Determination of Total Volatile Components of *Cucumis melo* L. Variety Cantaloupensis

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The volatiles of orange-fleshed *Cucumis melo* L. var. Cantaloupensis were studied using the Freon 11 extraction method and a combination of multidimensional gas chromatography (MDGC) and a gas chromatography-mass spectrometry (GCMS). The total profile of identified compounds is reported, including the sulfur compounds that play an important role in the overall aroma profile of melon fruit according to the sniffing port analysis.

INTRODUCTION

The volatiles of *Cucumis melo* L. var. Cantaloupensis have been reported by several investigators (Yabumoto et al., 1977; Kemp et al., 1972, 1973), who have described the typical melon aroma as mainly due to linear saturated and unsaturated aldehydes issued from linolenic and linoleic acid degradation.

Buttery et al. (1982) have reported that the typical aroma of C. melo var. C. inodorous cv. Honeydew is due to (Z)-6-noneyl acetate, (Z,Z)-3,6-nonadienyl acetate, (Z)-3-nonenyl acetate, 3-methyl-2-butenyl acetate, and a sulfur compound, ethyl (methylthio)acetate. This was the first time that a sulfur compound was mentioned to contribute to the typical aroma of C. melo. In 1989 Homatidou et al. reported eight more sulfur compounds contributing to the aroma of C. melo L. var. Cantaloupensis, which are methyl (methylthio)acetate, 3-(methylthio)propane-1-nitrile, 3-(methylthio)propan-1-ol, ethyl (methylthio)acetate, 2-(methylthio)ethyl acetate, methyl 3-(methylthio)propanoate, ethyl 3-(methylthio)propanoate, and 3-(methylthio)propyl acetate. The above-mentioned thio compounds except 3-(methylthio)propane-1-nitrile and 3-(methylthio)propan-1-ol were also reported independently by Wyllie and Leach (1990).

The above results prompted us to investigate further the volatiles of *C. melo* L. var. Cantaloupensis by using multidimensional gas chromatography and GCMS to identify as much as possible components in the volatiles, thus obtaining further information for the constituents responsible for the typical aroma of melon.

RESULTS AND DISCUSSION

The volatiles of ripe fresh C. melo L. var. Cantaloupensis were extracted with Freon 11 and were subjected to MDGC and GCMS analysis; the identified compounds appear in Table I. A lot of volatile components, as indicated in Table I, were detected for the first time in C.melo L. var. Cantaloupensis, and this is probably due to the nondestructive extraction at low temperature and the very efficient capillary chromatographic system used for the analysis. Minor components containing heteroatoms such as sulfur compounds and trace peaks generally do not give significant mass spectrum for their identification, and a big sample injection causes overloading of the column and lack of resolution. MDGC solved these problems by enrichment of trace components. Other advantages of the system are the possibility for a first preliminary run (master GC) with simultaneous sniffing of the eluents and the fact that peaks or regions of organoleptic interest can be switched into the other column (slave GC) of the system by proper programming of the valve.

Compounds that have not previously reported as constituents of the volatile components of *C. melo* L. var. Cantaloupensis have been identified and belong to aromatic and aliphatic esters, aliphatic and aromatic alcohols, aliphatic and aromatic aldehydes and ketones, γ and δ -lactones, phenols, aliphatic acids, terpenoids, nitriles, and sulfur compounds (Table I).

Nine sulfur compounds have been identified as contributing to the aroma of C. melo L. var. Cantaloupensis, from which 2-(methylthio)ethanol is reported for the first time. The common precursor for all of the sulfur compounds is probably methionine, through a biogenetic pathway involving methionine deamination and α -keto 4-(methylthio)butanoic acid decarboxylation. The only exception is 3-(methylthio)propane-1-nitrile, which is formed after the myrosinase activity upon 2-(methylthio)ethyl glucosinolate (Schreier, 1982). The existence of myrosinase activity in C. melo L. var. Cantaloupensis, both in endocarp and in seeds, has been recently reported (Bois et al., 1992). This activity seems to increase during the maturation of the fruit.

