

Volatile Compounds in the Skin and Pulp of Queen Anne's Pocket Melon

CHRISTOPHE AUBERT^{*,†} AND MICHEL PITRAT[§]

Centre Technique Interprofessionnel des Fruits et Légumes (Ctifl), route de Mollégès, F-13210 Saint-Rémy-de-Provence, France, and Institut National de la Recherche Agronomique (INRA), Station de Génétique et d'Amélioration des Fruits et Légumes, B.P. 94, F-84143 Montfavet cedex, France

The quantitative distribution of volatile compounds in the skin and pulp of Queen Anne's pocket melon [*Cucumis melo* var. *dudaim* (L.) Naudin] has been investigated. Volatile compounds were extracted by liquid–liquid microextraction (LLME) using chloroform and analyzed by GC-FID and GC-MS. Sixty volatiles, including 20 esters, 15 alcohols, 7 lactones, 7 aldehydes and ketones, 6 sulfur compounds, and 5 C₆ compounds, have been identified. Among them, 38 were reported for the first time in pocket melon, 10 of them have been, however, labeled “tentatively identified”. The results showed that the levels of volatiles in skin were significantly higher than those observed in pulp. Eugenol, the major constituent in skin (15.3%), thioether esters, and lactones were thought to contribute significantly to the unique aroma of the pocket melon. Finally, the distribution of lactones was also found to be different in skin and pulp according to their carbon chain length.

KEYWORDS: *Cucumis melo*; pocket melon; aroma; volatile compounds; pulp; skin

INTRODUCTION

Cucumis melo L., commonly called melon, belongs to the Cucurbitaceae family and to the genus *Cucumis*. Probably originating from Africa, melons were imported to Europe from Persia and the Caucasus approximately 3000 years ago (1). Since the pioneer works done by Naudin in 1859 (2), who proposed nine tribes for cultivated melons and one for wild forms, several infraspecific classifications of melon have been proposed (3). Within this diversity, a unique type of melon has always been recognized, and Linné proposed the species *Cucumis dudaim*, which was later classified as *C. melo* var. *dudaim* (L.) Naudin. It is characterized by small reddish yellow fruits with ochre stripes and a round or slightly oval shape, with a velvety skin. Queen Anne's pocket melon, sometimes called the Fragrant melon, has a very unique fragrant, exotic, and musky smell, generally described as a mix of cantaloupe, pineapple, and a hint of jasmine. Although they are attractive in their velvety rinds, these melons have a whitish, flaccid, and insipid pulp and are barely edible. They are generally cultivated for ornamental or aromatic uses from Turkey and the Caucasus to Afghanistan. In Victorian times, these melons were carried in the pocket as a body fragrance. It is said that Queen Anne herself carried one, which would explain why common names such as Her Majesty's melon have been applied. Although the melon volatiles have been extensively investigated (4–18), the aroma composition of Pocket Melon is limited to the study of Shu et

al. (19), and to our best knowledge, no study on the volatiles in the skin has appeared in the literature. The aim of this work was to investigate the distribution of the volatiles in the skin and pulp of Queen Anne's pocket melon.

MATERIALS AND METHODS

Solvent and Chemicals. Analytical grade chloroform (Chromasolv Plus, 99.9%) and *n*-propyl gallate (≥98%) were, respectively, from Sigma and Fluka. Ammonium sulfate (NH₄)₂SO₄ (puriss. p.a., ≥99%) and *n*-alkane standards (C₈–C₄₀) were from Riedel-de Haën. Reference compounds were obtained from Sigma-Aldrich (no. 1–3, 5–13, 15–20, 22, 24–27, 29, 31, 33, 39, 42–44, 46, 47, 49–52, 55, and 60), Interchim (no. 4, 23, 35, 38, 40, 41, 48, 53, and 58), and Fluka (no. 21, 45) (see Table 1 for compound numbers).

Samples. Fully ripe Queen Anne's pocket melons (*C. melo* var. *dudaim*) were produced under plastic tunnel by the experiment unit of INRA in Montfavet (Vaucluse, France). Immediately after harvest, the skin of the fruits (~20) was delicately removed using a paring knife, and flesh cubes (~1 cm³) were prepared. Skin and flesh cubes were then immediately frozen with liquid nitrogen and stored at –20 °C until analysis.

