results in a higher rate of protein free radicals formation, as already mentioned; (2) lipid hydroperoxide decomposition either as a complex [LOOH···PH] or as LOOH; both decompositions would promote protein free radical formation and subsequent protein polymerization in accordance with the proposed mechanism; the decreases of the PV of the lysozyme–lipid emulsions subjected to freeze-drying support this hypothesis.

Lysozyme biological activity decreases after treatment with peroxidizing lipids. Previous works (Kanner and Karel, 1976; Funes et al., 1980) have shown a loss of such activity upon treatment with peroxidizing L or their breakdown volatile products. Our inability to detect changes due to freeze-drying—which induces a higher lysozyme polyermization—would indicate that such loss is not associated with cross-linking.

Practical consequences of this work are that (1) usual methods to measure lipid oxidation (such as PV and TBA reaction) in lipid-containing systems subjected to freeze-drying may show erroneously low values and (2) the freeze-drying process can affect the quality of food systems in which lipid oxidation has already been initiated.

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# Additional Aroma Components of Honeydew Melon

Ron G. Buttery,* Richard M. Seifert, Louisa C. Ling, Edwin L. Soderstrom, Joseph M. Ogawa, and Jean G. Turnbaugh

In relation to the attraction of certain insect pests the volatile components of honeydew melon have been reinvestigated. Volatiles were isolated both by Tenax adsorbent trapping and by vacuum steam distillation continuous extraction. Major aroma compounds identified that had not been previously reported in melons include (*Z*)-6-nonenyl acetate, (*Z*,*Z*)-3,6-nonadienyl acetate, (*Z*)-3-nonenyl acetate, 3-methyl-2-butenyl acetate, and ethyl (methylthio)acetate ($CH_3SCH_2COOEt$). Odor threshold determinations indicted that (*Z*)-6-nonenyl acetate could be an additional important contributor to the total aroma for humans.

It is known that certain insects such as *Drosophila* spp. and *Nitidulid* spp. carry spores of *Monilinia* spp. and *Rhizopus* spp. that can cause diseases of fruit and nut crops (Tate and Ogawa, 1975; Wilson and Ogawa, 1979). It was observed that if honeydew melons are available that the same insects are strongly attracted to the ripe melons. It was felt that an improved knowledge of the volatiles of honeydew melons could provide basic information useful in understanding which chemical compounds are involved in the attraction of the insects to the various fruits. Such information might also make possible the formulation of synthetic attractive mixtures potentially useful for the trapping of these insects for both population estimation and possible control.

The volatile components of muskmelon and watermelon have been studied by Kemp and co-workers (Kemp, 1975; Kemp et al., 1972a,b, 1973, 1974). The volatiles of these and other melons including honeydew melon have also been studied by Yabumoto and Jennings (1977) and Yabumoto et al. (1978). None of these earlier studies, however, particularly concentrated on honeydew melon volatiles, and it was felt that further studies, aimed only at honeydew melon volatiles, were desirable.

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710 (R.G.B., R.M.S., L.C.L., and J.G.T.), Stored Products Insect Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Fresno, California 93727 (E.L.S.), and University of California, Davis, Davis, California 95616 (J.M.O.).

## EXPERIMENTAL SECTION

**Materials.** Ripe fresh honeydew melons (*Cucumis inodorus* Naud.) were obtained from experimental fields at the University of California, Davis, and from local markets.

Authentic chemical compounds were generally obtained from reliable commercial sources or synthesized by established methods. (*Z*)-6-Nonenal was synthesized as

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described by Seifert (1981) and used as a starting material to synthesize (*Z*)-6-nonenol and its acetate. (*E,Z*)-3,6-Nonadienol was synthesized by the method outlined by Kemp et al. (1974). Ethyl (methylthio)acetate ($CH_3SCH_2COOEt$) was prepared by treating ethyl bromoacetate with sodium methylmercaptide. All authentic compounds were repurified by gas–liquid chromatography (GLC) separation, and their identity was checked by spectral means.

**Isolation of Volatiles Using Tenax Traps.** The traps as described previously (Buttery et al., 1982) were made from Pyrex glass and contained a 1.3 cm diameter by 7 cm long (1.7 g) column of Tenax GC adsorbent (60–80 mesh; Applied Science Laboratory). The melon (1 g) was sliced into pieces (ca. 15 × 5 × 5 cm) and placed in a 5-L flask, and the activated trap was attached to the neck of the flask with standard taper joints. Purified air (500 mL/min) was drawn through the flask and then through the Tenax trap by applying reduced pressure to the outlet of the trap. The isolation was carried out for 8 h at room temperature (25 °C). The trap was then removed and the trapped material eluted with freshly distilled diethyl ether. The ether extract was then concentrated to a small volume by using a warm water bath and low hold up distillation columns.

