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Evaluation of Aroma Compounds Contributing to Muskmelon Flavor in Porapak Q Extracts by Aroma Extract Dilution Analysis

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The flavor of the Miyabi variety of Japanese muskmelon was extracted according to the Porapak Q column method (PQM) and evaluated by using aroma extract dilution analysis (AEDA) method. The overall odor of the PQM extracts was perceived as having a natural muskmelon-like odor, suggesting that the PQM was able to extract volatile compounds in muskmelon fruit without degradation of original flavor. Forty-six odorant compounds [Kovats index (KI), $961 \leq KI \leq 2605$] were found by GC-sniffing in PQM extracts, confirming the effectiveness of PQM in trapping a wide range of volatile compounds in muskmelon flavor. The 46 odorants could be divided into three groups on the basis of their odor attributes: fruity note ($KI < 1300$); green, grassy, or cucumber-like note ($1300 < KI < 2020$); and sweet note ($KI > 2020$). When the original extracts were diluted in AEDA analysis, seven odorants could still be detected by GC-sniffing at a flavor diluation (FD) factor of 128 or above: one had a fruity note (compound **3**); four had a cucumber-like, green, or grassy note (compounds **12**, **17**, **21**, and **23**); and two had a sweet note (caramel-like or yakitori-like) (compounds **32** and **34**).

KEYWORDS: Melon aroma; sniffing-GC; aroma extract dilution analysis; Porapak Q column

INTRODUCTION

Numerous studies conducted on the aromas of muskmelons have focused mainly on identifying and determining their volatile compounds (*1*−*5*), but few studies have focused on the contributors to the overall muskmelon (*6*−*8*). In these studies 11 odorants were detected from muskmelon concentrates by a solvent extraction method (*6*) and distillation method (*8*). Ethyl 2-methylpropionate and methyl 2-methylbutyrate were the most intense odorants. However, in our previous study (*9*), the Porapak Q column method (PQM) showed a good ability to trap a wide range of volatile compounds (low to high boiling point compounds) at room temperature without degrading the melon flavor. Our previous results implied that many more odorant compounds contributing to muskmelon flavor might be revealed in PQM extracts.

Usually, the odor attributes in volatile compounds can be detected by sniffing experiments with panelists using a gas chromatography (GC)−olfactory or GC−sniffing method (*6*, *10*, *11*). The significance of each aroma compound can be evaluated by its potency using an aroma extract dilution analysis (AEDA), described by Grosch et al. (*12*). In the present study, the AEDA method was carried out to evaluate the aroma compounds contributing to muskmelon flavor in the Miyabi variety. The effectiveness of the PQM method in trapping a wide range of volatile compounds in muskmelon flavor was presented by GC−sniffing.

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MATERIALS AND METHODS

Plant Materials. Fresh ripe muskmelons (*Cucumis melo* L. cv. Miyabi) (Yokohama Ueki Corp. Co., Ltd., Yokohama, Japan) were obtained from Kochi-Haruno Agricultural Cooperative Association in Japan. Muskmelon fruits were harvested at 55 days after anthesis and then ripened at 25 °C for 1 week. The firmness and °Brix of the mesocarps of the fruits averaged 0.64 kg/cm^2 and 14.53, respectively. Three fruits were cut vertically, and the mesocarp tissues (100 g) of each of the three fruits (total weight = 300 g) were mixed together, homogenized using a blender (Sanyo SM-KW 50, Sanyo Electronic Co., Ltd., Osaka, Japan), and centrifuged at 10000*g* for 20 min. The supernatant was then used for volatile extraction. Four replicates were done. For each replicate, three new fruits of the same batch were used.

Porapak Q Column Concentration Method. We followed the processes described by Shimoda et al. (*13*). A glass column (2 × 10 cm) packed with Porapak Q (polydivinylbenzene, 50−80 mesh, Waters Co., Ltd., Milford, MA) was regenerated by washing with 100 mL of

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diethyl ether, 100 mL of methanol, and 100 mL of deionized water. The sample was passed through the column, and then the column was washed with 100 mL of deionized water. The absorbed compounds were eluted with 100 mL of diethyl ether. The eluate was dried over anhydrous sodium sulfate for 6 h after the addition of an internal standard (10 μL of 0.1% cyclohexanol), and the solvent was evaporated to ~50 μL under a nitrogen stream before 1 μL was drawn for sniffing−GC analysis.