Isolation of Volatiles. Samples were analyzed according to the liquid–liquid microextraction (LLME) method previously described by Aubert et al. (20) with some modifications. Twenty-five grams of pulp (or skin), 50 mL of *n*-propyl gallate (10 mM), 15 g of (NH₄)₂SO₄, and 10 μL of 2-octanol (3.32 μg/mL) (internal standard) were homogenized in a Waring blender for 1 min. The mixture was centrifuged (21000g, 5 min, 4 °C), and the supernatant was filtered through a Whatman paper filter (grade 41). Forty milliliters of supernatant was introduced into a 50-mL screw-capped conical centrifuge tube (34 × 98 mm glass borosilicate) containing a magnetic stir bar (15 × 6 mm). Two hundred microliters of chloroform was added, and the mixture was extracted for 60 min under magnetic stirring

* Corresponding author (telephone +33 490 92 05 82; fax +33 490 92 48 87; e-mail aubert@ctifl.fr).

[†] Centre Technique Interprofessionnel des Fruits et Légumes (Ctifl).

[§] Institut National de la Recherche Agronomique (INRA).

Table 1. Concentrations^a of Volatiles in the Skin and Pulp of Queen Anne's Pocket Melon

no.	compound	RI ^b	assignment ^c	F ^d	p ^e	skin	pulp
esters							
1	ethyl butanoate	[1061]	A	159.7	***	900.0 ± 94.7	206.5 ± 7.5
2	ethyl 2-methylbutanoate	[1065]	A	3711.8	***	746.0 ± 16.8	98.3 ± 7.6
3	butyl acetate	[1080]	A	5.2		123.9 ± 8.1	110.5 ± 6.2
7	3-methylbutyl acetate	1123	A	9.8	*	280.7 ± 25.6	334.9 ± 15.6
8	ethyl pentanoate ^f	1134	A	56.8	**	9.3 ± 0.4	13.6 ± 0.9
13	ethyl hexanoate	1236	A	838.7	***	56.1 ± 3.1	3.7 ± 0.1
14	3-methyl-2-butenyl acetate ^f	1251	B	2.3		14.2 ± 1.0	12.9 ± 1.1
15	hexyl acetate	1275	A	0.9		183.0 ± 7.8	175.9 ± 9.9
19	(Z)-3-hexenyl acetate	1321	A	7.5		34.5 ± 2.0	28.8 ± 3.0
23	3-acetoxy-2-butanone ^f	1389	A	1585.2	***	842.8 ± 35.5	26.8 ± 1.8
28	2,3-butanediol diacetate (1) ^f	1495	B	333.8	***	1714.3 ± 144.9	181.5 ± 11.4
30	ethyl 3-hydroxybutanoate ^f	1521	B	549.9	***	67.7 ± 3.8	15.7 ± 0.7
32	2,3-butanediol diacetate (2) ^f	1532	B	623.9	***	749.0 ± 47.9	56.3 ± 3.8
34	ethyl 3-acetoxy-2-methylbutanoate ^f	1569	B	2179.1	***	206.0 ± 6.8	22.3 ± 0.7
36	3-acetoxy-2-butanol (1) ^f	1581	B	1279.8	***	370.7 ± 16.5	29.4 ± 1.1
37	3-acetoxy-2-butanol (2) ^f	1597	B	345.3	***	28.9 ± 2.1	6.2 ± 0.3
42	benzyl acetate ^f	1733	A	246.5	***	61.5 ± 2.6	94.3 ± 2.5
43	phenylethyl acetate ^f	1787	A	474.7	***	71.9 ± 4.4	14.6 ± 1.2
47	3-phenylpropyl acetate ^f	1944	A	84.3	***	73.3 ± 4.1	123.9 ± 8.6
51	cinnamyl acetate ^f	2153	A	23.9	**	52.5 ± 1.2	71.1 ± 6.5
	total			1182.4	***	6586.2 ± 235.1	1627.0 ± 84.5
sulfur compounds							
24	methyl 2-(methylthio)acetate ^f	1412	A	775.6	***	25.8 ± 1.2	5.3 ± 0.3