**Isolation by Vacuum Steam Distillation Continuous Extraction.** This was carried out on the melon (6 kg), sliced into ca. 4 × 4 × 4 cm pieces, by using a 12-L flask and 4 L of odor-free water with a Likens-Nickerson steam distillation continuous extraction head with hexane as the solvent. The isolation was carried out under reduced pressure (100 mmHg) with the melon at ca. 51 °C. The method is essentially the same as that used by the authors for other materials [cf. Buttery and Kamm (1980)]. The hexane extract obtained was dried by freezing out the water and concentrated under reduced pressure (100 mmHg) to give the volatile oil, which was stored at −20 °C with a trace of ethyl antioxidant 330 (1,3,5-trimethyl-2,4,6-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)benzene).

**Capillary GLC–Mass Spectral (GLC–MS) Analysis.** The GLC column used was a 150 m × 0.64 mm i.d. Pyrex glass capillary coated with Carbowax 20-M.

The column was held at 50 °C for 30 min after injection and then temperature programmed from 50 to 170 °C at 1 °C/min and held at the upper limit with a He inlet pressure of 16 psi. A single-stage Lewellyn-Littlejohn silicone rubber membrane molecular separator was used to couple the end of the capillary to the mass spectrometer (a modified CEC 21-620 cycloidal type). Electron ionization was 70 eV.

**Packed Column GLC–Infrared Spectral Analysis.** The GLC column was 3 m long × 0.64 cm o.d., stainless steel, packed with 80–100-mesh Chromosorb G-DMCS coated with 2% Carbowax 20-M. The column was held at 35 °C for 20 min and then temperature programmed at 2 °C/min from 35 to 170 °C and held at the upper limit. Samples were collected in 3 mm o.d. × 14 cm Pyrex tubes. The infrared absorption spectra were measured as thin films between ultramicro salt plates or as solutions in $CCl_4$ in ultramicrocavity cells by using a reflecting beam condensor with a Perkin-Elmer Model 197 instrument.

**Odor Threshold Determinations.** These were carried out following the procedure described by Guadagni and Buttery (1978) using odor-free Teflon squeeze bottles equipped with Teflon tubes.

## RESULTS AND DISCUSSION

Two different methods were used to isolate the volatile components of honeydew melon. The first involved sweeping purified air over the freshly sliced ripe melon and trapping the volatiles in the exit air stream on a Tenax adsorbent trap. The volatiles were then eluted from the trap with diethyl ether. The second method used vacuum steam distillation continuous extraction with hexane as solvent. The concentrates from each method were used for separate GLC–MS analyses. Isolation of some of the more major components by packed column GLC with batch analysis by infrared absorption spectra was also carried out with the vacuum steam volatile concentrate.

Table I lists the compounds identified in the present work, together with some idea of each component's relative concentration in the concentrate based on GLC peak areas. The amount of volatile oil found by vacuum steam distillation continuous extraction was of the order of 2 parts per million (ppm) of the original melon. Most of the compounds in Table I had been identified in the previous studies on melons (Kemp, 1975; Kemp et al., 1972a,b, 1973, 1974; Yabumoto and Jennings, 1977; Yabumoto et al., 1978). Three important compounds identified in the present studies, which had not been reported in melons before, were (*Z*)-6-nonenyl acetate, (*Z,Z*)-3,6-nonadienyl acetate, and (*Z*)-3-nonenyl acetate. The free alcohols (*Z*)-3-nonenol, (*Z*)-6-nonenol, and (*Z,Z*)-3,6-nonadienol had been previously identified in muskmelon and watermelon by Kemp (1975). Significant amounts of these free alcohols were not found in the present work with ripe honeydew melon by using the Tenax trapping method. Greater amounts were found by using the vacuum steam distillation continuous extraction method. The large excess of water used in the latter method may have facilitated the removal of the alcohols.

The related potent odorant (*Z*)-6-nonenal, previously identified in melons by Kemp et al. (1972a) and long known to have a characteristic melon aroma, was not found in detectable amounts in the present work. It is possible, however, that the conditions used in the present study were not the most suitable for this compound.

