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Headspace Examination of Volatile Emissions from Ripening Papaya (*Carica papaya* L., Solo Variety)

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Organic compounds released from intact papayas at each of four ripeness stages were concentrated by Tenax trapping-ether desorption and were identified by capillary gas chromatography-mass spectrometry. Linalool, benzyl isothiocyanate, and phenylacetonitrile were released in significant amounts at all four ripeness stages, but linalool production increased dramatically as the fruit progressed from one-fourth to full ripeness. Free benzyl isothiocyanate levels also increased with fruit ripening, but phenylacetonitrile release fluctuated across the four fruit stages, showing no clear correlation with ripeness. Numerous esters and monoterpenes were only detected in emissions from fully ripe fruit. In initial flight tunnel bioassays with Oriental fruit flies, ripe papaya emissions were found to enhance significantly the attractiveness and oviposition stimulation of a perforated yellow sphere fruit model.

Effective attractants for females of the various tephritid fruit flies would be useful tools in monitoring fly populations, in determining female mating levels in sterile mass release or male annihilation projects, and in directly suppressing population levels. One promising source of such attractants is the mix of volatile compounds released by plant hosts of such flies, particularly that part of the host which is the preferred oviposition site for gravid females.

Papaya fruit (*Carica papaya* L.) is readily infested by several of the Hawaiian tephritids, especially the Oriental fruit fly *Dacus dorsalis* Hendel and the Mediterranean fruit fly *Ceratitis capitata* Wiedmann. The melon fly *Dacus cucurbitae* Coquillet will also infest papaya when given no choice of other host but is less of a problem under field conditions (Liquido et al., 1989). Infestation levels in Hawaiian papaya orchards have been shown to be directly related to the ripeness stage of the individual fruit (Seo et al., 1982, 1983; Liquido et al., 1989). Seo and co-workers attributed this relationship to the deterrent effect of benzyl isothiocyanate released from unripe fruit latex when the skin is punctured by the female fly's ovipositor. They suggested that ripe fruit are more heavily infested because the riper portions of the fruit release less benzyl isothiocyanate producing latex when punctured and because other attractive compounds produced by riper fruit may override the deterrent effect of whatever benzyl isothiocyanate is still released. Liquido et al. (1989) suggested that the greater attractiveness of riper fruit, rather than a decrease in the deterrent properties of ripening fruit, is the key factor in explaining the variation of infestation rates with fruit ripeness. This preference for riper post color break fruit is supported by recent field observations of *D. dorsalis* adult fly distribution among papaya fruit at various ripeness stages in an unharvested orchard near Hilo (D.M.L.). Liquido and co-workers (Liquido, N. J., Chan, H. T., 1989, personal communication; Liquido and Cunningham, 1990) have also studied the correlation between physical and chemical properties of ripening papayas and infestation rates by oriental and melon fruit flies.

The commercial quarantine commodity treatment procedure for Hawaiian papayas involves both a fruit selection procedure and treatment of harvested fruit in a two-stage hot water bath sequence (Couey and Hayes, 1986). Proper application of this procedure reduces fruit fly infestation to Probit 9 levels, or no more than 3 survivors/100 000 treated insects at the 95% confidence level. An important parameter in this procedure is stage of fruit

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Table I. Hunter Colorimeter Values for Papaya Samples

	Hunter colorimeter *b* value	
maturity class	blossom end	most yellow area
mature green	12.88–15.40 x = 14.34 SD = 0.83	14.59–20.26 x = 18.27 SD = 1.93
color break	15.34–19.34 x = 16.92 SD = 1.40	18.45–24.39 x = 20.99 SD = 1.92
one-fourth	17.25–22.35 x = 19.26 SD = 1.96	17.95–25.75 x = 23.26 SD = 2.69
fully ripe	29.71–34.16 x = 32.34 SD = 1.38	fruit full yellow-orange

ripeness; fruit riper than one-fourth ripe will not ensure a Probit 9 product after two-stage hot water treatment. Ripeness classification is a subjective process, requiring considerable experience, so Couey and co-workers developed colorimetric standards for classifying fruit into ripeness stages (Couey et al., 1984; Couey and Hayes, 1986). Present APHIS (Animal Plant Health Inspection Service, USDA) standards for fruit to be treated require a Hunter (colorimeter) *b* value of 23.4 or lower at the blossom end of the fruit and 27.4 or lower at the most yellow area of the fruit.

