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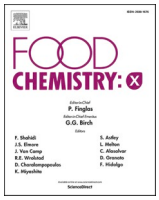

Characterization of aroma compounds in *Rosa roxburghii* Tratt using solvent-assisted flavor evaporation headspace-solid phase microextraction coupled with gas chromatography-mass spectrometry and gas chromatography-olfactometry

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ABSTRACT

Rosa roxburghii Tratt (RRT) has become popular owing to its high vitamin C content. Volatiles are important factors that affect the quality of RRTs and their processed products. In this study, volatile compounds were extracted using headspace-solid phase microextraction (HS-SPME) and solvent-assisted flavor evaporation (SAFE); 143 volatile compounds were identified by gas chromatography-mass spectrometry (GC–MS), and RRT from different origins were well distinguished based on principal component analysis. 45 odor-active components were identified using gas chromatography-olfactometry (GC-O). Through quantitative descriptive analysis (QDA), there were prominent "grassy" and "tea-like" attributes in RRT. Partial least-squares regression (PLSR) revealed that Longli RRT was greatly related to "tea-like" and "woody" attributes. Among the volatiles identified, alcohols and esters were considered the dominant volatile compounds of RRT, 4-methoxy-2,5-dimethyl-3(2H)-furanone was the most prominent compound. This study enriches the flavor chemistry theory of RRT and provides a scientific basis for optimizing the aroma of RRT and its processed products.

Introduction

Rosa roxburghii Tratt (RRT) is the fruit of the perennial deciduous shrub Saosi flower of the Rosaceae family, which is mainly produced on the Yunnan-Guizhou Plateau and the western plateau of Sichuan. The flowers, leaves, fruits and seeds of RRT are used as medicine. RRT fruit contains a variety of essential amino acids as well as superoxide dismutase, and its vitamin C content is 5–759 times higher than that of common fruits and vegetables (Gong et al., 2016). Studies have shown that RRT has many functions including antioxidant, anti-inflammatory, anti-aging and immune regulation (Li et al., 2022). With the increasing demand for health foods, RRT has received increasing attention because of its potential health benefits; however, fresh RRT fruit is rarely eaten directly due to the astringent taste, so it is often processed into juice drinks, fruit vinegar, wine, jam, oral tonics and other foods (Ikram, Remedios, Rachid, & Enrique, 2022; P.R. et al., 2022; Du, Ma, & Tian, 2018; Yu et al., 2022). In addition, aroma is one of the most appreciated characteristics of any fruits including RRT and can influence the sensory experience and acceptability, selection preference of consumers (Defilippi, Manrquez, Luengwilai, & Gonzlez-Agero, 2009; Selli, Gubbuk, Kafkas, & Gunes, 2012), it is very important in evaluating the nutritional value and freshness of food (Huang et al., 2022). Aroma-rich foods are often more attractive, which can greatly stimulate the purchasing power of consumers. Therefore, the analysis of volatile components can help to understand the flavor of RRT and control the quality of its products; however, research related to RRT has mostly focused on the

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development of functional foods and the analysis of its bioactive function and only a few studies have considered the flavor of RRT.

The earliest RRT flavor research scholars, such as Liang, found more than 130 ingredients after studying the flavor substances of fresh RRT fruits harvested from Guiyang and Longli and characterized more than ten types of substances, including leaf alcohol and its esters, butyl benzoate, linalool, octanol, and *trans*-2-hexenol as important compounds in RRT (Liang, Han, Chen, & Shi, 1992). Lin et al. analyzed the changes in flavor substances before and after the fermentation of RRT, and the results showed that 98 volatile components were detected in RRT before fermentation (Lin, Wang, Yang, Yang, & Zhang, 2020). Zhou et al. studied the free volatiles and O-glycosidic bonding volatiles in wild RRT juice from Exi Xuan'en and identified 38 free volatile components (Zhou, Ma, Zhu, Chen, & Pan, 2015). Niu et al. studied volatile compounds of RRT using gas chromatography-olfactometry and odor activity values, and the results showed that ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methylbutyrate, and ethyl hexanoate were present with much higher odor activity values (OAVs) than other compounds (Niu et al., 2021). Qingyang Li et al. compared the volatile components of RRT at different altitudes and picking periods in Guizhou (Li et al., 2022). Li et al. used HS-SPME combined with SAFE to identify and analyze potential aroma-presenting compounds in RRT (Li, Huang, Tang, Li, & Lou, 2021). Huang et al. studied the volatile components in RRT from five locations in Guizhou using HS-SPME (Huang, Li, Hardie, Tang, & Li, 2022). Most of these studies have attached importance to the volatile components of RRT using a single extraction technique, but there is less information on odor-active compounds derived from a combination of two complementary extraction techniques.

Headspace solid-phase microextraction is a nonsolvent selective extraction method that has been widely used for food flavor analysis, whereas solvent-assisted flavor evaporation (SAFE) is an extraction technique that can efficiently extract and separate volatile compounds. SAFE is an aroma extraction method that enriches food volatile components by freezing at low temperatures under high vacuum conditions with appropriate solvents (Engel, Bahr, & Schieberle, 1999), and the aroma of the extracts obtained by applying both methods to flavor analysis can best represent the true aroma of food. Molecular sensory science is a technology that combines human perception ability with modern instruments to qualitatively, quantitatively and describe flavor at the analytical level (Veronika & Peter, 2007). It has been widely used in the flavor research of many foods like millet huangjiu and flat peach juices (Tan, Wang, Zhan, & Tian, 2021; Ye, Wang, Zhan, Tian, & Liu, 2022). In previous studies, we identified and analyzed the volatilities of RRT from different production areas (Huang, Li, Haridie, Tang, Li, 2022). Based on this, we extracted volatile components from RRT in two complementary ways, and further identified potential odor active compounds in RRT by GC-O, which will help improve the theoretical flavor system of RRT as well as flavor maintenance and optimization in the processing of RRT products.

Materials and methods

Materials and reagents

Rosa roxburghii Tratt (Guinong No.5) were collected from four production areas in Guizhou Province (Shuicheng County, SC; Zunyi City, ZY; Longli County, LL; and Dafang County, DF) in August 2021; the plants were artificially planted and the fruits were fresh without mold or spoilage and had reached commercial maturity. During the ripening period in the four production areas, well-developed RRT plants with similar growth were selected and fruit of uniform size, normal shape, and with no pests, diseases, or damage were randomly collected from all parts of the plant and mixed. Sodium chloride (95% purity) and ethanol (95% purity), were supplied by Guizhou Boaoruijie Biotechnology Co. Ltd. (Guizhou, China), Cyclohexanone (99% purity) and C7-C30 *n*-alkanes were obtained from SigmaAldrich Chemical Co., Ltd..