EXPERIMENTAL PROCEDURES

Ripe fresh C. melo L. var. Cantaloupensis samples grown from authenticated seeds were obtained from the greenhouses of VIORYL S.A. (agricultural and chemical company) and analyzed immediately after harvesting.

The majority of the compounds found in the extract of C. melo L. var. Cantaloupensis were identified by their mass spectra, compared with the mass spectra of authentic samples from either the VIORYL S.A. private sample collection library or the NBS library. Trace compounds were confirmed by coinjection with authentic samples. The new sulfur compounds were compared with authentic samples from reliable commercial sources or prepared by classical organic synthesis methods as follows: 2-(methylthio)ethanol was synthesized from reaction of 2-chloroethanol with sodium methylthioate; 2-(methylthio)ethyl acetate was synthesized from acetylation of 2-(methylthio)ethanol by acetic anhydrite in pyridine; 3-(methylthio)propanenitrile was synthesized by reaction of 3-chloropropanenitrile with sodium methylthioate. 3-(Methylthio)propan-1-ol was purchased from PFW Co. Benzyl cyanide was purchased from Janssen Chimica. Methyl (methylthio)acetate and ethyl (methylthio)acetate were purchased from Janssen. Methyl 3-(methylthio)propanoate and ethyl 3-(methylthio) propanoate were purchased from Frutarome Co.

