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Research Article

GC-O-MS Analysis of Aroma-Active Compounds of Chinese Almonds Obtained by Different Pretreatment Methods

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Almonds (*Prunus dulcis*) are an ancient and commercially valuable crop from Asia. In this experiment, solid-phase microextraction (SPME), solvent-assisted flavor evaporation (SAFE), and simultaneous distillation extraction (SDE) were used to pretreat the almonds from China. The almond extract was then analyzed by aroma extract dilution analysis (AEDA) with gas chromatography-olfactory-mass spectrometer (GC-O-MS). SPME and SAFE were identified as the better extraction methods, and 52 aroma-active compounds of almonds, such as 1-nonanal, eugenol, and linalool, were identified based on odor activity values (OAVs). Aroma recombination and omission experiments showed that "sweet," "fruity," and "fatty" are the main aroma attributes of Chinese almonds, with a strong relationship with hexanal, heptaldehyde, 1-nonanal, etc. This experiment provides new insights into the sensory contribution of Chinese almond volatiles and helps Chinese almond producers characterize and market the aroma profile of Chinese almond varieties.

1. Introduction

Almonds (*Prunus dulcis*) are an ancient crop from Asia [1] and are one of the most important tree nut crops in commercial production [2]. It is now grown in California (U.S.), the Mediterranean, Central Asia, and Australia [3]. Gradziel [4] proposed that the almonds now cultivated were selected from species originally listed as *Amygdalus communis* L. (syn. *Prunus communis* Archang), and their ability to adapt to harsh climates has allowed them to survive in ecosystems ranging from the Taklamakan Desert in western China to the Mediterranean Sea.

Recent studies on almonds have focused on their biological activity and nutritional composition. Current pharmacological studies represent that almond has several biological activities, including prebiotic, antimicrobial, antioxidant, anti-inflammatory, anticancer, hepatoprotective, cardiometabolic protection, nootropic, anxiolytic, sedative-hypnotic, and nervous-improving effects [5]. Musarra-Pizzo et al. [6] described for the first time that natural almond skin exerts both anti-HSV type-1 and antimicrobial activity and could potentially be used in a topical formulation. Studies on the composition and characterization of almond macronutrients and micronutrients have shown that it has many nutritious ingredients such as fatty acids, lipids, amino acids, proteins, carbohydrates, vitamins, and minerals, as well as secondary metabolites [7]. Roncero et al. [8] found that almonds contain a considerable amount of high-quality protein, mainly globulins, essential minerals, and fiber and are low in sugar, in addition to containing many phytochemicals with potential health benefits. However, less research has been performed on almond's volatile organic compounds.

Franklin and Mitchell [3] discussed the use of raw and heat-processed almonds, volatile compounds resulting from heating, aroma qualities associated with various odorants, and flavor development and off-flavors in almonds due to rancidity. Lipan et al. [9] identified 35 volatile compounds in almonds, and the key compounds for the roasting process were 2,5-dimethyl-pyrazine, furfural, and trimethyl pyrazine. King et al. [10] used descriptive sensory analysis to evaluate the sensory characteristics of 83 raw, whole

almonds and demonstrated that the sensory characteristics of almond varieties of different origins differed. Oliveira et al. [11] found that benzaldehyde and 3-methyl-1-butanol are the main aroma compounds of raw almonds. However, no studies on the aroma substances of Chinese almonds have been found.

As is well known, in the United States, almonds are primarily grown in California, which contributes to approximately 80% of the global almond supply [10]. Almonds cultivated in China, although of the same variety as those in California, differ in their aroma and chemical composition due to the different climate. Sathe et al. [12] demonstrated that the fat content and fatty acid composition of almonds depend not only on the genotype but also on the location and climatic conditions of the growing season. King et al. [10] also demonstrated that different varieties of almonds in California also have different sensory attributes. In this study, high-quality almonds growing in the Kashgar region of Xinjiang, China, which is the largest almond-growing area in China, were selected [13].

GC-MS was the main technology used for flavor or volatile profile determination [14, 15]. AEDA is a highly efficient quantitative gas chromatography olfactometry (GC-O) procedure for determining the potency of odorants in food extracts [16]. In recent years, because the AEDA combined with the GC-O-MS method is more effective for the detection of aroma compounds, many people have adopted this method [17–20]. In this experiment, almonds were pretreated with SPME, SAFE, and SDE, and the extracts were analyzed by AEDA with GC-O-MS to obtain the aroma-active compounds of almonds. The aroma profile of almonds was obtained by aroma recombination and omission experiments. This experiment provides new insights into the sensory contribution of Chinese almond volatiles and helps Chinese almond producers to characterize and market the aroma profile of Chinese almond varieties.

2. Materials and Methods

2.1. Samples and Chemicals. Almond: Kashgar, Xinjiang; analytically pure NaCl, NaOH, glucose, and tartaric acid: Sinopharm Chemical Reagent Beijing Co., Ltd; solid-phase extraction column Cleanert PEP-SPE (1000 mg packing): Agela & Phenomenex, Inc.; D-gluconolactone, polyvinylpyrrolidone, and C_6–C_{30} n-alkanes: Sigma-Aldrich (Shanghai) Trading Co., Ltd.; aroma standards: Sigma-Aldrich (Shanghai) Trading Co., Ltd. and Honeywell Fluka™.

