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Article

Evaluation and Comparison of Pear Flower Aroma Characteristics of Seven Cultivars

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Abstract: Due to its ornamental and medicinal value, pear flower has been historically loved and used in China. However, the current understanding of their odor-active compounds and aroma profiles is rather limited. This work aimed to evaluate and compare the overall aroma profile of pear flowers; the volatiles in flowers of seven pear cultivars (Anli, Bayuesu, Golden, Brown peel, KorlaXiangli, Lyubaoshi, Xizilü) were analyzed using solid-phase microextraction–gas chromatography-mass spectrometry (SPME-GC-MS). A total of 93 volatile compounds were identified and quantified within the amount of volatiles in the range of 62.7–691.8 μg kg^{-1} (FW) and showed high and significant variability in different cultivars. Anli and Brown peel flowers showed a relatively higher volatile abundance, while KorlaXiangli flowers were significantly lower than other cultivars. Although the composition of volatiles depended on the existence of different chemical classes, the odor activity values (OAVs) and odor descriptions showed some aldehydes were part of their main peculiarities and were considered as the basic active odorants that presented strong intensity of citrus and floral odor. Moreover, multivariate analysis showed the pear flower of different cultivars could be arranged in different clusters by the identified odorants. This study provides first-hand knowledge regarding pear flower aroma profiles, and that the cultivar differences were critical for the overall pattern.

Keywords: pear flowers; active odorants; OAVs; multivariate analysis; aroma profiles

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1. Introduction

Pear (*Pyrus* L.) grows in moderate climate zones with many cultivars and is believed to have originated in the western mountainous area of China. It is widely distributed and cultivated in the north and south of China, with the largest production and richest germplasm resources in the world. Pear fruit has been used not only as a fruit but also as a traditional medicine with antitussive, anti-inflammatory, and diuretic effects for 1700 years [1].

The flower is an important part of plants. Except for the ornamental value, the aromatic substances released by flowers have high sensory and physiological values and even affect people's appetite and mental state [2]. In China, people have used pear flowers to describe the beauty of artistic conception and they are regularly depicted in poems. The edible flower is often rich in nutrients, bringing food a good flavor or pleasant sensory effect. Therefore, *Osmanthus* and *Dendranthema* flowers have been famously used to make cakes by Chinese people since the Tang dynasty. Presently, in Yunnan province, a kind of pear flower is also used as an edible material favored by ethnic minorities. The medicinal effects of analgesic and diastolic of pear flower mean it is widely used in folk medicine treatments as a component of herbal mixtures [3]. According to the well-known traditional Chinese medical book Bencao Gangmu [4], pear flower could even eliminate black speckle, as a necessary material for women to whiten their skin in ancient days. Today, we know that the high content of arbutin is a representative active factor for whitening skin [1,5].

In the flowering process, the transition from vegetative to reproductive growth greatly affects fruit development and seed quality [6,7]. Previous studies have revealed that the most diverse and abundant volatiles are released predominantly from flowers as species-specific olfactory cues for attracting insect pollinators to ensure plant reproductive success [8,9]. Numerous insects possess olfactory receptors that respond specifically to plant volatiles and recognize the plant species [10]. The volatile compounds are the main constituents responsible for the aroma quality as a key factor attracting people and have a high correlation with the overall liking of the flower. Furthermore, many of the classes of volatile compounds isolated from plants show anti-microbial and anti-herbivore activity [8,11–13]. Based on their biosynthetic origin, volatiles can be divided into several classes, including terpenoids, fatty acid derivatives, and amino acid derivatives, involving complex enzymatic reactions [14]. The emission of volatiles is determined not only by genetics but can also be greatly influenced by environmental factors, such as light intensity, temperature, humidity, concentrations of CO_2 and O_3, and nutrient status [15–17]. The studies of flowers mainly focus on the morphology of ornamental aspects [18], however, aromatic properties are often neglected and some important odor-active compounds are gradually lost due to artificial selection and genetic erosion [19]. Previous studies on pear flowers have mainly concerned the structure and physiology aspects as well [3,20]; only Zhang et al. [21] has reported on the volatiles in KorlaXiangli flower to date.

To better understand the flavor of pear flowers, here we identify the volatile compounds and characterize the aroma profiles of seven pear cultivars. By combining the odor description, OAVs, aroma profiles, and multivariate analysis, the active odorants and aroma characteristics of pear flowers were evaluated.

2. Materials and Methods

2.1. Plant Material

Fresh flowers of seven pear cultivars (Anli, Bayuesu, Golden, Brown peel, KorlaXiangli, Lyubaoshi, Xizilü) were picked from the pear germplasm resources nursery at Hebei Normal University of Science and Technology (39.71° N, 119.185° E), China, in 2021. The entire nursery was managed with the same conditions of irrigation, pruning, and disease control. Forty flowers at the full-bloom stage from three mature trees of each cultivar were sampled and packed into polyethylene bags, sealed, labeled, and quickly transferred to the laboratory for analysis. The species of seven pear cultivars and sample collection date is shown in Table 1.

Table 1. The species of seven pear cultivars and sample collection date.

Cultivars	Species	Sampling Time
Anli	a	7 April
Bayuesu	b	14 April
Golden	c	21 March
Brown peel	c	14 April
KorlaXiangli	d	21 March
Lyubaoshi	c	14 April
Xizilü	c	14 April

a, Pyrus ussuriensis Maxim; b, Pyrus bretschneideri Rehd; c, Pyrus pyrifolia Nakai; d, Pyrus sinkiangensis Yü.

2.2. Extraction of Volatile Compounds

The volatile components of the pear flower were absorbed by HP-SPME method. 2.0 g samples were weighed accurately and loaded into 20 mL headspace vials, then sealed. The 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) was used for SPME. The fiber was preconditioned and cleaned thermally at 270 °C for 1 h in GC injector according to prescriptions. Before extraction, the samples were conditioned at 50 °C for 30 min for equilibrium, then the fiber was exposed to a capped headspace vial for 30 min at 50 °C. All the extraction processes were performed by Agilent PAL 3 autosampler (Santa Clara, CA, USA). Three replicates of each cultivar were carried out.

2.3. GC-MS Analysis

After adsorption, the fiber was withdrawn and introduced into the GC heated injector port for desorption at 230 °C for 5 min at splitless mode. Then volatile compounds were separated and identified on an Agilent 7890A-5975C gas chromatograph-mass spectrometer (Santa Clara, CA, USA), equipped with a HP-5MS capillary column (30 m × 320 μm × 0.25 μm). Helium (purity 99.999%) was employed as the carrier gas with a flow rate of 1.0 mL min^{-1}. The temperature program started at 50 °C maintained for 2 min and was increased to 200 °C at a rate of 10 °C·min^{-1} maintained for 7 min. The mass spectrometry conditions included: ion source temperature was 230 °C; electronic impact mode (EI) at 70 eV; scanned in the m/z range of 50–550 amu with a speed of 2.9 scan s^{-1} in a full scan mode.

