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Identification of Aroma-Active Compounds in Jiashi Muskmelon Juice by GC-O-MS and OAV Calculation

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ABSTRACT: To identify aromatic compounds in Jiashi melon juice, gas chromatography−mass spectrometry−olfactometry (GC-MS-O) analysis was used. Odor activity values (OAVs) were also calculated on the basis of the qualitative and quantitative analysis of volatile compounds. Results showed that 42 volatiles were identified, among which 4 compounds, namely, diethyl carbonate, isophorone, 2-butoxyethyl acetate, and menthol, were identified or tentatively identified for the first time as volatiles in melon fruit. Twelve compounds, namely, (2E,6Z)-nona-2,6-dienal, (3Z,6Z)-nona-3,6-dien-1-ol, ethyl butanoate, ethyl 2-methylbutyrate, ethyl 2-methylpropanoate, (Z)-non-6-enal, (E)-2-nonenal, heptanal, methyl 2-methylbutyrate, nonanal, hexanal, and 2-methylpropyl acetate, were identified as the potent odorants of Jiashi melon juice by both OAV and detection frequency analysis (DFA). In addition, seven odorants were detected by all of the panelists and showed higher OAVs, indicating that DFA and OAV resulted in relatively similar "Jiashi" melon aroma patterns.

KEYWORDS: melon, aroma-active compound, GC-MS, detection frequency analysis, gas chromatography−olfactometry, odor activity value

■ INTRODUCTION

Jiashi melon (*Cucumis melo* var. *reticulatus*, Hami melon) is a late-maturing, climacteric aromatic[1] and one of the most appreciated muskmelon varieties planted in the Xinjiang Uyghur Autonomous Region of China for both the foreign and local markets. Among many factors influencing the melon quality, odor-active compounds are major determinants of consumers' acceptance. The aromatic profile of Jiashi muskmelon is thermally sensitive,[2] strictly bottlenecking its industrial development. Therefore, studying its aroma-active compounds can provide an important index in seeking new nonthermal processing technologies to improve the merchandise rate of Jiashi muskmelon.

Melon aroma is determined by unique combinations of aroma-active compounds and strongly dependent on the cultivar and physiological behavior of the fruit.[3,4] With the development of modern analytical techniques to evaluate volatiles, the melon volatile compound profile has been extensively investigated,[4] and more than 250 volatile compounds have been identified in different varieties of melon,[5] most of which are esters, aldehydes, alcohols, ketones, and sulfur-derived compounds. These studies[3−12] on melon aroma have found that different types of melon could result in different volatile profiles (e.g., esters, especially acetates, are dominant in climacteric melon types, whereas nonaromatic varieties often have much lower levels of total volatiles and lack the volatile esters), which may be due to the fact that melon is a highly polymorphic species that comprises a broad array of wild and cultivated genotypes differing in fruit traits such as climactericity, sugar and acid contents, and secondary metabolites associated with aroma.[9] However, these relevant research works mostly focused on the qualitative analysis of volatiles of melon fruits, with relatively fewer investigations on odor-active component identification.[6,8,10−12] In addition, to the best of our knowledge,

no studies on the odor-active compounds of Jiashi muskmelon have been found.

Gas chromatography−olfactometry (GC-O) and odor activity value (OAV) calculation are the commonly used methods for odor-active compound identification. GC-O is based on the use of human assessors as sensitive and selective detectors to ascertain odor-active volatile compounds in a sample extract.[13] Among three categories of GC-O methods (detection frequency, dilution to threshold, and direct intensity), detection frequency analysis (DFA) is much simpler, more repeatable, and time-saving because it accounts for the variable sensitivities of assessors, and independent panels are even able to generate similar aromagrams without being trained prior to the analysis.[14] This method was originally used to identify taints in mineral water packaged in polyethylene-lined cardboard cartons.[15] Currently, it has been applied for the selection of odor-active compounds in many foods.[13,16] The OAV, which was defined by Rothe and Thomas[17] as the ratio of the concentration of the flavor compound to its odor threshold (OT), is a parameter widely used to obtain odor patterns starting from quantitative compositions. In this method, compounds with a ratio value ≥1 are considered to be responsible for aroma, and the greater their OAV is, the more they contribute to the aroma profile. Even though the use of this value has been criticized,[18] because it neglects the effect among odorants in a mixture, it has been used widely in the determination of potency of odor-active compounds in many foods.[19,20]

The present study aimed (i) to characterize and quantify volatiles of Jiashi muskmelon juice by HS-SPME-GC-MS, (ii)

to identify odor-active compounds of Jiashi muskmelon juice, and (iii) to compare odor patterns obtained from OAV and DFA and discuss their advantages and deficiencies.

