fold diluted solutions were taken to perform the HS-SPME. Then, 2 mL of each of the HTE stock solutions, as well as four-fold diluted and 10-fold diluted solutions were taken to perform the HS-SPME. Next, 2 mL of each of the RD, two-fold diluted, and four-fold diluted solutions were used to perform the HS-SPME, and the aroma components were extracted. The HS-SPME procedure was carried out as described by Su et al [9].

#### 2.3.2. Sniffing and detection

The method for sniffing the odors using GC-olfactometry involved a four-way flow divider at the end of the GC column, which split the sample (split ratio, 2:1) to the mass detector and olfactometer. While sampling, five trained personnel sat at the outlet of the olfactometer, and recorded and described the odors they smelled. According to the aroma intensity, the aroma compounds were divided into four grades—1, 2, 3, and 4—from weak to strong. A sniffing aro-magram could then be formed according to the grades. Each sample was assessed three times by each judge. The sniffing time for each run was 50 minutes.

Identification was achieved from comparisons of mass spectra obtained from the samples with those present in the NIST05 MS Library database. Retention index was calculated using the software system named AMDIS. Identification was considered tentative when it was based only on the mass spectra data. Compounds that were identified by confirming their mass spectrogram to that of authentic chemicals, purchased from commercial sources, were considered to be positively identified.

The method for determining the key compounds involved increasing the dilution ratio, resulting in a gradual reduction of the aroma intensity and species. The detected aroma compounds were noted; the compounds of a higher frequency, detected using GC-olfactometry, were regarded as the key aromas. At the same time, the content of the aroma compounds was detected by GC-MS, and each compound was given an odor activity value (OAV), which is the ratio of the concentration and the threshold. Hence, the key aroma compounds in rose-based products were finally determined by HS-SPME–GC-MS and GC-olfactometry.

#### 2.3.3. Quantification

The standard addition method was used in this analysis. The working solutions of aroma standards at five different levels of concentration were added to the samples prior to the HS-SPME. Quantitative analysis was finished by the calibration curve acquired. Stock standard solutions of the analytes were prepared in ethanol and stored at 4°C under refrigeration. Furthermore, working solutions were prepared by dilution of the stock solutions with ethanol in the appropriate quantities.