The qualitative identification of compounds was assigned by the retention indices (RI) determined through a C6-C30 n-alkane mixture (Sigma-Aldrich, St. Louis, MO, USA) and matching their recorded mass spectra using the NIST14 databases performed by automated mass spectral deconvolution and identification system (AMDIS). The semi-quantitative analysis of volatile compounds was performed was carried out as reported by Ye et al. [2] with some modifications. The compound 3-octanol (Sigma-Aldrich, St. Louis, MO, USA) was chosen as the internal standard equivalent by the GC peak area under the same conditions.

2.4. Statistical Analysis

Principal components analysis (PCA) were carried out by SIMCA 14.1, and hierarchical cluster analysis (HCA) were conducted using ClustVis, a web-based platform (www.biit.cs.ut.ee/clustvis/, accessed on 25 March 2022). Significant levels were obtained by Duncan's new multiple range tests ($p < 0.05$) by DPS 7.05. The other data were compiled and analyzed using Microsoft Office 2016. All data represented the means of three experiments performed in triplicate.

3. Results

3.1. Total Ion Chromatograms

The GC chromatograms of the volatile compounds emitted by pear flowers are shown in Figure 1. The comparison of typical profiles of chromatograms demonstrated that the Anli flower had the largest abundance of several main volatiles, while most volatile peaks in KorlaXiangli were significantly smaller than other cultivars. Moreover, there was a visible difference between the spectral appearances that could be observed within different cultivars.

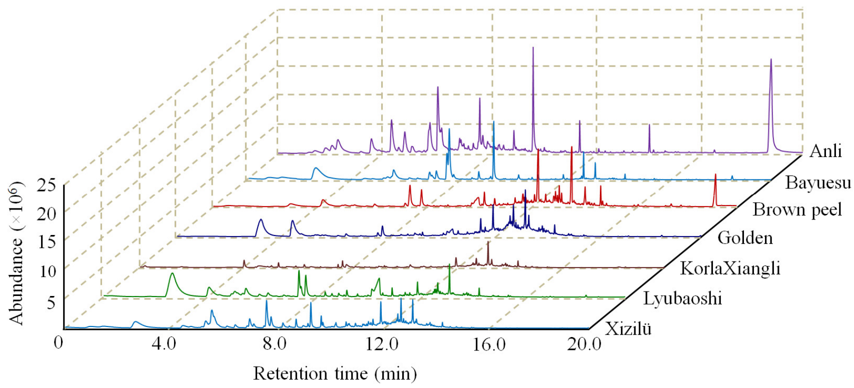

Figure 1. Chromatographic profiles obtained by SPME-GC-MS of volatile compounds in pear flower of seven cultivars.

3.2. Identification of the Volatile Compounds

The volatile compounds identified in pear flowers are shown in Table 2. A total of ninety-three volatile compounds were identified and quantified in the flowers of seven pear cultivars, including alcohols (15), arenes (6), phenols (3), aldehydes (13), terpenoids (13), ketones (4), heterocycle (1), alkanes (10), and esters (28). Lyubaoshi flower contained the highest number of 52 volatiles, while Golden flower exhibited the least (39 compounds); the other flowers of Bayuesu, Xizilü, Anli, Brown peel, and KorlaXiangli contained 51, 47, 46, 42, and 42 volatile chemicals, respectively. The chemical classes of ester, alcohol, aldehyde, and terpene showed the largest numbers in most cultivars. Naphthalene, 1-decanal, 2-pentyl-furan, 2-ethyl hexanol, 1-nonanal, 1-nonanol, methyl 2-ethyl hexanoate, (*E*)-geranyl acetone, 6-methyl-5-hepten-2-one, (*Z*)-3-hexenyl acetate, methyl 2-hydroxy-3-methyl pentanoate, benzaldehyde, hydroquinone, 2-phenylethanol, 2-nitrophenol, 1-octanol, and all alkanes were the common volatiles existed in each cultivar.

Table 2. The volatile compounds detected in the flower of seven pear cultivars and their semi-quantitative determination (μg kg^{-1} FW).