Other prominent compounds in Table I that had not been previously reported in melons were 3-methyl-2-butenyl acetate and $CH_3SCH_2COOEt$. The latter compound was only found by using the vacuum steam distillation isolation method.

The authors were able to synthesize reasonable amounts of (*Z*)-6-nonenol (and acetate) but could only synthesize the *E,Z* form of 3,6-nonadienol (and acetate). Only very small quantities of (*Z*)-3-nonenol (and acetate) were obtained by *E–Z* isomerization (using benzenethiol) from the *E* isomer.

The Kovats' GLC retention index for authentic (*Z,Z*)-3,6-nonadienol and acetate were estimated from that of the *E,Z* isomer and the difference found for (*E*)- and (*Z*)-3-nonenols and acetates.

**Odor Thresholds of Melon Components.** Insects and humans probably differ considerably in their sensitivity to the different aroma components of honeydew melon. The odor thresholds (*T*), in water solution, of a number of the compounds in Table I had been determined previously at this laboratory by Guadagni (1970) for humans. The odor thresholds of the unsaturated $C_9$ alcohols and their acetates were determined during the present study. These thresholds are listed in Table II. From this data and the amounts of each compound present it is reasonable to expect that a number of compounds are major contributors to honeydew melon aroma as far as humans are concerned. These include ethyl 2-methylbutyrate ($T$ = 0.3 ppb), ethyl butyrate ($T$ = 1 ppb), ethyl hexanoate ($T$ = 1 ppb), hexyl acetate ($T$ = 2 ppb), 3-methylbutyl acetate ($T$ = 2 ppb), benzyl acetate ($T$ = 2 ppb), (*Z*)-6-nonenyl

Table I. Compounds Identified in Volatiles of Honeydew Melon

| compound[a] | characteristic MS ions, m/e[c] | Kovats' GLC index[d] | approx rel % in volatile oil: Tenax trap | approx rel % in volatile oil: vac stm distill. |
|---|---|---|---|---|
| | Aliphatic Esters | | | |
| methyl acetate | 43, 74, 42, 59 | 570 | | |
| ethyl acetate | 43, 45, 61, 70, *88* | 870 | 7 | |
| propyl acetate | 43, 61, 73, 59, 87 | 980 | 1 | 0.2 |
| methyl butyrate | 43, 74, 71, 59, 87, *102* | 990 | 2 | 0.8 |
| isobutyl acetate[b] | 43, 56, 73, 41, 61, 86 | 1020 | 11 | 4 |
| ethyl butyrate[b] | 43, 71, 88, 60, 45 | 1040 | 2 | 4 |
| ethyl 2-methylbutyrate[b] | 57, 41, 102, 85, 74, 115 | 1040 | 2 | 5 |
| butyl acetate[b] | 43, 56, 73, 61, 55, 87 | 1070 | 2 | 6 |
| 3-methylbutyl acetate[b] | 43, 70, 55, 73, 61, 87 | 1120 | 17 | 7 |
| pentyl acetate | 43, 70, 61, 55, 73, 58 | 1170 | 1 | 0.1 |
| ethyl hexanoate[b] | 43, 88, 99, 60, 71, 73 | 1230 | 1 | 1.9 |
| 3-methyl-2-butenyl acetate | 43, 68, 67, 53, 71, 86 | 1250 | 2 | 2 |
| hexyl acetate[b] | 43, 56, 61, 84, 69, 73 | 1270 | 2 | 2 |
| (*Z*)-3-hexenyl acetate[b] | 43, 67, 82, 54, 73 | 1310 | 1 | 0.4 |
| heptyl acetate[b] | 43, 56, 70, 61, 69, 98 | 1370 | 0.1 | 0.1 |
| nonanyl acetate | 43, 56, 70, 61, 83, 98 | 1560 | 2.5 | 0.2 |
| (*Z*)-3-nonenyl acetate | 43, 54, 67, 81, 95, 124 | 1590 | 5 | 0.3 |
| (*Z*)-6-nonenyl acetate | 43, 67, 95, 82, 81, 124 | 1620 | 4 | 1 |
| (*Z,Z*)-3,6-nonadienyl acetate[e] | 43, 79, 93, 67, 122, 107 | 1660 | 5.5 | 2.5 |
| | Others | | | |
| nonanal | 57, 44, 98, 82, 114, *142* | 1390 | 5 | 2 |
| benzaldehyde | 77, 105, 106, 51, 50, 39 | 1520 | 0.1 | 0.1 |
| $CH_3SCH_2COOEt$[b] | 61, *134*, 88, 45, 47, 70 | 1430 | | 2 |
| benzyl acetate[b] | 43, 108, 91, 79, 65, *150* | 1710 | 2.5 | 4.4 |
| (*Z*)-6-nonenol | 67, 41, 55, 68, 82, 95 | 1710 | <0.1 | 0.5 |
| (*Z,Z*)-3,6-nonadienol[e] | 67, 41, 55, 93, 79, 109 | 1730 | <0.1 | 1 |