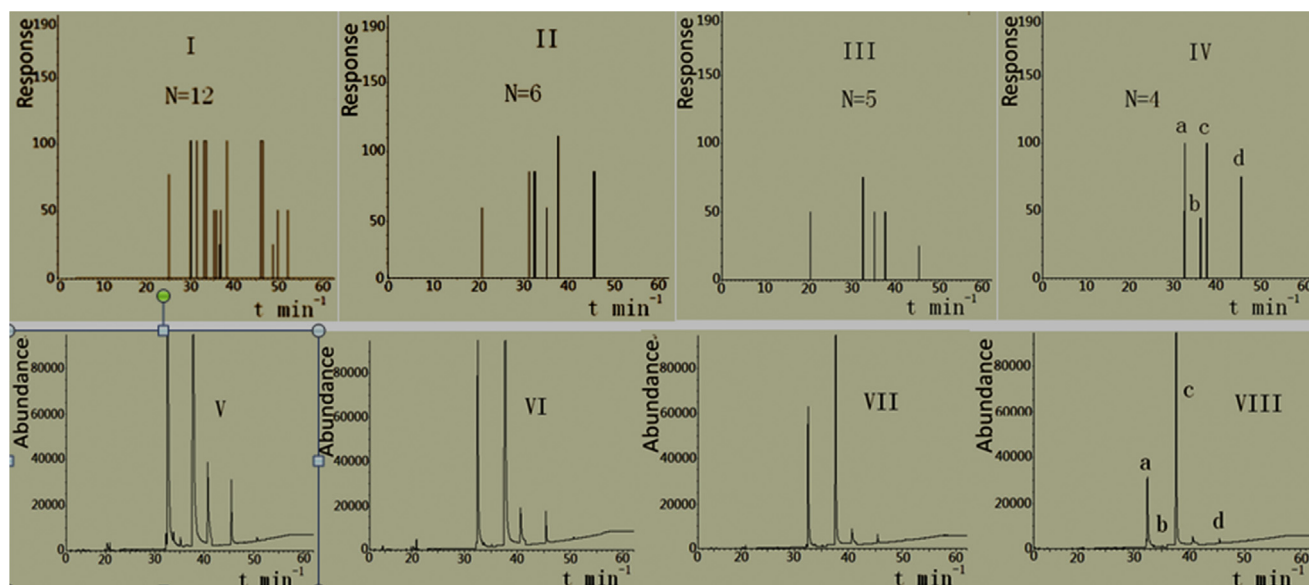


Figure 1 – Consensus aromagram and gas chromatogram of the cell saps. (A–D) Sniffing aromagrams of the rose cell sap, the 2-fold diluted rose cell sap, the 4-fold diluted rose cell sap, and the 10-fold diluted rose cell sap. One line means the type of aroma; the longer the line, the stronger the aroma; the thicker the line, the longer the time the aroma is present in the consensus aromagram. (E–H) Gas chromatograms of the rose cell sap, the two-fold diluted rose cell sap, the four-fold diluted rose cell sap, and the 10-fold diluted rose cell sap. a = citronellol; b = geraniol; c = β-phenyl ethyl alcohol; d = eugenol; N = number of aroma compounds.

Table 1 – Frequency table of detected aroma components in rose-based products.

Compound	Retention index ( $R_t$ )	Qualitative mass	Odor description	LTE <sup>a</sup>	HTE <sup>a</sup>	RD <sup>a</sup>
β-Myrcene	826.50	41, 93, 69	Pleasant, light balsam scent	5	0	0
D-Limonene	1252.47	68, 93, 136	Similar to the lemon flavor	0	5	0
Linalool	1637.48	71, 93, 121	Lilac, lily of the valley, rose flower, woody, fruity flavor	0	15	20
Citronellyl acetate	1702.37	69, 95, 123	Fresh fruit aroma with lemon scent	5	5	10
Heptadecane	1700.00	57, 71, 85		0	0	0
α-Terpineol	1725.82	59, 93, 121	Clove aroma	0	10	0
Citronellol	1784.51	69, 82, 95	Sweet rose	20	15	20
Nerol	1812.57	69, 41, 93	Delightful rose and orange blossom aroma, peaceful, kind of fruity lemon	0	0	0
Geraniol	1847.58	69, 93, 123	Sweet rose aroma	20	15	15
β-Phenyl ethyl alcohol	1901.07	91, 92, 122	Rose aroma	20	15	20
Nerolidol <sup>b</sup>	1976.35/2020.68	69, 93, 107	Weak clear mellow sweet orange blossom flavor, such as rose, lily of the valley, & apple blossoms	0	5	0
Methyl eugenol	2000.48	178, 163, 147	Sweet cloves-fennel aroma, such as carnations, lasting, a mild spice tea	0	0	5
n-Heneicosane	2100.00	77, 85, 296		0	0	0
Nonadecane	1900.00	71, 85, 268		0	0	0
Eugenol	2190.84	164, 103, 149	Strong aroma of cloves	20	15	20
Rose oxide <sup>b</sup>	1531.48/1539.12	54, 69, 139	Sweet floral aroma, such as roses & nuances of fresh bay leaves	5	10	10
Methyl geranate	1725.69	69, 41, 114		0	0	0

HTE = high-temperature extract; LTE = low-temperature extract; RD = rose drink.

<sup>a</sup> Numbers represent the frequency of the detection of the aroma compounds.

<sup>b</sup> The compound has its isomeride.

