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Volatile Flavor Components of *Annona atemoya* (Custard Apple)

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The volatiles of fresh custard apple (*Annona atemoya* cv. African Pride) were separated by simultaneous steam distillation-solvent extraction and analyzed by capillary gas chromatography and capillary gas chromatography-mass spectrometry. All of the compounds identified were mono- or sesquiterpenes with α - and β -pinene, germacrene D, and bicyclogermacrene constituting the major components. No marked change in the composition of the volatiles was observed during ripening.

The family Annonaceae contains a considerable number of fruits of economic significance (Idstein et al., 1984). Of these, *Annona atemoya* dominates the commercial market in Australia where it is known as custard apple. A tropical fruit originating in the lowland tropics or subtropics of South America, they have a light green bumpy skin and a flesh that is white, juicy, and aromatic.

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Despite the characteristic desirable flavor of this fruit, no detailed analysis of the volatiles responsible or of their changes during ripening have been reported. The fruit investigated in this study was one of the two varieties generally grown commercially in Australia, the hybrid *A. atemoya* cv. African Pride.

EXPERIMENTAL SECTION

Fruits. Fresh custard apple fruits (*A. atemoya* cv. African Pride) adjudged to be mature but green were purchased from commercial sources in Sydney, Australia. They were stored at room temperature (average 15-16 °C), and individual fruits were extracted at various intervals

during the ripening process.

Isolation of Volatiles. Custard apple pulp (250 g), separated from skin and embedded seeds, was blended with water (180 mL) for 30 s and then extracted for 1.5 h in a Likens-Nickerson apparatus (Likens and Nickerson, 1964) with pentane (5 mL) as solvent. The extract was concentrated to 0.2 mL on a micro Kuderna-Danish apparatus at a bath temperature of 50 °C.

Gas Chromatography. A Pye-Unicam GCV fitted with a split injection system (SGE, Victoria, Australia) and heated fid was used. Analyses were carried out on either a 25 m × 0.33 mm (i.d.) BP-20 (SGE) or a 30 m × 0.26 mm (i.d.) SE-30 (J&W) fused silica column. The injection split ratio was 1:80. The column temperature was held at 70 °C for 2 min and then increased at 4 °C/min to a final temperature of 200 °C. Nitrogen (flow rate 10 cm/s) was used as carrier gas. Detector and injector temperatures were 240 and 210 °C, respectively. Injection volumes of 1–2 µL were used.

Gas Chromatography–Mass Spectrometry. GC–MS was carried out on two different systems. One consisted of a Shimadzu gas chromatograph fitted with a SCOT column (FFAP, 85 m × 0.5 mm (i.d.), SGE) coupled to a modified MS-12 mass spectrometer. Spectra were recorded every 6 s and were processed by a V. G. Digispec data system. Ionization potential was 70 eV, ion source temperature 200 °C, and accelerating voltage 8000 V. The column temperature was programmed from 70 to 200 °C at 3 °C/min.

The second system was composed of a Kratos MS25 mass spectrometer linked to a DS-50 data system and coupled to a Carlo Erba gas chromatograph fitted with a fused silica capillary column (BP-1, 25 m × 0.33 mm (i.d.), SGE). The temperature program was 70 °C for 2 min and then 4 °C/min to 220 °C. Operating parameters of the mass spectrometer were as follows: ionization potential, 70 eV; ion source temperature, 200 °C; scan speed, 1 s/decade.

RESULTS AND DISCUSSION

The volatile flavor components identified in custard apple together with quantitative data are shown in Table I.

Identification of components was made by comparison of mass spectra and retention times either with authentic compounds or with literature data (Heller and Milne, 1980; Stenhagen et al., 1974; Vernin et al., 1984; Jennings and Shibamoto, 1980).

A total of 33 peaks of relative concentration 0.1% or greater were found in the extract when analyzed on a polar capillary column, while a further 14 were present in trace amounts (<0.1%). Including all the major components, 38 have been reliably identified and a further 2 tentatively identified. All those identified were terpenoid in nature, in contrast to the considerable variety of nonterpenoid esters, alcohols, and carbonyls identified in the botanically related *Annona cherimolia* (cherimoya) (Idstein et al., 1984) and *Annona muricata* (soursop) (MacLeod and Pieris, 1981; Paull et al., 1983).