[a] Mass spectrum (complete spectrum) and Kovats' GLC retention index are consistent with those of authentic compounds except for the compounds followed by footnote *e*. [b] Infrared (IR) absorption spectrum is also consistent with that of authentic samples. [c] Not necessarily the most intense ions but those considered characteristic for that compound. Ions are listed in descending order of intensity with the molecular ion (if found) in italic type. [d] Kovats' GLC index for the Carbowax 20-M Pyrex capillary column. [e] Mass spectral data are identical with those of the authentic *E,Z* isomer. Kovats' index is consistent with that expected for the *Z,Z* isomer based on comparison of (*E*)- and (*Z*)-3-nonenols and acetates.

Table II. Odor Thresholds of Some Compounds That Have Been Identified in Melons

| compound | odor threshold (*T*) in ppb[c] of water |
|---|---|
| (*Z*)-6-nonenal | 0.005 |
| ethyl isobutyrate | 0.1 |
| ethyl 2-methylbutyrate | 0.3 |
| (*Z*)-6-nonenol | 1 |
| ethyl butyrate | 1 |
| ethyl hexanoate | 1 |
| (*Z*)-6-nonenyl acetate | 2 |
| benzyl acetate | 2 |
| hexyl acetate | 2 |
| 3-methylbutyl acetate | 2 |
| (*E,Z*)-3,6-nonadienol[a] | 3 |
| ethyl acetate | 5 |
| 2-methylbutyl acetate | 11 |
| (*E,Z*)-3,6-nonadienyl acetate[a] | 15 |
| $CH_3SCH_2COOEt$ | 25 |
| (*E*)-3-nonenyl acetate[b] | 60 |
| isobutyl acetate | 65 |
| butyl acetate | 66 |
| nonyl acetate | 200 |
| benzaldehyde | 350 |

[a] Found in melons as the *Z,Z* form. [b] Found in melons as the *Z* form. [c] Parts of compound (v/v) per billion ($10^9$) parts of water.

acetate (*T* = 2 ppb), and possibly (*E*)-6-nonenol (*T* = 1 ppb) and (*Z,Z*)-3,6-nonadienol (*T* = 3 ppb for *E,Z* isomer).

Although not detected in the present work, (*Z*)-6-nonenal (*T* = 0.005 ppb) has a characteristic potent melon-like aroma as far as humans are concerned. Only a minute concentration of (*Z*)-6-nonenal, below the level of detection used in the present work, would be necessary to contribute considerably to the melon aroma for humans.

The (*Z*)-6-nonenyl acetate found in moderate amounts in honeydew melon, in the authors' opinion, has a pleasant honeydew melon like aroma and is reasonably potent.

Except for the unsaturated $C_9$ acetates and alcohols, most of the compounds identified in Table I occur fairly commonly in fruits and are not that characteristic for melons. Only the three compounds (*Z*)-6-nonenyl acetate, (*Z*)-3-nonenyl acetate, and (*Z,Z*)-3,6-nonadienyl acetate seem particularly unique to the honeydew melon. It seems reasonable that humans and insects pick out particularly unique compounds to recognize each individual food.

**Synthetic Aroma Mixture.** A mixture of major aroma compounds was made up to test with humans and also with certain insects. This mixture contained the following compounds: 5 mL of ethanol, 50 μL of isobutyl acetate, 50 μL of ethyl butyrate, 60 μL of ethyl 2-methylbutyrate, 70 μL of butyl acetate, 80 μL of 3-methylbutyl acetate, 20 μL of ethyl hexanoate, 30 μL of hexyl acetate, 5 μL of nonyl acetate, 5 μL of (*Z*)-6-nonenyl acetate, 3 μL of (*E*)-3-nonenyl acetate, 100 μL of ethyl acetate, and 55 μL of benzyl acetate. A solution was made by dissolving 10 μL of this mixture in 500 mL of water. This diluted solution was compared to juice pressed from fresh ripe honeydew melon by panel methods [cf. Guadagni et al. (1972)]. The juice and synthetic mixture were presented to the judges in coded opaque Teflon bottles. The judges were asked which bottle had an odor most like honeydew melon. With 32 total judgments (from 16 judges) the juice from the melon was chosen 53% of the time and the synthetic mixture 47%. Using a different type of test each judge was asked to rate each sample from 1 to 5 where 5 is the most like honeydew melon aroma and 1 is the least. In this study (37 total judgments) the natural juice was rated an average of 4.6 and the synthetic 4.1. These studies indicated that