■ MATERIALS AND METHODS

Chemicals. n-Alkanes ($C_5–C_{20}$) used for the linear retention index (LRI) calculation were purchased from Chemical Ltd., Beijing, China. The reference standards for qualitative and quantitative analysis except ethyl carbonate, 3,5,5-trimethylcyclohex-2-en-1-one (isophorone), and 2-butoxyethyl acetate, for which no standards are available, listed in Table 1 were all obtained from Sigma-Aldrich, Milwaukee, WI, USA (no. 1′, 3′, 4′, 5′, 6′, 10′, 11′, 13′, 14′, 17′, 18′, 20′, 21′, 23′, 30′, 32′, 35′, 36′, 38′, 39′, 40′), J&K Chemical Ltd., Beijing, China (no. 2′, 7′, 9′, 12′, 16′, 19′, 24′, 25′, 26′, 29′, 31′, 34′, 37′, 41′, 42′), and Shanghai Oriental Limited Epristeride, Shanghai, China (no. 15′, 22′, 28′, 33′). Methanol (HPLC grade) served as the solvent for dissolving reference standards, and 1-heptanol used as the internal standard was purchased from J&K Chemical Ltd.. Sodium chloride used for volatile extraction and other reagents were all purchased from Beijing Chemical Reagent Co., Beijing, China.

Melon Samples and Juice Preparation. Jiashi muskmelon was harvested with the best edible quality according to the experience of melon farmers (using a combination of different harvest indices, including about three-fourths firm and dry skin netting development, aroma emission detected by the human nose, dark green skin color, development of an annular ring in the peduncle, and peduncle suberization) and free from any quality deterioration or decay. Immediately after harvest, the fruits were transported to the laboratory by air. The melon pieces ($1 \times 2 \times 3$ cm^3) were prepared and frozen according to the method proposed by Ma[21] and immediately vacuum-sealed in aluminum foil compound bags (20×15 cm^2) and stored at -18 °C after being fully mixed. Before use, three bags of melon cubes (~80 g) used as triplicates were thawed overnight at 4 °C in a domestic refrigerator and then melon juice was prepared according to the method described by Chen.[2]

Extraction of Volatiles from Jiashi Muskmelon Juice Using Manual SPME. A manual SPME (Supelco, Inc., Bellefonte, PA, USA) with a 50/30 μm polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR) SPME fiber was used for volatile extraction after the fiber had been conditioned at 250 °C for 30 min. Immediately after juicing, melon juice (8 mL) was quickly transferred into a 15 mL headspace bottle containing 2.4 g of NaCl and then equilibrated in a laboratory stirrer/hot plate (model PC-420, Corning Inc. Life Science, Acton, MA, USA) at 40 °C for 20 min. Then, a stainless steel needle, housing the SPME fiber, was placed through the hole to expose the fiber at the position of 1 cm over the liquid surface for 20 min with magnetic stirring at 100 rpm.

GC-MS Analysis. After extraction, the SPME device was inserted into the injection port of an Agilent 7890A gas chromatograph coupled with an Agilent model 5975C series mass spectrometer and desorbed for 3 min in a split/splitless GC injection port, which was equipped with an inlet linear specific for SPME use (Agilent Technologies). The analytical conditions were as follows: to improve the resolution of volatile compounds, the volatiles were separated on two types of fused silica capillary columns with different polarities, namely, DB-5 (30 m × 0.25 mm i.d. × 0.25 μm; J&W Scientific, Folsom, CA, USA) and DB-Wax (30 m × 0.25 mm i.d. × 0.25 μm; J&W Scientific); the temperature of the injector and the MS interface temperature was set at 250 and 260 °C, respectively; the oven temperature programs of the DB-5 and DB-Wax columns were 40 °C (2 min), 3 °C/min to 100 °C, 5 °C/min to 200 °C, and 8 °C/min to 250 °C (2 min) and 40 °C (2 min), 3 °C/min to 200 °C, and 10 °C/min to 230 °C (5 min), respectively; the carrier gas was helium at 1.2 mL/min; the injector mode was splitless; the mass spectrum in the electron impact mode was generated at 70 eV; and temperatures for ion source and the quadrupole mass filter were 230 and 150 °C. Chromatograms were recorded by monitoring the total ion current in a 29−200 mass range.

Gas Chromatography−Olfactometry Frequency Analysis. A sniffing port (Sniffer 9000, Brechbühler, Switzerland) coupled to a GC-MS instrument (7890A-5875C, Agilent Technologies, Inc.) was used for odor-active compound characterization. At the exit of the capillary column, the effluents were split 1:1 (by volume) into a sniffing port and a MS detector by employing the Agilent capillary flow technology; the transfer line to the GC-O sniffing port was held at 280 °C; humidified air was added in the sniffing port at 60 mL/min to help maintain the olfactory sensitivity by reducing dehydration of mucous membranes in the nasal cavity. The sampling method and GC-MS conditions were the same as those described above for the GC-MS analysis.