2.2. Instruments. Olfactory detection port (ODP): GERSTEL GmbH & Co.KG; Solvent-assisted flavor evaporation (SAFE): Glasbläserei-Bahr; Agilent 6890A gas chromatograph (GC) system with a 5975C mass spectrometer (MS): Agilent Technologies, Inc.; DVB/CAR/PDMS headspace solid-phase microextraction head: Supelco®; DB-5 column (30 m/0.25 mm/0.25 μm), HP-INNOWax column (60 m/0.25 mm/0.25 μm): J&W Scientific, Inc.

2.3. Experimental Methods

2.3.1. Sample Pretreatment

(1) Solid-Phase Microextraction (SPME). Solid-phase microextraction (SPME) is a modern, nonexhaustive sample preparation technique. The advantages of this technique include simplicity, rapidity, improved sample clean-up, accurate analysis, and low organic solvent consumption [21]. For SPME, 2 g of almonds, 10 mL of distilled water, and 1 g of NaCl were weighed into an extraction flask and placed in a water bath at 60°C for 30 min. The SPME fiber head was then immersed in the sample at the same temperature for 30 min, and then the fiber head was transferred to the injection port and desorbed at 250°C for 5 min for GC-O-MS analysis.

(2) Solvent-Assisted Flavor Evaporation (SAFE). Solvent-assisted flavor evaporation (SAFE) allows the isolation of volatiles from either solvent extracts, aqueous foods, aqueous food suspensions, or even matrices with a high oil content [22]. For SAFE, 20 g almonds were accurately weighed in a 500 mL conical flask, 200 mL dichloromethane was added, and extracted on a magnetic stirrer for 1 h, then placed in a liquid separator funnel, the upper liquid was poured into a 500 mL round-bottomed flask, the lower liquid was retained and transferred to a conical flask, then 200 mL dichloromethane was added and extracted on a magnetic stirrer for 1 h. Transferred to the separator funnel, the upper liquid was transferred to the round-bottom flask again, and the lower liquid was retained. After the third transfer to the conical flask, 100 mL methylene chloride was added, and the upper liquid was combined into the round-bottom flask three times. The extract from three extracts is extracted in the SAFE device. The distillate was dried with anhydrous sodium sulfate and concentrated to 5 mL on a rotary evaporator, then further concentrated to 200 μL under a mild nitrogen stream. Concentrate fractions were stored at −20°C before GC-O-MS analysis.

(3) Simultaneous Distillation Extraction (SDE). When SDE is used correctly, it is often the best method for obtaining the highest recovery from a wide range of compounds [23]. For SDE, the almonds were heated in an oil bath at 100 ± 1°C for 3 hours while the dichloromethane was heated in a water bath at 50 ± 1°C. The extracted dichloromethane solution was then dried, concentrated, and stored using the same treatment as SAFE.

2.3.2. GC-O-MS Conditions. The GC column was equipped with HP-INNOWax and DB-5, and the carrier gas was helium with a flow rate of 1 mL/min. The injection volume was 1 μL without splitting. The programmed ramp-up procedure: the starting temperature was 40°C, held for 2 min, ramped up to 230°C at 4°C/min, and held for 15 min. The temperature of both the inlet and the auxiliary heating zone was 250°C. The samples were separated by the column and then entered into the mass spectrometry and ODP for detection at a ratio of 1 : 1, respectively. The ion source was

an electron ionization (EI) source with an electron energy of 70 eV and a mass scan range of 30–350 m/z. The temperature of the mass spectrometry interface was 250°C and the ion source temperature was 230°C.

2.3.3. Identification and Quantification of the Aroma-Active Compounds. The mass spectra corresponding to each peak were searched using the NIST20 spectral library with a retention similarity of 70% or more, and the concentrations of each component were calculated using the external standard quantitation method.

The chromatographic scans were also performed on the mixed C_6–C_{30} n-alkane specimens to calculate the retention indices of the aroma components and to compare them with the corresponding literature values. The linear retention index (RI) was calculated as follows:

$$\mathrm{RI} = 100n + 100 \times \frac{t_i - t_n}{t_{n+1} - t_n}, \tag{1}$$

where n is the carbon number of n-alkanes; t_i is the retention time of the target compound, min; t_n is the retention time of C_n, min; and t_{n+1} is the retention time of C_{n+1}, min.

The aroma standards were mixed and diluted into 4 different concentrations, and the samples were accurately injected under the same chromatographic conditions as the measured components, and the peak areas were measured to establish the standard curves of each component.

2.3.4. Aroma Extract Dilution Analysis (AEDA). Each concentrated extract was gradually diluted with dichloromethane to 1 : 3, 1 : 9, 1 : 27 . . . 1 : 2187. Each diluted sample (1 μL) was analyzed by ODP using HP-INNOWax and DB-5 columns under the above GC conditions, and each sample and each of its dilutions were analyzed by sniffing by three sniffers trained in sniffing for more than six months, and the dilution factors of compounds with fragrance detected by two or more sniffers were calculated until no aromatic compound could be detected. The flavor dilution (FD) factor for each compound represents the maximum perceived dilution. Each odor is identified by comparing its odor, retention index (RI), and mass spectrum with the odor of the real compound.

2.3.5. Sensory Analysis. Sensory analysis was performed in the sensory laboratory of the Shanghai Institute of Technology (Shanghai, China) according to the methods specified in the National Standard ISO 8589-2007. The panel consisted of 15 trained professors and students (7 males and 8 females, aged 20–40) to evaluate the aroma extract of almonds. After training and the sensory triangle test, 10 professional panelists were selected on merit to perform the sensory analysis.