Instruments and equipment

The following equipment was used for processing and analysis: TQ8040NX Gas Chromatograph Mass Spectrometer (Shimadzu, Japan); AOC-6000 autosampler with a PAL automatic solid-phase microextraction device and a 1 cm-50/30 μm DVB/CAR/PDMS fiber head (CTC, Switzerland); DF-101S heat-gathering thermostatic heating magnetic stirrer (Gongyi Yuhua Instrument Co. Ltd.); custom solvent-assisted flavor evaporation device (Glasbläserei Bahr, Germany); custom Weyl distillation column (Jiangsu San Aisi Scientific Instruments Co. Ltd); and a DL-5 M low-speed, large-capacity refrigerated centrifuge (Hunan Pingfan Technology Co.).

Methods

Preparation of RRT juice

Fresh and ripe RRT fruits without mold or spoilage were selected, and after removing the calyx and stems, washing, draining and cutting, the juice was extracted. The juice was centrifuged at 4,000 r/min for 5 min at 5 ℃, and the supernatant was extracted and frozen at −20 ℃.

Quantitative sensory descriptive analysis of RRT juice

Ten evaluation panel members (5 males and 5 females aged 22–30 with no smoking history) with experience in sensory analysis tasting were recruited from the fruit wine brewing team at Guizhou Institute of Technology. The sensory evaluation team underwent a month-long sensory training on the 54 aroma kit (Le Nez du Vin®, France) until they could identify each aroma with an accuracy of 95% or higher. The group members discussed the aroma attributes of RRT and selected seven aroma attributes to describe the overall aroma characteristics of RRT and used some of the aroma suites as references for sensory evaluation (chemical standards were substituted if no suitable reference could be found in the aroma suites), including grassy, woody, honey, caramel, tea-like, pear, and floral aromas. The intensity of the seven sensory attributes was assessed with a 5-point intensity scale from 1 (very weak) to 5 (very strong), and each sample was evaluated in triplicate. Five concentration ranges of butanol were configured in this analysis (1 = 640 ppm, 2 = 1280 ppm, 3 = 1920 ppm, 4 = 2560 ppm, 5 = 3200 ppm) and the five concentration levels were carefully selected so that the aroma intensity of these concentrations of butanol could cover the aroma intensity range of all samples. Before the quantitative sensory descriptive analysis, the panilasts sniffed the strength of five butanols as a reference and remembered the strength of each concentration.

Headspace-solid phase microextraction

A mixture of 8 mL RRT juice and 2 μL cyclohexanone (internal standard) was measured into a 20 mL headspace vial, dissolved by adding 2.88 g NaCl and sealed with a PTFE spacer. The mixture was equilibrated at 40 ℃ for 15 min, extracted at 40 ℃ for 30 min, and then inserted into the GC inlet to desorb in nonsplit mode for 2 min.

Solvent-assisted flavor evaporation

Based on a previous method (Li et al, 2021), the raw juice of RRT (200 mL) was measured into a 500 mL partition funnel, 10 μL of cyclohexanone was added, the extracts were combined after three extractions with dichloromethane (50 mL, 40 mL, and 30 mL), 5 g of anhydrous sodium sulfate was added to the extracts for water removal and the extracts were then filtered. The mixture was extracted at 40 ℃ and 1.0×10^{-4} Pa using a SAFE device, concentrated to approximately 2 mL by Weyl distillation, and then concentrated to 500 μL using liquid nitrogen prior to measurement.

GC–MS analysis

The chromatographic conditions were as follows: column, InertCap Wax capillary column (60 m × 0.25 mm, 0.25 μm ID, strong polarity); ramp-up procedure, 40 ℃ for 3 min, increase to 230 ℃ at 3 ℃/min, and

hold for 2 min; carrier gas, He; flow rate, 1.88 mL/min; inlet temperature, 240 ℃; injection volume, 2 μL; split ratio, 5:1; and solvent delay time, 3.8 min.

The mass spectrometry conditions were as follows: electron bombardment (EI) ion source; electron energy, 70 eV; ion source temperature, 230 ℃; mass spectrometry interface temperature, 250 ℃; and mass scan range (m/z), 29 ~ 500amu.

Volatile compounds were identified by comparison with spectra from the National Institute of Standards and Technology mass spectrometry library (Version 14.0); identification was based on greater than 85% similarity, and the retention indices (RIs) were calculated by analyzing a series of *n*-alkanes (C7-C30) under the same chromatographic conditions. The volatile compounds were quantified using cyclohexanone as the internal standard, and the relative content of each compound was obtained by comparison with the area of the internal standard.

GC-O analysis of RRT aroma extraction fractions

GC-O analysis was performed on a Shimadzu TQ8040NX gas chromatograph-mass spectrometer equipped for olfactometry.

The OSME method was based on the method of Qian et al. (Klesk, & Qian, 2003). The time-intensity (OSME) test was performed on an InertCap Wax capillary column. 15 days before the official GC-O analysis, GC-O exercises were carried out on panlists every day. Fruits including RRT were used as exercise samples for GC-O analysis, each time with different duration of about 40–65 min. Meanwhile, 54 aroma kit were used everyday to exercise the cognitive ability and intensity judgment of various aroma types. Six panelists (3 males and 3 females, aged 22–28) with extensive GC-O experience were selected from the quantitative sensory descriptive analysis evaluation team to record retention time, aroma description, and aroma intensity (AI) range from 1 (weakest) to 5 (strongest) for RRT juice. The strength reference is consistent with Section 2.3.2. To reduce the sensory and mental fatigue that can arise during sniffing, each sniffing session was performed by different personnel in a segmented sequence. Each component was repeated 3 times for each person, for a total of 9 evaluations, and the average value of the three evaluators was taken as the final aroma intensity value.

Statistical analysis

A one-way ANOVA was performed on all analyzed data using SPSS 21.0 with a $p < 0.05$ indicating a significant difference. PCA was performed using SIMCA 14.1, and the heatmap was performed using TB tools. PLSR analysis was performed using Unscrambler version 9.8 (CAMOASA, Oslo, Norway). All results in the article are presented in (SAFE, HS-SPME) presentation order. Two replicates were analyzed for SAFE extraction samples, and three replicates were analyzed for all remaining experiments.