Detection frequency analysis using a panel of four judges (two males and two females aged between 20 and 35 years) was applied to obtain the odor pattern of Jiashi muskmelon juice following the methodology described by Yu.[22] In total, eight GC-O runs were performed (two runs for each assessor). The detection frequency (DF) for an odor with the same retention index and a similar description was summed. At the sniffing port any odorant that had total detection frequencies ≥2 was arbitrarily considered to have aroma potential activity.

Quantitative Analysis. Quantitative data of the identified compounds were obtained from calculating their relative quantitative correction factors (RQCFs) by using the "single point correction method" similar to the standard addition method. The GC conditions were the same as described earlier for the GC-MS analysis, but the mass acquisition mode was changed to a selected ion monitor (SIM) and solvent delay time was set to 3 min to avoid the harm of solvent in standard solutions to the filament. Selected ions (m/z) used for quantitative purposes are shown in Table 1. The procedure of obtaining RQCFs was as follows: a volume of 8 mL of Jiashi muskmelon juice was analyzed by SPME-GC-MS/SIM, yielding the quantifying ion peak area of each identified compound. The same volume of Jiashi muskmelon juice containing defined amounts of mixed authentic standards and internal standard (to eliminate the run-to-run variation) was again analyzed to obtain a new quantifying ion peak of each identified compound and quantitative correction factor of internal standard. RQCF of each volatile was obtained by using the equation

$$f_i{}' = \frac{f_{wi}}{f_{ws}} = \frac{m_i/{}_\Delta A_i}{m_s/A_s} = \frac{A_s m_i}{{}_\Delta A_i m_s} \tag{1}$$

where $f_i{}'$ is RQCF of identified compound (i); m_s and m_i are the known contents of authentic standard (i) and internal standard (s), respectively; A_s is the quantifying ions peak area of internal standard (s) and ${}_\Delta A_i$ is the quantifying ions peak area of identified compound (i) changed before and after addition of the standard solution to juice sample.

To quantify the identified volatiles of Jiashi muskmelon juice, a volume of 8 mL of juice containing the same content of internal standard as that in RQCF calculation was prepared and then subjected to GC-MS/SIM analysis. The concentration was calculated using the formula

$$m_i = f_i{}' \times A_i \times \frac{m_s}{A_s} \tag{2}$$

where m_i is the content of compound (i) and m_s is the known level of internal standard (s); A_i and A_s are quantifying ion peak areas of identified compound (i) and internal standard (s), respectively; and $f_i{}'$ is the RQCF of identified compound (i). The quantifying ion peak area of each component in selected ion chromatograms was determined in triplicate, and the average value was calculated. Finally, the concentration of each identified volatile in the Jiashi muskmelon juice was expressed in nanograms per milliliter of juice. To quantify compounds without standard reference including ethyl carbonate, isophorone, and 2-butoxyethyl acetate, RQCFs of the same class of compounds, namely, ethyl propionate, 2,2,6-trimethylcyclohexan-1-one, and 3-acetyloxybutan-2-yl acetate (2,3-butanediol diacetate), were used.

Table 1. Qualitative and Quantitative Results of Volatiles in the Headspace of Jiashi Muskmelon Juice