Gas Chromatography Analysis and Sniffing. A Shimadzu GC-17A (Shimadzu Co., Ltd., Kyoto, Japan) was equipped with a Y connector outside a capillary column, allowing the effluent to be split between a sniffing port and a flame ionization detector (FID). The column was a DB-Wax fused silica capillary column (60 m × 0.25 mm i.d.; J&W Scientific, Folsom, CA), and the oven temperature was held at 40 °C for 10 min and then increased by 3 °C/min to 220 °C, at which it was held for 30 min. The injector and detector temperatures were 230 and 250 °C, respectively. The flow rate of helium carrier gas was 30 cm/s. The split ratio of the injector was 1:20. Values of the relative amounts of volatile compounds were calculated by dividing the GC peak area by the internal standard area. These relative amount values were repeated four times. For the sniffing experiments, three panelists sniffed directly from the sniffing port, and the odor attributes of each compound were repeated by different panelists on separate runs. The expression of each aroma compound was decided by agreement between at least two of the panelists.

Aroma Extract Dilution Analysis. The significance of each aroma compound in muskmelon flavor was evaluated by its potency using an AEDA method of Grosch et al. (*12*). The aroma extract (50 μL) collected from PQM was used and diluted with diethyl ether in the ratio $1:2^n$, where *n* was selected from 1, 2, 3, or *n* as the dilution factor. Aliquots of 1 μL of a diluted sample were analyzed by GC−sniffing as previously described.

Gas Chromatography—Mass Spectrometry (GC-MS) Analysis. A Shimadzu GC-MS-QP5050 (Shimadzu Co., Ltd., Kyoto, Japan) was used to identify the volatile compounds in muskmelon fruit. The injector and interface temperatures were 230 °C. The column and oven conditions for GC-MS analysis were as described for sniffing−GC analysis. The resulting GC-MS data for the volatile compounds were identified by comparison of their mass spectra with those of a corresponding reference standard (Bedoukian Research Inc., Danbury, CT) and those of the NIST library (Shimadzu, Kyoto, Japan, 1998). The volatile compounds were also estimated in accordance with a modified Kovats method (*14*) and compared with reported values.

RESULTS AND DISCUSSION

The odor descriptions and flavor dilution (FD) factors of the odorant compounds in PQM extracts of Miyabi muskmelon fruit are shown in **Table 1**. Forty-six odorants (961 < KI ≤ 2605) were found in PQM extracts that had a natural muskmelon-like odor. Many more odorants found in the PQM extracts showed that PQM was able to extract and trap a wide range of volatile compounds at room temperature without degradation of original flavor. Odor attributes of volatile compounds in muskmelon flavor could be divided into three groups, and they were also found to closely associate with their Kovats index. Compounds **1**−**6** (KI < 1300) were perceived as having fruity odor attributes (like melons and apples), whereas most of the compounds **7**−**31** (1300 < KI < 2020) were perceived as having a green or cucumber-like odor. Most of the compounds **32**−**46** (KI > 2020) were perceived as having a sweet odor such as caramel-, cake-, and milk coffee-like odor; interestingly, they have never been reported in muskmelon flavor extracted by a solvent extraction method (*6*), simulataneous distillation−extraction method (*7*), or distillation method (*8*). The first occurrence of these odorant compounds in PQM extracts suggests the effectiveness of the PQM method in trapping a wide range of volatile compounds in muskmelon flavor.