Results and discussion

Volatiles of RRT

The heatmap shown in Fig. 1 is based on the volatile substances identified in the RRT juice by the two extraction methods with the color normalization scale from 0 to 1 representing the content of volatile substances in the RRT samples from none to high. As shown in the figure, 90 volatile compounds were identified by SAFE extraction, and 101 volatile compounds were identified by HS-SPME extraction. A total of 143 volatiles were identified, including 21 alcohols, 55 esters, 4 acids, 18 aldehydes, 13 ketones, 10 terpenes, 19 aromatics and 3 furans. 49 compounds were identified by the two methods, including 6 alcohols, 24 esters, 4 acids, 3 aldehydes, 2 ketones, 1 terpenes and 9 aromatic compounds., and the types of volatile substances contained in the four production areas for the respective methods were DF (52, 77), ZY (64, 82), LL (72, 82), and SC (53, 79). As shown in Table 1, the relative

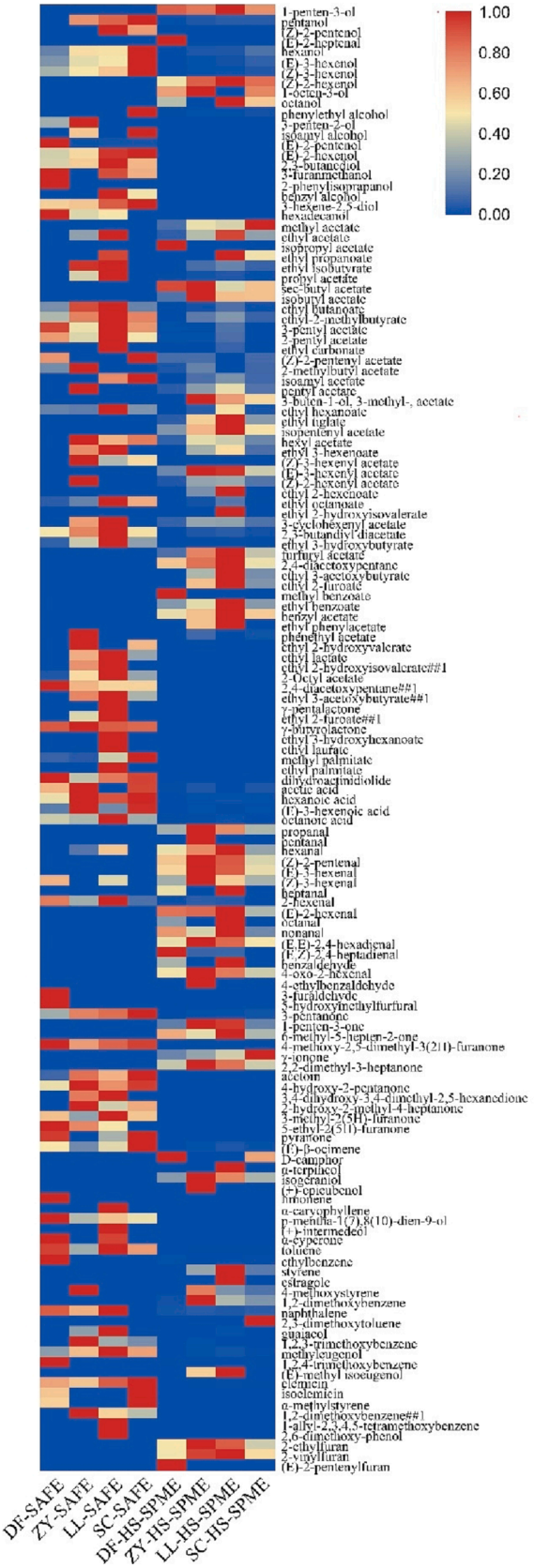

Fig. 1. Heatmap of the volatile substance of RRT juice.

Table 1
Relative content of volatile compounds in RRT.

Num.		**SAFE**/(μg•L^{-1})				**HS-SPME**/(μg•L^{-1})			
		DF	ZY	LL	SC	DF	ZY	LL	SC
1	Alcohols	256477.70 ± 12571.30^{d}	305827.63 ± 50083.12^{c}	419885.32 ± 13521.10^{b}	678155.59 ± 20141.48^{a}	4390.29 ± 172.49^{d}	11437.42 ± 590.91^{c}	13270.24 ± 430.22^{b}	29242.79 ± 1932.95^{a}
2	Esters	53120.51 ± 2781.97^{c}	141089.16 ± 23223.40^{b}	106879.45 ± 4568.62^{a}	109239.99 ± 5402.21^{c}	5335.24 ± 324.91^{d}	24857.77 ± 928.29^{b}	33043.12 ± 580.82^{a}	12352.63 ± 453.10^{c}
3	Acids	16115.98 ± 857.29^{c}	53497.31 ± 8732.66^{a}	26677.45 ± 894.05^{b}	57030.21 ± 5250.35^{a}	80.29 ± 6.79^{c}	454.77 ± 23.38^{a}	351.47 ± 25.87^{b}	394.53 ± 51.80^{b}
4	Aldehydes	62189.48 ± 3863.52^{a}	5506.58 ± 896.59^{c}	21452.19 ± 751.28^{b}	2503.34 ± 84.56^{d}	27910.50 ± 1330.81^{b}	38721.27 ± 591.61^{a}	38759.41 ± 485.11^{a}	15253.12 ± 738.73^{c}
5	Ketones	639624.54 ± 26207.93^{b}	519409.38 ± 84523.23^{c}	654086.62 ± 20958.67^{b}	714938.33 ± 25634.72^{a}	2600.26 ± 191.56^{c}	5523.87 ± 102.64^{a}	5885.63 ± 270.59^{a}	3338.85 ± 203.45^{b}
6	Terpenes	6512.54 ± 553.14^{a}	1189.72 ± 197.45^{d}	5444.23 ± 174.21^{b}	2805.85 ± 142.16^{c}	44.04 ± 3.08^{d}	85.72 ± 30.08^{b}	105.98 ± 8.24^{a}	64.12 ± 16.38^{c}
7	Aromtics	83337.11 ± 3363.45^{d}	91202.72 ± 14898.70^{b}	123465.18 ± 3863.39^{a}	106072.69 ± 4086.53^{c}	181.83 ± 10.78^{d}	1821.00 ± 76.50^{a}	1289.90 ± 66.52^{b}	596.82 ± 46.64^{c}
8	Furans	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	432.26 ± 24.36^{d}	770.16 ± 145.04^{a}	695.73 ± 37.39^{b}	329.48 ± 40.12^{c}

Note: Different lowercase letters represent significant differences ($p < 0.05$).

contents of volatile substances in RRT juice, in descending order, were SC, LL, ZY and DF; the volatile substances in RRT juice included alcohols, esters, acids, aldehydes and ketones, terpenoids, aromatics and furans, the study showed that esters were the most diverse compounds in RRT, which is the same as the previous study, except that this study showed that aldehydes were the most abundant compounds in RRT, whereas the previous study showed that esters and aldehydes were the most abundant compounds in RRT (Huang, Li, Haridie, Tang, & Li, 2022). This composition may reflect hydroperoxide decomposition during the storage of RRTs.