Before the experiment, each panelist underwent 4 hours of sensory training. The panelists were asked to evaluate the aroma characteristics of the almond spices obtained by different extraction methods and finally discussed identifying six aroma notes (fatty, sweet, balsam, fruity, floral, and green) and the corresponding reference compounds: nonanal for fatty, benzaldehyde for sweet, guaiacol for balsam, hexanol for fruity, linalool for floral, and hexanal for green. Also, a 9-point intensity scale was used to assess the intensity of each fragrance note.

2.3.6. Odor Activity Values (OAVs). The OAV represents the olfactory activity value of the flavor active compound, OAV >1 means that the compound has a direct effect on the aroma, and it is generally accepted that the higher the OAV the greater the contribution of the volatile compound to the aroma of the sample. The calculation formula is as follows:

$$\mathrm{OAV} = \frac{c}{T}, \tag{2}$$

where c is the concentration of the compound, mg/kg; T is the threshold of the compound in water, mg/kg.

2.3.7. Aroma Recombination and Omission. Aroma compounds with OAV ≥1 in almonds were mixed in a blank substrate according to their actual concentration as a recombinant model and compared with unpretreated almond aroma. The team evaluated the intensity of six different aroma types in the samples to determine the effect of these aroma compounds on the overall aroma contribution of almonds.

Aroma omission could verify the contribution of a single compound to the aroma of almonds. The experiment needed to use the aroma deletion model of a certain aroma compound and two complete aroma recombination models. All samples were labeled with a three-digit code and presented in a random order to the panelists for the sensory triangle test. The panelists were required to distinguish the deletion model from the two complete aroma recombination models and describe the differences in the deletion model aromas.

2.3.8. Statistical Analysis. The NIST20 database was used for GC-MS analysis. The concentration of volatiles was submitted to analysis of variance (ANOVA) by SPSS version 26.0. Microsoft Office Excel 2016 was used for data processing and radar plotting. Principal component analysis (PCA) was performed using the Dynamic PCA plug-in to evaluate the regularity and difference among tested samples.

3. Results and Discussion

3.1. Identification of Almond Volatile Components Extracted by SAFE, SPME, and SDE. Volatile aroma compounds were isolated from almonds using three different extraction methods (SAFE, SPME, and SDE). A total of 75 aroma compounds were identified by GC-O and GC-MS analysis of the extracted volatile compounds, including 15 aldehydes, 15 alcohols, 12 esters, 7 acids, 5 terpenes, 4 ketones, and 17 other types of compounds (Table 1). 49, 36, and 47 aroma compounds were detected by three extraction methods, SAFE, SPME, and SDE, respectively.

Table 1: Identification and FD factors for volatile aroma compounds of almonds.

No.	Component	RI[a]		Reference RI		Odor[b]	FD factor[c]			Identification
		HP-INNOWax	DB-5	HP-INNOWax	DB-5		SAFE	SPME	SDE	
Aldehydes										
1	Hexanal	1089	805	1081	805	Fresh, green	243	—	243	MS, RI, O, S
2	trans-2-Pentenal	1136	757	1134	N	Green	—	—	3	MS, RI, O, S
3	Heptaldehyde	1189	901	1186	901	Fatty, green	81	—	—	MS, RI, O, S
4	2-Hexenal	1222	854	1226	N	Fatty, green	—	—	3	MS, RI, O, S
5	1-Nonanal	1392	1104	1403	1101	Fatty, rose	2187	243	2187	MS, RI, O, S
6	2-Furaldehyde	1439	818	1439	833	Sweet, woody	243	9	9	MS, RI, O, S
7	(2E, 4E)-Hepta-2,4-dienal	1493	1011	1497	1017	Fatty, green	3	—	—	MS, RI, O, S
8	Benzaldehyde	1525	973	1530	961	Sweet, cherry	729	81	—	MS, RI, O, S
9	trans-2-Nonenal	1533	1162	N	1168	Fatty, green	—	—	729	MS, RI, O, S
10	5-Methylfurfural	1571	978	1558	957	Sweet,	27	9	9	MS, RI, O, S
11	Phenylacetaldehyde	1641	1045	1663	1044	Green, sweet, floral	729	243	2187	MS, RI, O, S
12	2,4-Decadienal	1826	1319	N	1309	Fatty	—	—	81	MS, RI, O, S
13	Pyrrole-2-carboxaldehyde	2022	1031	N	N	Musty, beef	1	1	—	MS, RI, O, S
14	(E)-Cinnamaldehyde	2041	1266	N	1266	Sweet, candy	—	3	1	MS, RI, O, S
15	Cinnamaldehyde	2045	1260	N	N	Sweet, honey	3	—	—	MS, RI, O, S
Alcohols										
16	2-Methyl-3-buten-2-ol	1039	600	1048	N	Grass, greasy	—	—	3	MS, RI, O, S
17	1-Penten-3-ol	1154	671	N	716	Green	—	—	3	MS, RI, O, S
18	Pentanol	1246	760	1244	761	Greasy, sweet	243	—	—	MS, RI, O, S
19	1-Hexanol	1346	858	1340	869	Greasy, fruity	729	—	—	MS, RI, O, S
20	3-Hexen-1-ol	1378	845	1363	858	Green, leaf	9	—	—	MS ,RI, O
21	2-Ethylhexanol	1480	1028	1490	1028	Orange, floral	3	—	—	MS, RI, O, S
22	2,3-Butanediol	1531	800	1553	767	Fruity, creamy	3	—	—	MS, RI, O, S
23	Linalool	1538	1098	1551	1099	Floral	2187	243	2187	MS, RI, O, S
24	Alpha-Terpineol	1691	1192	1718	1195	Rosin, floral	9	1	9	MS, RI, O, S
25	2,2,6-Trimethyl-6-vinyltetrahydro-2h-pyran-3-ol	1732	1163	N	N	Floral, honey	729	81	81	MS, RI, O, S
26	Nerol	1789	1228	1757	1228	Sweet, neroli	—	—	3	MS, RI, O, S
27	Benzyl alcohol	1880	1045	1898	1032	Floral	243	27	8	MS, RI, O, S
28	2-Phenylethanol	1906	1117	1872	1117	Floral	81	27	27	MS, RI, O, S
29	3-Phenyl-1-propanol	2040	1232	N	1232	Sweet, balsam	3	—	—	MS, RI, O, S
30	2-Propen-1-ol, 3-phenyl-	2280	1312	N	1312	Sweet, balsam	27	—	—	MS, RI, O, S
Ketones										
31	3-Hexanone	1063	783	N	784	Sweet, fruity	—	—	9	MS, RI, O, S
32	2-Butanone	1287	1362	908	N	Sweet, greasy	9	—	—	MS, RI, O, S
33	Acetophenone	1653	1078	1671	1065	Sweet, spicy	243	—	—	MS, RI, O, S
34	Damascone	1817	1362	1830	N	Sweet, fruity	—	—	81	MS, RI, O, S
Esters										
35	Dihydrofuran-2(3H)-one	1633	917	N	908	Creamy, greasy	9	1	1	MS, RI, O, S
36	Gamma-Caprolactone	1704	1047	N	N	Grass, coconut	3	—	—	MS, RI, O, S
37	Benzyl acetate	1726	1170	1689	1165	Sweet, floral	—	9	—	MS, RI, O, S
38	Methyl salicylate	1775	1198	1751	1198	Sweet, beer	27	27	27	MS, RI, O, S

