Sulfur-Containing Compounds in the Aroma Volatiles of Melons (*Cucumis melo*)

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The incidence of six thioether esters, methyl (methylthio)acetate, ethyl (methylthio)acetate, 2-(methylthio)ethyl acetate, methyl 3-(methylthio)propanoate, ethyl 3-(methylthio)propanoate, and 3-(methylthio)propyl acetate, considered to be of importance to the aroma profiles of *Cucumis melo* fruit, has been surveyed in a wide range of cultivars. Their presence and concentrations appear to be under genetic control since there are marked differences between cultivars. The concentrations of these compounds have been determined in a number of cultivars and some have been shown to have odor values which indicate that they contribute to the overall aroma perception of the ripe fruit.

INTRODUCTION

Cucumis melo is a polymorphic species that has been divided into seven different varieties (Whitaker and Davis, 1962): *C. melo* var. Canteloupensis Naud, *C. melo* var. Flexuosus Naud, *C. melo* var. Conomon Mak, *C. melo* var. Chito Naud, *C. melo* var. Dudaim, *C. melo* var. Reticulatus Naud, and *C. melo* var. Inodorus Naud. The dessert melons of commercial importance, for example, muskmelon, honeydew, and casaba, belong predominantly to the latter two varieties. The large number of cultivars within these two varieties exhibits a wide variation in flavor and aroma characteristics, and these are important in influencing consumer preferences. However, the aroma profiles of only a very small number of melon cultivars have been investigated (Kemp et al., 1971, 1972a,b, 1973, 1974; Yamaguchi et al., 1977; Horvat and Senter, 1987; Yabumoto et al., 1978). The varieties and cultivars investigated were shown to contain typical mixtures of fruit esters dominated quantitatively by ethyl acetate, isobutyl acetate, 2-methylbutyl acetate, ethyl butanoate, and ethyl-2-methylbutanoate. The presence of sulfur-containing compounds in extracts from these melons was inferred by sensory evaluation, but only dimethyl disulfide was positively identified (Yabumoto et al., 1977). Honeydew was further investigated by Buttery et al. (1982), who confirmed the identity of many of the compounds previously reported in this cultivar. They also demonstrated the presence of a range of C-9 unsaturated esters and alcohols, some of which have a characteristic melonlike odor. These presumably originated as a result of lipoxygenase activity. These workers also reported the presence of ethyl (methylthio)acetate in the extract obtained from this melon by simultaneous distillation extraction at atmospheric pressure. Additional thioether esters, found in melon aroma extracts obtained by widely differing extraction techniques, have subsequently been reported (Homatidou et al., 1989; Wyllie et al., 1990). Homatidou also identified the sulfur-containing compounds 3-(methylthio)propanenitrile and 3-(methylthio)-propanol. It is anticipated that this sulfur-containing group of compounds will play an important role in the overall aroma profile of melon fruit if they are present in concentrations greater than their odor thresholds.

As part of a survey of melon flavor and aroma characteristics, the occurrence of six thioether esters in the fruit of a large range of melon cultivars of distinctly different morphological characteristics has been undertaken.

EXPERIMENTAL PROCEDURES

Melons used in this study were grown from authenticated seed obtained from commerical seed producers. All were grown in the open on a single site. The cultivars included in Table I were grown in the ground using standard horticultural practices; those in Table II were grown using a run-to-waste hydroponic system fed with a controlled nutrient mix. Cultivars whose fruit exhibit abscission (full slip) at maturity were harvested at this stage. Some cultivars whose fruit do not show this characteristic (non-slip) were picked when judged fully ripe by an experienced horticulturist. Fruit was stored at 4 °C and extracted within 48 h.

Melon samples were taken from a whole fruit by cutting it into longitudinal sections, removing the edible portion (middle mesocarp) and cutting this into small cubes (ca. 5 × 5 cm). This sample (typically 500–1000 g) together with distilled water (500 mL) was then placed into a simultaneous distillation extraction (Likens–Nickerson) apparatus and extracted for 1.5 h using pentane as the extracting solvent. For quantitative work a weighed quantity of the internal standard (butyl hexanoate) was added to the distillation flask together with the melon flesh. The extract was concentrated (1 mL) in a Kuderna–Danish flask attached to a Snyder column using a bath temperature of 45 °C. The concentrated extract was chromatographed using either a Pye Unicam GCV or a Hewlett-Packard 5890 chromatograph fitted with two columns [SGE, Victoria, Australia; 25 m × 0.3 mm i.d. OV101 and 25 m × 0.32 mm i.d. BP20 (Carbowax)] inserted into a single split injector (split ratio 1:30) and terminated at independent flame ionization detectors. Chromatographic conditions were as follows: initial temperature, 70 °C for 2 min; program rate, 4 °C/min; final temperature, 200 °C; injector temperature, 220 °C; detector temperature, 220 °C; carrier gas, N_2 (GCV), H_2 (5890). Data outputs from both detectors were collected and processed using a dual-channel computing integrator (DAPA Scientific Pty. Ltd., Perth, Australia). For the GC effluent sniffing experiments the outlet from a column (OV101, 25 m × 0.32 mm i.d.) was divided 1:1 using an outlet splitter (SGE) with one arm connected to an FID detector and the other to a Teflon sniffing port. The selective detection of sulfur compounds utilized a similar splitter with one arm connected to the FID and the other to the FPD (Pye Unicam GCV). Chromatographic conditions were as described above. The identities of the compounds described in this paper were confirmed by mass spectral and retention index data as previously described (Wyllie et al., 1990; Homatidou et al., 1989).