Alcohols are an important group of volatile compounds in RRT. 16 alcohols were detected by the SAFE method with 6 common to all four origins, and 11 were detected by the HS-SPME method with 7 common to the four origins. A range of 256477.70 μg•L^{-1} (DF) to 663291.01 μg•L^{-1} (SC) was detected by the SAFE method and 4390.29 μg•L^{-1} (DF) to 29242.79 μg•L^{-1} (SC) was detected by the HS-SPME method, indicating that the SAFE method is better for the extraction of alcohols, which is consistent with the results of Gong et al. (Gong, 2016). The alcohols mostly had mellow and fruity aromas (Fan & Qian, 2006), which significantly affected the overall flavor presentation of RRT. (Z)-3-hexenol was the most abundant alcohol in the RRT volatiles and has a pleasant flavor of fresh grass and herbs (Buettner, 2019). Higher alcohols such as isoamyl alcohol and phenylethyl alcohol, which are the main components of hetero or higher alcohols, have also been detected in RRT juice. These volatile alcohols typically originate from the oxidation of fatty acids and the degradation of amino acids (Schwab Wilfried, 2008) and can also be synthesized by yeast via the amino acid anabolic pathway (Wang, Chen, Yang, Wang, & Wang, 2019). The type and content of these higher alcohols influence the aroma and taste of RRT juice, and because they have good solvent properties (Li, 2020)), they can dissolve other volatile components and thus give RRT juice a distinctive flavor.

Esters are the most volatile substances in RRT. The esters in RRT are usually derived from straight chain or branched chain carboxylate esters derived from fatty acid and amino acid pathways (Gong, 2016; Li, Wang, Li, & Li, 2019; Liu, Wang, Han, & Sun, 2016). These esters can give RRT a floral and fruity aroma. Some esters may be perceived due to the reinforcement of other substances and therefore may contribute to the flavor of RRT even when below threshold levels of detection (Meilgaard, 1975). The SAFE and HS-SPME extraction methods identified 37 and 41 esters, respectively, in RRT juice; all RRTs were rich in ethyl esters with SAFE extracting more ethyl esters than HS-SPME (6499.66 μg•L^{-1} in DF, 37413.05 μg•L^{-1} in ZY, 83387.29 μg•L^{-1} in LL and 17743.33 μg•L^{-1} in SC). The higher levels of ethyl esters typically presented a pleasant cheesy, fruity flavor that made the RRT juice more aromatic, and this may partly explain the similarity of the fruity aroma characteristics in the RRT juice of the four production areas. Hexenyl (Z)-3-acetate was the most abundant ester in RRT, reaching 53003.98 μg•L^{-1} in the ZY samples extracted using the SAFE method and giving the juice a fruity, green and hyacinth-like aroma profile.

The formation of aldehydes and ketones is generally due to the oxidation of alcohols or the reduction of acids, although some are derived from sugar metabolism (Zapata, Mateo Vivaracho, Cacho, & Ferreira, 2010). 14 and 22 aldehydes and ketones were identified by the SAFE and HS-SPME methods, respectively. The aldehydes and ketones were the most abundant volatile substances in the RRT juice, among which the SAFE method identified from 524915.96 μg•L^{-1} (ZY) to 7117441.67 μg•L^{-1} (SC), which indicated that the SAFE method was better at extracting aldehydes and ketones. The most abundant compound in this category was 4-methoxy-2,5-dimethyl-3(2H)-furanone, which had the highest content of all the volatile compounds in RRT. Studies have shown that after the intermediate 4-hydroxy-5-methyl-2-methylene-3(2H)-furanone is generated by hexose diphosphate, strawberry ketone oxidoreductase catalyzes the substrate to form furanone (Wu, Zhang, Qiang, Zhang, & Zhan, 2021), and methyltransferase converts furanone to methoxy furanone (Silvia, 2021). 4-Methoxy-2,5-dimethyl-3(2H)-furanone has a pleasant caramel, roasted aroma, and therefore may be one of the compounds responsible for the “caramel” aroma in RRT. The aldehydes and ketones (Z)-3-hexenal, 2-hexenal and hexenal have a cool, grassy flavor, and therefore may be the flavor basis for the grassy aroma characteristic of RRT.

Acids, terpenoids and aromatic substances were also identified in RRT. The acid contents of RRTs from different production areas were similar, but (E)-3-hexenoic acid, a decomposition product from the autoxidation of (E)-2-hexenal, was much higher in ZY and SC than RRTs from DF and LL (Fischer & Grosch, 1991). Octanoic acid was present at similar levels in three of the regions, except for a slightly higher level in LL, where it can give off a cheese and cream-like flavor at low concentrations. Guaiacol was detected in RRT from ZY, and LL was considered to have ’smoky’ aroma profile in this study.

Principal component analysis of volatile components of RRT from different production areas

To better investigate the differences in the volatile components of RRT juice extracted by the two methods, a PCA biplot model was constructed for the volatile aroma components. The gray circles in the figure represent the volatiles identified in the RRT juice, and the hexagons with different colors represent the RRT juice samples from different production areas. As shown in Fig. 2, the volatile aroma components of RRT