Table 1: Continued.

No.	Component	RI[a]		Reference RI		Odor[b]	FD factor[c]			Identification
		HP-INNOWax	DB-5	HP-INNOWax	DB-5		SAFE	SPME	SDE	
39	Gamma-nonanoic lactone	2032	1344	N	1344	Fruity, creamy	243	—	—	MS, RI, O, S
40	Pentadecanoic acid, ethyl ester	2144	1880	N	1880	Honey, sweet	—	—	9	MS, RI, O, S
41	Methyl palmitate	2208	1921	N	1921	Greasy, waxy	27	3	3	MS, RI, O, S
42	Ethyl palmitate	2247	1975	2250	1975	Waxy	—	9	9	MS, RI, O, S
43	Dihydroactinidiolide	2355	1479	N	1486	Plum	81	81	27	MS, RI, O, S
44	Methyl oleate	2439	2106	N	2100	Fatty	—	—	3	MS, RI, O, S
45	Ethyl linoleate	2523	2164	N	2155	Fatty, fruity	—	—	27	MS, RI, O, S
46	Benzyl benzoate	2636	1750	2655	1775	Sweet, balsam	—	81	—	MS, RI, O, S
Terpenoids										
47	Myrcene	1164	992	1169	992	Spicy, balsam	—	81	81	MS, RI, O, S
48	Styrene	1260	911	N	889	Sweet, balsam	243	—	9	MS, RI, O, S
49	Alpha-pinene	1489	1377	1022	940	Woody, honey	—	—	9	MS, RI, O, S
50	Beta-caryophyllene	1594	1162	1614	1420	Sweet, woody	—	—	27	MS, RI, O, S
51	Delta-cadinene	1754	1526	1764	1526	Musk, grass	—	9	9	MS, RI, O
Acids										
52	Acetic acid	1446	1020	1427	625	Spicy, sour	9	1	—	MS, RI, O, S
53	Hexanoic acid	1833	1020	1831	1020	Sour, fatty	81	27	9	MS, RI, O, S
54	Heptanoic acid	1941	1076	N	1076	Sour	27	9	3	MS, RI, O, S
55	Octanoic acid	2049	1186	2039	1186	Fatty, waxy	27	27	—	MS, RI, O, S
56	Nonanoic acid	2153	1283	2192	1293	Waxy, cheese	27	9	2187	MS, RI, O, S
57	Benzoic acid	2438	1170	N	1170	Balsam	27	3	1	MS, RI, O, S
58	Myristicin	1681	1762	N	1516	Waxy, fatty	—	—	3	MS, RI, O, S
Other										
59	2-Pentylfuran	1230	993	1231	993	Fruity, green	—	—	243	MS, RI, O, S
60	2,5-Dimethylpyrazine	1324	928	N	928	Cocoa, roasted	9	—	—	MS, RI, O, S
61	2,6-Dimethylpyrazine	1328	723	N	N	Cocoa, roasted	9	9	—	MS, RI, O, S
62	Pyrazine,2-ethyl-6-methyl-	1384	834	N	991	Potato	27	—	—	MS, RI, O, S
63	2,3,5-Trimethylpyrazine	1403	840	1411	1005	Nuts	9	81	—	MS, RI, O, S
64	Linalool oxide	1468	1080	N	N	Mud, floral	81	27	—	MS, RI, O
65	1-(Furan-2-yl)ethanone	1500	911	N	N	Sweet, balsam	—	—	1	MS, RI, O, S
66	Guaiacol	1856	1089	1884	1089	Balsam, vanilla	243	—	—	MS, RI, O, S
67	2-Methoxy-4-methylphenol	1953	1207	1982	1157	Clove	9	81	—	MS, RI, O, S
68	1-(1H-Pyrrol-2-yl)ethanone	1968	1072	N	1060	Musty, nuts	3	3	3	MS, RI, O, S
69	Phenol	1998	1005	N	1072	Plastic	1	1	—	MS, RI, O, S
70	Eugenol	2161	1348	2120	1356	Sweet, balsam	2187	243	729	MS, RI, O, S
71	4-Ethylphenol	2167	1178	N	1178	Beaver	—	—	81	MS, RI, O, S
72	4-Ethyl-2-methoxyphenol	2191	1309	N	1270	Woody, green	—	243	729	MS, RI, O, S
73	2,4-Di-tert-butylphenol	2298	1512	N	1502	Phenol	27	27	9	MS, RI, O, S
74	Indole	2444	1295	N	1292	Animal, floral	—	—	81	MS, RI, O, S
75	Vanillin	2568	1396	N	1394	Sweet, vanilla	729	—	9	MS, RI, O, S