25	ethyl 2-(methylthio)acetate ^f	1452	A	365.2	***	652.0 ± 55.1	43.0 ± 2.5
29	2-(methylthio)ethyl acetate ^f	1505	A	191.1	***	102.5 ± 5.2	56.1 ± 2.6
35	ethyl 3-(methylthio)propanoate ^f	1571	A	1611.0	***	97.1 ± 3.9	7.6 ± 0.3
38	3-(methylthio)propyl acetate ^f	1633	A	11.7	*	57.8 ± 4.1	67.5 ± 2.8
41	3-(methylthio)propanol ^f	1719	A	1.5		4.5 ± 0.1	4.8 ± 0.4
	total			532.8	***	935.3 ± 56.2	179.6 ± 7.8
alcohols							
5	2-methylpropanol ^f	[1093]	A	1653.6	***	26.2 ± 0.3	8.5 ± 0.7
11	2-methylbutanol	1208	A	2420.5	***	84.3 ± 1.5	22.8 ± 1.6
18	4-heptanol ^f	1308	A	1282.7	***	35.9 ± 1.5	5.7 ± 0.2
26	1-octen-3-ol	1454	A	8708.8	***	51.2 ± 0.9	1.2 ± 0.1
27	heptanol ^f	1458	A	894.9	***	76.1 ± 2.6	21.9 ± 1.7
33	octanol ^f	1561	A	1489.4	***	1140.2 ± 45.4	122.4 ± 4.6
39	α-terpineol	1696	A	788.8	***	47.1 ± 2.3	9.4 ± 0.3
44	benzyl alcohol	1880	A	346.4	***	164.5 ± 12.7	25.5 ± 2.2
45	2-phenylthanol	1915	A	389.3	***	26.7 ± 1.6	7.3 ± 0.7
49	3-phenyl-1-propanol	2048	A	70.3	**	365.3 ± 27.3	216.5 ± 14.1
52	eugenol	2170	A	2700.9	***	2302.4 ± 68.7	225.1 ± 8.6
55	cinnamyl alcohol ^f	2286	A	38.6	**	184.1 ± 12.2	125.5 ± 10.9
56	chavicol	2342	B	5084.7	***	589.8 ± 12.1	83.6 ± 2.4
57	isoeugenol ^f	2350	B	635.8	***	25.4 ± 1.6	2.7 ± 0.1
59	methoxyeugenol ^f	2543	B	2146.0	***	104.3 ± 3.0	21.4 ± 0.9
	total			2149.2	***	5223.2 ± 156.1	899.3 ± 41.5
aldehydes and ketones							
6	(E)-2-pentenal ^f	[1096]	A	267.0	***	23.8 ± 1.6	7.6 ± 0.7
10	heptanal ^f	1190	A	641.7	***	34.9 ± 2.1	4.4 ± 0.3
16	3-hydroxy-2-butanone	1287	A	3319.7	***	203.7 ± 5.0	24.0 ± 2.0
17	octanal ^f	1291	A	469.6	***	26.0 ± 1.6	46.8 ± 0.6
20	6-methyl-5-hepten-2-one ^f	1341	A	203.7	***	3.5 ± 0.1	2.0 ± 0.2
31	benzaldehyde	1526	A	2265.6	***	154.9 ± 5.2	11.1 ± 0.8
60	vanillin ^f	2563	A	255.0	***	18.3 ± 1.8	2.1 ± 0.1
	total			7659.0	***	465.2 ± 6.0	98.0 ± 4.1
C₆ compounds							
4	hexanal	[1087]	A	607.7	***	389.4 ± 23.4	56.3 ± 1.0
9	(Z)-3-hexenal ^f	1146	A	581.4	***	23.2 ± 1.6	1.2 ± 0.1
12	(E)-2-hexenal	1220	A	413.6	***	260.2 ± 22.0	1.9 ± 0.2
21	hexanol	1355	A	6621.0	***	490.1 ± 9.9	20.7 ± 1.3
22	(Z)-3-hexen-1-ol	1387	A	1526.7	***	37.6 ± 1.3	6.9 ± 0.3
	total			3330.3	***	1200.5 ± 33.3	86.9 ± 2.8
lactones							
40	γ-hexalactone ^f	1703	A	5196.4	***	209.7 ± 3.5	41.3 ± 2.1
46	γ-octalactone ^f	1917	A	535.6	***	128.8 ± 7.4	27.3 ± 1.7
48	δ-octalactone ^f	1966	A	534.0	***	8.4 ± 0.5	1.7 ± 0.1
50	γ-decalactone ^f	2144	A	424.7	***	94.5 ± 4.0	36.6 ± 2.7
53	δ-decalactone ^f	2195	A	232.4	***	8.6 ± 0.4	3.7 ± 0.4
54	(Z)-7-decen-5-olide ^f	2259	B	88.7	***	5.8 ± 0.3	3.4 ± 0.3
58	γ-dodecalactone	2372	A	2365.7	***	30.0 ± 0.6	11.3 ± 0.2
	total			2008.5	***	485.6 ± 12.0	125.3 ± 7.1
	total			1763.3	***	14900.5 ± 467.3	3020.9 ± 147.4