Volatile Compounds	RT	RI	LRI	μg kg^{-1} FW							Identification
				Anli	Bayuesu	Brown Peel	Golden	KorlaXiangli	Lyubaoshi	Xizilü	
Alcohols											
3-Hexenol	1.35	853	858	3.89	-	-	-	-	-	-	MS, RI
1-Hexanol	1.64	861	867	2.04a	-	-	-	0.87b	-	-	MS, RI
5-Methyl-1-hexanol	3.97	924	930	7.47	-	-	-	-	-	-	MS, RI
1-Heptanol	5.11	985	975	-	0.55c	-	-	0.12b	-	1.03a	MS, RI
2-Ethyl hexanol	6.86	1023	1029	5.20a	2.04b	1.47c	2.42b	0.46d	0.29d	2.64b	MS, RI
1-Octanol	7.83	1065	1068	-	1.79b	1.58c	1.35d	0.29e	0.27e	15.63a	MS, RI
2-Phenylethanol	9.06	1117	1114	106.92a	4.19e	48.53b	25.65c	2.65f	4.28e	17.07d	MS, RI
(*Z*)-3-Nonenol	9.81	1157	1153	-	0.81	-	-	-	-	-	MS, RI
1-Nonanol	10.05	1178	1175	6.09c	9.70b	1.88e	3.13d	0.15g	0.47f	12.97a	MS, RI
2-Propyl-1-heptanol	10.58	1195	1194	6.03	-	-	-	-	-	-	MS
2-Butyl-1-octanol	11.73	1264	1277	-	-	-	-	-	1.76a	0.87b	MS, RI
1-Decanol	11.86	1273	1272	-	2.87a	-	-	-	-	3.32a	MS, RI
1-Dodecanol	14.40	1452	1460	-	-	5.53	-	-	-	-	MS, RI
2-Hexyl decanol	15.31	1509	1504	14.40b	-	-	-	-	37.00a	-	MS, RI
1-Tetradecanol	17.41	1672	1676	-	-	4.55	-	-	-	-	MS, RI
SubTOTAL				148.15a	21.96f	63.54b	32.55e	4.54g	44.07d	53.54c	
Arenes											
p-Benzoquinone	3.09	909	905	49.43a	-	0.96c	3.04bc	-	3.55b	-	MS, RI
Benzyl nitrile	9.46	1144	1143	0.53c	-	35.65a	-	0.58c	-	7.40b	MS, RI
Naphthalene	10.46	1189	1191	1.98c	1.26d	1.92c	5.36a	1.29d	0.27e	2.71b	MS, RI
Hydroquinone	11.82	1271	1241	55.31a	2.20de	31.15b	19.50c	2.64de	0.33e	4.13d	MS, RI
2-Methyl naphthalene	12.33	1302	1315	0.44c	0.21d	-	0.66b	0.21d	1.91a	-	MS, RI
2,6-Ditert-butylquinone	14.91	1479	1472	-	-	8.29a	4.95b	-	1.51c	-	MS, RI
SubTOTAL				107.7a	3.66f	77.97b	33.51c	4.72f	7.57e	14.23d	
Phenols											
Phenol	5.50	992	984	1.11a	0.20c	-	-	0.07d	1.03b	0.23c	MS, RI
2-Nitrophenol	9.41	1138	1136	-	0.34c	1.68b	1.70b	0.15c	25.03a	0.63c	MS, RI
5-Methyl-2-nitrophenol	11.52	1253	1250	-	-	1.15b	-	-	2.12a	-	MS, RI
SubTOTAL				1.11d	0.54e	2.83b	1.7c	0.22f	28.18a	0.23f	
Aldehydes											
2-Hexenal	1.31	847	853	4.21	-	-	-	-	-	-	MS, RI
Benzaldehyde	5.09	967	961	45.68a	0.29f	7.13c	18.57b	4.27d	3.22e	2.78e	MS, RI
1-Octanal	6.11	1005	1004	-	9.78b	-	2.28c	2.23c	1.47c	22.43a	MS, RI
Phenyl acetaldehyde	7.22	1048	1042	-	-	6.83a	6.36a	1.23c	2.74b	-	MS, RI
(*E*)-2-Octenal	7.65	1065	1056	-	-	-	-	-	0.31	-	MS, RI
1-Nonanal	8.72	1104	1102	5.42e	46.53b	10.32d	8.75e	1.32f	37.29c	49.76a	MS, RI
(*E*)-2-Nonenal	9.92	1162	1162	-	21.72a	-	-	-	-	0.44b	MS, RI
1-Decanal	10.72	1204	1208	3.25d	81.26a	3.16d	7.67c	0.74d	1.40d	43.68b	MS, RI
(*E*)-2-Decenal	11.70	1265	1260	-	0.29	-	-	-	-	-	MS, RI
2-Phenyl-2-butenal	11.94	1278	1276	-	-	-	-	-	1.24	-	MS, RI
1-Undecanal	12.43	1309	1310	-	2.05b	-	0.67c	-	0.44d	3.03a	MS, RI
1-Dodecanal	13.97	1411	1412	0.72a	0.59b	-	-	-	-	-	MS, RI
1-Hexadecanal	19.59	1807	1822	0.86b	-	-	0.98a	-	-	-	MS, RI
SubTOTAL				60.14c	162.5a	28.03e	45.27d	9.8f	48.1d	122.12b	
Terpenoids											
β-Ocimene	7.57	1052	1051	-	0.20b	-	-	-	3.07a	-	MS, RI
(*E*)-Citral	8.26	1082	1078	-	13.09a	0.59c	-	-	-	3.04b	MS
Linalool	8.63	1101	1098	4.13b	45.37a	-	-	-	0.39c	-	MS, RI
(*E*,*Z*)-Alloocimene	9.33	1130	1131	-	0.25	-	-	-	-	-	MS, RI
(*E*,*E*)-Alloocimene	9.58	1149	1142	-	0.18	-	-	-	-	-	MS, RI
2-Ethenyl-1,1-dimethyl-3-methylenecyclohexane	9.74	1168	-	2.07	-	-	-	-	-	-	MS