**Table 2 – Content and OAV of main aroma compounds in LTE.**

Compound	Amount (µg/L)	Threshold (µg/L)	OAV	RD
Rose oxide	265.20	0.5 [1]	530.40	0.83
Linalool	15.96	6 [1,2]	2.66	1.51
$\alpha$ -Terpineol	24.10	280 [1]	0.09	5.96
Citronellol	8399.64	40 [1]	209.99	0.066
Nerol	115.26	300 [1]	0.38	7.44
Geraniol	142.46	75 [1]	1.90	0.79
$\beta$ -Phenyl ethyl alcohol	332,467.62	1000 [2]	332.47	11.70
Eugenol	4674.86	6 [2]	779.14	0.98

LTE = low-temperature extract; OAV = odor activity value; RD = rose drink.

### 3. Results and discussion

#### 3.1. Results of LTE

LTE is the extract from rose at 25°C; the sniffing aromagram, the gas chromatogram, and the dilution ratio of the LTE are shown in Figure 1, which shows that as the degree of dilution of the LTE gradually increases, the species of aroma compounds decreases. Twelve types of compounds were detected in the LTE stock solution, whereas only four kinds of substances were detected in the 10-fold diluted solution. Table 1 shows that the higher the frequency of the detected aroma in the sample, the greater the contribution of that aroma. The results for the LTE show that the frequency of  $\beta$ -benzyl ethanol, citronellol, eugenol, and geraniol were

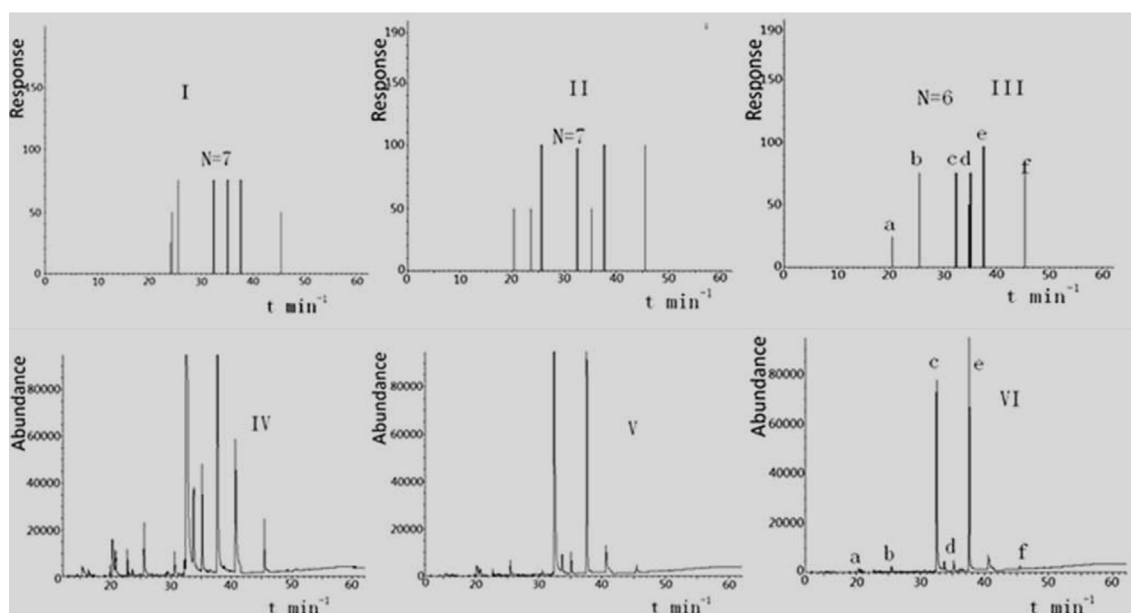
higher—indicating that these four compounds have the largest contribution to the aroma of the LTE.

