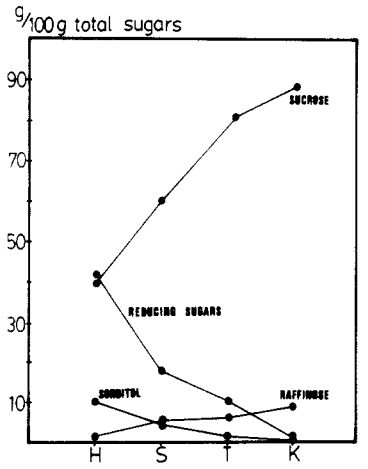

Figure 3. Variation of the principal sugar components in the different parts of the almond fruit.

López Herrera, 1952; Saura-Calixto et al., 1981).

A comparison of sugar composition, expressed in grams of sugar per 100 g of total sugars, in the different parts of almond fruit (Figure 3) shows an increase of oligosaccharides (raffinose and sucrose) from the outside to the inside of the fruit, i.e., from the hull to the kernel, while reducing sugars and sorbitol decrease appreciably.

Registry No. Sucrose, 57-50-1; raffinose, 512-69-6; sorbitol, 50-70-4.

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Received for review January 13, 1984. Revised manuscript received March 20, 1984. Accepted May 21, 1984.

Free and Bound Terpene Compounds in Papaya (*Carica papaya*, L.) Fruit Pulp

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Papaya (*Carica papaya*, L.) fruit pulps were prepared by three different methods: (i) by adding sodium azide; (ii) by adding mercurous ion; (iii) without any enzyme inhibition during homogenization of ripe fruits. After solvent extraction of the pulps, containing added internal standard, quantitative capillary gas chromatography of the concentrated extracts showed that the concentrations of benzyl isothiocyanate, linalool, two isomer furanoid linalool oxides, and terpene hydrocarbons were much lower as a result of enzyme inhibition with Hg^{2+}. Consequently, linalool does not occur in free form in the ripe fruit but is formed by enzymic activity during fruit processing due to cell disruption. The identities of the volatiles were confirmed by capillary gas chromatography–mass spectrometry.

The aroma composition of papaya (*Carica papaya*, L.) fruit has been extensively investigated by different authors (Katague and Kirch, 1965; Flath and Forrey, 1977; MacLeod and Pieris, 1983; Idstein and Schreier, 1984), leading to the identification of major volatiles such as benzyl isothiocyanate, terpene hydrocarbons like (*E*)- and (*Z*)-ocimene, limonene, sabinene, and (*Z*)-neoalloocimene, and terpene alcohols like linalool, α-terpineol, nerol, and geraniol as well as the (*E*)- and (*Z*)-linalool oxides. In all these studies, fruit tissue homogenization was a part of the sample preparation sequence. In this paper, it will be demonstrated that some terpene compounds are formed during fruit pulp processing due to disruption of cell structure and do not occur in their free forms in the fruit.

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EXPERIMENTAL SECTION

Sample Preparation and Extraction. Three different methods of sample preparation were employed for pulp preparation from selected fresh papaya (*C. papaya*, L., var. Solo) fruits of the same fully ripened stage. After the fruit was peeled and the seeds were carefully removed, 1.5-kg portions of intact fruit tissue were added to each of the following: (i) 1.5 L of an 0.05 M aqueous NaN_3 solution containing 50 mL of 0.1 M phosphate buffer (pH 7.5); (ii) 1.5 L of an 0.1 M aqueous $HgCl_2$ solution; (iii) 1.5 L of distilled water. After homogenization, dilution (1:3 w/w) with distilled water, and addition of an internal standard (150 μg/kg 2-octanol), the diluted pulps were individually subjected to solvent extraction using a pentane–dichloromethane mixture (2:1) (Drawert and Rapp, 1968). Each extract was carefully concentrated by distillation (Vigreux

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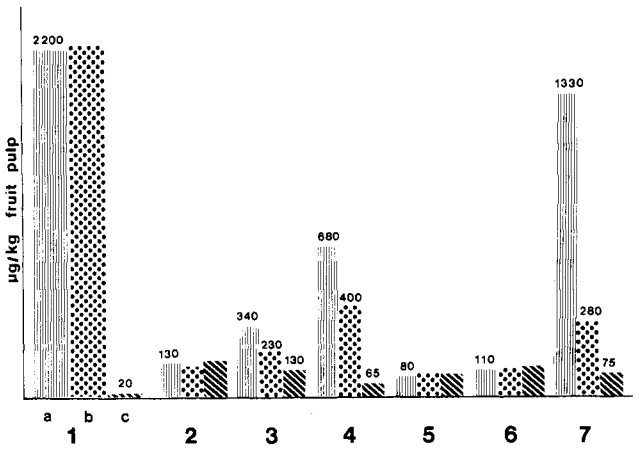

Figure 1. Amounts (µg/kg) of some major volatiles in pulp of ripe papaya fruit: (a) without enzyme inhibition; (b) buffered (pH 7.5) and NaN_3 addition; (c) Hg^{2+} inhibition during fruit homogenization. 1 = linalool; 2 = α-terpineol; 3 = (*Z*)-linalool oxide, furanoid; 4 = (*E*)-linalool oxide, furanoid; 5 = benzyl alcohol; 6 = γ-octalactone; 7 = benzyl isothiocyanate.

column, 45 °C) to 1 mL for subsequent capillary gas chromatography and combined capillary gas chromatography–mass spectrometry.