Table 2. *Cont.*

Volatile Compounds	RT	RI	LRI	μg kg^{-1} FW							Identification
				Anli	Bayuesu	Brown Peel	Golden	KorlaXiangli	Lyubaoshi	Xizilü	
Methyl geranate	12.71	1327	1326	-	3.7	-	-	-	-	-	MS, RI
Eugenol	13.27	1364	1359	-	0.40c	2.16b	20.15a	-	0.42c	-	MS, RI
(*E*)-β-Damascone	14.11	1421	1420	-	-	-	-	-	0.81	-	MS, RI
(*E*)-Geranyl acetone	14.60	1456	1454	12.00a	2.93b	-	-	0.63c	2.21b	2.29b	MS, RI
α-Curcumene	15.09	1492	1483	-	-	-	-	0.63b	0.50b	2.02a	MS, RI
α-Farnesene	15.35	1512	1507	2.62c	-	-	18.09a	2.89c	1.48d	4.04b	MS, RI
Geranyl linalool	23.85	2024	2034	72.36	-	-	-	-	-	-	MS, RI
SubTOTAL				93.17a	66.11b	2.16e	38.24c	4.16e	8.88d	11.39d	
Ketones											
6-Methyl-5-hepten-2-one	5.64	997	991	15.51a	2.28b	1.52bc	1.94b	0.22c	0.34c	1.38bc	MS, RI
Acetophenone	7.92	1072	1068	0.91a	-	-	-	-	0.32b	-	MS, RI
4-Oxoisophorone	9.56	1148	1147	-	-	-	-	-	0.52	-	MS, RI
Benzophenone	16.88	1630	1625	-	-	2.95	-	-	-	-	MS, RI
SubTOTAL				16.42a	2.28c	4.47b	1.93c	0.22e	1.18d	1.38d	
Alkanes											
Dodecane	10.65	1198	1200	1.39e	2.93d	0.86f	3.36c	-	5.67b	4.99a	MS, RI
Tridecane	12.27	1298	1300	7.40a	0.90d	4.20c	5.69b	0.41e	0.81d	7.69a	MS, RI
Farnesane	13.46	1376	1376	19.32a	0.63e	2.82d	7.98b	0.54ef	0.36f	7.19c	MS, RI
Tetradecane	13.80	1400	1400	28.98b	4.86e	11.68c	43.44a	7.92d	1.91f	28.12b	MS, RI
n-Octylcyclohexane	14.24	1443	1447	8.30c	0.75e	-	10.36a	1.49d	1.11de	9.65b	MS, RI
3-Methyltetradecane	14.76	1471	1470	13.17b	0.54c	193.53a	8.22bc	1.78c	8.22bc	7.01bc	MS, RI
Pentadecane	15.20	1500	1500	58.71b	15.65e	19.01d	78.29a	16.83e	0.68f	30.77c	MS, RI
3-Methylpentadecane	15.94	1572	1571	21.92a	0.51d	18.29b	5.29c	0.59d	0.63d	1.13d	MS, RI
n-Nonylcyclohexane	15.95	1558	1556	4.16b	0.66g	2.68c	7.76a	1.41e	1.07f	2.35d	MS, RI
Hexadecane	16.50	1600	1600	21.82bc	5.46c	213.03a	39.79b	3.53c	0.41c	5.76c	MS, RI
SubTOTAL				185.16c	32.88e	466.10a	210.17b	34.49e	20.87f	104.67d	
Heterocycle											
2-Pentyl-furan	5.76	1001	996	4.91a	0.55e	1.49d	3.08c	0.15f	4.33b	0.51e	MS, RI
Esters											
3-Methyl-2-oxovalerate	4.61	958	957	-	-	0.61c	4.11b	0.32d	5.10a	-	MS, RI
Methyl 2-hydroxy-3-methyl pentanoate	5.80	1003	994	31.21a	2.04d	0.84g	1.44e	0.97f	5.06c	7.98b	MS, RI
(*Z*)-3-Hexenyl acetate	6.27	1011	1005	21.11a	-	2.60b	2.30c	0.08d	2.22c	2.69b	MS, RI
Hexyl acetate	6.59	1021	1011	3.39	-	-	-	-	-	-	MS, RI
Methyl 2-ethyl hexanoate	7.25	1049	1043	10.31a	1.82e	2.90d	2.79d	0.32f	3.87c	7.68b	MS, RI
Ethyl 2-hydroxy-4-methyl valerate	7.98	1077	1078	1.35c	13.36a	-	-	-	-	3.38b	MS, RI
Ethyl 2-ethylhexanoate	8.94	1104	1109	-	0.77	-	-	-	-	-	MS, RI
(*Z*)-3-Hexenyl iso-butyrate	9.63	1151	1145	-	-	-	-	-	-	0.58	MS, RI
2-Phenylethyl formate	10.20	1173	1176	4.76a	-	1.00c	0.75c	-	2.88b	-	MS, RI
(*E*)-3-Hexenyl iso-butyrate	10.42	1183	1186	-	-	-	-	-	-	1.3	MS, RI
Ethyl octanoate	10.60	1198	1196	-	0.82	-	-	-	-	-	MS, RI
(*Z*)-3-Hexenyl valerate	11.17	1232	1235	-	0.60c	0.98b	-	0.13e	0.22d	11.12a	MS, RI
Hexyl 2-methylbutyrate	11.26	1237	1239	-	0.60b	-	-	0.53b	-	2.57a	MS, RI
(*E*)-2-Hexenyl valerate	11.31	1240	1243	-	-	1.19	-	-	-	-	MS, RI
Phenethyl acetate	11.64	1260	1256	-	-	1.63c	4.14a	0.52d	0.58d	0.90b	MS, RI
Propyl benzoate	12.09	1287	1284	-	-	1.55	-	-	-	-	MS, RI
Ethyl nonanoate	12.25	1297	1294	-	0.24	-	-	-	-	-	MS, RI
2-Methyl-phenylmethyl formate	12.41	1307	1271	-	-	-	-	0.72	-	-	MS
(*Z*)-3-Hexenyl 2-methylcrotonate	12.70	1327	1325	0.65b	-	-	-	-	0.46c	3.08a	MS, RI
Hexyl tiglate	12.81	1333	1331	-	-	-	-	-	-	0.6	MS, RI
(*E*)-2-Hexenyl tiglate	12.90	1340	1338	-	-	-	-	0.25b	-	0.52a	MS, RI
Butyl benzoate	13.51	1380	1377	2.28a	-	-	1.78c	0.22d	2.05b	-	MS, RI
(*Z*)-3-Hexenyl hexanoate	13.56	1383	1382	-	-	-	-	-	-	0.49	MS, RI
Ethyl decanoate	13.75	1396	1392	-	0.29	-	-	-	-	-	MS, RI
Ethyl laurate	16.43	1595	1597	-	0.38	-	-	-	-	-	MS, RI
2-Ethyl hexyl benzoate	17.98	1722	1735	-	-	5.63	-	-	-	-	MS, RI
Methyl palmitate	21.76	1935	1928	-	-	1.67a	1.09b	0.28c	-	-	MS, RI
Ethyl palmitate	23.54	2010	1996	-	1.36	-	-	-	-	-	MS, RI
SubTOTAL				75.08a	22.3c	20.6c	18.39c	4.35d	22.43c	43.52b	

RT, Retention time (min); RI, retention index calculated on the basis of compound RT and RTs of an alkane (C6–C30) standard mixture; LRI, retention index given on HP-5MS column by identification software NIST Mass Spectral Search Program on the basis of literature data; "-", no peak detected. Different letters in the same row indicate significant differences among cultivars ($p < 0.05$).

As shown in Figure 2, the total amount of volatiles in pear flowers was significantly different within the seven cultivars. Anli flower contained the highest amount (691.8 μg kg^{-1}) close to Brown peel (667.2 μg kg^{-1}), while KorlaXiangli showed the lowest amount of volatiles (62.7 μg kg^{-1}), approximately the eleventh of Anli flower; the total amount of

Golden flower (384.8 μg kg^{-1}) was close to Xizilü (351.6 μg kg^{-1}); cultivars Bayuesu and Lyubaoshi flower were 312.8 and 185.6 μg kg^{-1}, respectively. Among these volatile chemicals, terpenoids such as geranyl linalool, linalool, and α-farnesene had relative high amounts in Anli, Bayuesu, and Golden flower; 3-methyltetradecane and hexadecane were the major volatiles that accounted for 29.0% and 31.9% in Brown peel flower; 2-phenylethanol, 1-decanal, pentadecane, 1-nonanal were the most abundant volatiles in Anli flower (15.5%), Bayuesu (26.0%), Golden (20.3%), and Lyubaoshi (20.1%), respectively. In addition, a range of esters (fatty acid-derived volatiles) was detected in each cultivar, while the levels were relatively lower compared to other chemicals. Even where volatiles in different pear flowers involved the same chemical classes, the amount varied greatly (Figure 2): alkanes existed in almost all cultivars as the background volatiles with a remarkable amounts level; alcohols mainly existed in the flowers of Brown peel, Anli, and Lyubaoshi; arenes were the dominant volatiles in Brown peel, Anli, and Golden flower; and the highest amount of aldehyde appeared in Bayuesu, Xizilü, and Lyubaoshi flower.

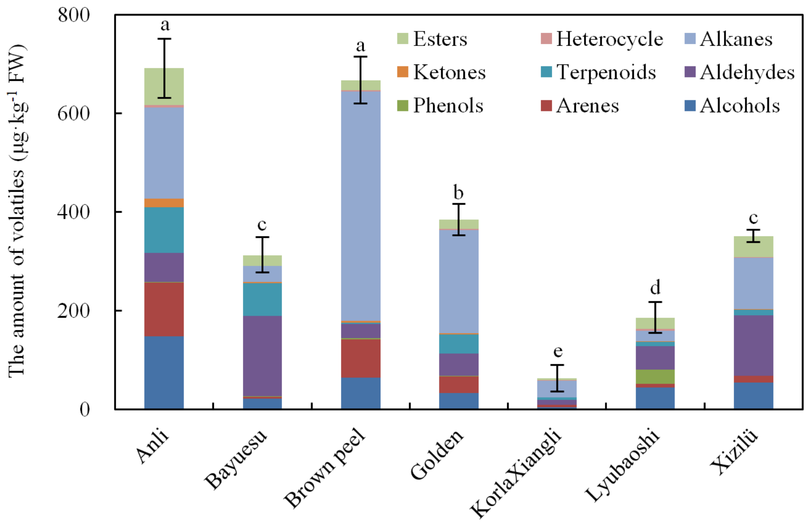

Figure 2. The amount of different volatile chemical classes in flowers of seven pear cultivars. The error bars represent the standard deviation (n = 3). Different letters above the column indicate statistical differences between cultivars ($p < 0.05$), the same as below.