Table I. Volatiles of Cucumis melo L. Variety Canteloupensis

compounds	previously reported	methods of identification
	Aliphatic Esters	
methyl acetate	Yabumoto et al. (1977); Buttery et al. (1982)	MS, RT
ethyl acetate	Yabumoto et al. (1977); Buttery et al. (1982); Wyllie and Leach (1990) Vabumoto et al. (1977); Buttery et al. (1982); Wyllie and Leach (1990)	MS, RT
propyl acetate methyl butencete	Yabumoto et al. (1977); Buttery et al. (1962); Wyllie and Leach (1990) Vabumoto et al. (1977); Buttery et al. (1982)	MS, RT MS PT
2-methylpropyl acetate	Yabumoto et al. (1977); Kemp et al. (1972, 1973); Buttery et al. (1982); Wyllie and Leach (1990)	Ms, RT
methyl 2-methylbutanoate	Yabumoto et al. (1977); Wyllie and Leach (1990)	MS, RT
ethyl propanoate	Yabumoto et al. (1977); Wyllie and Leach (1990)	MS, RT
ethyl 1-butanoate	Yabumoto et al. (1977); Kemp et al. (1973); Buttery et al. (1982); Wyllie and Leach (1990)	MS, RT
butyl 1-acetate	Yabumoto et al. (1977); Kemp et al. (1972, 1973); Buttery et al. (1982); Wyllie and Leach (1990)	MS, RT
ethyl (E)-2-butenoate ^a ethyl 2-methylbutanoate	Yabumoto et al. (1977); Kemp et al. (1972, 1973); Buttery et al. (1982);	MS, RT MS, RT
3-methylbutyl egetate	Wyllie and Leach (1990) Kemp et al. (1973): Buttery et al. (1982)	MC DT
nronyl butanoate	Yabumoto et al. (1977)	MS, RT MS RT
ethyl 3-methylbutanoate		MS. RT
amyl acetate	Yabumoto et al. (1977); Buttery et al. (1982)	MS. RT
methyl hexanoate	Yabumoto et al. (1977)	MS, RT
3-methyl-2-butenyl acetate	Buttery et al. (1982)	MS, RT
butane-2,3-diol monoacetate		MS, RT
allyl pentanoate ^a		MS, RT
2-methyl-2-butenoate ^a		MS, RT
propyl pentanoate		MS, RT
ethane-1,2-diol diacetate		MS, RT
ethyl hexanoate	Yabumoto et al. (1977); Kemp et al. (1972, 1973); Buttery et al. (1982); Wullia and Leach (1990)	MS, RT MS, RT
3(Z)-hexenyl acetate	Yabumoto et al. (1977); Kemp et al. (1972, 1973); Buttery et al. (1982); Willie and Leach (1990)	MS, RT
hexyl acetate	Yabumoto et al. (1977); Kemp et al. (1972, 1973); Buttery et al. (1982); Wyllie and Leach (1990)	MS, RT
propane-1.2-diol diacetate	Wyllie and Leach (1990)	MS. RT
(Z)-3-hexenyl pentanoate		MS, RT
butane-2,3-diol diacetate (2 peaks)	Wyllie and Leach (1990)	MS, RT
ethyl (E,E)-2,4-hexadienoate ^a		MS, RT
1-octen-3-yl acetate		MS, RT
ethyl 3-hydroxyhexanoate	Buttom et al. (1090)	MS, RT MS, DT
nonyi acetate	Buttery et al. (1962)	MS, RT MS PT
(Z, Z)-3 6-nonedienvl acetate	Kemp et al. (1973)	MS, RT
ethyl hexadecanoate	Kemp et al. (1972)	MS, RT
1-methylethyl dodecanoate		MS, RT
	Aliphatic Alcohols	10 50
(Z)-3-hexenol	Yabumoto et al. (1977)	MS, RT
1-hexanol	Yabumoto et al. (1977); Kemp et al. (1973); Wyllie and Leach (1990)	MS, RT MS, DT
3-nexanol 2.2 dimethyl 2 hytenol		MS, RI MS PT
1.octen-3-ol	Kempetal (1973)	MS, RT
1-nonen-4-ol		MS. RT
1-octanol	Kemp et al. (1972, 1973)	MS, RT
(<i>Z</i> , <i>Z</i>)-3,6-nonadien-1-ol	Buttery et al. (1982)	MS, RT
(Z)-6-nonenol	Kemp et al. (1972); Buttery et al. (1982)	MS, RT
nonanol	Kemp et al. (1972, 1973)	MS, RT
h aman al	Aldehydes, Ketones	MC DT
nexanai 2. othylbutanol		MO, TT MO DT
2-emplorialian 6-methyl-3-hentenone		MS, RI MS RT
6-methyl-5-hepten-2-one		MS. RT
3-octanone		MS, RT
3-hydroxy-2-butanone		MS, RT
(Z)-6-nonenal		MS, RT
nonanal	Kemp et al. (1972); Buttery et al. (1982)	MS, RT
aecanai		MS, RT MS, RT
h	Aromatic Aldehydes, Ketones	
Denzaldenyde phonylocotoldenyde	remp et al. (1972); Buttery et al. (1982)	MS, KT Mg DT
phenylacetaldenyde anisaldehyde		MS RT
(E)-cinnamic aldehyde		MS. RT
heliotropine		MS, RT
acetovanillone		MS, RT
		MS, RT

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Table I (Continued)

compounds	previously reported	methods of identification
benzyl acetate phenyl ethyl acetate	Aromatic Esters Kemp et al. (1973); Buttery et al. (1982); Wyllie and Leach (1990) Kemp et al. (1973); Wyllie and Leach (1990)	MS, RT MS, RT
ethyl benzoate ethyl phenyl acetate		MS, RT MS RT
phenyl propyl acetate	Kemp et al. (1973); Wyllie and Leach (1990)	MS, RT
	Aromatic Alcohols	
benzyl alcohol phenyl ethyl alcohol phenyl propyl alcohol (<i>E</i>)-cinnamic alcohol		MS, RT MS, RT MS, RT MS, RT
	Lactones (γ, δ)	
γ -octalactone δ -nonalactone γ -nonalactone γ -decalactone δ -undecalactone		MS, RT MS, RT MS, RT MS, RT MS, RT
b-undecalactone		W15, IV I
eugenol	Phenois	MS RT
eugenor	Alimbadia Asida	1410, 101
butanoic acid dodecanoic acid tetradecanoic acid hexadecanoic acid (Z)-9-octadecenoic acid octadecanoic acid	Aliphatic Acids	MS, RT MS, RT MS, RT MS, RT MS, RT MS, RT
	S Compounds	-, .
dimethyl disulfide	S Compounds Buttery et al. (1982)	MS, RT MS_RT
methyl (methylthio)acetate 3-(methylthio)propan-1-ol ethyl (methylthio)acetate 2-(methylthio)ethyl acetate methyl 3-(methylthio)propanoate ethyl 3-(methylthio)propanoate 3-(methylthio)propyl acetate	Wyllie and Leach (1990); Homatidou et al. (1990) Homatidou et al. (1990) Wyllie and Leach (1990); Homatidou et al. (1990)	MS, RT MS, RT MS, RT MS, RT MS, RT MS, RT MS, RT
	Terpenoids (Hydrocarbons and Oxygen Derivatives)	
β -pinene 1,8-cineol limonene γ -terpinene p-menth-1-en-8-ol verbenone		MS, RT MS, RT MS, RT MS, RT MS, RT MS, RT
terpinyl acetate		MS, RT MS, RT
geranyl acetone	$V_{\text{comp}} \rightarrow c [(1070, 1070)]$	MS, RT
p-ionone dihydroactinidiolide	remp et al. (1972, 1973)	MS, RT MS, RT
•	Cyano Compounds	,
benzyl cyanide 3-(methylthio)propanenitrile	oyuno compoundo	MS, RT MS, RT
^a Tentative.		