[a]RI is calculated according to formula (1). Reference RI is from this website (https://webbook.nist.gov/). N, not found. [b]Odor characteristics were detected at the olfactory probe port. [c]Flavour dilution factor. —, not detected; MS, characterization by MS spectroscopy; RI, identification of the compound by comparing the RI value with the real compound; O, characterization of its odor by comparing it with the real compound by GC-O; S, characterization of the detected profile of the compound by comparing it with the standard.

In the SAFE method, the FD factors of 49 aroma compounds ranged from 1 to 2187 according to AEDA. Among these compounds, 16 aromas with FD factors ≥243 (Table 1) were tentatively considered important aroma compounds for almonds. Among them, nonanal (fatty, rose), linalool (floral), and eugenol (sweet) had the highest FD factor of 2187. Meanwhile, five compounds ranked second with an FD factor of 729, including benzaldehyde (sweet, cherry), phenylethyl aldehyde (green, sweet, floral), hexanol (oily, fruity), and 2,2,6-trimethyl-6-vinyltetrahydro-2H-furan-3-ol (honey, floral). Eight other aroma compounds, including hexanal (fresh, green), furfural (sweet, woody), pentanol (oily, sweet), benzyl alcohol (floral), acetophenone (sweet, spicy), gamma-nonanoic lactone (coconut, cream), styrene (sweet, balsam), and guaiacol (balsam, vanilla) had FD factors of 243. In addition, 15 other aromas had high FD factors (≥27), and they may also be important key aroma compounds in almonds.

For the SPME method, only five aromas with FD factors ≥243 were detected (Table 1), including nonanal (fatty, rose), phenylethyl aldehyde (green, sweet, floral), linalool (floral), eugenol (sweet, balsam), and 2-methoxy-4-vinyl phenol (woody, fresh). In contrast, 2-methoxy-4-vinyl phenol (woody, fresh) was not detected in the SAFE isolates, indicating that a single extraction method (SAFE or SPME) was not sufficient to isolate all characteristic aroma-active components from almonds.

In the SDE method, the samples were heat-treated, and some aroma compounds such as 2-pentenal, 2-hexenal, 2-nonenal, 2,4-decadienal, 2-methyl-3-buten-2-ol, 1-penten-3-ol, nerolidol, 3-hexanone, β-damascenone, and pentadecanoic acid (Table 1) may be produced during heating or their concentrations may increase enough to be detected. Among these aromatic compounds, 2-nonenal with an FD factor of 729 may be derived from heptanal, as it is available in the SAFE method. During heating, some compounds may decompose or react with other compounds to produce new compounds with different thresholds [24].

3.2. Sensory Differences between Different Extraction Methods of Almonds. The panelists evaluated and compared the intensity of six aromas: fatty, sweet, balsam, fruity, floral, and green aromas in almond extracts obtained from different pretreatment methods and unpretreated almonds. A mapping model for sensory evaluation in principal component analysis was developed using the external preference mapping method.

As can be seen from Figure 1, PC1 contributed 76.89% and PC2 contributed 21.98%; these two principal components provided almost all the information of the data, amounting to a total of 98.87%. The almond extracts obtained with SPME and SAFE methods were close to unpretreated almonds (UNP), close to the fruity aroma, so they had very similar odors. On the contrary, the aroma of the almond extract obtained with the SDE method was different from UNP and close to fatty. It can be seen from these results that SDE is not suitable for the separation of aroma compounds in almonds.

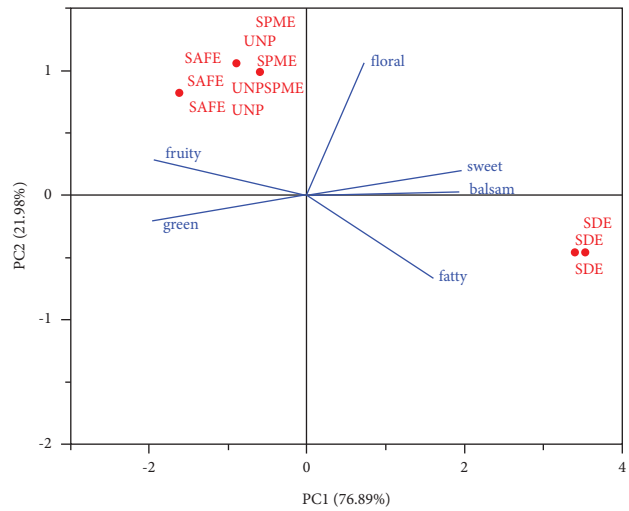

Figure 1: Principal component analysis of almond aromas from different pretreatment methods.