The first group of odorant compounds (KI < 1300) that were perceived as having the fruity odor attributes was considered to be responsible for the fruity aroma in muskmelon. Ethyl 2-methylpropionate (compound **1**) was the only compound that possessed the fresh characteristic muskmelon aroma in PQM extracts of Miyabi muskmelon; therefore, it was undoubtedly responsible for the muskmelon flavor. Methyl 2-methylbutyrate (compound **2**) was also considered to contribute to the Miyabi muskmelon aroma due to its relatively high FD factor (FD factor = 16), and it was reported to contribute to muskmelon flavor (*6*−*8*). Ethyl butyrate (compound **3**) was found to have a relatively high FD factor (FD = 256), suggesting that it contributes greatly to the Miyabi muskmelon aroma. However, this result did not coincide with the study of Schieberle et al. (*6*), in which ethyl butyrate had a considerably lower FD factor compared with ethyl 2-methylpropionate and methyl 2-methylbutyrate. This discrepancy might be attributable to the differences in the extracting methods of compounds or in the varieties used. Butyl acetate (compound **4**) was also detected by GC−sniffing as having grape-like odor attributes. This compound has been extracted from melons and identified in many studies (*1*, *5*, *7*, *15*−*17*), but none of these studies included sniffing experiments, although in the study of Schieberle et al. (*6*) with AEDA, butyl acetate was not perceived. Our finding of butyl acetate by GC−sniffing in Miyabi muskmelon is the first to reveal the odor of butyl acetate in muskmelon flavor.

Compound **7** was identified on the basis of its Kovats index, MS, and odor description as 2-acetyl-1-pyrroline. This compound is a key compound of fragrant rice (*18*) and has also been isolated and identified from popcorn (*19*). This compound is quite unstable and may well have been lost during the extraction methods used; muskmelon aroma has rarely been associated with fragrant rice aroma (2-acetyl-1-pyrroline), so it might have been ignored and not reported in fruit or vegetable flavors in previous studies. However, it was perceived in PQM extracts by GC−sniffing in our study, and its FD factor was relatively high (FD factor = 32), so 2-acetyl-1-pyrroline may contribute to the aroma of Miyabi muskmelon. In addition, 2-acetyl-1-pyrroline is a nitrogen-containing compound, so our result indicated the ability of PQM to extract nitrogen-containing compounds from fruit flavor.

The second group of odorant compounds, which were perceived as having cucumber-like, green, or grassy odor attributes, was considered to be responsible for the grassy note of muskmelon. Ethyl (methylthio)acetate (compound **12**), and ethyl 3-(methylthio)propionate (compound **16**), which are sulfur compounds, could be also extracted by the PQM method and were perceived as having cucumber-like or green fruity odor attributes. Ethyl (methylthio)acetate has been isolated and identified from 18 melon varieties by Wyllie and Leach (*7*), so it must be a common odorant among melon varieties. In the present study, this compound also showed a relatively high FD factor (FD factor = 128), confirming its importance for muskmelon flavor. (*E*,*Z*)-2,6-Nonadienal (compound **17**), the cucumber-like odor, also showed a relatively high FD factor (FD factor = 128). It was found to be a principal odorant in cucumber (*7*) but not in muskmelon (*6*). Schieberle et al. (*6*) identified (*E*,*Z*)-2,6-nonadienal in two cucumber varieties and found that it differs considerably in concentration and contribution to the overall aroma among varieties. Therefore, we speculated that the contribution of (*E*,*Z*)-2,6-nonadienal to muskmelon aroma might also greatly differ among varieties, so it could be undoubtedly reported to be responsible for Miyabi muskmelon flavor. (*Z*)-3-Nonen-1-ol (compound **20**) and (*Z*)-

Table 1. Potent Odor Compounds in the Extract from Muskmelon Fruit (*C. melo* L. Cv. Miyabi) by Porapak Q Column Concentration Method (PQM)