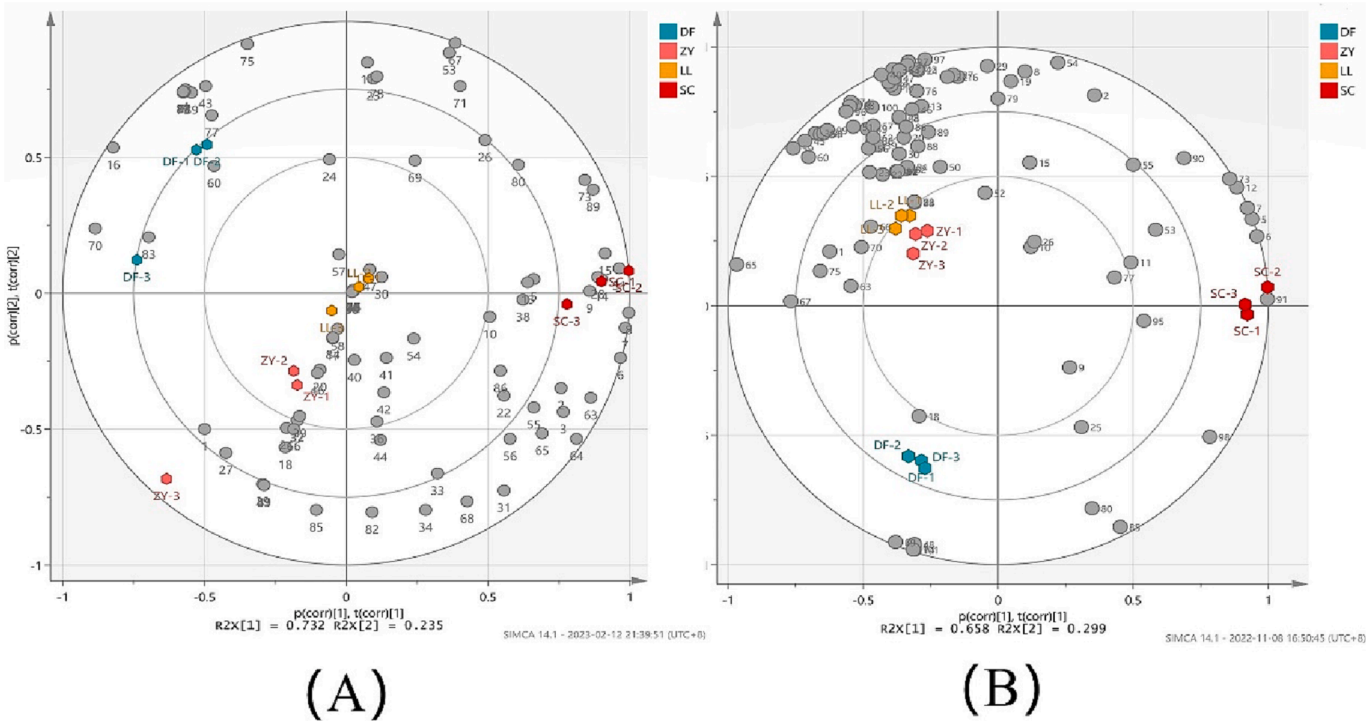

Fig. 2. PCA analysis of the volatile substance of RRT juice (A: extracted by SAFE; B: extracted by HS-SPME).

varied significantly between the different production areas. The graph shows that the RRT components from the four production areas extracted by the SAFE method were divided into three separate clusters, but the volatiles of RRT from the LL and ZY production areas were similar. The RRT extracted using the HS-SPME method was also divided into three clusters with the volatiles from the ZY and LL production areas clustered together and the volatiles from DF and SC clustered together, which was consistent with the PCA results of the SAFE method. The PCA plots of the two extraction methods show that the volatile substances extracted by the two extraction methods are similar, although the PCA plots show that there are significantly more volatile aroma components in the RRT from the DF and ZY production areas than in the other production areas, and the esters (17–53) have a significant influence on this difference. For the SAFE extraction of RRT from SC, the compounds with a greater contribution included (Z)-3-hexenol (14), (E)-2-hexenol (E)-2-hexenol (15), ethyl isobutyrate (28), ethyl caprylate (51), and (E)-2-hexenol (9). For the HS-SPME extraction of RRT from SC, hexanol (5) and (E)-3-hexenol (6) had high contributions; combined with Fig. 1, it can be seen that the DF RRT contained fewer volatile substances, particularly *sec*-butyl acetate (18), which showed a relatively high correlation with the DF RRT extracted by the HS-SPME method. Environmental factors including altitude, temperature and sunlight, as well as rainfall, have an impact on fruit development and composition (Li et al., 2022). However, the data showed that the mean temperature differences between the four producing areas in 2019 were small, and the study suggests that rainfall may be the cause of the differences in volatile compounds between the four producing areas (Huang, Li, Haridie, Tang, & Li, et al., 2022).

Quantitative sensory descriptive analysis of RRT juice

Sensory descriptors were constructed for RRT from the four production areas. As shown in Fig. 3, seven attributes were used to describe the aroma profile of RRT, including 'grassy', 'woody', 'honey', 'tea-like', 'pear', 'caramel' and 'floral'. The results showed that the sensory description of RRT varied somewhat between the different production areas. Among the aromatic characteristics of RRT, the 'grassy' attribute

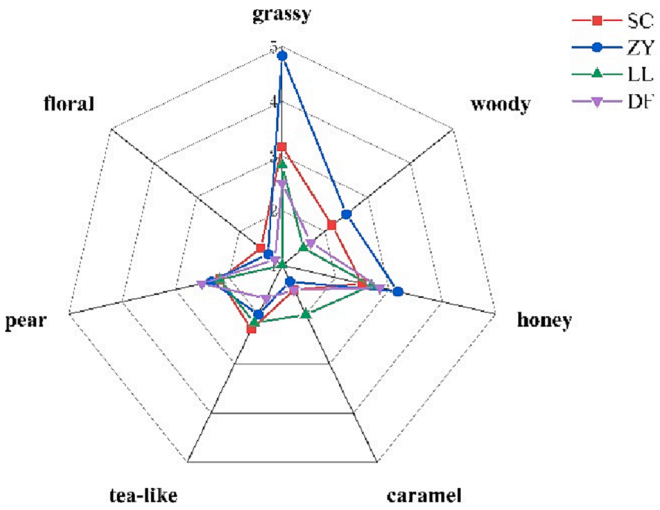

Fig. 3. Quantitative descriptive analysis of RRT juice.

was the most prominent, and the 'honey' and 'pear' attributes also played major roles, followed by the 'tea-like', 'woody', 'caramel' and 'floral' attributes, there are some differences between previous studies that showed The aroma was predominately 'fruity' combined with 'floral' attributes, however, it exhibited similar scores in "woody" attribute (Huang, Li, Haridie, Tang, & Li, 2022), this may be due to the fact that RRT contains different levels of volatile compounds at different times of harvest, leading to different sensory properties.. The "grassy" attribute and "woody" attributes of RRT were significantly higher in ZY than those of other production areas, but the "caramel" attribute was lower than those of other production areas. However, the sensory attributes of DF RRT juice were all low. DF RRT and LL RRT scored similarly on the 'woody' attribute, and the 'pear' attribute scores were similar for RRT from all four production areas.