Once the content of the aroma compounds in the LTE were detected by HS-SPME–GC–MS, the OAV values were calculated (Table 2). In the cell saps, the  $\beta$ -benzyl ethanol, citronellol, and eugenol contents were higher, whereas the OAV values of citronellol,  $\beta$ -phenyl ethyl alcohol, and rose oxide were higher (OAV > 100). Although the  $\beta$ -benzyl ethanol content was much higher than that of citronellol, the sensory threshold of  $\beta$ -benzyl ethanol was also higher, which led to a slight difference in the activity of citronellol and  $\beta$ -benzyl ethanol; therefore, their smelling intensity was almost the same. The odor threshold referred to in past research [1,2] gives the threshold value in water; however, the threshold will change in different substrates. The OAV value of rose oxide is larger than that of geraniol, but during the actual smelling the odor of geraniol was clear, and the frequency of occurrence was high; hence, it is also regarded as a key aroma in the LTE.

According to the smelling results and the OAV value, the key aroma compounds of the LTE were  $\beta$ -benzyl ethanol, citronellol, eugenol, and geraniol.

#### 3.2. Results of HTE

HTE is the extract from rose at a high temperature. The 100% pure HTE, four-fold diluted and 10-fold diluted samples were analyzed in this work; the sniffing aromagram and gas chromatogram are shown in Figure 2. As observed for the LTE, as the degree of dilution of the HTE gradually increased, the species of aroma that were detected fell from 14 to 6 or 7 (Figure 2). By contrast, compared with the LTE, the number of aroma species that were detected in the HTE increased, which



**Figure 2 – Sniffing aromagram and gas chromatogram of the high-temperature extract (HTE). (A–C) Sniffing aromagrams of rose hydrosol, the four-fold diluted HTE, the 10-fold diluted HTE. One line means the type of aroma; the longer the line, the stronger the aroma; the thicker the line, the longer time the aroma is present in the consensus aromagram. (D–F) Gas chromatograms of the HTE, the four-fold diluted THE, and the 10-fold diluted HTE. a = rose oxide; b = linalool; c = citronellol; d = geraniol; e =  $\beta$ -phenyl ethyl alcohol; f = eugenol; N = number of aroma compounds.**

**Table 3 – Content and OAV of the main aroma compounds in the HTE.**

Compound	Amount ( $\mu\text{g/L}$ )	Threshold ( $\mu\text{g/L}$ )	OAV	RD
Rose oxide	1296.96	0.5 [1]	2593.92	0.67
Linalool	357.94	6 [1,2]	59.66	1.34
$\alpha$ -Terpineol	216.80	280 [1]	0.77	3.44
Citronellol	6834.90	40 [1]	170.87	0.92
Nerol	485.04	300 [1]	1.62	4.56
Geraniol	767.62	75 [1]	10.23	0.83
$\beta$ -Phenyl ethyl alcohol	55,341.24	1000 [2]	55.34	2.33
Eugenol	2198.80	6 [2]	366.47	1.66

HTE = High-temperature extract; OAV = odor activity value; RD = rose drink.

**Table 4 – Content and OAV of the main aroma compounds in RD.**

Compound	Amount ( $\mu\text{g/L}$ )	Threshold ( $\mu\text{g/L}$ )	OAV	RD
Rose oxide	16.1	0.5 [1]	32.2	1.04
Linalool	5.57	6 [1,2]	0.93	0.95
$\alpha$ -Terpineol	41.5	280 [1]	0.15	2.75
Citronellol	147.14	40 [1]	3.68	0.92
Nerol	N.D.	300 [1]	—	—
Geraniol	5.2	75 [1]	0.07	2.59
$\beta$ -Phenyl ethyl alcohol	13,351.15	1000 [2]	13.35	1.99
Eugenol	73.29	6 [2]	12.22	4.89