^a Values expressed in μg/kg equivalent of 2-octanol are given as average ± standard deviation (n = 3). ^b Linear retention index based on a series of n-hydrocarbons. Values in brackets are interpolated. ^c A, identified by mass spectrum and linear retention index of reference compounds; B, tentatively identified by mass spectrum and linear retention index similar to mass libraries or published data. ^d F_(0.05; 1; 4) = 7.7; F_(0.01; 1; 4) = 21.2; F_(0.001; 1; 4) = 74.1. ^e *, p < 0.05; **, p < 0.01; ***, p < 0.001. ^f Newly identified in *C. melo* var. *dudaim*.

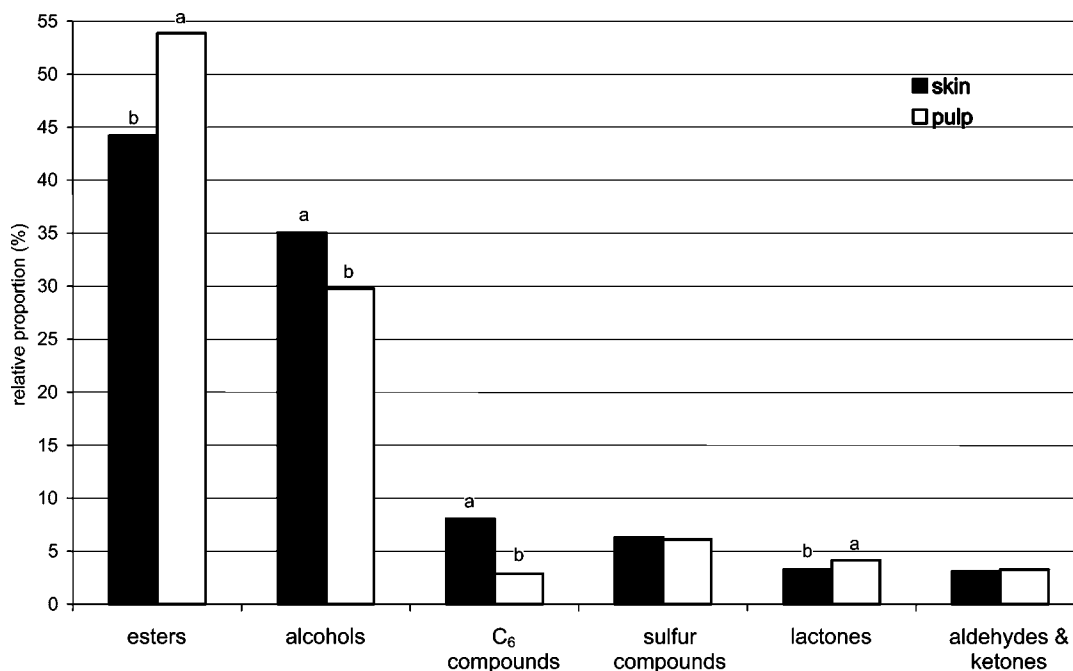


Figure 1. Relative proportion (percent) of the main classes of volatile compounds in the skin and pulp of Queen Anne's pocket melon. Vertical bars with different letters are significantly different ($p < 0.05$).

at room temperature. After removal of the magnetic stir bar, the tube was sonicated for 1 min in a Branson Ultrasonic Cleaner 5510 and centrifuged (1000g, 5 min, 4 °C). Chloroform extract was then recovered with a 50 μ L syringe, transferred to a 100 μ L vial, and immediately injected in GC-MS and GC-FID.

GC-FID Conditions. A Varian 3800 gas chromatograph equipped with a DB-Wax Etr (J&W Scientific) capillary column (30 m \times 0.25 mm i.d., 0.25 μ m film thickness) was used. The flow of hydrogen 5.7 (Linde) carrier gas was 1.3 mL/min. The oven temperature was kept at 40 °C for 3 min, then programmed to 250 °C at 5 °C/min, and kept at 250 °C for 15 min. Injections (1 μ L) were performed using a 1079 PTV injector from Varian under the following injection program: initially 20 °C for 0.1 min, then programmed to 250 °C at 200 °C/min, and kept at 250 °C. Injections were performed using a CombiPAL autosampler equipped with a Peltier cooling rack at 4 °C (CTC Analytics). The flame ionization detector was kept at 250 °C. The levels of the volatile compounds were expressed as 2-octanol equivalents (assuming all of the response factors were 1). The concentrations are to be considered as relative data because recovery after extraction and calibration factors related to the standard were not determined.