Isolation of Volatiles. One C. melo fruit Alpha HF1 Tezier, approximately 700 g, was peeled, the seeds were removed, and the fruit was sliced to $1 \cdot \text{cm}^3$ small pieces; 250 g of pure fruit was immersed into 250 mL of Freon 11 and left for 24 h at 0 °C. The whole frozen mixture was crushed, and the fruit with the water phase was separated and reextracted with 250 mL of cold Freon 11. The two organic phases were collected, dried over sodium sulfate, and concentrated at 36 °C and 760 mmHg in a small Vigreux column (20-cm height and 1-cm diameter), leaving about 1 cm³ of solution ready for the analysis.

MDGC Analysis. The MDGC system used is a prototype of Varian International designed by Dr. H. Kern according to the analytical requirements of VIORYL S.A. It consists of two gas chromatographs, a Varian 3400 (master) and a Varian 3600 (slave) coupled through a VALCO four-port valve (VALCONE E", with core temperature 275 °C) and a transfer line. The valve can be controlled through the relay section of the master GC, and the transfer line can be heated and the temperature controlled through the auxiliary section of the master GC. The flow stream eluting from the first megabore column can be switched either to the FID or master GC or to the column of the slave GC for further separation or enrichment. In the second case an empty fused silica tube (1 m long, 0.32 mm i.d.) is used.

The master GC is equipped with the following: split-splitless injector; FID; 30-m, 0.53 mm i.d. FSOT column coated with DB-1 (methyl silicon 1.5 μ m film thickness from Varian); 90 °C air actuator for the valve; effluent sniffing port (placed between the end of the megabore column and the FID); and two-channel integrator Varian 4290.

The slave GC is equipped with the following: split-splitless injector; 30-m 0.32 mm i.d. FSOT column coated with RSL 150 [poly(dimethylsiloxane) 1 μ m film thickness, Heliflex RSL 150 from Altech Associates]; four detectors (two FIDs, one FPD, one TSD) with the possibility of direct operation in each detector or simultaneous operation in two different detectors through splitter (splitting ratio 1:1); option for subambient temperature operation; two-channel integrator Varian 4290.

The two gas chromatographs can operate independently or in collaboration.

The system gives the possibility of a first preliminary run in the master GC with simultaneous sniffing of the eluents. In this run peaks or even regions of organoleptic interest can be specified. In a second run regions of interest can be switched into the column of the slave GC by proper programming of the valve. At the same time the slave GC is kept cool by using liquid nitrogen or liquid carbon dioxide.

To begin the analysis, a first run of the sample was made in the analytical column of 3600 using FID and FPD detectors. In the first run, only three S compounds were detected.