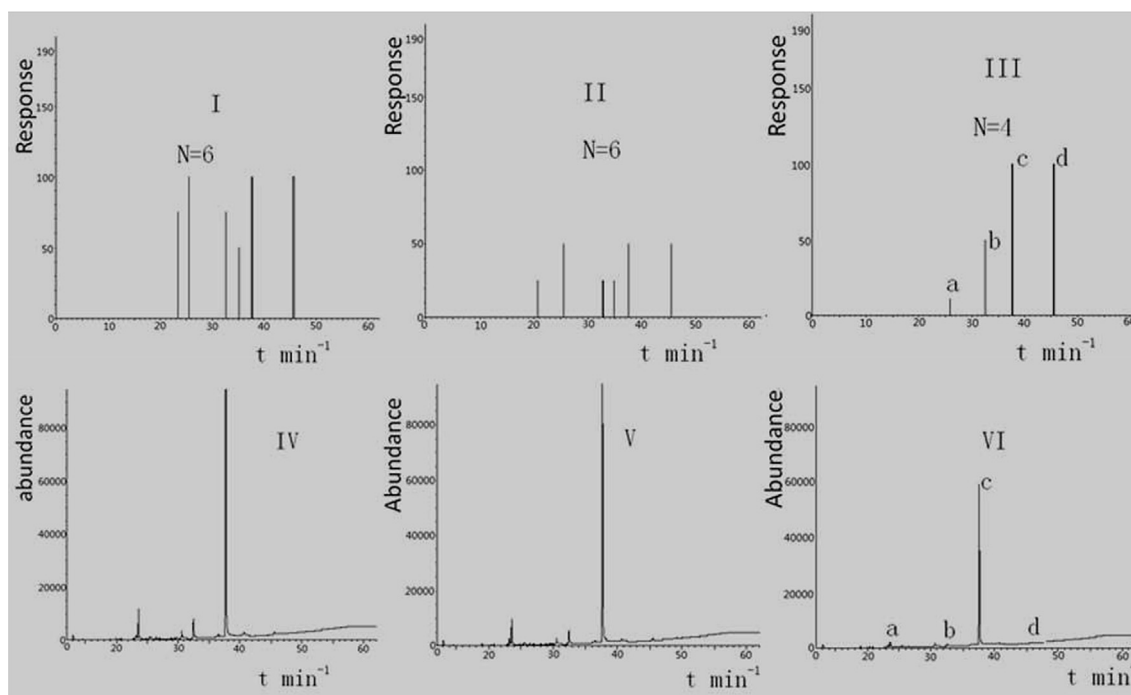
N.D. = not detected; OAV = odor activity value; RD = rose drink.

was consistent with the results of the gas chromatogram. In the 10-fold diluted LTE solution, four types of compounds were detected, whereas there were six in the HTE. After quantitation by HS-SPME–GC–MS and the related standard materials, it was determined that the six compounds were rose oxide, linalool, citronellol, geraniol,  $\beta$ -phenyl ethyl alcohol, and eugenol.

The smelling statistical results (Table 1) show that, in all diluted solutions, the smelling frequency of linalool, citronellol, geraniol,  $\beta$ -phenyl ethyl alcohol, and eugenol were higher, followed by that of rose oxide. Therefore, the most important compounds in the HTE were  $\beta$ -phenyl ethyl alcohol, citronellol, geraniol, eugenol, linalool, and rose oxide. Because linalool and rose oxide were not detected in the LTE using

olfactometry and their boiling point temperature is higher, it may be that the higher temperature makes it conducive for both aroma components to be detected.