As a first step in exploring the interaction between female fruit flies and papaya fruit as it ripens, a study of the volatile compound profile typical of intact papaya fruit at several ripeness stages was undertaken.

The volatile aroma complex from papaya fruit has been examined by several research groups. Some were primarily concerned with benzyl isothiocyanate generation (Ettlinger and Hodgkins, 1956; Gmelin and Kjaer, 1970; Tang, 1971, 1973), while others examined total volatiles concentrates from ripe fruit pulp (Katague and Kirch, 1965; Flath and Forrey, 1977; MacLeod and Pieris, 1983; Heidlas et al., 1984; Schreier et al., 1985; Idstein and Schreier, 1985; Winterhalter et al., 1986). Schreier's group (Heidlas et al., 1984; Schreier et al., 1985) demonstrated that very little free linalool or benzyl isothiocyanate is present in intact papaya tissue, by inactivating the papaya enzyme systems with mercuric chloride and then preparing volatiles concentrates.

EXPERIMENTAL SECTION

Papaya Samples. Fruit were collected from a commercial papaya orchard near Hilo, HI. Six fruit were selected by experienced judges for each of four ripeness categories: mature green (MG; 3.06 kg total); color break (CB; 3.14 kg); one-fourth ripe (QR; 3.18 kg); fully ripe (FR; 2.96 kg). Colorimetric measurements of fruit skin color were taken on a Hunter Labscan LS5100 colorimeter (Hunter Associates, Inc.). Hunter *b* values for the sample are listed in Table I.

Headspace Trapping System. The sample chamber consisted of a 12-L round-bottomed flask fitted with a large flanged neck. A mating flanged head with two O-ring compression seals was used to close the chamber. Breathing quality air from a gas cylinder was passed through a large activated charcoal column into the sample chamber through 6.4-mm-o.d. Teflon tubing, which extended through an O-ring seal down to the bottom of the sample flask. A Tenax adsorbent packed glass trap was attached to the O-ring-sealed exit port of the flanged head.

Tenax traps were fabricated from 25-mm-o.d. borosilicate glass tubing (ca. 12.5 cm long), with short stubs of 6.4-mm (inlet) and 9.5-mm (exit) tubing at the ends. Stainless steel Swagelok caps with ceramic-filled Teflon ferrules were used to close the trap ports. A 6.5-g bed (2.2 cm dia × 10 cm long) of 60/80-mesh Tenax-GC adsorbent was held in each trap by a sealed-in fritted glass disk at the inlet end and by a plug of glass wool at the trap exit. Tenax adsorbent was extracted with acetone and then with diethyl ether, in a Soxhlet extractor for consecutive 6-h periods. The adsorbent was dried and then was packed into the trap bodies. Before use, each trap was conditioned at 195 °C for at least 5 h (oxygen-free purified nitrogen sweep gas), cooled to room temperature, and capped.

Sampling Procedure. The purged sample chamber was opened, and six papayas at a given ripeness stage were inserted. A purified air flow of 1 L/min was established, and a clean Tenax trap was inserted into the exit port of the chamber. After 2 h, the trap was removed and immediately capped. Before any papaya samples were placed in the clean chamber, a 2-h blank sample was collected under the same conditions.

Internal Standard Addition and Volatiles Desorption. Individual traps were clamped vertically, and ether solutions of two internal standards (cycloheptanone and cyclodecanone) were applied to the frit in the trap inlet (25 μL of each solution, containing 15 μg of the respective standard). The trap was then inverted and reverse-flushed with 75 mL of distilled ether (1 ppm Antioxidant 330, Ethyl Corp). The ether washings were concentrated by careful distillation to ca. 0.02 mL.