no.[a]	compound	CAS Registry No.	match quality[b] DB-5	DB-Wax	LRI[c] DB-5	DB-Wax	quantitative ion[d]	RQCF[e]	C[f]	RSD (%)[g]	ID[h]
1	methyl acetate	79-20-9	81	83	653	834	74	0.51	158	3.96	LRI, MS, S
2	ethyl acetate	141-78-6	91	90	704	900	43	0.23	1.10e3	6.14	LRI, MS, S
3	ethyl propanoate	105-37-3	87	91	713	963	29	2.31	361	8.06	LRI, MS, S
4	2-methylbutan-1-ol	34713-94-5	91	86	739	1207	57	1.32	45.7	5.95	LRI, MS, S
5	ethyl 2-methylpropanoate	97-62-1	94		746		43	0.74	94.0	7.04	LRI, MS, S
6	2-methylpropyl acetate	110-19-0	93	83	752	1019	56	0.20	67.0	2.27	LRI, MS, S
7	methyl 2-methylbutanoate	868-57-5	93	90	764	1008	57	28.41	25.6	9.12	LRI, MS, S
8	diethyl carbonate*	105-58-8	91		776		91		5.26	8.77	LRI, MS, T
9	hexanal	9012-63-9	95	94	801	1020	44	0.29	17.8	6.39	LRI, MS, S
10	ethyl butanoate	105-54-4	93	94	804	1041	71	13.14	3.85e3	5.63	LRI, MS, S
11	butyl acetate	123-86-4	81	81	816	1077	43	12.44	117	8.36	LRI, MS, S
12	ethyl 2-methylbutanoate	7452-79-1	97	83	842	1056	57	0.38	115	8.89	LRI, MS, S
13	(E)-hex-2-enal	73543-95-0	94	87	844	1229	41	3.13	31.2	1.51	LRI, MS, S
14	hexan-1-ol	25917-35-5	83	86	851	1357	56	2.81	4.44	9.11	LRI, MS, S
15	3-methylbutyl acetate	29732-50-1	93	90	876	1122	43	0.19	14.3	5.37	LRI, MS, S
16	2-methylbutyl acetate	624-41-9	95	93	880	1126	70	0.16	38.8	5.50	LRI, MS, S
17	heptanal	85-86-9	81	87	903	1185	41	1.02	283	7.41	LRI, MS, S
18	benzaldehyde	317-34-0	96		943		77	0.75	13.1	4.23	LRI, MS, S
IS[i]	1-heptanol	111-70-6	97	93	953	1450	70	1.00	20.5	4.05	LRI, MS, S
19	oct-1-en-3-ol	57-71-6	83	91	958	1456	57	0.11	0.54	9.15	LRI, MS, S
20	6-methylhept-5-en-2-one	409-02-9	81	83	978	1348	43	0.23	3.22	9.54	LRI, MS, S
21	octanal	823-40-5	91	87	1005	1302	56	0.60	1.02	6.99	LRI, MS, S
22	[(Z)-hex-3-enyl] acetate	3681-71-8	83	90	1007	1325	67	0.10	0.29	6.03	LRI, MS, S
23	hexyl acetate	88230-35-7	84	87	1009	1275	84	0.15	1.65	8.48	LRI, MS, S
24	2-ethylhexan-1-ol	704-76-7	87	86	1040	1497	57	0.35	9.02	7.40	LRI, MS, S
25	2,2,6-trimethylcyclohexan-1-one	62861-88-5	89	87	1045	1334	82	0.10	0.38	8.86	LRI, MS, S
26	2-phenylacetaldehyde	122-78-1	93		1049		91	0.64	3.32	9.06	LRI, MS, S
27	3,5,5-trimethylcyclohex-2-en-1-one*	78-59-1	86	91	1056	1419	8		11.4	5.66	LRI, MS, T
28	3-acetyloxybutan-2-yl acetate	1114-92-7	87	90	1069	1534	43	3.52	4.40	7.32	LRI, MS, S
29	2-butoxyethyl acetate*	112-07-2	90		1078		57		2.55	8.67	LRI, MS, T
30	(Z)-non-6-enal	2277-19-2	94	93	1096	1462	41	0.45	9.16	8.58	LRI, MS, S
31	nonanal	75718-12-6	91	95	1104	1401	57	0.10	4.36	7.68	LRI, MS, S
32	(2E,6Z)-nona-2,6-dienal	557-48-2	83	91	1110	1599	41	3.56	327	5.73	LRI, MS, S
33	(3Z,6Z)-nona-3,6-dien-1-ol	56805-23-3	86	83	1141	1736	67	5.69	201	8.23	LRI, MS, S
34	(E)-2-nonenal	30551-15-6	89	95	1147	1549	43	0.26	10.1	2.41	LRI, MS, S
35	phenylmethyl acetate	140-11-4	94	90	1162	1745	108	0.53	1.99	7.37	LRI, MS, S
36	nonan-1-ol	28473-21-4	83	87	1170	1664	56	0.13	1.84	5.55	LRI, MS, S
37	5-methyl-2-propan-2-ylcyclohexan-1-ol*	89-78-1	87	83	1171	1519	71	0.89	0.91	9.26	LRI, MS, S
38	decanal	75718-12-6	84	90	1209	1453	43	2.14	1.56	0.85	LRI, MS, S
39	2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	98-55-5	95	93	1211	1605	59	0.79	0.38	6.61	LRI, MS, S
40	2,6,6-trimethylcyclohexene-1-carbaldehyde	52844-21-0	90	87	1254	1638	137	1.09	2.15	4.52	LRI, MS, S
41	(5E)-6,10-dimethylundeca-5,9-dien-2-one	689-67-8	95	91	1365	1840	43	0.09	0.54	4.61	LRI, MS, S
42	(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one	127-41-3	90	83	1442	1895	121	12.04	0.21	8.77	LRI, MS, S

[a]Volatiles are shown according to their order of appearance in the chromatogram on DB-5 column. [b]Match quality on DB-5 and DB-Wax columns given by NIST library. [c]Retention index on DB-5 and DB-Wax columns according to equation proposed by Vandendool and Kratz.[25] [d]Obtained from the mass spectra of reference standards in the electron impact model at 70 eV. Those of compounds without reference standard were from NIST library. [e]Relative quantitative correction factor equals the ratio of quantitative factor of identified components to that of internal standard (1-heptanol). [f]Concentration is expressed in nanograms per milliliter of juice, and data listed are the mean of three assays. [g]Relative standard deviation ($n = 3$); all RSDs were <10%. [h]ID, volatiles were identified according to abbreviations: LRI, comparing linear retention indices (LRI) on two columns (DB-5 and DB-Wax) with those in the literature; S, confirmed with standard reference; MS, mass spectrum comparisons with those in NIST library; T, tentatively identified because no standard reference was available. [i]Internal standard.