3.3. Odor Types, Description, and OAVs

The OAVs showed only a limited number of volatiles that could be detected at concentrations high enough to be perceived (OAV ≥ 1), which were considered as contributors responsible for the overall flower odor (Table 3). Due to the OAVs, 1-nonanal, 1-octanal, 1-decanal, phenethyl acetate, methyl 2-hydroxy-3-methyl pentanoate, and hexyl acetate were the key active odorants to the aroma of Anli flower; 1-octanal, 1-nonanal, 1-decanal, (*E*)-2-nonenal, 1-decanol were the most active odorants in Bayuesu and Xizilü flower; phenethyl acetate, 1-nonanal, 1-octanal, eugenol were the odor-active compounds to the aroma of Golden flower; 1-nonanal, phenethyl acetate, and phenyl acetaldehyde were the key contributors to Brown peel flower; 1-octanal, 1-nonanal, phenethyl acetate were the key contributors to KorlaXiangli flower; and 1-nonanal, phenethyl acetate, (*E*)-β-damascone were the active odorants in Lyubaoshi flower. To sum up, volatile aldehydes, particularly 1-octanal, 1-nonanal, 1decanal, and (*E*)-2-nonenal were present in higher amounts than their threshold and were considered the key factors to form the odor of pear flower. However, most esters and alcohols showed less importance due to their amounts being below the threshold.

Table 3. Odor thresholds in water, odor descriptions, and OAVs of the studied aroma volatiles determined in pear flowers.

Code	Volatile Compounds	Threshold ($\mu g\ kg^{-1}$) [1]	OAVs [2]							Odor Description
			Anli	Bayuesu	Golden	Brown Peel	KorlaXiangli	Lyubaoshi	Xizilü	
A 1	1-Octanal	0.7 [22]		13.97	3.25		3.19	2.1	32.04	Aldehydic, citrus-like
A 2	1-Nonanal	1 [23]	5.42	46.53	8.75	10.32	1.32	37.29	49.76	Aldehydic, citrus, floral
A 3	1-Dodecanal	2 [22]	0.36	0.29						Aldehydic, soapy, floral, citrus
A 4	1-Undecanal	12.5 [23]		0.16	0.05			0.03	0.24	Aldehydic, waxy, citrus
A 5	1-Decanal	3 [24]	1.08	27.09	2.56	1.05	0.25	0.47	14.56	Aldehydic, waxy, citrus
C 1	(*E*)-Citral	32 [23]		0.41		0.02			0.1	Citrus, lemon-like
C 2	2-Ethyl hexanol	270,000 [22]	<0.01	<0.01	<0.01	<0.01			<0.01	Citrus, fatty, floral
C 3	6-Methyl-5-hepten-2-one	68 [25]	0.23	0.03	0.03	0.02	<0.01	<0.01	0.02	Citrus, green, lemongrass
E 1	2-Ethyl hexyl benzoate	NE								Ethereal
Fa 1	1-Dodecanol	16 [26]				0.35				Fatty
Fa 2	(*E*)-2-Octenal	3 [23]						0.1		Fatty, cucumber
Fa 3	Butyl benzoate	NE								Fatty, balsamic
Fa 4	Propyl benzoate	NE								Fatty, fruity, balsamic nutty
Fa 5	Benzophenone	NE								Fatty, balsamic, geranium, rose
Fa 6	(*E*)-2-Nonenal	0.9 [27]		24.13					0.49	Fatty, green, citrus
Fa 7	1-Heptanol	3 [22]		0.18			0.04		0.34	Fatty, spicy, oily, solvent
Fa 8	(*E*)-2-Decenal	3 [24]		0.1						Fatty, waxy, mushroom
Fa 9	1-Decanol	0.1 [22]		28.7					33.18	Fatty, waxy
Fl 1	(*E*)-Geranyl acetone	60 [28]	0.2	0.05			0.01	0.04	0.04	Floral, magnolia
Fl 2	2-Phenylethanol	750 [28]	0.14	0.01	0.03	0.06	<0.01	0.01	0.02	Floral, floral-rose
Fl 3	Geranyl linalool	NE								Floral, floral-rose
Fl 4	Acetophenone	65 [22]	0.01					<0.01		Floral, bitter almond
Fl 5	Linalool	6 [23]	0.69	7.56				0.07		Floral, citrus, lemon
Fl 6	2-Methyl naphthalene	3 [23]	0.15	0.07	0.22		0.07	0.64		Floral, fatty, oily
Fl 7	β-Ocimene	34 [29]		0.01				0.09		Floral, green, lavender, mango
Fl 8	2-Phenylethyl formate	270 [30]	0.02		<0.01	<0.01		0.01		Floral, green, rose, hyacinth
Fl 9	Phenethyl acetate	0.25 [23]			16.56	6.51	2.07	2.34	3.61	Floral, honey, rose, honey
Fl 10	1-Nonanol	50 [23]	0.12	0.19	0.06	0.04	<0.01	0.01	0.26	Floral, waxy, rose, orange
Fr 1	(*E*)-β-Damascone	0.05 [23]						16.17		Fruity, floral-rose note
Fr 2	(*Z*)-3-Hexenyl iso-butyrate	NE								Fruity, apple, pear
Fr 3	Hexyl acetate	2 [31]	1.69							Fruity, pear
Fr 4	Methyl 2-hydroxy-3-methyl pentanoate	2.4 [32]	13.01	0.85	0.6	0.35	0.41	2.11	3.32	Fruity, estery caramellic
Fr 5	Ethyl 2-hydroxy-4-methyl valerate	NE								Fruity, blackberry
Fr 6	Benzaldehyde	350 [23]	0.13	<0.01	0.05	0.02	0.01	0.01	0.01	Fruity, almond
Fr 7	2-Pentyl-furan	5.8 [33]	0.85	0.09	0.53	0.26	0.03	0.75	0.09	Fruity, green bean
G 1	(*E*)-3-Hexenyl iso-butyrate	500 [26]							<0.01	Green, green-fruity
G 2	3-Hexenol	70 [23]	0.06							Green, green leafy-like
G 3	2-Hexenal	17 [23]	0.25							Green, green leafy-like
G 4	1-Hexanol	500 [23]	<0.01				<0.01			Green, green leafy-like
G 5	(*Z*)-3-Hexenyl acetate	16 [31]	1.32		0.14	0.16	0.01	0.14	0.17	Green, green banana-like
G 6	Hexyl 2-methylbutyrate	22 [29]		0.03			0.02		0.12	Green, green apple
G 7	(*Z*)-3-Hexenyl valerate	NE								Green
G 8	Phenyl acetaldehyde	4 [34]			1.59	1.71	0.31	0.68		Green, honey, hyacinth type
H 1	α-Curcumene	NE								Herbal, herbal
O 1	Ethyl 2-ethylhexanoate	50 [29]		0.02						Orris
Ph 1	Phenol	5900 [22]	<0.01	<0.01			<0.01	<0.01	<0.01	Phenolic, pungent
Pu 1	Eugenol	6 [23]		0.07	3.36	0.36		0.07		Pungent, spicy, clove
Pu 2	Naphthalene	21 [23]	0.09	0.06	0.26	0.09	0.06	0.01	0.13	Pungent, dry resinous
Pu 3	Hydroquinone	5000 [35]	0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	Pungent
Pu 4	*p*-Benzoquinone	NE								Pungent
T 1	(*E*,*Z*)-Alloocimene	NE								Terpenic
T 2	(*E*,*E*)-Alloocimene	NE								Terpenic