Concentrate Separation. Trapped volatile concentrates were separated with 60 m × 0.32 mm (i.d.) DB-1 fused silica columns (J&W Scientific; bonded cross-linked methylsilicone). These were installed in Hewlett-Packard 5830A/5840A gas chromatographs (flame ionization detector) or in a Finnigan MAT 4500 quadrupole gas chromatograph/mass spectrometer (electron impact mode; emission current −0.30 mA; multiplier −1225 V; scan 1 s, 33–350 m/z). Column ovens were held at 50 °C for 0.1 min and then were programmed from 50 to 250 °C at 4 °C/min. Column head pressures were 159 kPa (23 psi; GC/FID) or 103 kPA (15 psi; GC/MS). A DB-Wax column (identical dimensions and source; maximum temperature 230 °C; bonded cross-linked poly(ethylene glycol)) was used on occasion in the GC/FID to separate and integrate peak areas of components whose GC peaks overlapped on the DB-1 phase.

Component Identification and Quantitation. Component identifications were obtained by comparison of experimental mass spectral and retention index data (normal hydrocarbon reference scale) with those of authentic compounds.

Quantitation data were based upon GC peak area measurements obtained with the Hewlett-Packard GC/FID units. Papaya component peak areas were converted to micrograms of component trapped after calculating micrograms/area count, using an average of the two internal standard area counts (15 μg of each added).

RESULTS AND DISCUSSION

Components identified in headspace concentrates from intact papaya fruit samples at four ripeness stages are listed in Table II. Retention index values were determined with the DB-1 capillary column (see the Experimental Section). Calculated amounts of each compound trapped (total micrograms from 120 L of air headspace) are also included. All identifications are based upon both mass spectral matching and retention index correlation with authentic samples. Quantitation is based upon GC/FID peak integration data, so accuracy is potentially limited by a number of factors, including partial overlap or coelution of two or more components, possible sample discrimination during injection, peak broadening or partial adsorption of more polar components, and differences in FID response factors among the sample components. Other potential sources of both qualitative and quantitative errors are Tenax trap breakthrough by some components, decomposition on the adsorbent, artifact introduction, and partial loss of more volatile constituents during ether wash concentration. Finally, since fruit from one orchard was used in this study, the inherent diversity of biological material ensures that the quantitative data in Table II are only applicable in detail to the specific fruit examined.

The greatest number of components was collected from the fully ripe fruit. Numerous esters and monoterpenes

Table II. Papaya Headspace Components

component	retention index ref	retention index exptl	amount trapped,[a] μg MG	CB	QR	FR
Alcohols						
linalool oxide A	1056	1056			0.06	4.51
linalool oxide B	1070	1070			0.03	4.07
linalool	1083	1084	0.58	1.84	2.09	191.78
4-terpineol	1159	1160				0.12
Aldehydes						
hexanal	772	772	0.14	0.43	0.15	0.21
heptanal	876	877	0.04	0.14		0.03
benzaldehyde	926	927	0.02	0.04	0.03	0.24
octanal[b]	979	979	0.13	0.59	0.25	0.12
nonanal	1082	1082	0.63	2.27	0.71	0.51
decanal[b]	1184	1184	0.35	1.17	0.42	0.32
Esters						
ethyl acetate	600	602	4.35	5.33	1.37	3.32
ethyl butyrate[b]	780	779				0.01
prop-2-yl butyrate[b]	823	823				0.05
methyl hexanoate	906	906				0.05
γ-hexalactone	1003	1003				0.04
methyl octanoate	1107	1107				0.05
ethyl benzoate	1143	1143				0.05
methyl salicylate[b]	1166	1167	0.02	0.03	0.01	0.01
butyl hexanoate[b]	1173	1175				0.06
ethyl octanoate[b]	1180	1180				0.09
γ-octalactone	1210	1209				0.08
methyl geranate[b]	1301	1302				0.06
triacetin[b]	1307	1306	0.02	0.01	0.01	0.02
butyl benzoate	1346	1348				0.07
3-methylbutyl benzoate[b]	1411	1411				0.03
Heteroatoms						
methyl thiocyanate	665	665	0.03	0.05	0.05	0.03
phenylacetonitrile	1088	1088	0.88	2.34	1.86	2.34
benzyl isothiocyanate	1318	1317	0.29	0.57	0.39	2.19
Hydrocarbons						
myrcene	981	982				0.60
α-phellandrene	996	996				0.03
terpene (136)		1000				0.09
α-terpinene	1008	1008				0.02
β-phellandrene	1018	1019				0.02
limonene	1020	1020				0.08
(*Z*)-β-ocimene	1026	1026				0.37
(*E*)-β-ocimene	1037	1037				0.35
γ-terpinene	1048	1048				0.01
terpinolene	1077	1078				0.12
sesquiterpene (204)		1366		0.06	0.05	0.02
sesquiterpene (204)		1385		0.09	0.07	0.01
sesquiterpene (204)		1386		0.07	0.04	0.01
sesquiterpene (204)		1400		0.03		
caryophyllene[b]	1414	1416	0.24	0.56	0.43	0.15
germacrene D[b]	1473	1473		0.17	0.08	0.02
pentadecane[b]	1500	1500	0.07		0.09	0.04
sesquiterpene (204)		1514	0.04	0.08	0.03	
Ketones						
pentane-2,4-dione[b]	753	753	0.01			
4-hydroxy-4-methyl-pentan-2-one	811	811	0.15	0.20	0.04	0.05
heptan-2-one	865	864				0.03
6-methylhept-5-en-2-one	961	961		0.01		0.02
geranylacetone[b]	1429	1428		0.10	0.10	0.07