Analysis of odor-active substances identified by GC-O

A total of 45 odor-active compounds were identified by GC-O analysis in combination with the OSME method, of which 26 were identified by the SAFE method and 37 were identified by the HS-SPME method. The aroma description of each compound was also determined during the olfactory analysis (Table 2). The odor-active compounds included 3 alcohols, 16 esters, 2 acids, 15 aldehydes and ketones, 3 aromatics and 6 unknown compounds. The compound with the highest aroma intensity among the volatiles identified by both extraction methods was 4-methoxy-2,5-dimethyl-3(2H)-furanone, and other compounds with high AI values in DF RRT were (Z)-3-hexenol (4.3, 1.7), furfuryl alcohol (4.7, -), ethyl 2-methylbutyrate (3.2, 3.2), ethyl caprylate (4.3, 3.8) and (Z)-3-hexenal (3.8, 2.5); although the furfuryl alcohol had an aroma intensity of up to 4.7, this compound was detected only using the SAFE method. Compounds with large AI values in ZY RRT included ethyl 2-methylbutyrate (4.7, 3.7), ethyl octanoate (4.3, 4.7), (Z)-3-hexenal (4.3, 1.8), and ethyl butyrate (3.8, 2.8); compounds with large AI values in LL RRT included ethyl 2-methylbutyrate (4.8, 4.8), ethyl octanoate (4.8, 5), and (Z)-3-hexenol (4.5, 2); and compounds with large AI values in SC RRT included (Z)-3-hexenol (4.5, 3), ethyl 2-methylbutyrate (4.5, 3), and 2,3-butanediol, but they were detected only by the SAFE method. As seen from Table 2, the aroma active compounds in RRT are almost all esters or aldehydes and ketones, which indicates that these types of compounds play an important role in the aroma of RRT. Combined with the aroma description and sensory evaluation, it was found that these two types of compounds mainly impart ‘floral’ and ‘fruity’ aromas to RRT.

4-Methoxy-2,5-dimethyl-3(2H)-furanone had the highest AI values (5) among the RRT compounds from all four production areas as identified by both extraction methods and was also shown to be an important aroma component in pineapple rind (Zheng et al., 2013) and in the strawberry variety ’Princess Rose’ (Wang et al., 2018), with a very strong roasted flavor. Huang et al. (Huang, Li, Haridie, Tang, & Li, 2022) also identified significant amounts of 4-methoxy-2,5-dimethyl-3(2H)-furanone in RRT juice, which is considered to be one of the most important aroma active compounds in RRT. In addition, volatiles including ethyl butyrate, ethyl 2-methylbutyrate, ethyl caprylate, 1-methylheptyl acetate, hexanoic acid, 4-methoxy-2,5-dimethyl-3(2H)-furanone and so on were identified in two extraction methods from four sites and had high AI values, so they may also be important contributors to the aroma of RRT. Hexanoic acid and (E)-3-hexenoic acid are two acids identified as odor-active compounds in RRT juice, which give the juice a fruity and cheesy aroma. Hexanoic acid had a high AI value in both ZY and SC RRT, and (E)-3-hexenoic acid was detected exclusively in ZY and SC RRTs but also had a high AI value, suggesting that the cultivation conditions in ZY and SC may be more suitable for the formation of acids in RRT. Guaiacol was the only aromatic substance identified as an odor-active compound that was not detected in DF RRT juice but had high AI values in RRT from the other three locations. Guaiacol has a burnt, smoky aroma. 1-Penten-3-one was detected only in ZY and LL by HS-SPME and can be produced via the lipoxygenase pathway (Wang, Li, Hu, & Zhao, 2022); it has a slightly irritating odor, therefore, it may not be conducive to the pleasant aroma of RRT. 2-Hydroxy-2-methyl-4-heptanone and 3-methyl-2(5H)-furanone are ketone-like odor-active compounds common to the four production areas, and their formation may be related to the high carotenoid content in RRTs; studies suggest that methyl ketones may be formed by fatty acid oxidation (Sato, 2022; Ye, He, He, Zhang, Liu, Zhang., 2022). Six unknown compounds were also detected that were sniffed but not identified, probably due to a low threshold of perception by humans; these compounds mainly presented ‘green’ and ‘fruity’ aromas and were therefore tentatively judged to be low threshold trace aldehyde esters.

Correlation analysis of odor-active compounds, sensory attributes and RRTs from different origins

A partial least squares regression (PLSR)-biplot model was developed to further investigate the correlation between the identified odor-active compounds and the sensory attributes of the RRT samples from different origins. The blue circles in the figure represent RRT samples from different production areas, whereas the red circles represent the six sensory attributes and the odor-active compounds identified by GC-O. In the graph, odor-active substances that are closer to the sensory attributes indicate a stronger correlation between the substances and the sensory attribute; conversely, a greater distance between the substance and the attribute indicates a weaker correlation. From the distribution in Fig. 4, it can be seen that the differences in odor-active substances in the RRTs from the four production areas (extracted by the SAFE method) are significant, and LL RRT appears to be associated with the aroma characteristics of ‘caramel’, ‘woody’ and‘tea-like’ as are the odor-active compounds such as 2-pentyl acetate (12) and 3-methyl-2(5H)-furanone (21). The “grassy” attribute and odor-active compounds such as furanone acetate (13), acetic acid (14) and (E)-3-hexenoic acid (15) were closely correlated, whereas the DF RRT was closely correlated with 4-methoxy-2,5-dimethyl-3 (2H)-furanone (19), which seems weakly correlated with most of the other aroma attributes. the high content of hexanal and (Z)-3-hexenal in the samples all explains the “grassy” attributes in the RRT. Using the HS-SPME method, DF RRT showed a strong correlation with (E, Z)-2,4-heptadienal (22), whereas SC RRT was highly correlated with the “floral” attribute, and ZY RRT juice showed a strong correlation with the “grassy” attribute. LL RR was highly correlated with several odor-active compounds such as ethyl butyrate (5), ethyl caprylate (11), and 4-methoxy-2,5-dimethyl-3(2H)-furanone (28). These compounds give RRT its fruity and sweet aroma characteristics. The contents of other volatile substances in the RRT juices from different production areas also had various similarities and differences.