0021-8561/92/1440-0253$03.00/0

Table I. Incidence of Thioether Esters in a Range of *C. melo* Cultivars[a]

cultivar	methyl (methylthio)acetate	ethyl (methylthio)acetate	2-(methylthio)ethyl acetate	ethyl 3-(methylthio)propanoate	3-(methylthio)propyl acetate
Accent	–	+++	+++	–	–
Alice	–	–	++++	–	tr
Arava	–	–	+	–	+++
Baladi	–	+	++++	–	tr
Chando	–	+	+++	–	–
Delicious	–	–	+++++	–	–
Earlisweet	–	++	++++	–	tr
Eden Gem	–	–	++	–	++
Eurasia	–	–	–	–	+++
Fiata	–	++	+++	+	++
Galia	–	–	++++	–	–
Haom	–	tr	++++	tr	+++
Harmony	–	+++	–	–	–
Honeyball	–	tr	–	–	–
Jumbo	tr	++	+++	++	++
Makdimon	–	–	+++	–	++++
Noy Yarok	–	+	tr	+++	+
Parisienne	–	++	++++	tr	tr
Petra	–	–	++++	–	+
Resistant Joy	–	+	++++	–	tr
Schoons	–	++	+++	–	–
Sharom	tr	+	++++	tr	++
Sprite	–	–	+	–	++
Sunrise	–	++	+++	–	–
Viva	–	–	–	–	–
Yokneam	–	tr	++++	–	+

[a] +, relative intensity. tr, trace. –, not detected.

Table II. Concentrations[a] of Thioether Esters in Melon Cultivars

cultivar	methyl (methylthio)-acetate	ethyl (methylthio)-acetate	2-(methylthio)-ethyl acetate	methyl 3-(methylthio)-propanoate	ethyl 3-(methylthio)-propanoate	3-(methylthio)-propyl acetate
Alice	14	11 (<1)	355	15	nd	200 (7)
Grande Gold	2	nd	24	nd	3 (<1)	32 (1)
Haom	46	122 (5)	1072	24	150 (21)	1404 (47)
Makdimon	6	nd	67	9	12 (2)	421 (14)
Noy Yarok	nd	nd	nd	26	48 (7)	11 (<1)
Parisienne	98	354 (14)	498	9	27 (4)	142 (5)
Yokneam	30	255 (10)	1412	nd	16 (2)	233 (8)
Petra	73	73 (3)	513	11	18 (3)	317 (11)

[a] All values are in micrograms per kilogram of melon flesh. nd, not detected. Values in parentheses are odor values.

RESULTS AND DISCUSSION

Table I provides a semiquantitative description of the distribution of five of the six thioether esters in 26 cultivars. Methyl 3-(methylthio)propanoate has not been included since it never appears in greater than trace amounts in any cultivar. The aroma extracts were all obtained by the same simultaneous distillation method from fruit harvested when fully mature and grown under similar conditions. Marked qualitative and quantitative differences between the various cultivars can be seen. 2-(Methylthio)ethyl acetate and 3-(methylthio)propyl acetate are the dominant compounds in many of the cultivars, with ethyl (methylthio)acetate also occurring frequently. It appears that the formation of these compounds is under genetic control because of the obvious cultivar-dependent nature of their occurrence. The structure of these compounds suggests that they may be derived from methionine, possibly via the biochemical pathway utilized for the generation of the ripening hormone ethylene (Yang and Hoffman, 1984). If this is the case, then their generation and concentrations may very well be dependent on fruit maturity and harvest time. Homatidou et al. (1989) suggest that the sulfur compounds are generated from thioglucosinolates by a mechanism similar to that demonstrated for radishes and similar fruits (Schreier, 1984). While this pathway would be expected to give rise to compounds such as 3-(methylthio)propanenitrile and 3-(methylthio)propanol, the origin of the thioether esters by this mechanism is less obvious. We have been unable to identify either of the two above compounds in our extracts obtained by simultaneous distillation extraction. Table II lists the concentrations of the six thioether esters in a smaller number of cultivars. These concentrations were determined by comparison with an internal standard (butyl hexanoate), which was added to the melon flesh before extraction. No corrections for FID detector response or extraction efficiency have been made. All of the melons described here were grown hydroponically under controlled nutrient regimes and were harvested when fully ripe. The odor unit (U_O) values (Guadagni et al., 1966) are also shown where the threshold value of the thioether ester has been reported. In almost all cases the odor unit value of at least one of the thioether esters is of sufficient magnitude to indicate that they would make a marked contribution to the overall aroma profile of the fruit. This is particularly so for the cultivars Haom, Yokneam, Petra, and Parisienne. The cultivar Parisienne had the highest total yield of aroma volatiles (60 mg/kg) of all those tested and was generally assessed to have a very strong and characteristic melon aroma. Haom, Petra, and Yokneam had somewhat lower total yields of volatiles (44, 40, and 45 mg/kg, respectively) but were still considered to be highly aromatic fruit. On the other hand, Alice had a markedly lower aroma concentration (10 mg/