Chromatographic conditions of the above-mentioned runs are as follows: oven (3600); initial temperature, 45 °C; hold time, 10 min; temperature I, 100 °C, rate 2 °C/min; temperature II, 220 °C, rate 4 °C/min; hold time, infinity; injector, 200 °C (splitless); detector (FID), 280 °C; detector (FPD), 250 °C; sample volume, 0.5 μ L (5% w/w); carrier gas, He, 1.5 mL/min.

However, the sniffing of eluents in the master GC indicated that more components seem to be characteristic, so a procedure of enrichment by heart cutting was decided.

Chromatographic conditions for the first run in the master GC were as follows: oven (3400); initial temperature, 60 °C; initial hold time, 10 min; temperature I, 120 °C, rate 3 °C/min; temperature II, 220 °C, rate 5 °C/min; hold time, infinity; injector, 200 °C (splitless); detector (FID), 280 °C; sample volume, 10 μ L (5% w/w); carrier gas, He, 3.0 mL/min; transfer line temperature, 200 °C. The procedure was repeated three times for each fraction, keeping the oven of the slave GC at -3 °C (using liquid CO₂ during trapping).

At the end of the third run the 3600 oven was heated from -3 to 45 °C at a rate 50 °C/min and with the same chromatographic conditions previously mentioned. The same run was made in both FID and FPD.

Nine S compounds were detected in total.

A full description of the MDGC system and how it works is given in Homatidou et al. (1990).

GCMS Analysis. A Hewlett-Packard 5970 A system equipped with a EI source was used. Special attention was paid to the regions of the chromatogram where the MDGC analysis showed the presence of sulfur compounds.

For the analysis the following multistep program was used:

initial temp, °C	initial time, min	rate, °C/min	final temp, °C	final time, min	total time, min
60	3.0	3.0	80	1.0	10.67
		3.0	150	0.0	34.00
		5.0	200	0.0	44.00
		10.0	230	20.0	67.00

Conditions were as follows: injector temperature, 200 °C; transfer line, 230 °C; SE-30 fused silica capillary column, 12 m 0.2 mm i.d.; carrier gas, He; sample volume, 1 μ L; split ratio, 1:100.