Conclusions

In this study, the volatile components in RRT were extracted by the combination of HS-SPME and SAFE, which can more comprehensively characterize the volatile compounds in RRT. The results indicated that 143 volatile substances were identified in RRT juice from four production areas by using GC–MS analysis, and a total of 45 odor-active substances were identified by OSME combined with GC-O analysis. Esters, alcohols and aldehydes were the three most important types of volatile substances in the RRT aroma. The results of the principal component analysis showed that the volatile substances differed significantly between different locations, with LL and SC RRTs being the richest in volatile substances. The results of the quantitative descriptive analysis showed that the ‘grassy’, ‘honey’ and ‘tea-like’ attributes were the most prominent aroma characteristics in RRT, and the ‘grassy’ attribute was most prominent in ZY RRT. GC–MS combined with GC-O showed that 4-methoxy-2,5-dimethyl-3(2H)-furanone was one of the most important compounds in RRT, as it had the highest content and AI value and thus gave the ‘caramel’ and ‘roasted’ aromas to RRT. 2-Methylbutyric acid ethyl ester, octanoic acid ethyl ester, butyric acid ethyl 2-methylbutyrate, ethyl caprylate, ethyl butyrate and (Z)-3-hexenol are also important odor-active substances in RRT. In addition, it is inferred from the GC-O results that acids may partially explain the difference in the flavor of RRTs from ZY and SC compared with those from DF and LL. The PLSR analysis of odor-active compounds, sensory attributes and the correlation analysis of RRTs from different production areas revealed that there were some prominent aroma characteristics and a strong correlation with certain volatile substances in RRT from different production areas; for example, LL RRT was strongly correlated with the ‘tea-like’ attributes of guaiacol. The results of this study enrich the theoretical basis of flavor chemistry in RRT, provide a new understanding of the aroma qualities of RRT, and provide a basis for the optimization of RRT

Table 2
Aroma-active compounds in RRT identified by GC-O.

No.	compounds	RI		CAS[b]	Description of aroma	Identification[c]	Aroma Intensity[d]				Aroma intensity[ed]			
		Inertcap wax	literture[a]				DF	ZY	LL	SC	DF	ZY	LL	SC
1	Leaf alcohol	1380	1389	928–96-1	Vegetables, grassy	MS, R, A	4.3	4.3	4.5	4.5	1.7	1.8	2.0	3.0
2	2,3-Butanediol	1352	1541	513–85-9	Markers-like, fruity, buttery	MS, R, A	2.7	4	4.5	4.3	—	—	—	—
3	Furaneol	2026	2028	3658–77-3	Caramel-like, bakery	MS, R, A	4.7	5.0	5.0	5.0	—	1.5	—	1.8
4	Ethyl isobutyrate	964	961	97–62-1	Fruity, sweet	MS, R, A	—	3.5	4.0	3.0	1.2	1.7	2.0	1.7
5	Isobutyl acetate	1013	1014	110–19-0	Fruity, sweet	MS, R, A	—	—	—	—	—	2.0	1.7	1.5
6	Ethyl butyrate	1034	1039	105–54-4	Fruity, sweet	MS, R, A	1.7	3.8	4.0	2.0	2.2	2.8	3.7	2.5
7	Ethyl 2-methylbutyrate	1049	1051	7452–79-1	Fruity, sweet	MS, R, A	3.2	4.7	4.8	4.5	3.2	3.7	4.8	3.0
8	3-pentyl acatate	1067	—	620–11-1	Fruity	MS, A	3.5	2.3	3.8	2.5	1.7	2.2	4.0	2.2
9	Ethyl Hexanoate	1230	1233	123–66-0	Sweet, fruity, pineapple	MS, R, A	—	—	1.7	—	—	1.7	2.3	1.5
10	Ethyl 3-hexenoate	1301	1304	2396–83-0	Mushroom, sweet	MS, R, A	—	1.5	—	1.2	1.7	2.5	3.2	1.8
11	(E)-3-hexenyl acetate	1329	1321	3681–82-1	Banana, hyacinth	MS, R, A	—	—	—	—	1.2	2.8	2.7	1.7
12	Ethyl lactate	1338	1337	97–64-3	Acidic odor, cheese	MS, R, A	—	—	2.2	—	—	—	—	—
13	Ethyl caprylate	1426	1425	106–32-1	Bouquet, fruity	MS, R, A	4.3	4.3	4.8	4.7	3.8	4.7	5.0	4.7
14	1-methylheptyl acetate	1522	1493	2051–50-5	Fruity, green pepper	MS, R, A	2.3	2.7	3.3	2.5	4.3	3.5	4.5	3.7
15	Furaneol acetate	2251	—	4166–20-5	Dry red date, caramel	MS, A	—	1.3	—	—	—	—	—	—
16	Furfuryl acetate	1539	1539	623–17-6	Herbal, sweet	MS, R, A	—	—	—	—	1.3	2.3	2.5	1.2
17	2,4-diacetoxy pentane	1543	—	7371–86-0	Vegetables, grassy	MS, A	—	—	—	—	1.2	2.0	2.2	1.2
18	hexyl 3-methyl-2-butenoate	1561	1572	17627–41-7	Fruity, cheese	MS, R, A	—	—	—	—	1.7	—	1.8	—
19	Phenethyl acetate	1818	1822	103–45-7	Rose, honey	MS, R, A	—	—	—	—	—	1.3	1.0	1.0
20	Hexanoic acid	1836	1841	142–62-1	Orange, lemon, fatty	MS, R, A	1.3	3.0	2.8	3.0	2.0	2.3	2.5	2.5
21	3-Hexenoic acid	1947	1948	1577–18-0	Acidic odor, sweat, cheese	MS, R, A	—	3.0	—	2.5	—	—	—	—
22	Hexanal	1081	1083	66–25-1	Vegetables, green	MS, R, A	—	—	1.3	—	1.7	2.0	3.0	1.5
23	(E)- 3-hexenal	1140	1140	69112–21-6	Green apple	MS, R, A	—	—	—	—	2.0	2.7	2.8	2.0
24	(Z)- 3-hexenal	1143	1146	6789–80-6	Vegetables, green	MS, R, A	3.8	—	3.7	—	2.5	2.7	2.7	2.5
25	(E)-2- hexenal	1224	1224	6728–26-3	Green, banana, cheese	MS, R, A	3.8	—	3.7	—	1.3	1.5	1.7	—
26	(E, Z)-2,4-heptadienal	1466	1459	4313–2-4	Green, leaves, vegetables	MS, R, A	—	—	—	—	1.7	1.0	1.5	1.0
27	Benzaldehyde	1532	1534	100–52-7	Sweet, fruity	MS, R, A	—	—	—	—	—	1.5	3.0	2.0
28	2,5-Dimethyl benzaldehyde	1711	1705	5779–94-2	Tea-like, mint, fresh	MS, R, A	—	—	—	—	1.7	2.0	1.8	1.0
29	2,4-Dimethyl benzaldehyde	1820	/	15764–16-6	Floral, tea-like	MS, A	—	—	—	—	1.7	1.3	2.0	1.8
30	Ethyl vinyl ketone	1022	1020	1629–58-9	Green, pungent odor	MS, R, A	—	—	—	—	—	1.5	1.5	—
31	4-*sec*-butoxy-2-butanone	1585	—	57545–63-8	Cucumber	MS, A	—	—	—	—	2.3	1.7	2.8	1.5
32	3-Pentanone	978	978	96–22-0	stink	MS, R, A	—	2.3	2.5	2.8	—	4.0	2.0	2.0
33	4-Methoxy-2,5-dimethyl-3(2H)-furanone	1595	1604	4077–47-8	Bakery, caramel, cocoa	MS, R, A	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
34	1,2-Dimethoxy benzene	1726	1727	91–16-7	Herbal, incense, vanilla	MS, R, A	—	—	—	—	1.7	1.7	1.8	1.8
35	2-hydroxy-2-methyl-4-heptanone	1666	—	54862–91-8	Acidic odor, cheese	MS, A	1.2	2.7	2.3	2.2	2.0	4.0	4.0	4.0
36	3-methyl-2(5H)-furanone	1723	1713	22122–36-7	Vegetables, leaves, mint	MS, R, A	2.7	2.8	3.0	3.0	4.0	2.0	2.8	4.0
37	Naphthalene	1747	1749	91–20-3	insects	MS, R, A	—	—	—	—	1.0	1.0	1.3	1.5
38	Guaiacol	1858	1859	1990–5-1	Empyreuma, smoky	MS, R, A	—	3.3	3.7	3.0	—	2.0	4.0	2.0
39	Methyl eugenol	2013	2031	93–15-2	Raw soybean	MS, R, A	—	—	—	—	—	2.3	2.5	1.5
40	Unknown compound 1	1373	—	—	grassy	MS, A	2.5	2.3	2.8	1.3	4.3	3.8	5.0	2.5
41	Unknown compound 2	1440	—	—	milk	MS, A	—	—	—	—	1.8	1.2	2.0	—
42	Unknow compound 3	1444	—	—	fruity	MS, A	—	1.5	2.5	1.3	—	1.0	1.2	—
43	Unknown compound 4	1497	—	—	Vegetables, herbal	MS, A	2.0	1.5	1.3	1.2	—	—	—	—
44	Unknown compound 5	1597	—	—	Sweet, fruity	MS, A	1.0	2.2	1.0	1.0	—	—	—	—
45	Unknown compound 6	1728	—	—	Leaves, herbal	MS, A	—	—	—	—	—	—	1.2	—