Table 3. *Cont.*

Code	Volatile Compounds	Threshold (μg kg^{-1}) [1]	OAVs [2]							Odor Description
			Anli	Bayuesu	Golden	Brown Peel	KorlaXiangli	Lyubaoshi	Xizilü	
Wa 1	1-Octanol	110 [23]		0.02	0.01	0.01	<0.01	<0.01	0.14	Waxy, citrus
Wa 2	(Z)-3-Nonenol	NE								Waxy, cilantro
Wa 3	1-Tetradecanol	NE								Waxy
Wa 4	Hexadecanal	NE								Waxy
Wa 5	Ethyl octanoate	5 [36]		0.16						Waxy, pineapple, apple
Wa 6	Ethyl laurate	400 [26]		<0.01						Waxy, fruity
Wa 7	Ethyl nonanoate	377 [23]		<0.01						Waxy, fatty oily, fruity, wine
Wa 8	Ethyl decanoate	23 [26]		0.01						Waxy, coconut
Wa 9	Ethyl palmitate	2000 [23]		<0.01						Waxy, balsamic
Wo 1	4-Oxoisophorone	NE								Woody, citrus, musty
Wo 2	α-Farnesene	87 [37]	0.03		0.21		0.03	0.02	0.05	Woody, green vegetative nuance

[1] The threshold was obtained through the literature; [2] OAV of one volatile compound was the ratio of its concentration to its odor threshold. NE, not estimated.

Based on odor description, eight main odor types were grouped (Table 3), described as fruity, aldehydic, green, floral, citrus, fatty, waxy, and pungent. Remarkably, several compounds had multiple flavors benefiting the flower's fragrance. Total amounts of different odor types suggested, the floral, fruity, pungent, and green types showed most abundance in Anli, Golden, and Brown peel flowers; the aldehydic, waxy and floral types showed higher values in Bayuesu and Xizilü flowers; aldehydic type was present higher in Lyubaoshi, but all types were significantly lower in KorlaXiangli flowers (Figure 3). This finding suggested Bayuesu and Xizilü flowers had stronger aroma intensity of aldehydic and waxy, but the odor strength of KorlaXiangli was much weaker than the other cultivars.

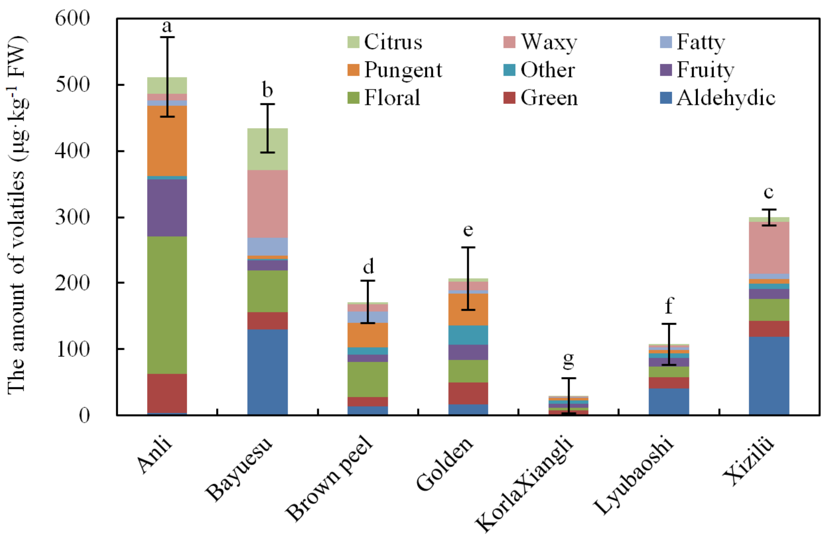

Figure 3. The concentrations of different odor types in the flowers of seven pear cultivars.

3.4. Aroma Profiles Analysis

To visually and effectively explain the aroma features of pear flowers, the radar maps based on the accumulated OAVs were applied (Figure 4). Although most odor types were distributed in each cultivar, the radar maps indicated that the odor behaved significantly differently: Bayuesu flower was similar to Xizilü, which showed aldehydic, waxy, and fatty series were the predominant flavor characters, but the green odor was rich in Bayuesu; the profile of Brown peel flower was close to Golden flower and showed high intensity in aldehydic, waxy and fatty; and Lyubaoshi flower showed high abundance in aldehydic and fruity series.

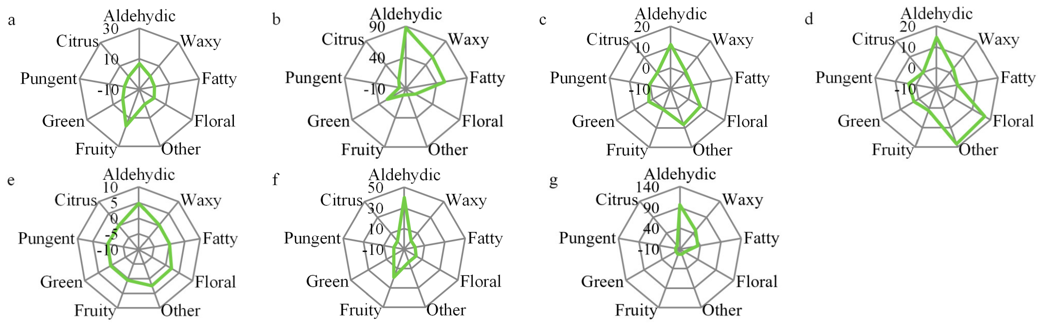

Figure 4. Radar map of aroma series based on the accumulated OAVs. (**a**) Anli, (**b**) Bayuesu, (**c**) Brown peel, (**d**) Golden, (**e**) KorlaXiangli, (**f**) Lyubaoshi, and (**g**) Xizilü.