Calculation of OAVs. OAVs were calculated according to the equation

$$OAV = C_i / OT_i \tag{3}$$

where C_i is the concentration of the odorant (i) calculated from eqs 1 and 2 and OT_i is its corresponding odor threshold in water found in the literature.[23,24] Compounds with OAV ≥1 were considered to be potential contributors to Jiashi muskmelon aroma profile.

Compound Identification. The identification of volatile compounds was made by comparing their linear retention indices (LRI) and MS fragmented patterns with those of standard compounds and published data (acceptable variability of the retention index between the calculated and one from reference was <20), as well as by comparing their mass spectra with the MS library of NIST08 (match quality ≥ 80). LRI was calculated using a mixture of *n*-alkanes, C_5-C_{20}, as standards according to the method of Vandendool and Kratz.[25] Compounds without reference volatiles were considered to be tentatively identified.

Statistical Analysis. Quantitative analysis was carried out in triplicate, and statistical analysis of the ANOVA (using Tukey procedure) was conducted to indicate the variability using OriginPro v8.0 (OriginLab Inc., Northampton, MA, USA).

■ RESULTS AND DISCUSSION

Characterization and Quantification of Volatiles. Altogether, 42 volatiles including esters, short-chain alcohols, aldehydes, and ketones were detected in Jiashi muskmelon juice (Table 1); 39 of these compounds were unequivocally identified on the basis of their reference volatiles, whereas diethyl carbonate, isophorone, and 2-butoxyethyl acetate were considered to be tentatively characterized because their standard reference is not available. Most of these volatiles are well-known in melon fruits, but four volatiles, namely, diethyl carbonate (no. 8′), isophorone (no. 27′), 2-butoxyethyl acetate (no. 29′), and 5-methyl-2-propan-2-ylcyclohexan-1-ol (menthol) (no. 37′) were identified for the first time as melon volatiles. In addition, (*E,Z*)-3,6-nonadiene, (*Z*)-nonen-6-ol, (*Z*)-nonen-3-ol, methyl butyrate, eucalyptol, thymol, furan aldehydes, sulfur-containing compounds and acids reported as aroma composition of Hami melon and other cantaloupe varieties[2,3,21,26,27] were not found in Jiashi muskmelon juice. These differences could be attributed to geographical and cultivar variations as well as different aroma extraction methods used.

Among 42 volatiles, esters, including 11 acetates and 6 nonacetate esters, were dominant quantitatively (accounting for 40.48%). Other quantity-predominant compounds were alcohols (28.57%) and aldehydes with a nine-carbon straight chain (19.05%). As was the case in this study, Gonda et al.[9] concluded that volatiles in melon fruits were mainly esters with floral and fruity note as well as aldehydes and alcohols possessing green melon-like and grassy smells.

A single-point correction method combined with GC-MS/SIM analysis was used to quantify odorants in original Jiashi muskmelon juice. Compared with the MS calibration factor determination reported by Chetschik et al.,[28] this method took account of the distribution coefficient variation among volatiles during the SPME procedure other than differences among their response factors to the MS detector. In addition, compared with the fast quantitative determination method for melon aroma by Verzera,[26] in which data acquisition was based on total ion chromatograms, the quantification analysis based on selected ion chromatograms in this study was more sensitive and selective because SIM can eliminate the interference of high background values.

As shown in Table 1, the RQCFs of volatiles in Jiashi muskmelon juice varied greatly from 28.41 to 0.10, indicating that volatiles vary greatly in the distribution coefficients during SPME procedure as well as in response factors to MS detector. Among all of these volatiles, esters are the most abundant compounds (acetates account for 81.37% in concentration and nonacetate esters account for 4.47%), followed by aldehydes (10.14%) and alcohols (3.80%). It was clear from the

quantitative and qualitative data that esters dominated not only quantitatively but also qualitatively. This was in accordance with the conclusion of previously published study,[29] in which volatile esters were reported to be prominent in volatiles of climacteric aromatic melon varieties. In addition, consistent with the previous study[30] in which ethyl butanoate was characterized as the most abundant volatile of 'Miyabi' melon, ethyl butanoate was also detected to have the highest concentration (accounting for 55.5%) in Jiashi muskmelon juice.

Aroma-Active Compounds Obtained from DFA. In DFA, the detection frequency of the odor-active compound at the sniffing port was used as a measure for the intensity of a compound, and compounds detected more frequently were concluded to have a greater relative importance.[16] As shown in Table 2, a total of 21 events were determined to have aroma