In general terms, though not in detailed composition, custard apple volatiles are similar to those found in mangoes (MacLeod and Snyder, 1985; Engel and Tressl, 1983) in which mono- and sesquiterpene hydrocarbons comprise the majority of the total volatiles. The compounds α -pinene, β -pinene, limonene, bornyl acetate, germacrene D, and bicyclogermacrene together constitute some 80% of the total extract. Oxygenated terpenes were present in minor quantities, with the sesquiterpene alcohols globulol, spathulenol, and the cadinols being the most

Table I. Volatile Flavor Components of Custard Apple

peak no. ^a	compound ^b	peak area, ^c %	Kovat's index ^d
1	α -pinene	25.6	1032
2	camphene	0.9	1078
3	β -pinene	21.0	1120
4	myrcene	3.0	1169
5	α -phellandrene	0.8	1177
6	α -terpinene	0.4	1201
7	limonene	7.4	1208
8	β -phellandrene	0.4	1208
9	1,8-cineole	0.1	1215
10	β -cis-ocimene*	0.1	1245
11	β -trans-ocimene	2.1	1258
12	<i>p</i> -cymene	0.3	1282
13	terpinolene	0.2	1294
14	α - <i>p</i> -dimethylstyrene	tr.	
15	α -cubebene	0.1	1467
16	α -copaene	0.3	1501
17	linalool	0.2	1539
18	β -cubebene	0.1	1548
19	<i>trans-p</i> -meth-2-en-1-ol?	0.1	
20	bornyl acetate	3.0	1581
21	β -elemene	0.2	1596
22	terpinen-4-ol	0.4	1605
23	caryophyllene	0.2	1617
24	aromadendrene	0.1	1628
25	sesquiterpene C ₁₅ H ₂₄	tr	
26	<i>cis</i> -meth-2-en-1-ol ?	tr	
27	sesquiterpene C ₁₅ H ₂₄	tr	
28	isopinocarveol	tr	1668
29	humulene	tr	1677
30	α -terpineol	0.3	1685
31	borneol	tr	1694
32	viridifluorene	tr	1701
33	germacrene D	10.9	1719
34	bicyclogermacrene	14.6	1745
35	δ -cadinene	1.0	1766
36	sesquiterpene C ₁₅ H ₂₂	tr	
37	unidentified C ₁₅ H ₂₀	tr	
38	unidentified C ₁₅ H ₂₂	tr	
39	sesquiterpene C ₁₅ H ₂₄	tr	
40	sesquiterpene C ₁₅ H ₂₆ O	tr	
41	globulol	0.3	2073
42	viridiflorol	tr	2084
43	spathulenol	1.0	2118
44	T-cardinol*	0.2	2165
45	T-muurrolol*	0.2	2180
46	δ -cadinol*?	0.1	
47	α -cadinol*	0.7	2224

^a Order of elution from a FFAP GC column. ^b Components were identified by comparison of their mass spectra and retention indices with those of pure reference substances or in oils of known composition except for those denoted by an asterisk where literature data were used. ^c tr < 0.1%. ^d Determined on a BP-20 fused silica column under conditions described in the Experimental Section.

notable. These may arise via the biogenetic transformations of germacrene D and bicyclogermacrene as postulated for hops (Tressl et al., 1983) since these precursors are present in relatively large concentrations in the extract.

Comparison of the custard apple volatiles from fruit judged to be mature but unripe, through to fruit considered overripe, showed no significant changes in the relative proportions of the components. Although not determined quantitatively in this study, the total amounts of volatiles present in fruit at different stages of ripening do not appear to be subject to gross changes. This is in accord with the observations for mango (MacLeod and Snyder, 1985). The postharvest changes in the composition of *A. atemoya* cv. African Pride have been reported (Wills et al., 1984), but no measurements of the variations in flavor volatiles were made. The changes in the production of flavor volatiles of *A. muricata* L. (soursop) during ripening have been investigated (Paull et al., 1983), but the completely

different composition of the volatiles from this fruit compared to those from custard apple precludes valid comparison.