GC-MS Conditions. A Varian 3800 gas chromatograph was used with the same DB-Wax Etr capillary column as above. The oven and injector temperature programs were as above. Injections (1 μ L) were performed using a CombiPAL autosampler (CTC Analytics). The flow of helium 6.0 (Linde) carrier gas was 1 mL/min. A Saturn ion-trap mass spectrometer was used. Mass spectra were recorded in electronic impact (EI) ionization mode. The ion trap, the manifold, and the transfer line temperatures were set, respectively, at 150, 45, and 250 °C. Mass spectra were scanned in the range m/z 30–350 amu at 1 s intervals. Compounds were first identified using NIST/EPA/NIH MS Search 2.0 and our own mass spectra libraries. Identities of most were then confirmed by comparison of their linear retention indices and EI mass spectra with those of reference compounds.

RESULTS AND DISCUSSION

Recently described as a fast, precise, and sensitive method for the analysis of volatile compounds in fruits and vegetables (20), the LLME is a single-step extraction that reduces solvent consumption, avoids the concentration process, and therefore minimizes the risk of a thermal degradation of the volatiles. **Table 1** shows the volatile compounds extracted from the skin

and pulp of Queen Anne's pocket melon by LLME using chloroform. Sixty compounds were separated, identified, and quantified by GC-MS and GC-FID including 20 esters, 15 alcohols, 7 lactones, 7 aldehydes and ketones, 6 sulfur compounds, and 5 C₆ compounds. The linear retention index (RI), the F test, and the p value of the ANOVA performed between the levels of volatiles recovered in the skin and pulp are also given in **Table 1**. The relative abundances (percent) of the main volatile classes in the skin and pulp are shown in **Figure 1**.

As shown in **Table 1** and **Figure 1**, esters and alcohols were qualitatively and quantitatively the main components, accounting for 80 and 84% of the total volatiles quantified, respectively, in skin and pulp. Except for 5 of the 60 volatiles, namely, butyl acetate (3), 3-methyl-2-butenyl acetate (14), hexyl acetate (15), (*Z*)-3-hexenyl acetate (19), and 3-(methylthio)propanol (41), the differences observed between the levels of volatiles in the skin and pulp were found to be statistically different ($p < 0.05$), and the results show that generally higher concentrations were recovered in the skin. Indeed, except for 3-methylbutyl acetate (7), ethyl pentanoate (8), octanal (17), 3-(methylthio)propyl acetate (38), benzyl acetate (42), 3-phenylpropyl acetate (47), and cinnamyl acetate (3-phenyl-2-propenyl acetate) (51), the levels of volatiles were found to be from 1.5 to 140 times higher in the skin than in the pulp. Nevertheless, as shown in **Figure 1**, the distributions of the volatiles in skin and pulp were slightly different. Esters made up 44 and 54% of the total of volatile compounds, respectively, in the skin and pulp, followed by alcohols (respectively, 35 and 30%), whereas C₆ compounds accounted for 8% in skin and <3% in the pulp.

As mentioned above, esters were the main compounds identified in both samples, in particular aliphatic esters (96 and 81% of the total esters, respectively, in skin and pulp). Among them, the levels of acetoxy and hydroxy esters were particularly predominant in skin. Although these compounds are generally found in tropical fruits such as passion fruit, pineapple, and mango (21–23), 2,3-butanediol diacetate (28, 32) and its precursor 3-acetoxy-2-butanol (36, 37) have been previously identified in Japanese melon (cv. Golden Crispy) (8) and in *C.*