Table 2. Odor-Active Compounds of Headspace Jiashi Muskmelon Juice Characterized by Detection Frequency Analysis

no.[a]	compound[b]	DF[c]	odor[d]
5	ethyl 2-methylpropanoate	8	fruity, sweet
10	ethyl butanoate	8	sweet, fruity
12	ethyl 2-methylbutanoate	8	cantaloupe-like, fruity, melon
19	oct-1-en-3-ol	8	earthy, mushroom
30	(*Z*)-non-6-enal	8	honeydew melon, fruity
32	(2*E*,6*Z*)-nona-2,6-dienal	8	cucumber-like, green melon
33	(3*Z*,6*Z*)-nona-3,6-dien-1-ol	8	cucumber
34	(*E*)-2-nonenal	8	melon, fresh
Un	unkown[e]	8	grassy
17	heptanal	7	citrus
40	2,6,6-trimethylcyclohexene-1-carbaldehyde	6	mint
20	6-methylhept-5-en-2-one	5	floral
31	nonanal	5	melon
23	hexyl acetate	4	fruity
7	methyl 2-methylbutyrate	3	fruity, sweet
26	2-phenylacetaldehyde	3	floral
27	3,5,5-trimethylcyclohex-2-en-1-one	3	camphor-like
6	2-methylpropyl acetate	2	floral
9	hexanal	2	green, leaf
18	benzaldehyde	2	aromatic, almond-like, sweet
39	2-(4-methylcyclohex-3-en-1-yl) propan-2-ol	2	clove-like, ferment

[a]Numbers correspond to those in Table 1. [b]Each compound was identified by comparing it with an authentic standard based on the following criteria: (i) matching retention time on the same column; (ii) mass spectrum on NIST 08 database; (iii) description of its odor description; (iv) injection of reference standards. [c]Sum of times detected by four assessors. [d]Odor description as perceived by panelists during detection frequency method. [e]Compound which was not identified by MS due to its concentration being too low.

impact using the detection frequency method, based on our arbitrary criteria (detection frequency ≥ 2), and most of them were related to melon, cucumber, fruity, and floral descriptors. Among these compounds, 20 were qualified on the basis of mass spectra, RI, odor descriptors, and reference standards, whereas one compound (no. Un′), noted by all judges, was not detected by GC-MS, which might due to its concentration being below the detection limit of the method used. Nine components, namely, ethyl 2-methylpropanoate, ethyl butanoate, ethyl

2-methylbutyrate, 1-octen-3-ol, (Z)-non-6-enal, (2E,6Z)-nona-2,6-dienal, (3Z,6Z)-nona-3,6-dien-1-ol, (E)-2-nonenal, and an unknown compound, were detected by all assessors, indicating that they contributed more actively to the aroma profile of Jiashi muskmelon. These nine odorants, except for mushroom-like 1-octen-3-ol and the unknown compound, were all previously reported as potent contributors to the aroma profile of C. melo L. fruits.[10,29] Alcohols and aldehydes with nine carbon atoms, dominated by (Z)-non-6-enal, (E)-2-nonenal, and (3Z,6Z)-nona-3,6-dien-1-ol, which smelled "cantaloupe-like, cucumber-like", were identified by many researchers as the characteristic components of the family Cucurbitaceae.[7,31,32] The unknown compound was tentatively identified as (E,Z)-2,4-octadienal according to its LRIs on DB-5 (1080) and DB-Wax (1350) columns as well as its "grassy" odor attribute described by the assessors. There is a need for further studies to identify this component using more effective extraction methods and highly sensitive detection techniques. It is worth noting that camphor-like isophorone was identified for the first time in melon fruits. It was speculated that this odorant might play an indispensable role in the distinct aroma profile of Jiashi muskmelon.

DF percentage of each class of odorants (the ratio of DF sum of each class odorants to that of all aroma-active compounds detected by DFA) revealed that cucumber-like aldehydes gave the greatest contribution to aroma profile of Jiashi muskmelon juice (42.2%), followed by sweet and fruity esters (28.4%), grassy alcohols (15.5%), and floral ketones (6.9%). From the results above, a conclusion could be tentatively drawn that the overall aroma profile of Jiashi muskmelon consisted of aroma compounds with various sensory attributes, none of which might bear the typical aroma of the food itself. However, aroma reconstitution and omission test are needed in further studies to verify this preliminary conclusion.

Odor-Active Components Identified by OAV Calculation. As shown in Table 3, 21 of 42 compounds were present in amounts equal to or greater than their odor threshold, demonstrating that they were responsible for Jiashi muskmelon aroma. Among these potent odorants, cucumber-like (2E,6Z)-nona-2,6-dienal had the highest OAV (3.27e4), followed by (3Z,6Z)-nona-3,6-dien-1-ol (2.01e4), ethyl butanoate (3.85e3), ethyl 2-methylbutyrate (1.15e3), ethyl 2-methylpropanoate (940), (Z)-non-6-enal (458), ethyl acetate (220), and (E)-2-nonenal (126), leading to the conclusion that odor-active compounds of Jiashi muskmelon obtained from OAV calculation were mainly composed of unsaturated aldehydes and alcohols with nine carbon atoms as well as branched esters dominated by acetate, propyl and butyl esters. These results correlated well with the conclusion drawn by Kemp et al. that aldehydes with nine carbon atoms and some volatile esters played a key role in the melon-like and fruity note of muskmelon.[6] Other unsaturated aldehydes and alcohols with nine carbon atoms in Table 3 were also reported in previous studies as major contributors to "grassy" and "green melon" notes of muskmelon and cantaloupe.[3,32] Consistent with this study, ethyl 2-methylpropanoate, exhibiting a sweet and fruity aroma, was deemed to be the primary odorant of muskmelons (C. melo).[11] However, sulfur-containing compounds such as methyl (methylthio)acetate, ethyl (methylthio)acetate, ethyl 3-methylthiopropionate, and methyl 3-methylthiopropionate, which were detected to be the primary odorants of other muskmelon cultivars,[3,8] as well as 4-hydroxy-2,5-dimethyl-3(2H)-furanone found with the highest dilution factor in 'Miyabi' muskmelon,[33] failed to be identified as impact odorants