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Registry No. α -Pinene, 80-56-8; camphene, 79-92-5; β -pinene, 127-91-3; myrcene, 123-35-3; α -phellandrene, 99-83-2; α -terpinene, 99-86-5; limonene, 138-86-3; β -phellandrene, 555-10-2; 1,8-cineole, 470-82-6; β -cis-ocimene, 3338-55-4; β -trans-ocimene, 3779-61-1; *p*-cymene, 99-87-6; ferpinolene, 586-62-9; α -*p*-dimethylstyrene, 1195-32-0; α -cubebene, 17699-14-8; α -copaene, 3856-25-5; linalool, 78-70-6; β -cubebene, 13744-15-5; *trans-p*-menth-2-en-1-ol, 35376-40-0; bornyl acetate, 76-49-3; β -elemene, 33880-83-0; terpinen-4-ol, 562-74-3; caryophyllene, 87-44-5; aromadendrene, 72747-25-2; *cis*-menth-2-en-1-ol, 29803-82-5; isopinocarveol, 6712-79-4; humulene, 6753-98-6; α -terpineol, 98-55-5; borneol, 507-70-0; viridiflorene, 21747-46-6; germacrene D, 23986-74-5; bicyclogermacrene, 24703-35-3; δ -cadinene, 483-76-1; globulol, 489-41-8; viridiflorol, 552-02-3; spathulenol, 6750-60-3; T-cadinol, 5937-11-1; T-munrolol, 19912-62-0; δ -cadinol, 36564-42-8; α -cadinol, 481-34-5.

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Binding of Alkanone Flavors to β -Lactoglobulin: Effects of Conformational and Chemical Modification

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Purified β -lactoglobulin B (β -Lg) readily bound the alkanones 2-heptanone, 2-octanone, and 2-nonanone. The β -Lg possessed one primary binding site per monomer, and the binding constants (K) were 150, 480, and 2400 M⁻¹ for these compounds, strongly suggesting hydrophobic interactions. Modification of the structure of β -Lg with urea, reduction of disulfide bonds, or ethylation reduced binding of these flavor compounds reflecting the importance of native structure in determining binding affinities.

The interactions of flavors with food components affect the perceived flavors of foods (Solms, 1973; Franzen and Kinsella, 1975; Kinsella, 1981; Damodaran and Kinsella, 1980). The concentration of a flavor in saliva may determine the perceived intensity of food flavors (McNulty and Karel, 1973), and food components that affect the rate or extent of transfer of a flavor component from a food to the saliva influence the perceived flavor.

Proteins can bind flavor compounds and affect the flavor of foods (Gremli, 1974; Beyeler and Solms, 1974; Arai et al., 1970; Franzen and Kinsella, 1974, 1975; Kinsella, 1980; Damodaran and Kinsella, 1981a,b). Some information is available concerning the extent and nature of flavor binding by soy proteins (Damodaran and Kinsella, 1980; Wilson, 1985). Research aimed at understanding the fundamental mechanisms of flavor binding to other proteins is needed. Relationships between the conformational states of proteins, their surface properties, and flavor-binding behavior need to be established before flavor-binding phenomena can be effectively controlled to optimize the impact of added flavors or minimize off-flavors.

β -Lactoglobulin (β -Lg) is a useful model protein for studying flavor protein interactions because its conformation and physical properties are well-defined (McKenzie, 1971; Creamer et al., 1983). In addition, β -Lg possesses

measurable binding properties for nonpolar compounds; hence, we selected β -Lg to study its capacity for binding alkanone flavors.

MATERIALS AND METHODS

Materials. β -Lactoglobulin (β -Lg) was purified from commercial β -lactoglobulin (1 \times crystallized and lyophilized; Lot 111F-8025, Sigma, St. Louis, MO) by the anion-exchange chromatographic method of Piez et al. (1961). Spectral-grade isooctane was purchased from Fisher Scientific Co. (Fairlawn, NJ). Pure (>98%) 2-nonanone, 2-octanone, and 2-heptanone were obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals used were reagent grade. Distilled, deionized water was used in all experiments.

Protein Solutions. The concentrations of native β -Lg in solution were determined with an absorptivity of 9.55 at 278 nm for a 1% solution (McKenzie, 1971). The concentrations of ethyl-esterified and sulfite-reduced proteins were determined by the method of Lowry et al. (1951) using pure β -Lg as a standard. Protein solutions at concentrations of 1% were made in 20 mM phosphate buffer, pH 6.7, containing sodium azide (0.02%). All protein solutions were passed through a Millipore (Bedford, MA) type AP prefilter before use to remove undissolved materials.

Measurement of Binding. The interaction of alkanones with β -Lg was studied by an equilibrium dialysis method as described by Damodaran and Kinsella

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