[a] From air, at 1 L/min for 120 min. [b] Previously unreported in papaya.

appeared only in this headspace sample, mostly at low levels. Myrcene and the two ocimenes were the most abundant of the monoterpenes.

Many constituents were released by papaya at less ripe stages as well. Linalool was found in all headspace samples, although a pronounced increase in release rate was found in the transition from one-fourth ripe to fully ripe fruit. The homologous series of C_6–C_{10} aliphatic aldehydes reached headspace concentration maxima at one-fourth ripeness, while the diverse group of ketones showed little pattern.

Benzyl isothiocyanate was collected from all four ripeness stages, peaking at full ripeness, but the headspace concentration of phenylacetonitrile, the other major nitrogen compound, exhibited no clear correlation with fruit ripeness. The amounts of phenylacetonitrile found in intact fruit emissions were surprisingly large, especially in comparison with the corresponding benzyl isothiocyanate levels. Previous studies of volatiles from blended ripe papaya flesh (Flath and Forrey, 1977; MacLeod and Pieris, 1983; Idstein and Schreier, 1985; Schreier et al., 1985) have found very little phenylacetonitrile relative to benzyl isothiocyanate (1–4%), unless the pulp was boiled at atmospheric pressure in air. The nitrile then predominated (4.5–11.8 times more than the isothiocyanate). Benzyl glucosinolate in papaya is degraded enzymatically by myrosinase when the fruit tissue is injured to yield predominately benzyl isothiocyanate (Tang, 1971). Glucosinolates may also give nitriles, thiocyanates, and amines, the relative amounts depending upon the specific glucosinolate and upon the enzyme systems present in a specific plant (Robinson, 1980). Nitriles may also be generated from the corresponding glucosinolate by thermal degradation (MacLeod et al., 1981); this is the likely explanation for the high nitrile/isothiocyanate ratio in papaya volatile concentrates prepared at 1 atm. In the present headspace study, thermal degradation of a portion of the glucosinolate present near the surface of the papaya fruit under tropical orchard conditions provides a reasonable explanation for the moderate amounts of phenylacetonitrile detected.

At least seven sesquiterpene hydrocarbons could be detected in concentrates from the four batches of fruit, but with the exception of caryophyllene and germacrene D, concentrations were too low and mass spectra too weak to provide reliable identifications.