3.3. Quantification and OAV Calculation of Volatile Aroma Compounds in Almonds. The SAFE and SPME extracts of almonds with FD factor ≥1 were analyzed using AEDA combined with the GC-MS technique.

The highest concentration of 2,3-butanediol can be seen in Table 2, but the calculated OAV is lower due to the higher threshold value. On the other hand, certain compounds have lower thresholds, and therefore have higher OAV despite relatively low concentrations, such as nonanal, linalool, etc. Compounds with OAV greater than 1 are referred to as effective aroma substances. Compounds with OAV greater than 10 are said to be important, and there are 41 major aroma compounds with OAV ≥1. There are 34 major aroma compounds with OAV ≥10, among which nonanal, phenylacetaldehyde, linalool, gamma-nonanoic lactone, benzyl benzoate, myrcene, eugenol, 2-methoxy-4-vinyl phenol, and vanillin were the most important aroma compounds in almonds and the OAV of these aroma compounds was more than 1000.

Aroma compounds with higher OAV usually also have higher FD factors. Among the almond aroma compounds, nonanal had the highest FD factor of 2187 and the highest OAV due to its low threshold (0.0011 μg/kg). Therefore, nonanal was considered the most important contributor among almond aroma compounds. In addition, hexanal, phenylacetaldehyde, linalool, gamma-nonanoic lactone, benzyl benzoate, myrcene, and vanillin all had high values of FD factor and OAV, indicating a strong correlation between the two methods. However, there are some exceptions such as eugenol and 2-methoxy-4-vinyl phenol, which have higher OAV but lower FD factor, while benzaldehyde, hexanol, and guaiacol have higher FD factor but lower OAV.

Other studies have found classes of volatile compounds in almonds that generally overlap but vary widely in relative compound abundance. For example, Agila and Barringer [25] found that the most abundant volatile compounds in almond samples were methanol and ethanol and speculated that these may be fatty acid breakdown products, while most

Table 2: Standard curve, coefficient of determination r^2, concentration, threshold, and OAV of almond volatile aroma compounds.

No.	Compound	Standard curve	r^2	c (μg/g)	Threshold[a] (μg/kg)	OAV
1	Hexanal	$y = 1.54666x - 0.02886$	0.9918	0.07275	0.0615	1183
3	Heptaldehyde	$y = 0.57836x - 0.00682$	0.9821	0.00989	0.028	353
5	1-Nonanal	$y = 2.6632128x - 0.19841$	0.9511	0.55342	0.0011	503109
6	2-Furaldehyde	$y = 0.10991x - 0.01622$	0.9792	0.00916	9.562	1
7	(2E, 4E)-Hepta-2,4-dienal	$y = 0.10168x - 0.00125$	0.9916	0.00073	10	<1
8	Benzaldehyde	$y = 0.05102x - 0.03634$	0.9872	0.07365	0.75089	98
10	5-Methylfurfural	$y = 0.03503x - 0.00015$	0.9999	0.00118	1.11	1
11	Phenylacetaldehyde	$y = 0.06255$x	0.9999	0.00843	0.004	2108
13	Pyrrole-2-carboxaldehyde	$y = 0.14937x - 0.01649$	0.967	0.0233	65	<1
14	(E)-Cinnamaldehyde	$y = 0.34739x + 0.0321$	0.9922	0.0874	6	15
15	Cinnamaldehyde	$y = 0.4567x - 0.012204$	0.9817	0.08279	0.75	110
18	Pentanol	$y = 0.11171$x-0.00096	0.9938	0.00402	0.1502	27
19	1-Hexanol	$y = 0.08612x - 0.000744$	0.9773	0.00025	0.0056	45
21	2-Ethylhexanol	$y = 0.10393x - 0.000297$	0.9968	0.00547	1.63	3
22	2,3-Butanediol	$y = 12.16106x + 40.3216$	0.9986	56.16106	100	<1
23	Linalool	$y = 0.09112x - 0.000975$	0.9964	0.03252	0.00022	147818
24	Alpha-terpineol	$y = 0.0266x - 0.000039$	0.9866	0.00052	1.2	<1
25	2,2,6-Trimethyl-6-vinyltetrahydro-2h-pyran-3-ol	$y = 0.03784x - 0.00455$	0.9997	0.06427	—	—
27	Benzyl alcohol	$y = 0.15829x - 1.21264$	0.9982	0.51272	2.54621	201
28	2-Phenylethanol	$y = 0.034x - 0.0046$	0.9999	0.01211	0.56423	21
29	3-Phenyl-1-propanol	$y = 0.86018x - 0.66414$	0.9952	0.000062	0.42	<1
30	2-Propen-1-ol, 3-phenyl-	$y = 0.05957x - 0.0012$	0.9922	0.00055	0.077	7
32	2-Butanone	$y = 8.78174x - 0.82822$	0.9861	13.92862	30	464
33	Acetophenone	$y = 0.03849x - 0.00094$	0.9639	0.00154	0.0063	244
35	Dihydrofuran-2(3H)-one	$y = 0.1861x - 0.000003$	0.9988	0.448601	2	224
36	Gamma-caprolactone	$y = 0.04958x - 0.0004353$	0.9994	0.0010521	0.26	4
37	Benzyl acetate	$y = 0.14146x + 0.0027$	0.9966	0.00694	0.364	19
38	Methyl salicylate	$y = 0.0843x - 0.0000453$	0.9985	0.00282	0.04	71
39	Gamma-nonanoic lactone	$y = 0.38231x - 0.02268$	0.9733	0.04854	0.0097	5004
41	Methyl palmitate	$y = 7.07063x - 0.15572$	0.9978	0.23741	2	119
42	Ethyl palmitate	$y = 0.7441x - 0.04646$	0.998	0.29917	2	150
43	Dihydroactinidiolide	$y = 0.3682x + 0.01537$	0.9991	0.10462	—	—
46	Benzyl benzoate	$y = 0.87324x + 0.06805$	0.9881	1.45144	0.341	4256
47	Myrcene	$y = 0.3814x + 0.002134$	0.9976	0.003904	0.0012	3253
48	Styrene	$y = 1.86973x - 0.01247$	0.9966	0.02997	0.065	461
52	Acetic acid	$y = 1.17119x - 1.01273$	0.9975	0.041341	99	<1
53	Hexanoic acid	$y = 0.20842x - 0.12782$	0.996	0.10747	0.89	121
54	Heptanoic acid	$y = 0.19076x - 0.00841$	0.9758	0.02493	0.91	27
55	Octanoic acid	$y = 0.72953x - 0.02963$	0.9972	0.2109	3	70
56	Nonanoic acid	$y = 0.32846x + 0.20217$	0.9993	0.31828	8.8	36
57	Benzoic acid	$y = 0.5697x - 0.03939$	0.9742	0.30163	1	302
60	2,5-Dimethylpyrazine	$y = 0.14038x - 0.0031$	0.9971	0.00336	1.75	2
61	2,6-Dimethylpyrazine	$y = 0.00381x + 0.0000183$	0.8607	0.00038	0.718	<1
62	Pyrazine,2-ethyl-6-methyl-	$y = 0.01872x - 0.0000776$	0.9999	0.00012	0.04	3
66	Guaiacol	$y = 0.02988x - 0.00064$	0.9989	0.00013	0.0015	87
67	2-Methoxy-4-methylphenol	$y = 10.6493x + 2.0852$	0.9999	2.4725	1.485	166
68	1-(1H-Pyrrol-2-yl)ethanone	$y = 0.04444x - 0.00136$	0.9999	0.00735	58.58525	<1
69	Phenol	$y = 0.96119x - 0.01652$	0.9734	0.02673	58.58525	<1
70	Eugenol	$y = 7.0991x - 0.11311$	0.9969	0.95176	0.0025	380704
72	4-Ethyl-2-methoxyphenol	$y = 1.6767x - 0.03218$	0.99315	0.50738	0.03	16913
73	2,4-Di-tert-butylphenol	$y = 0.73954x - 0.02205$	0.9735	0.20284	0.5	406
75	Vanillin	$y = 0.75289x - 0.29449$	0.988	0.40208	0.053	7586