[a] represents the retention index from literature on the website - https://webbook.nist.gov/chemistry/, — RI of the compound can not be found from the literature ; .
[b] —,the CAS of the unknown compound is not available.
[c] MS respresented that the mass spectrum agreed with those of the authentic compound; RI represented that the compound was confirmed by retention index of the compound standard ; A meant that the compound was identified by the reported aroma ; .
[d] Aroma intensity of volatiles extracted by SAFE; [e] Aroma intensity of volatiles extracted by HS-SPME; — represents that the compound was not perceived at the sniffing port.

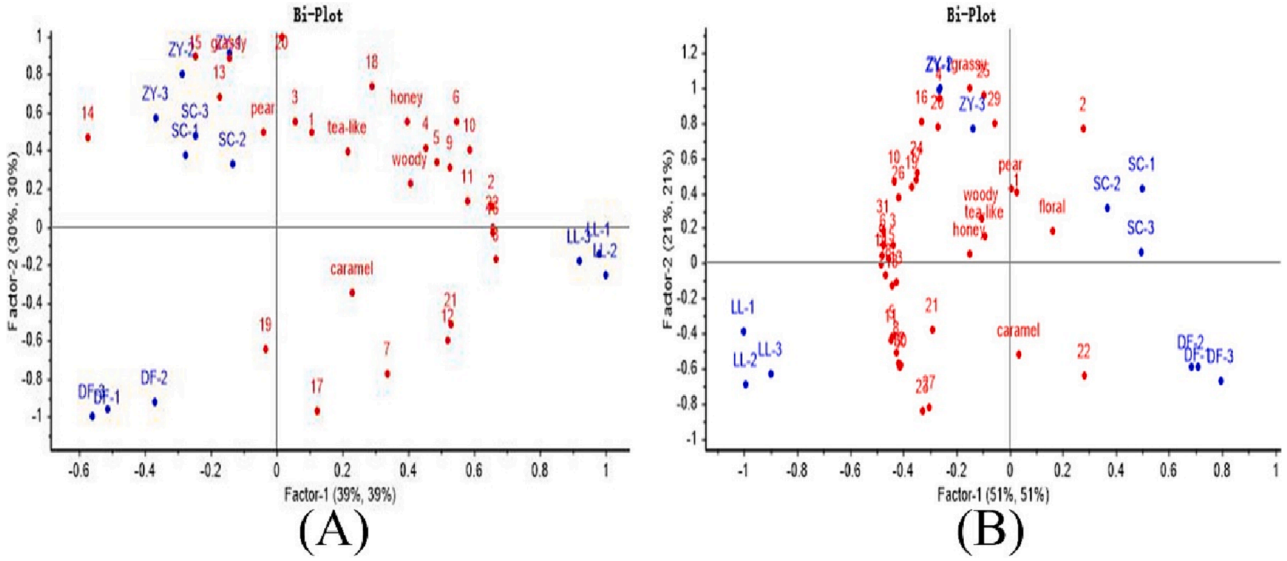

Fig. 4. PLSR analysis of the volatile substance of RRT juice (A: extracted by SAFE; B: extracted by HS-SPME).

products; however, the chemical nature of some unknown compounds detected in the study and the identification of key aroma compounds in RRT are still issues in the study of RRT volatile substances that need further exploration.

CRediT authorship contribution statement

Xiaofang Sheng: Writing – original draft, Writing – review & editing, Investigation, Methodology, Software, Formal analysis. **Mingzheng Huang:** Validation, Conceptualization, Funding acquisition. **Tingting Li:** Conceptualization, Methodology, Supervision. **Xin Li:** Validation, Conceptualization. **Shunyou Cen:** Conceptualization. **Qinyang Li:** Investigation, Methodology, Conceptualization. **Qun Huang:** Conceptualization. **Weiyuan Tang:** Validation, Conceptualization, Funding acquisition.

Declaration of Competing Interest

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Data availability

No data was used for the research described in the article.

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