compd no.	KI[a]	odor description	compound name	PQM peak area ratio[b]	PQM flavor dilution factor
1	961	melon-like	ethyl 2-methylpropionate	0.076	16
2	1010	apple-like	methyl 2-methylbutyrate	0.940	16
3	1036	grape-like	ethyl butyrate	0.250	256
4	1072	grape-like	butyl acetate	1.647	4
5	1128	green, fruity		nd[c]	8
6	1233	sweet, fragrant	ethyl hexanoate	tr[d]	4
7	1330	fragrance rice-like	2-acetyl-1-pyrroline	nd	32
8	1380	green, cucumber-like	(*Z*)-3-hexen-1-ol	0.122	16
9	1389	milk-like		0.042	16
10	1420	soybean powder-like		nd	8
11	1445	milk-like		nd	8
12	1447	cucumber-like	ethyl (methylthio)acetate	0.081	128
13	1452	sweet		nd	32
14	1455	soy sauce-like, fatty		0.183	8
15	1530	cucumber-like		0.035	64
16	1570	fruity, green	ethyl 3-(methylthio)propionate	0.149	2
17	1582	cucumber-like	(*E*,*Z*)-2,6-nonadienal	0.318	128
18	1625	sweet, green		0.429	32
19	1670	sweet,green		0.026	2
20	1682	sweet, green	(*Z*)-3-nonen-1-ol	0.085	4
21	1714	sweet, green	(*Z*)-6-nonan-1-ol	0.486	256
22	1752	scented rice-like		0.795	4
23	1759	grassy boiled leaf-like	(*Z*,*Z*)-3,6-nonadienol	tr	512
24	1765	green		0.109	16
25	1813	honey-like	2-phenethyl acetate	0.226	8
26	1882	fruity		tr	8
27	1902	sweet		0.069	8
28	1915	fruity, green	2-phenethyl alcohol	3.330	4
29	1938	sweet		nd	32
30	1996	rice-like		0.369	32
31	2005	seaweed-like		tr	2
32	2028	caramel-like	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	0.142	128
33	2050	caramel-like		0.408	64
34	2060	yakitori-like		0.065	256
35	2065	caramel-like		0.207	4
36	2070	bamboo shoot-like, fragrant		tr	64
37	2078	caramel-like		tr	2
38	2144	medicaine-like		tr	32
39	2147	cake-like		tr	8
40	2193	sweet		0.031	4
41	2206	cake-like		0.018	32
42	2234	shiitake mushroom-like		1.329	4
43	2301	sweet		0.021	2
44	2495	detergent-like		0.477	16
45	2571	milk coffee-like		0.092	64
46	2605	milk coffee-like		0.110	64

[a] Kovats index on DB-Wax. [b] GC peak area of compound/GC peak area of internal standard. Values are the means of four replicates. [c] Not determined. [d] Trace.

6-nonen-1-ol (compound **21**), which are the principal odorants in muskmelon flavor (*3*, *4*), were perceived as having sweet green odor attributes and detected in relatively high concentrations (expressed in peak area ratio). (*Z*)-6-Nonen-1-ol also showed a relatively high FD factor (FD factor = 256), suggesting it is one of the potent odorants in Miyabi muskmelon flavor. A small amount of (*Z*,*Z*)-3,6-nonadienol (compound **23**), having a grassy boiled leaf-like odor, was detected with GC-FID and showed the highest FD factor (FD factor = 512); therefore, it was also considered to be one of the important compounds in Miyabi muskmelon.

The last group of odorant compounds that were perceived as having sweet, caramel-like, cake-like, or milk coffee-like odor attributes was considered to be responsible for the sweet note of muskmelon. These compounds might also have a pronounced influence on the muskmelon's aroma when the fruit is eaten and then compounds are released (*20*). Among these compounds, compounds **32**−**34**, **36**, **39**, **41**, **45**, and **46** possessed relatively high FD factors (FD factor ≥ 32), so they also appear to be potent odorants in Miyabi muskmelon. Most of these compounds, except 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (compound **32**), could not be identified now; they were first reported in muskmelon flavor by AEDA in our study and were also detected in trace amounts, and no other studies had previously extracted or identified them, so it would be very difficult to identify and compare them now.

Although several odorant compounds were unidentified in Miyabi muskmelon, the data obtained by AEDA from this study give new information that there still are other important odorant compounds in muskmelon flavor, especially the sweet odorant compounds that have never been reported for muskmelon flavor. One limitation of detecting these compounds in other previous studies may be attributed to the limitation of the flavor extraction method. The present study reveals the excellence of PQM in extracting volatile compounds in muskmelon flavor and its efficiency of trapping a wide range of volatile compounds, suggesting it can become an interesting extraction method for melon flavor analysis in the future.

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