[a]The threshold of aromatic substances is their threshold in water (reference to odor threshold). —, not available from the reference. Compounds detected by both methods were quantified by SAFE.

other experiments showed that the most abundant odor-active compound in almonds was benzaldehyde [26–29].

3.4. Aroma Profile. The panelists evaluated the intensity of the fatty, sweet, balsam, fruity, floral, and green aroma types of the reconstituted model with untreated almonds and determined that these aromas had a significant impact on the overall aroma. Aroma intensities ranged from 0 (undetectable) to 8 (strong), and a radar plot was created using these sensory scores (Figure 2).

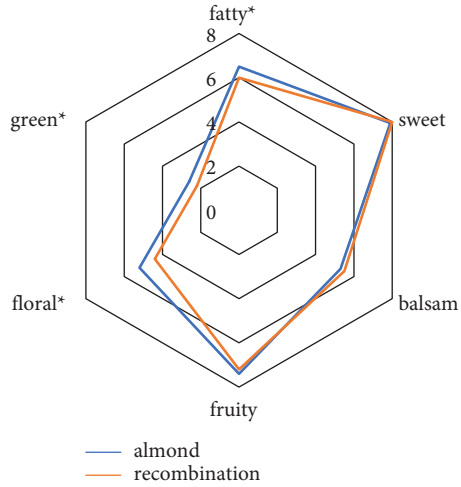

Figure 2: Aroma profile of real almond and aroma recombination model. ∗The scent was significantly different between samples ($p < 0.05$).

Table 3: Effect of individual aroma compounds on the aroma profile of almonds.