3.5. Multivariate Analysis

In this study, PCA was carried out using OAVs and samples as the variables, to obtain an overview of relationships among different cultivars in terms of their active odorants. As can be seen from Figure 5, the first principal component (PC1) and second principal component (PC2) were extracted, it was noted that the linear combination of PC1 and PC2 explained 62.1% of the total variance of pear flower samples, indicated that these two factors were considered sufficient for further discussion. The information on relationships between the active odorants and the PCs was provided according to the location of plotted data. As shown, the seven cultivars can be classified into four clusters: cultivars Golden and Brown peel flowers were group together in the first quadrant, the relevant odorants were naphthalene, β-ocimene, phenethyl acetate, α-farnesene, eugenol, 2-methyl naphthalene, and phenyl acetaldehyde; the second group contained Bayuesu and Xizilü flowers located in the left of the third quadrant, with high amounts of the chemicals 1-decanol, 1-heptanol, 1-undecanal, 1-octanal, 1-decanal, 1-nonanal, hexyl 2-methylbutyrate, (*E*)-citral, 1-octanol, 1-nonanol, (*E*)-2-nonenal, and linaloolhe as the relevant odorants, which meant that these cultivars were positive related with the aldehydic, waxy, and fatty series; KorlaXiangli and Lyubaoshi flower were clustered as the third group distributed mostly near the origin, revealed that the flowers were slightly in floral, pungent and green series; cultivars Anli flower situated individual in the lower right corner of the fourth quadrant clustered as the forth group, related to the typical chemicals of methyl 2-hydroxy-3-methyl pentanoate, (*E*)-geranyl acetone, 1-hexanol, 6-methyl-5-hepten-2-one, (*Z*)-3-hexenyl acetate, acetophenone, 2-phenylethanol, benzaldehyde, 2-pentyl-furan, and 2-phenylethyl formate, indicated this cultivar was rich in floral, fruity, and green odor.

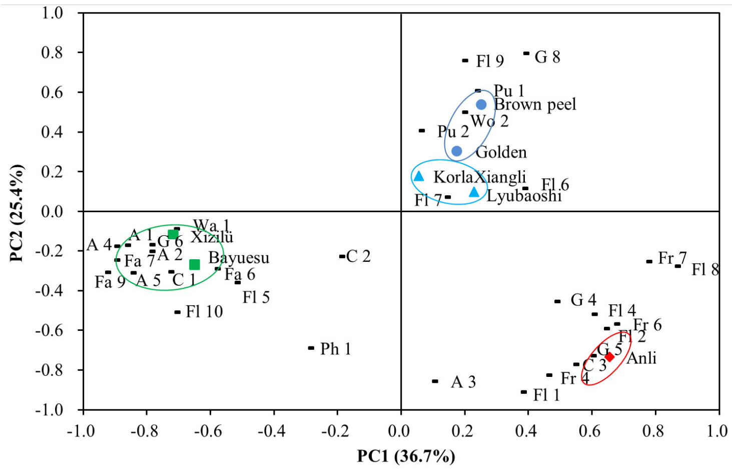

Figure 5. PCA scores plots result using seven pear flower samples as score values and OAVs of selected odorants as loading values. The odorant names correspond to the codes of compounds listed in Table 3.

Application of HCA can interpret the results in an intuitive graphical way; cultivars in the same cluster displayed similar characters, whereas the samples in different clusters were relatively different. In this study, the OAV data were also subjected to HCA and used to explore the heterogeneity between cultivars (Figure 6). Results showed the aroma profiles of different pear flowers were appropriately divided into two main branches, then were again subdivided into three groups. According to the dendrogram and heatmap obtained, Bayuesu had the highest odor-active compound similarity to the Xizilü flower and these were grouped in one cluster, with Golden and Brown peel flowers grouped in a second cluster, and Lyubaoshi and KorlaXiangli flowers grouped in the third cluster. The observed clusters can be explained by the similar odor type of the identified odorants. HCA also showed the highest diversity among the Bayuesu and KorlaXiangli flowers due to the significant differences in the kinds and amounts of the identified volatiles. Futhermore, the results suggested that the aroma of Anli flower was distinct from the other six cultivars. These results were consistent with the PCA analysis, indicating HCA was a powerful tool to validate the rationality of the clustering and reveal the characteristics of each group [38].

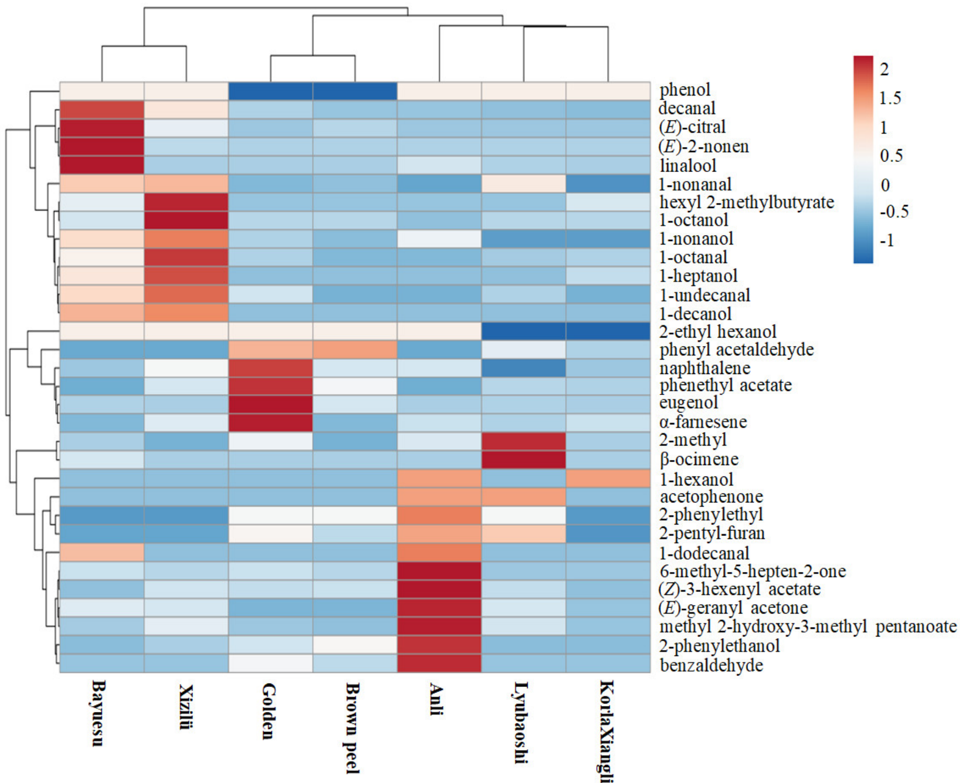

Figure 6. Hierarchical clustering heatmap of selected odorants in flowers of seven pear cultivars. Each colored cell on the map corresponds to OAVs of the odorants, with the samples in the rows and compounds in the columns.