the synthetic mixture, although not identical, was very similar to the natural honeymelon juice as far as humans are concerned.

Studies with insects are incomplete but preliminary results indicate that *Drosophila* spp. have an overwhelming preference for the natural honeydew melon aroma compared to that of the synthetic mixture. It is probable that the insects respond quite differently than humans to the honeydew melon volatile components. It is interesting that Jacobson et al. (1971) found synthetic (*E*)-6-nonenyl acetate to be a strong attractant for the female melon fly (*Dacus cucurbitae*).

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# Headspace Components of Passion Fruit Juice

Chu-Chin Chen,* May-Chien Kuo, Lucy S. Hwang, James Swi-Bea Wu, and Chung-May Wu

Volatile components of three varieties of passion fruit (*Passiflora edulis* Sims., *Passiflora edulis* forma *flavicarpa*, and their commercial hybrid) were collected from the headspace of passion fruit juice by using Tenax-GC as the adsorbing material. Individual components were identified by mass spectrometry and their retention indices. About 60 volatile components have been identified in one single chromatograph, including esters, alcohols, aldehydes, ketones, terpene compounds, and other miscellaneous compounds. The comparison of the flavor of three varieties of passion fruits indicated that the hybrid passion fruit would be the best for the manufacturing of passion fruit juice.

Passion fruit is a tropical fruit native to tropical America but now grown in most of the tropical and subtropical countries of the world. At present, there are three varieites of passion fruit grown in Taiwan, the purple passion fruit (*Passiflora edulis* Sims.), the yellow passion fruit (*Passiflora edulis* forma *flavicarpa*), and the hybrid passion fruit originated from the crossbreeding of yellow and purple passion fruit (♂ yellow × ♀ purple, $F_1$), each with a different harvesting period during late March to early December. The harvesting period of purple passion fruit begins in late March and ends in eary August, the yellow passion fruit period begins in late July and ends in early December, and the hybrid passion fruit period begins in early June and ends in late November. The purple passion fruit is purple skinned with an average weight about 35 g and grew uncultivated in the mountainous areas not exceeding 3000 ft above sea level of this island; pollination is done by the insects. The yellow passion fruit is yellow skinned with an average weight about 82 g and is commercially cultivated in the middle part of Taiwan but is unable to do the pollination itself. The hybrid passion fruit is purple skinned and self-pollination is possible. It has fruit size larger than that of the purple passion fruit but smaller than that of the yellow passion fruit (ca. 63 g). The cultivation of hybrid passion fruit is well established in the eastern region of Taiwan and may economically become an important agricultural product in the near future. Due to their exotic flavor, the passion fruit juice is now widely used in beverages and cordials. For the purpose of mass production of canned juice or concentrated juice, the understanding of the flavor components of passion fruit cultivated in this island becomes necessary. The studies of volatile components of purple and yellow passion fruit had been done separately in the past decade (Murray et al., 1972; Parliment, 1972; Winter and Klöti, 1972; Huet, 1973; Murray, 1977; Chan, 1980; Casimir et al., 1981) but the volatile components of hybrid passion fruit is still unknown, and the comparison of three varieties of passion fruit has not been done yet.

In recent years, adsorption polymers have been used for collection, concentration, and subsequent GC analyses in a wide variety of applications (Zlatkis et al., 1973; Murray, 1977; Charalambous, 1978; Buckholz et al., 1980). In this paper, we describe the use of Tenax-GC (a polymer of *p*-2,6-diphenylene oxide) as the adsorbing material to analyze and compare the headspace components of passion fruit juice.

Food Industry Research and Development Institute (FIRDI), Hsin-Chu, Taiwan, Republic of China (C.-C.C. and C.-M.W.), and Graduate Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan, Republic of China (M.-C.K., L.S.H., and J.S.-B.W.).

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