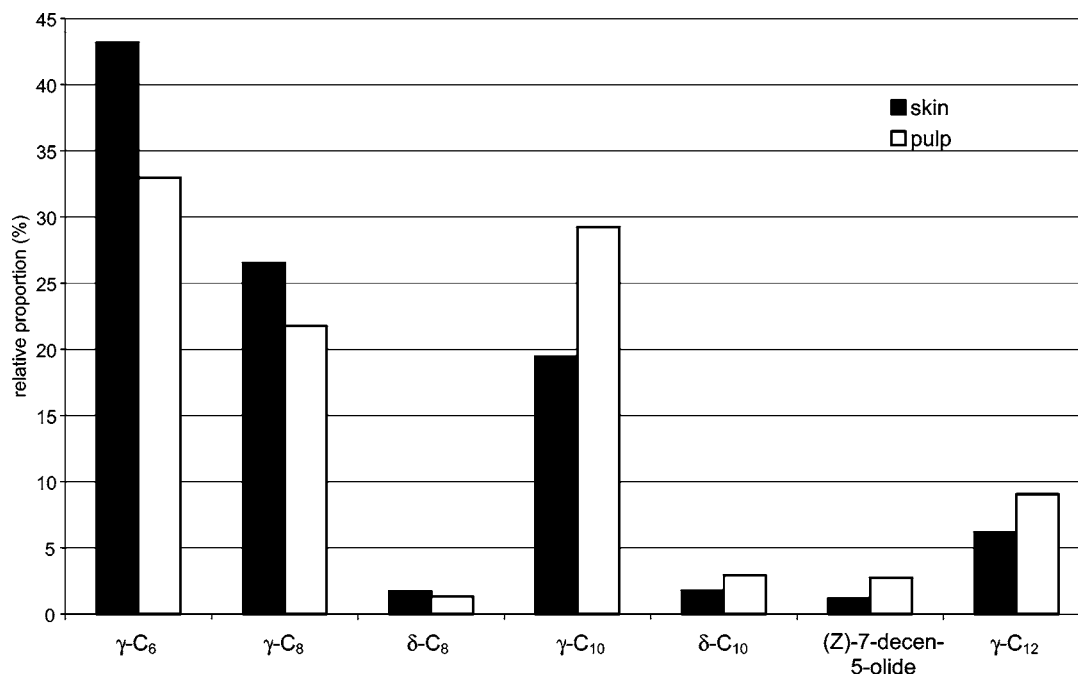


Figure 2. Average distribution of lactones in pulp and skin of pocket melon.

melo var. *cantalupensis* (cv. Alpha) (17), but this is the first time these compounds are identified in pocket melon. Engel et al. (22) and Umamo (24) have shown that the concentrations of hydroxy and acetoxy acid esters increased during pineapple ripening. Nevertheless, according to Wyllie and Leach (8), 2,3-butanediol diacetate has a sweet odor and appears to have high odor threshold and, therefore, would not be expected to have a significant role in the aroma of melon (cv. Golden Crispy). Because 2,3-butanediol diacetate possesses two asymmetric carbons, it has erythro and threo forms and a meso-form diastereoisomer. Therefore, it produces two peaks on a gas chromatogram. According to Wyllie and Leach (8), the most abundant peak (28) would be the D and/or L isomer, whereas the other (32) would be the meso isomer. Apart from acetoxy and hydroxy esters, ethyl butanoate (1), ethyl 2-methylbutanoate (2), butyl acetate (3), 3-methylbutyl acetate (7), and hexyl acetate (15) were the most abundant aliphatic esters in both samples. Earlier works also reported these compounds as being the most abundant in melon (*C. melo* var. *reticulatus* and *C. melo* var. *cantalupensis*) (11, 14, 18, 25) and that these compounds are necessary for the strong fruity notes in this fruit (4). Four aromatic esters have been also identified for the first time in pocket melon, although these compounds had been previously identified in other types of melon (17), except for cinnamyl acetate (51). As shown in Table 1, except for phenyl ethyl acetate (43), the levels of benzyl acetate (42), 3-phenylpropyl acetate (47), and cinnamyl acetate (51) were found to be significantly higher in pulp than in skin.

About alcohols, aromatic alcohols were found to be predominant in our extracts (72 and 78% of the total alcohols, respectively, in skin and pulp). Among them, eugenol (52) was found to be the major constituent of the skin (15.3%). Moreover, contrary to earlier work done by Shu et al. (19), eugenol was found to be the main aromatic alcohol in the pulp of pocket melon (7.4%) instead of chavicol (2.8%). Due to its concentration observed in skin and in pulp (respectively, 2300 and 225 ppb) and its very low odor threshold (6 ppb), it is obvious this compound should contribute significantly to the unique and exotic aroma of pocket melon. Finally, if most of the aromatic

alcohols have been previously identified in other types of melon (17) cinnamyl alcohol (55), isoeugenol (57), and methoxyeugenol (59) were identified for the first time in pocket melon.