Approximately 150–200 volatile components of ripe papaya pulp have been reported in the papers cited above. The much smaller number of components listed in Table II reflects two aspects of papaya headspace sampling: Volatiles are released by intact (but harvested) fruit in small amounts/unit time, and some of the reported volatile constituents are only generated in quantity from nonvolatile precursors on disruption of fruit tissue. The sampling procedure traps compounds released from the fruit surface; therefore, any changes in this surface should be reflected in corresponding changes in the mix and concentrations of trapped volatiles. As papaya fruit reaches full ripeness, the tissue softens and is more easily bruised, by contact either with adjacent parts of the tree or with firm surfaces after picking. The rapid increase in free linalool emissions observed in this study might be attributed in part to this softening process.

In initial flight tunnel bioassays (D.M.L., E.B.J.), female Oriental fruit flies were given a choice of two perforated 7.5-cm-diameter yellow polyethylene spheres (McInnis, 1989), one supplied with a purified air flow containing entrained volatiles from ripe intact papaya fruit and the other with a purified air blank. The flies showed significant differential attraction for and oviposition stimulation by the sphere emitting ripe papaya volatiles (x of 5.6 fly arrivals and 167.0 eggs/test) vs the air control sphere (x of 1.6 fly arrivals and 0.0 eggs/test). Fruit at different ripeness stages are being used in the same test procedure (E.B.J., D.M.L.), and identified components will be tested, both by themselves and in combination, for attractiveness. *D. dorsalis* antennal olfactory receptor

responses to individual papaya volatiles representative of the various fruit ripeness stages are also being recorded (D.M.L., E.B.J.). Details of these bioassay efforts will be published elsewhere.

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Registry No. Linalool oxide A, 34995-77-2; linalool oxide B, 5989-33-3; linalool, 78-70-6; 4-terpineol, 562-74-3; hexanal, 66-25-1; heptanal, 111-71-7; benzaldehyde, 100-52-7; octanal, 124-13-0; nonanal, 124-19-6; decanal, 112-31-2; ethyl acetate, 141-78-6; ethyl butyrate, 105-54-4; prop-2-yl butyrate, 638-11-9; methyl hexanoate, 106-70-7; γ-hexalactone, 695-06-7; methyl octanoate, 111-11-5; ethyl benzoate, 93-89-0; methyl salicylate, 119-36-8; butyl hexanoate, 626-82-4; ethyl octanoate, 106-32-1; γ-octalactone, 104-50-7; methyl geranate, 1189-09-9; triacetin, 102-76-1; butyl benzoate, 93-58-3; 3-methylbutyl benzoate, 94-46-2; methyl thiocyanate, 556-64-9; phenylacetonitrile, 140-29-4; benzyl isothiocyanate, 622-78-6; myrcene, 123-35-3; α-phellandrene, 4221-98-1; α-terpinene, 99-86-5; β-phellandrene, 555-10-2; limonene, 138-86-3; (*Z*)-β-ocimene, 3338-55-4; (*E*)-β-ocimene, 3779-61-1; γ-terpinene, 99-85-4; terpinolene, 586-62-9; caryophyllene, 87-44-5; germacrene D, 23986-74-5; pentadecane, 124-18-5; pentane-2,4-dione, 123-54-6; heptan-2-one, 110-43-0; 6-methylhept-5-en-2-one, 110-93-0; geranylacetone, 3796-70-1.

Factors Affecting the Thermal Degradation of *all-trans*-β-Carotene

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Thermal degradation compounds of *trans*-β-carotene were isolated by medium-pressure liquid chromatography and high-pressure liquid chromatography. The results of comparative tests on *trans*-β-carotene between a model system extrusion cooking process and heating at the same temperature were reported, and the effects of different medium conditions have been evaluated. Prolonged heating at 180 °C causes only limited breakdown of the molecule, but the presence of usual constituents of foods such as water or starch combined with mechanical mixing leads to much higher losses. The losses are increased by the presence of high pressure as is the case in extrusion cooking. Several hypotheses concerning the sequence of reactions involved in *trans*-β-carotene oxidative degradation are proposed.

Extrusion cooking is a widely used process in the food industry, and a variety of products are currently on the market, extensively developed at the level of organoleptic qualities (De la Guerivière et al., 1985). The color of

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