Capillary Gas Chromatography. A Carlo Erba Fractovap 4160 gas chromatograph equipped with FID and a J & W fused silica wide-bore CW 20 M capillary column (30 m, 0.31 mm) was used. On-column injection with an air-cooled injection system was employed. The temperature program was 50–240 °C at 2 °C/min. The flow rates for carrier gas were 0.5 mL/min He, for makeup gas 30 mL/min N_2, and for the detector gases 30 mL/min H_2 and 300 mL/min air, respectively. The detector temperature was 220 °C. Volumes of 0.2 µL were injected. Quantitative determinations (without consideration of FID response factors and extraction recoveries, i.e., calibration factors $F = 1.00$ for all compounds) were carried out by standard-controlled calculations using a Hewlett-Packard 3388A laboratory data system.

Capillary Gas Chromatography–Mass Spectrometry. A Varian 1400 gas chromatograph coupled by an open-split connection to a Finnigan MAT 44 mass spectrometer was used. The apparatus was equipped with a J & W fused silica wide-bore capillary column (CW 20 M, 0.31-mm i.d.). On-column injection with a water-cooled injection system was employed. The temperature program was 5 min isothermal at 60 °C and then 60–240 °C at 2 °C/min. The carrier gas was 1.0 mL/min He. The temperature of the ion source and all connection parts was 200 °C. The electron energy was 70 eV, current emission was 0.8 mA, and injection volumes were 0.1 µL.

RESULTS AND DISCUSSION

Recent results obtained with tea shoots (Takeo, 1981), grapes (Williams et al., 1982), and passion fruit (Engel and Tressl, 1983) revealed that some terpene compounds occur not only in free but also in bound forms in plants, i.e., as glycosides. Analogously, benzyl isothiocyanate has been recognized for a long time to be formed by enzymic splitting of glucosinolates during disruption of cell tissue in papaya (*C. papaya*, L.), e.g., during fruit processing (Tang, 1971). Looking for additional volatiles that might be produced as a result of cell structure disruption, we studied the quantitative composition of papaya flavor by capillary gas chromatography using three different methods of sample preparation, i.e., (i) with addition of NaN_3, (ii) with addition of Hg^{2+}, and (iii) without any enzyme inhibitor during fruit homogenization. Figure 1 shows the experimentally determined concentrations of some major volatiles in papaya fruit as a function of the sample preparation method. The identities of volatiles were confirmed by capillary gas chromatography–mass spectrometry. As expected, the concentration of benzyl isothiocyanate (Figure 1, no. 7) was markedly suppressed by enzyme inhibition. As to the linalool concentration (Figure 1, no. 1), enzymic inhibition with Hg^{2+} was even more effective; only 1% of the amount determined in the non-inhibited experiment was found with Hg^{2+} inhibition (Figure 1, no. 1c). In contrast, NaN_3 addition did not influence the concentration of linalool in the fruit pulp. Furthermore, the formation of furanoid linalool oxides (no. 3 and 4 in Figure 1) and of terpene hydrocarbons (not shown in Figure 1) was also influenced by enzymic inhibition during sample preparation. The concentrations of other flavor compounds, such as α-terpineol (Figure 1, no. 2), benzyl alcohol (Figure 1, no. 5), and γ-octalactone (Figure 1, no. 6), were not changed by Hg^{2+} or NaN_3 treatment of the pulp.

Analogous to the findings of Williams et al. (1982), linalool may be regarded as occurring in ripe papaya fruit in a glycosidic bound form, from which it is released by, e.g., endogenous β-glucosidase activity during cell disruption. After inhibition of the enzyme by Hg^{2+}, no linalool formation can be detected in the fruit pulp. This result has to be confirmed by corresponding enzymic studies as well as by investigations of the glycosidic precursors. Whereas at present the inhibition of formation of terpene hydrocarbons cannot be explained, three different precursors could be postulated for the effect of inhibitor treatment on formation of the furanoid linalool oxides. (a) 3,7-Dimethyloct-1-en-3,6,7-triol: This polyhydroxy compound cyclizes at ambient temperature and pH <7 to the linalool oxides. Buffering to pH 7.5 (in the experiment with NaN_3 addition; cf. Experimental Section) inhibits the cyclization step. (b) 6,7-Epoxydihydrolinaloyl glycoside: After hydrolytic cleavage by β-glucosidase spontaneous formation of the isomeric linalool oxides may occur. (c) (*E*)- and (*Z*)-linaloyl oxide glycosides: Due to the SH blocking activity of Hg^{2+} different enzymes taking part in monoterpene formation, e.g., lyases, could be inhibited. In accordance with this hypothesis, Hg^{2+} could inhibit the formation of linalool oxides from the precursors (a, b, c), whereas in the experiment with buffered (pH 7.5) pulp and NaN_3 addition, only the formation from precursor a would be inhibited.

Registry No. Benzyl isothiocyanate, 622-78-6; linalool, 78-70-6; (*Z*)-linalool oxide furanoid, 5989-33-3; (*E*)-linalool oxide furanoid, 34995-77-2; α-terpineol, 10482-56-1; benzyl alcohol, 100-51-6; γ-octalactone, 104-50-7; β-glucosidase, 9001-22-3.

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Received for review March 21, 1984. Accepted May 25, 1984.