With regard to C₆ compounds, aldehydes, and alcohols, products of the enzymatic breakdown of unsaturated fatty acids (26, 27), the results in Table 1 show that the skin had considerably higher concentrations than the pulp. These results were found to be consistent with those previously reported in the skin of tomato, mango, or peach (28–30). Due to the isolation method chosen (LOX deactivation with *n*-propyl gallate during homogenization) (20), it seems reasonable to suppose that the concentrations of these so-called green components are representative of the endogenous levels in the skin and pulp. Nevertheless, despite the precautions taken during the preparation of the samples, the possibility that this may partly be the result of tissue damage occurring during peeling cannot be excluded. Nevertheless, these results would suggest a higher activity of the enzymes involved in the LOX pathway in the skin, as previously reported in peach or in tomato (30, 31), and/or a higher concentration of fatty acids, especially linoleic and linolenic acids, in the skin.

In this study, six sulfur compounds have been identified for the first time in the skin and pulp of pocket melon and among them five thioether esters, methyl 2-(methylthio)acetate (24), ethyl 2-(methylthio)acetate (25), 2-(methylthio)ethyl acetate (29), ethyl 3-(methylthio)propanoate (35), and 3-(methylthio)propyl acetate (38). Except for 3-(methylthio)propyl acetate, levels of thioether esters have been found to be significantly higher in skin than in pulp. Ethyl 2-(methylthio)acetate was the major thioester observed in skin, whereas 3-(methylthio)propyl acetate and 2-(methylthio)ethyl acetate were dominant in pulp, as previously reported in pulp of other types of melons (10, 18). Due to their low odor threshold, the flavor impact of sulfur compounds on the aroma of many fruits (23, 32–35) or in wine (36–39) is now well established, particularly in melon (8, 10, 11, 13, 18). Some authors have reported that trace amounts of certain sulfur compounds such as ethyl 2-(methylthio)acetate have a major impact on the musky note of some melon aroma

(10, 11, 17), and therefore these compounds should play an important role to the aroma of pocket melon.

Seven lactones were identified in the skin and pulp of pocket melon (**Table 1**). Only lactones with even-numbered carbon chains have been identified, and as mentioned in **Figure 1** their total amounts accounted for 3 and 4%, respectively, in skin and pulp. Among them γ -C₆, C₈, C₁₀, and C₁₂ were the main lactones, and their levels were found to be 1.7–5.1 times higher in skin than in pulp. As indicated in **Figure 2**, their average distributions in skin and pulp were found to be different. Lactones with a carbon chain length up to C₈ were predominant in the skin, in particular, γ -hexalactone, whereas those with a ≥ 10 -carbon chain length were found to be the major lactones in the pulp, in particular, γ -decalactone. These compounds, well-known in other fruits, are particularly responsible for the spicy, floral, and fruity characteristics of peach, apricot, or plum aroma (40–44). Among lactones, γ -C₈, C₁₀, and C₁₂ are present at a concentration fairly above their odor threshold in both samples [γ -hexalactone, 1600 ppb; γ -octalactone, 7 ppb; δ -octalactone, 400 ppb; γ -decalactone, 11 ppb; δ -decalactone, 100 ppb; (Z)-decen-5-olide, 2000 ppb; γ -dodecalactone, 7 ppb] (42) and, therefore, are possible contributors to the particular aroma of Queen Anne's pocket melon. Except for γ -dodecalactone previously identified at a trace level in pocket melon (19), these lactones have been identified for the first time in this fruit.

With regard to aldehydes and ketones, except for octanal, their levels were found to be significantly higher in skin than in pulp. In addition to 2,3-butanediol diacetate, 3-acetoxy-2-butanol, and 3-acetoxy-2-butanone, 3-hydroxy-2-butanone (acetoin) was detected both in skin and in pulp. About this latter compound, it is noteworthy that its skin/pulp ratio was found to be similar to that observed for 2,3-butanediol diacetate and 3-acetoxy-2-butanol, that is, approximately 10, whereas that of 3-acetoxy-2-butanone was found to be higher (~30). Finally, if most of the aldehydes and ketones identified in this study have been previously detected in different types of melon (17) and/or in pocket melon (19), to our best knowledge this is the first time that 6-methyl-5-hepten-2-one and vanillin are reported, respectively, in pocket melon and in *Cucumis melo*.

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