kg), and only one of the sulfur esters had a concentration greater than its aroma threshold. This cultivar was generally considered to have little melonlike aroma character. There are distinct qualitative and quantitative intervarietal differences between the esters that constitute the majority of the aroma volatiles of melons (Wyllie et al., 1989). While these differences may account for the characteristic aroma associated with a particular variety or cultivar, it is likely that the thioether esters also play a significant role in this perception.

The important function of sulfur-containing compounds in the characteristic aroma of many fruits and vegetables is well established. Of particular interest to this case is the report by Dirinck et al. (1984) of the role of sulfur compounds in strawberry flavor. They have shown that for some varieties a correlation between sensory evaluation and total volatile concentration could be established. However, for other varieties the concentrations of sulfur-containing compounds such as dimethyl sulfide, methanethiol, methylthiol acetate, methylthiol butanoate, ethanethiol, and dimethyl disulfide must be taken into account. Those varieties having a more complete and intense pattern of sulfur-containing compounds were rated by the taste panel as having significantly more strawberry flavor intensity.

Methyl 3-(methylthio)propanoate, ethyl 3-(methylthio)propanoate, and 3-(methylthio)propyl acetate have been identified in pineapples (Takeoka et al., 1989); the first two appear in concentrations such that their odor unit values are of significance. Similarly, it has been observed that the concentration of the thiol, 4-methoxy-2-methyl-2-mercaptobutane, is highest in the most aromatic varieties of black currant buds (LeQuere and Latrasse, 1990). While the melons grown in this study have not been subjected to systematic sensory evaluation, our observations tend to suggest a similar role for sulfur compounds in melon aroma.

In a recent study, however, Schieberle et al. (1990) have shown that the aromagram obtained from the extract of a muskmelon (cultivar unspecified) contained 11 aroma-active compounds having significant flavor dilution factors. They concluded that methyl 2-methylbutanoate, (*Z*)-3-hexenal, (*E*)-2-hexenal, and ethyl 2-methylpropanoate were the primary odorants of the flavor of the melon investigated, with lesser contributions coming from (*Z*)-1,5-octadien-3-one and 1,8-cineole. Two of the odor-active compounds were not identified.

In our investigations of the aroma profile of over 30 *C. melo* cultivars we have not been able to detect the, presumably lipoxygenase-derived, hexenals or octadienone and these do not appear to have been reported by other workers. The nonenals, nonenols, and related compounds also produced by a similar enzymatic pathway have, on the other hand, been encountered more frequently (Kemp et al., 1972b; Buttery et al., 1982), although again we have detected these in only a few cultivars, all of which have low volatile concentrations. Possibly these compounds are present in concentrations below the limit of detection of the system used in our investigations. However, lipoxygenase activity appears to be dependent on cultivar, age, storage conditions, and sample location. For example, Kemp et al. (1973) have shown that the levels of lipoxygenase-derived products such as 2-nonenal, the 3- and 6-nonen-1-ols, and *n*-nonanol increase significantly during the storage of fruit in the frozen state. Further, Lester (1990) has demonstrated that the mature hypodermal tissue of at least one cultivar of netted muskmelon fruit has significantly higher lipoxygenase activity than that of the middle mesocarp.

These factors, together with the great variety of biological characteristics displayed by the very large number of *C. melo* cultivars, make generalizations about the key aroma compounds for this fruit difficult. It is therefore of the utmost importance that key factors that may influence flavor and aroma quality characteristics are reported. These include the authentication of germplasm, the husbandry, harvest and postharvest conditions, sampling and subsequent analytical procedures.

ACKNOWLEDGMENT

The help and advice of Mr. Brian Corliss, Connoisseur's Choice Ltd., in the growth and husbandry of the melons is gratefully acknowledged.

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Received for review September 3, 1991. Accepted November 21, 1991.