No.	Compound omitted from the complete recombinant	n^A	Description of aroma difference	Significance[B]
1	Hexanal	8	Reduced green aroma	**
3	Heptaldehyde	9	Almost no fatty and green aroma	**
5	1-Nonanal	9	Virtually no fat and fruity aroma	**
8	Benzaldehyde	4	nd	
11	Phenylacetaldehyde	8	Reduced green, sweet and floral aromas	**
14	(E)-Cinnamaldehyde	0	nd	
15	Cinnamaldehyde	4	nd	
18	Pentanol	2	nd	
19	1-Hexanol	4	nd	
23	Linalool	9	Almost no fat and green aromas	**
27	Benzyl alcohol	7	Slightly reduced floral aroma	*
28	2-Phenylethanol	3	nd	
32	2-Butanone	7	Slightly reduced sweetness	*
33	Acetophenone	7	Slightly reduced sweetness	*
35	Dihydrofuran-2(3H)-one	6	Slightly less fatty	*
37	Benzyl acetate	2	nd	
38	Methyl salicylate	4	nd	
39	Gamma-nonanoic lactone	8	Reduction of sweet and fruity aromas	**
41	Methyl palmitate	8	Reduces fatty notes	**
42	Ethyl palmitate	8	A slight reduction of fatty notes	**
46	Benzyl benzoate	9	Almost no sweet aroma	**
47	Myrcene	8	Reduction of sweet aroma	**
48	Styrene	9	Almost no sweetness	**
53	Hexanoic acid	6	Slightly reduced fat flavor	*
54	Heptanoic acid	3	nd	
55	Octanoic acid	4	nd	
56	Nonanoic acid	2	nd	
57	Benzoic acid	7	Slightly reduced sweetness	*
66	Guaiacol	7	Slightly reduced balsamic	*
67	2-Methoxy-4-methylphenol	8	Reduced balsamic	**
70	Eugenol	9	Almost no sweetness or balsamic notes	**
72	4-Ethyl-2-methoxyphenol	9	Almost no green aroma	**
73	2,4-Di-tert-butylphenol	4	nd	
75	Vanillin	9	Almost no sweet balsam	**

[A]Number of group members detected aroma differences through the sensory triangle test, a total of 10 participants. [B]Significance:**highly significant ($p < 0.01$); *significant ($p < 0.05$); nd, no difference.

The results are shown in Figure 2. There were no significant differences in the aroma profiles between the two samples, except for some minor differences reflected in the fatty, green, and floral aromas. This may be caused by these three unquantified compounds or may be due to undetected aroma compounds affecting the aroma. The aroma of almonds was mainly reflected in fatty, sweet, balsam, and fruity aromas with intensity scores of 6.5, 7.9, 5.3, and 7.4, respectively.

3.5. Omission Experiment. As shown in Table 3, first, hexanal was omitted from the recombination models, and 8 out of every 10 panelists were able to identify the different samples in the sensory triangle test and evaluate the lack of green notes. Heptaldehyde was then omitted and nine of the panelists identified the differences, with the omitted samples having less fatty and green notes than the recombination samples. When benzaldehyde was omitted, seven individuals identified a distinct floral aroma missing from the reconstituted sample. When γ-butyrolactone was omitted, six individuals identified the reconstituted sample as missing a distinct fatty aroma. The other aroma compounds (OAV $\geq$10) were then omitted one by one and the number of individuals who perceived aromatic differences was recorded (Table 3). Of these, the absence of benzyl alcohol, (E)-cinnamaldehyde, cinnamaldehyde, pentanol, hexanol, phenylethanol, benzyl acetate, methyl salicylate, heptanoic acid, octanoic acid, nonanoic acid, and 2,4-di-tert-butylphenol had a nonsignificant effect, and therefore it is presumed that these compounds were not the main aroma contributors. It can be assumed that volatile compounds such as heptanal, nonanal, linalool, and methyl palmitate may be associated with the "fatty note." Phenylacetaldehyde, γ-nonanoic lactone, benzyl benzoate, myrcene, styrene, and eugenol are highly associated with the "sweet" note. 2-Methoxy-4-methylphenol, eugenol, and vanillin may be closely associated with the "balsam" note. Finally, 1-nonanal and gamma-nonanoic lactone are the main contributors to the "fruity" note.

4. Conclusions

In this study, a total of 75 aroma compounds of Chinese almonds obtained by three different pretreatment methods were identified using an innovative method of aroma extract dilution analysis combined with GC-O-MS. The principal component analysis proved that solid-phase microextraction and solvent-assisted flavor evaporation were better pretreatment methods for the extraction of Chinese almond aroma compounds. Forty-one compounds with OAV $\geq$1 contributing to the aroma were obtained by FD factor and OAV calculation, among which 34 compounds with OAV $\geq$10 were considered important aroma compounds in Chinese almonds. Nonanal had the highest FD factor and OAV and was considered the most important contributing compound to the aroma of Chinese almonds. The compounds with OAV $\geq$10 were subjected to aroma recombination and omission experiments, and the results of the aroma recombination experiment showed that there was no significant difference between the aroma of the recombination model and that of the unpretreated Chinese almonds, and the results of the omission experiment showed that the aroma of most of the omitted models was significantly different from that of the complete aroma model. However, this study was only conducted for almonds from the Kashgar region of Xinjiang, China, and is not applicable to almonds from other regions. Future studies can use this as a basis for comparative analysis with almonds from other regions to draw more general conclusions.

Data Availability

The data supporting the results of this study can be obtained from the corresponding authors upon reasonable request.

Additional Points

This study identified the aroma compounds and characteristics of almonds produced in China. This knowledge could be applied in the future to almond growers and processors in China to enable them to correctly identify acceptable or unacceptable aromas in Chinese almonds, and possibly to the almond industry in China to provide standards to guide the development of industry standards to optimize Chinese almond aromas.

Consent

Written informed consent was obtained from all study participants.

Disclosure

Yuchen Gu is the first author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yuchen Gu was in charge of writing the original draft, data curation, formal analysis, and visualization. Tao Feng handled resources, reviewing and editing the manuscript, and supervision. Shiqing Song was in charge of investigation, resources, supervision, and reviewing and editing the manuscript. Lingyun Yao handled data curation and designed the study methodology. Min Sun was in charge of validation and software. Huatian Wang was in charge of supervision. Qian Liu and Chuang Yu designed the methodology.

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