Table 3. Potent Odorants in Headspace Jiashi Muskmelon Juice Identified by OAV Calculation

no.[a]	compound[b]	concentration[c] ($\mu g\ L^{-1}$)	threshold[d] ($\mu g\ L^{-1}$)	OAV
32	(2E,6Z)-nona-2,6-dienal	327.0	0.01	3.27e4
33	(3Z,6Z)-nona-3,6-dien-1-ol	201.0	0.01	2.01e4.
10	ethyl butanoate	3.85e3	1	3.85e3
12	ethyl 2-methylbutyrate	115	0.1	1.15e3
5	ethyl 2-methylpropanoate	94.0	0.1	940
30	(Z)-non-6-enal	9.16	0.02	458
2	ethyl acetate	1.10e3	5	220
34	(E)-2-nonenal	10.1	0.08	126
17	heptanal	283	3	94.3
3	ethyl propionate	361	10	36.1
42	(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one	0.21	0.007	30.0
38	decanal	1.56	0.1	15.6
7	methyl 2-methylbutyrate	25.6	2.5	10.2
16	2-methylbutyl acetate	38.8	5	7.76
15	3-methylbutyl acetate	14.3	2	7.15
31	nonanal	4.36	1	4.36
9	hexanal	17.8	4.5	3.96
13	(E)-2-hexenal	31.2	17	1.84
11	butyl acetate	117	66	1.77
21	octanal	1.02	0.7	1.46
6	2-methylpropyl acetate	67.0	66	1.02

[a]Numbers correspond to those in Table 1. [b]Each compound was identified by comparing it with an authentic standard on the basis of the following criteria: (i) matching retention time on DB-5 and DB-Wax column with that of references; (ii) consultation of the NIST08 database; (iii) injection of reference standards. [c]Concentrations were the same as those in Table 1 [d]Odor thresholds in water were taken from refs 23 and24.

of Jiashi muskmelon. This could be caused by differences of melon cultivars and culture conditions.

Figure 1, presenting the concentrations and contribution rates (OAV percentages) of each class of odorants, revealed
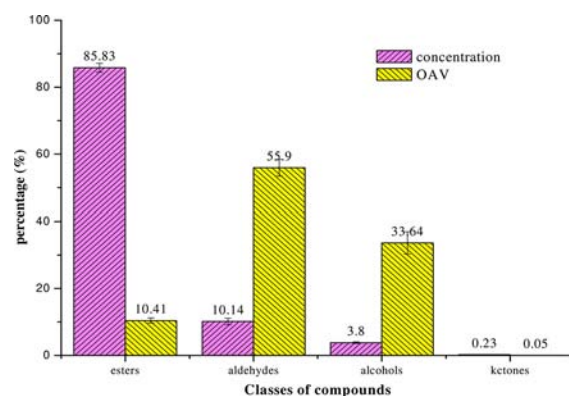

Figure 1. Concentration and OAV percentages of four classes of odorants in Jiashi muskmelon juice.

that although esters were superior in concentration, their contribution rate was only 10.41%, whereas alcohols and aldehydes were just the opposite. The total contents of aldehydes and alcohols were only 11 and 4% that of esters, respectively, but their contributions to aroma profile of Jiashi muskmelon are 5.4 and 3.2 times, respectively, as large as that of esters. This was

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due to the very low threshold values of aldehydes and alcohols. On the other hand, this result reinforced the fact that the peak profile obtained by any "chemical" detector alone did not necessarily reflect the sensory aromatic profile of a subject.