The content and OAV value of the main aroma components (Table 3) show that  $\beta$ -phenyl ethyl alcohol and citronellol were the most abundant in the HTE, but at a lower amount than that found in the LTE. Furthermore, the geraniol, rose oxide, and linalool contents in the HTE were much higher than those found in the LTE; this is perhaps attributable to the lower boiling point of  $\beta$ -phenyl ethyl alcohol and citronellol (the other 3 compounds have a higher boiling point). The aroma characters of the  $\beta$ -phenyl ethyl alcohol and citronellol were those of typical roses; hence, the rose aromas of the LTE were more prominent, whereas the aroma of the HTE was more complex. In the HTE, the OAV values of rose oxide,



**Figure 3 – Consensus aromagram and gas chromatogram of the rose drink (RD). (A–C) Sniffing aromagrams of the rose drinks, the two-fold diluted RD, and the four-fold diluted RD. One line means the type of aroma; the longer the line, the stronger the aroma; the thicker the line, the longer the time the aroma is present in the sniffing aromagram. (D–F) Gas chromatograms of the RD, the two-fold diluted RD, and the four-fold diluted RD. a = linalool; b = citronellol; c =  $\beta$ -phenyl ethyl alcohol; d = geraniol; N = number of aroma compounds.**

**Table 5 – Key aroma compounds of rose-based products.**

Sample	LTE	HTE	RD
Key aroma compounds	$\beta$ -Phenyl ethyl alcohol, citronellol, eugenol, geraniol	$\beta$ -Phenyl ethyl alcohol, citronellol, eugenol, geraniol, linalool, rose oxide	$\beta$ -Phenyl ethyl alcohol, citronellol, eugenol, linalool
HTE = high-temperature extracts; LTE = low-temperature extracts; RD = rose drink. Low-temperature extracts (LTEs), (HTEs), and rose drinks (RDs).			

linalool, citronellol, geraniol,  $\beta$ -phenyl ethyl alcohol, and eugenol were higher, which was consistent with the result of the smelling assessment.

Hence,  $\beta$ -phenyl ethyl alcohol and citronellol were important to the HTE, but eugenol, geraniol, linalool, and rose oxide can also affect its aroma quality.

### 3.3. Results of RD

After analyzing the RD using HS-SPME–GC-MS and GC-olfactometry, the sniffing aromagram and gas chromatogram (Figure 3) were obtained. According to Figure 3, the number of aroma component species was much smaller than that found in the LTE and HTE. In the four-fold diluted RD solution, only four kinds of aroma components were detected. There were four kinds of major aroma compounds in the RD (Table 1), which were different from those of LTE and HTE, as linalool replaced geraniol. Therefore, through sniffing, the main aroma compounds in the RD were found to be  $\beta$ -phenyl ethyl alcohol, eugenol, citronellol, and linalool.

As the number of aroma species detected in the RD was much smaller than that found in the LTE and HTE, their content in the RD must be less than that in the LTE and HTE, in agreement with the data shown in Table 4. Compared with Tables 2 and 3, the  $\beta$ -phenyl ethyl alcohol and citronellol contents in the RD were lower than those in the LTE and HTE. The contents of the geraniol and the linalool in the RD were almost the same. Affected by the odor threshold, the OAV value of linalool was much larger than that of geraniol, so at the same concentration linalool was more detectable, which proved that the smelling result in this study was reliable.

According to the result of the sniffing and OAV value, the main aroma compounds affecting the quality of the RD were  $\beta$ -phenyl ethyl alcohol, citronellol, eugenol, and linalool.

## 4. Conclusion

The main aroma components of rose-based products were investigated using HS-SPME–GC-MS combined with an olfactometry measurement. The OAV value was calculated according to the Guadagni theory, and the contribution to the flavor of the sample was compared. The results confirmed that the two methods can effectively determine the key aroma compounds of rose-based products (Table 5).

The key aroma compounds of the LTE are  $\beta$ -phenyl ethyl alcohol, citronellol, geraniol, and eugenol. The main aroma compounds in the HTE are  $\beta$ -phenyl ethyl alcohol, citronellol, geraniol, eugenol, linalool, and rose oxide. The four major aroma compounds in the RD are  $\beta$ -phenyl ethyl alcohol, eugenol, geraniol, and linalool.

## Conflicts of interest

All authors declare no conflicts of interest.

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