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Registry No. AcOMe, 79-20-9; AcOEt, 141-78-6; AcOPr, 109-60-4; CH₃(CH₂)₂CO₂Me, 623-42-7; AcOBu-i, 110-19-0; CH₃CH₂-CH(CH₃)CO₂Me, 868-57-5; CH₃CH₂CO₂Et, 105-37-3; CH₃(CH₂)₂-CO2Et, 105-54-4; AcOBu, 123-86-4; (E)-CH3CH=CHCO2Et, 623-70-1; CH₃CH₂CH(CH₃)CO₂Et, 7452-79-1; AcO(CH₂)₂CH(CH₃)₂, 123-92-2; CH₃(CH₂)₂CO₂Pr, 105-66-8; CH₃CH(CH₃)CH₂CO₂Et, 108-64-5; AcO(CH₂)₄CH₃, 628-63-7; CH₃(CH₂)₄CO₂Me, 106-70-7; AcOCH₂CH=C(CH₃)₂, 1191-16-8; AcOCH(CH₃)CH(OH)CH₃, 56255-48-2; CH₃(CH₂)₃CO₂CH₂CH=CH₂, 6321-45-5; CH₃(CH₂)₃-CO₂Pr, 141-06-0; AcO(CH₂)₂OAc, 111-55-7; MeO₂CCH₂CO₂Me, 108-59-8; CH₃(CH₂)₄CO₂Et, 123-66-0; (Z)-AcO(CH₂)₂CH=CHCH₂-CH₃, 3681-71-8; AcO(CH₂)₅CH₃, 142-92-7; AcOCH₂CH(CH₃)OAc, 623-84-7; (Z)-CH₃(CH₂)₃CO₂(CH₂)CH=CHCH₂CH₃, 35852-46-1; AcOCH(CH₃)CH(CH₃)OAc, 1114-92-7; (E,E)-CH₃CH=CH-CH=CHCO2Et, 2396-84-1; AcOCH(CH=CH2)CH2(CH2)3CH3, 2442-10-6; CH₃(CH₂)₂CH(OH)CH₂CO₂Et, 2305-25-1; AcO(CH₂)₈-CH₃, 143-13-5; (Z,Z)-AcO(CH₂)₂CH=CHCH₂CH=CHCH₂CH₃, 83334-93-4; H₃C(CH₂)₁₄CO₂Et, 628-97-7; H₃C(CH₂)₁₀CO₂Pr-i, 10233-13-3; (Z)-CH₃CH₂CH=CH(CH₂)₂OH, 928-96-1; CH₃(CH₂)₅-OH, 111-27-3; CH₃(CH₂)₂CH(OH)CH₂CH₃, 623-37-0; (CH₃)₃CH-(OH)CH₃, 464-07-3; CH₃(CH₂)₄CH(OH)CH=CH₂, 3391-86-4; CH₃(CH₂)₄CH(OH)CH₂CH=CH₂, 35192-73-5; CH₃(CH₂)₇OH, 111-87-5; (Z,Z)-CH₃CH₂CH=CHCH₂CH=CH(CH₂)₂OH, 53046-97-2; (Z)-CH₃CH₂CH=CH(CH₂)₅OH, 35854-86-5; CH₃(CH₂)₈OH, 143-08-8; CH₃(CH₂)₄CHO, 66-25-1; (CH₃CH₂)₂CHCHO, 97-96-1; (CH₃)₂CH(CH₂)₂C(O)CH₂CH₃, 624-42-0; (CH₃)₂C=CH(CH₂)₂C-(O)CH₃, 110-93-0; CH₃(CH₂)₄C(O)CH₂CH₃, 106-68-3; CH₃CH-(OH)C(O)CH₃, 513-86-0; (Z)-CH₃CH₂CH=CH(CH₂)₄CHO, 2277-19-2; CH₃(CH₂)₇CHO, 124-19-6; CH₃(CH₂)₈CHO, 112-31-2; PhCHO, 100-52-7; PhCH₂CHO, 122-78-1; MeOC₆H₄CHO, 50984-52-6; (E)-PhCH=CHCHO, 14371-10-9; AcOCH₂Ph, 140-11-4; PhCO₂Et, 93-89-0; PhCH₂OH, 100-51-6; Ph(CH₂)₂OH, 60-12-8; (E)-PhCH=CHCH₂OH, 4407-36-7; CH₃(CH₂)₂CO₂H, 107-92-6; CH₃(CH₂)₁₀CO₂H, 143-07-7; CH₃(CH₂)₁₂CO₂H, 544-63-8; CH₃(CH₂)₁₄CO₂H, 57-10-3; (Z)-CH₃(CH₂)₇CH=CH(CH₂)₇CO₂H, 112-80-1; CH₃(CH₂)₁₆CO₂H, 57-11-4; MeSSMe, 624-92-0; MeS(CH₂)₂OH, 5271-38-5; MeSCH₂CO₂Me, 16630-66-3; MeS-(CH₂)₃OH, 505-10-2; MeSCH₂CO₂Et, 4455-13-4; MeS(CH₂)₂OAc, 5862-47-5; MeS(CH₂)₂CO₂Me, 13532-18-8; MeS(CH₂)₂CO₂Et, 13327-56-5; MeS(CH₂)₃OAc, 16630-55-0; PhCH₂CN, 140-29-4; $MeS(CH_2)_2CN$, 54974-63-9; acetovanillone, 498-02-2; γ -octalactone, 104-50-7; δ-nonalactone, 3301-94-8; γ-nonalactone, 104-61-0; γ -decalactone, 706-14-9; δ -undecalactone, 710-04-3; eugenol, 97-53-0; β-pinene, 127-91-3; 1,8-cineole, 470-82-6; limonene, 138-86-3; γ-terpinene, 99-85-4; p-menth-1-en-8-ol, 98-55-5; verbenone, 80-57-9; β-cyclocitral, 432-25-7; terpinyl acetate, 80-26-2; geranyl acetone, 3796-70-1; β -ionone, 79-77-6; dihydroactinidiolide, 17092-92-1; heliotropine, 120-57-0.