4. Discussion

In this study, ninety-three volatile compounds were identified from the flower of different pear cultivars. Of those ninety-three chemicals, esters, alcohols, aldehydes, alkanes, and terpenoids were the dominant volatiles but showed noticeable differences in their amounts among different cultivars. Overall, volatiles in all cultivars were not distributed uniformly. This phenomenon was consistent with the previous studies on citrus flowers [39], Watermelon Rind [40], *Polianthes tuberosa* L. flower [41], and peach [42].

The aroma characteristics showed pear flower odor was complicated, involving a series of odor types. In this study, several aldehydes, alcohols, and terpenoids were commonly found in each cultivar and presented an intense citrus odor like orange or lemon, and floral fragrance like rose or hyacinth [42]. Moreover, a certain amount of fatty and waxy odorants with oil-like or balsam-like were found in Bayuesu and Xizilü flowers and several C6 aldehydes, alcohols, and related esters were detected in Anli flowers known as green leaf volatiles (GLVs) [11]. However, not all volatile compounds observed in pear flowers actually affect the aroma [17,43]; alkanes, as the major components of plant wax, were ubiquitous in all pear cultivars, always had less contribution to aroma but more for the fragrance lasting [42]. Somewhat unexpectedy, α-pinene, γ-terpinene, d-camphor, and geraniol generally exist in the flowers of plants but were hardly recognized in this experiment [44,45].

OAV was the ratio of concentration to the sensory threshold for a particular volatile compound [42]. The specific intensity and tendency of active odorants, which could be obtained by combining OAV and odor descriptor [43], provided further information on their important role in flavor [46]. In this study, OAVs of related odorants were further calculated to evaluate the aroma attributes of pear flowers. The lower threshold values of phenethyl acetate, 1-octanal, 1-nonanal, methyl 2-hydroxy-3-methyl pentanoate, 1-decanal, phenyl acetaldehyde, 2-pentyl-furan, and eugenol, typically showed higher OAVs and can be considered as the primary contributors of pear flower, presenting strong citrus, floral, and waxy notes [32]. Furthermore, some other volatiles with high OAVs were also observed in special cultivars, such as hexyl acetate, (*Z*)-3-hexenyl acetate correlated with the fruity and green odor detected in Anli flower; (*E*)-2-nonenal, 1-decanol, linalool correlated with fatty and floral odor [47] in Bayuesu and Xizilü flower; phenyl acetaldehyde and eugenol correlated with hyacinth and clove flavor [23] in Golden and Brown peel flower; and (*E*)-β-damascone correlated with floral and fruity notes [47,48] was just observed in Lyubaoshi flower. To sum up, the pear flower samples were characterized mainly by fruity, aldehydic, green, floral, citrus, fatty, waxy, and pungent notes. As reported previously [45,49,50], the relationship between aroma components and people's perception have always been a difficult and inadequately settled problem. For pear flowers, there is a lack of reference for evaluating the aromatic series and indicators for understanding the aroma characters. Still, in this study, there were obvious differences observed based on the aroma profiles conducted to compare the aroma attributes of different pear flowers. The aroma profiles indicated the major aroma characteristic between Bayuesu and Xizilü flower were much similar, with higher aldehydic, waxy, and fatty odor; the profile of Brown peel flowers were close to Golden pear, showing a high abundance in the series of aldehydic and floral flavor; and Lyubaoshi flower showed a high abundance of aldehydic and fruity series. These results further demonstrated that pear flowers mainly presented a sweet-orange or citrus-like flavor; these aroma descriptions were also consistent with the actual sensory characteristics. To the best of our knowledge, this was the first paper focused on preferred aromatic series and their components.

PCA is a multivariate technique used to analyze data in which observations were described by several inter-correlated quantitative dependent variables and samples [41]. HCA is suitable for classifying samples into different classes or clusters. Due to the significant similarities or differences in the quantities of the identified volatiles, Anli flower was discriminated from the others and existed as an independent category, indicating that the green leaf flavor was the preferred aromatic property; Bayuesu was similar to Xizilü flowers, discriminated from the others indicated that the citrus flavor was the preferred aroma correlated with the typical odorants of 1-octanal, 1-nonanal, 1-decanal with aldehydic odor [24,51]; in contrast, Bayuesu showed the highest diversity to KorlaXiangli flower because of the significant differences in the identified volatiles. It can be concluded that cluster analysis based on aroma components performed well in the discrimination of the flowers of different pear cultivars. To some extent, we also found the cluster results were consistent with the species difference (Table 1) and genetic relationship between cultivars. According to the obtained results, the PCA and HCA of volatile compounds can be successfully applied for the aroma evaluation of pear flowers, and the cultivars' differences were critical for the overall pattern.

As reported, several key volatile chemical compositions had previously been found to be bio-activator to plant species [52,53]. Here, we showed that the pear flower contained phenolic compounds such as hydroquinone, and its derivatives were investigated for their most important antioxidant-active and skin depigment [3]; linalool can repel agricultural pests such as aphid *Myzus persicae*; the C6 and C9 aldehydes and alcohols such as 3-hexenol, 2-hexenal, 1-hexanol were produced by plants in response to the physical injury and played an important role in plants defense strategies and pest resistance;11 furthermore, eugenol exhibits an excellent bactericidal activity against a wide range of organisms [54]. In addition, volatiles of 1-octanal, 1-nonanal, 1-dodecanal, 1-undecanal, 1-decanal, (*E*)-β-damascone, α-farnesene, benzaldehyde, 6-methyl-5-hepten-2-one, 2-hexenal were commonly found in diverse plants, and it had been recognized that the biological behavior responded by the blend of volatiles rather than individual compound [55].

5. Conclusions

This study determined the volatile compounds in the flowers of seven pear cultivars and demonstrated evidence of differences were statistical significance between cultivars. Considering the lower threshold perception and higher OAVs, volatile aldehydes were identified as key compounds for the pear flower in most cultivars. Being the basic active odorants, aldehydes displayed an intensely citrus and floral note. For the overall aroma profiles, pear flowers were characterized mainly by aldehydic, waxy, floral, and pungent series, moreover, it was essential for the attraction of insect pollinators to ensure plant reproductive success. Combined the aroma profiles and multivariate analysis, a clear classification of pear flowers was carried out. In conclusion, Bayuesu flower was very similar to Xizilü and grouped together; Golden flower was close to Brown peel and grouped together; Bayuesu showed the highest diversity to KorlaXiangli flower; and Anli flower showed many special elements to the other cultivars. Here, a comprehensive evaluation was performed to indicate the odor characteristics and relationship among the flower samples. Overall, these results support an important step in determining the role of aroma components and evaluated the characteristic aroma of pear flower, which could contribute to the cultivars distinction and further utilization of pear flower.

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