Comparison between Aroma Compound Profiles of Jiashi Muskmelon Juice Obtained from DFA and OAV. Comparative analysis on odor-active compounds obtained from DFA (Table 2) and OAV (Table 3) showed that 12 odorants, namely, (2E,6Z)-nona-2,6-dienal, (3Z,6Z)-nona-3,6-dien-1-ol, ethyl butanoate, ethyl 2-methylbutyrate, ethyl 2-methylpropanoate, (Z)-non-6-enal, (E)-2-nonenal, heptanal, methyl 2-methybutyrate, nonanal, hexanal, and 2-methylpropyl acetate, were detected as potent odorants of Jiashi muskmelon juice by both methods, and (2E,6Z)-nona-2,6-dienal and ethyl butanoate were revealed as the most important contributors to Jiashi muskmelon juice aroma profile. In addition, seven components that were detected by all panelists (except for mushroom-like 1-octene-3-ol), including (2E,6Z)-nona-2,6-dienal (cucumber-like), (3Z,6Z)-nona-3,6-dien-1-ol (cucumber), ethyl butanoate (sweet, fruity), ethyl 2-methylbutanoate (cantaloupe-like, fruity, melon), ethyl 2-methylpropanoate (floral, fruity, sweet), (Z)-non-6-enal (honeydew melon-like), and (E)-2-nonenal (melon-like, fresh), were also characterized with a larger OAV (>100). From the findings mentioned above, it could be concluded that DFA and OAV resulted in relatively similar aroma compound profiles of Jiashi muskmelon.

However, there were also some differences between aroma compound profiles obtained with these two methods. Nine components, namely, ethyl acetate (OAV = 220), ethyl propionate (36.1), (E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one (α-ionone) (30.0), decanal (15.6), 2-methylbutyl acetate (7.76), 3-methylbutyl acetate (7.15), (E)-2-hexenal (1.84), butyl acetate (1.77), and octanal (1.46), which were identified as the odor-active compounds by OAV, failed to be selected in DFA, whereas 8 odorants with OAV < 1, namely, 1-octene-3-ol (DF = 8), 2,6,6-trimethylcyclohexene-1-carbaldehyde (β-cyclocitral) (6), 6-methylhept-5-en-2-one (5), hexyl acetate (4), 2-phenylacetaldehyde (3), isophorone (3), benzaldehyde (2), and 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (α-terpineol) (2), were characterized by DFA to be potent odorants because their DF were all ≥2.

These above differences were due to the different application principles of DFA and OAV methods. Specifically, the reasons are as follows. (a) OAV calculation did not take into account the interactions between odorants in a mixture, such as suppression, synergism, and antagonism, and using water as a medium to OAV calculation did not exactly reflect the compound threshold both in the DFA and in the food matrix. Thus, components with OAV < 1 may be perceived by the panelists during the detection frequency method due to its cooperation with other compounds, whereas components with OAV ≥ 1 may fail to be noticed because of antagonistic effects. (b) OAV calculation was contrary to Stevens' law,[34] in which psychometric functions of odorants were considered as being sigmoidally shaped and each compound was shown to have a unique psychometric (concentration−response) function but not that substances with equal OAV have equal intensity, as OAV assumes. This was also confirmed in this study; for example, even though the OAV of ethyl acetate (220) was greater than that of (E)-2-nonenal (126), (E)-2-nonenal was noted by all panelists, whereas ethyl acetate failed to be detected during GC-O (Tables 2 and 3). (c) Biological variance during DFA, such as different respiratory rates[35] and saturation of receptors, could

lead to the missing of detection of a compound eluting immediately after a strong odor. As shown in Table 2, the missing of butyl acetate (RT = 9.363) and (E)-2-hexenal (RT = 11.608), which were characterized as potent odorants using OAV, might result from the high intensity (DF = 8) of ethyl butanoate (RT = 8.662) and ethyl 2-methylbutyrate (RT = 11.377), respectively (Figure 2).
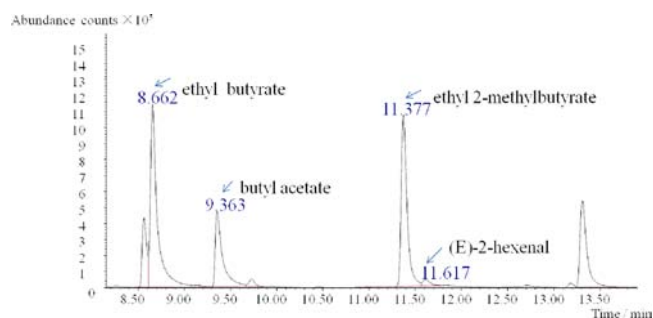

Figure 2. Partial total ion chromatogram (TIC) of Jiashi muskmelon juice identified by HS-SPME-GC-MS.

To sum up, representing the odor contribution of volatile by OAV is an approximation because its application still raises too many problems of details. But we must accept that OAV is one way to simplify the puzzle of food aroma complexity. As for DFA, it realized the combination of sensory evaluation and instrumental analysis in odor pattern study. However, it should not be considered as a routine analytical method because it is time-consuming. There is a need to learn more about the physiology of aroma perception, the relations between odorants, and about uncertain factors influencing DFA results to improve the accuracy of these two methods. In addition, future emphasis should be on conducting aroma recombination experiments and omission tests to verify the obtained results from these two methods and to compare the effectiveness of these two methods in characterizing the fingerprint of volatile flavor constituents of Jiashi muskmelon.

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Notes
The authors declare no competing financial interest.

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