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Volatile Flavor Components of Soursop (*Annona muricata*)

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Representative samples of the aroma volatiles of soursop—a tropical fruit—were obtained by means of a modified Likens and Nickerson apparatus by using 2-methylbutane as the solvent. Extracts were concentrated by a low-temperature–high-vacuum distillation procedure, and components of resultant essences were identified as far as possible by GC–MS using both EI mass spectrometry and CI mass spectrometry. Most aroma components were esters (~80% of the sample), and they constituted a chemically closely related series. Methyl hexanoate (~31%) and methyl hex-2-enoate (~27%) were the two most abundant components and together amounted to ~0.7 mg/kg of fruit.

There are very many tropical fruits which are little known in the Western World yet whose flavor would undoubtedly appeal to the Western palate. Very few of these fruits have been studied to determine the nature of the volatile components responsible for their characteristic flavor, although Alves and Jennings (1979) recently reported a preliminary survey of some fruits of the Amazon region. Soursop (*Annona muricata*) is a tropical fruit native to and common in tropical America and the West Indies, although it is grown in some other countries including Sri Lanka. It is a member of the annonaceous fruits which are sometimes collectively known as "custard apples" from the custard-like flavor of many. Soursop, however, is rather more acid and less sweet than most other members of the group. It can grow to a large size and may weigh up to 4 kg. Its very pleasant flavor is unique and the fruit has potential for development as a processed product. Normally the juicy, fibrous pulp is consumed as such, but it can be used to prepare an ice cream and it can be mixed with water and sugar to provide an extremely refreshing drink. This paper describes the results of a project aimed at determining the nature of the compounds mainly responsible for the characteristic soursop flavor.

EXPERIMENTAL SECTION

Fresh Soursop fruits were transported by air from Sri Lanka, but it was found essential to pack them in an ethylene adsorbent to prevent overripening and spoilage en route. Fruits were then ripened in the laboratory in an atmosphere of 0.1% ethylene in nitrogen for 24 h at 30 °C.

Sample Preparation. Fruit pulp (350 g), separated from embedded seeds, was mixed with water (100 mL) and extracted for 1.5 h in a Likens and Nickerson (1964) apparatus as modified by MacLeod and Cave (1975) by using 2-methylbutane (10 mL) as the solvent. At the end of this time the residue did not possess any appreciable aroma. The extract was concentrated to 0.5 mL by using the low-temperature–high-vacuum distillation procedure devised by MacLeod and Cave (1975). The resultant essence possessed a strong aroma characteristic of the fruit.

Gas Chromatography. Essences were examined by gas chromatography using a Pye-Unicam 204 instrument equipped with a heated FID. Most work was carried out by using an 18 ft × 4 mm i.d. glass column packed with 10% PEG 20M coated on 100–120 BSS mesh acid-washed Diatomite C. Nitrogen carrier gas was used (60 mL/min), and the best temperature program was 60 °C for 5 min, followed by an increase of 12 °C/min to 160 °C for the remainder of the run. Detector and injection temperatures were 250 °C, and typically 4 μL of sample was injected.

Gas Chromatography–Mass Spectrometry. Components in the essence were identified as far as possible by GC–MS using a Kratos MS 25 instrument linked online to a Kratos DS 50 data processing system. The same GC conditions as described above were employed but using helium as the carrier gas and a slightly lower flow rate (40 mL/min). A single-stage, all-glass jet separator was used at 250 °C. Both electron impact (EI) mass spectrometry and chemical ionization (CI) mass spectrometry were performed, and at various times the MPM unit and the retrospective single ion monitoring facility of the data system were employed to good advantage. Significant operating parameters of the mass spectrometer during EI work were as follows: ionization potential, 70 eV; ionization current, 100 μA; source temperature, 200 °C; accelerating voltage, 1.5 kV; resolution, 600; scan speed, 1 s/decade (repetitive throughout run). Identical conditions were employed during CI mass spectrometry except for the following: reagent gas, methane (or isobutane or ammonia); ionization potential, 100–110 eV; emission current, 5 mA.

Quantitative Assessment. Sample preparation and concentration were conducted with quantitative accuracy so that a known aliquot of the fruit sample was analyzed. Quantitative data were then derived both from the trace obtained from the TIC monitor during GC–MS and from the FID trace during routine GC. Known amounts of a selection of identified components (particularly the esters) were injected under the same analytical conditions to assess the response factors of the detectors to the various classes of identified compounds.

Odor Assessment. Aromas of the separated components of the essence were assessed at an odor port following GC using a Pye-Unicam 104 instrument. An outlet splitter set at 10:1 diverted the major fraction of the eluant through a heated line to the outside of the oven for aroma assessment by a total of three subjects. An injection volume of 10 μL was necessary.

RESULTS AND DISCUSSION

Various extraction methods using a number of different

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Table I. Volatile Flavor Components of Soursop Fruit

peak no.	component	t_R, min	% rel abund	μg/kg of fruit	odor quality
1	unknown	1.1	0.02	0.30	odorless
2	unknown	1.4	0.10	1.28	odorless
3	C_5 branched chain hydrocarbon	2.6	0.59	7.31	odorless
4	C_5 branched chain hydrocarbon	3.0	0.21	2.55	odorless
5	C_5 branched chain hydrocarbon	3.6	0.004	0.06	odorless
6	unknown	4.0	0.06	0.70	odorless
7	unknown	4.6	0.003	0.04	odorless
8	1,1-diethoxyethane	5.2	0.15	1.91	sickly
9	dichloromethane	6.0	5.72	59.51	sickly
10	unknown	6.8	0.002	0.03	rancid
11	methyl butanoate	7.2	4.44	54.83	fruity, ester
12	unknown	8.4	0.02	0.21	
13	chloroform	8.8	0.36	4.46	medicinal, sickly
14	ethyl butanoate	9.2	0.15	1.91	fruity, apples
15	toluene	9.6	0.03	0.34	
16	2-methylhexan-1-ol	9.9	0.15	1.91	green
17	methyl but-2-enoate	10.4	4.75	58.65	caramel
18	unknown	10.6	0.06	0.76	fruity
19	unknown	11.0	0.004	0.05	apples
20	ethyl but-2-enoate	11.4	0.06	0.71	fruity, faint caramel
21	methyl hexanoate	11.8	30.95	382.50	ester, pear drops
22	ethyl hexanoate	12.7	3.56	43.99	fruity
23	methyl hex-3-enoate	13.2	0.07	0.90	grass
24	styrene	13.6	0.01	0.17	faint nuts
25	methyl hex-2-enoate	14.0	26.70	329.90	ester, fruity
26	hexan-1-ol	14.8	3.30	40.80	raw nuts
27	ethyl hex-2-enoate	15.2	0.07	0.93	
28	*cis*-hex-3-en-1-ol	15.6	0.31	3.78	green, grass
29	methyl octanoate	16.4	0.52	6.38	roasted coconut
30	unknown	17.1	0.001	0.01	slight nuts
31	ethyl octanoate	17.6	1.44	17.85	coconut
32	unknown	18.6	0.39	4.78	brown
33	unknown	19.6	1.24	15.30	fruity
34	methyl oct-2-enoate	20.4	4.13	51.00	floral
35	S and N heterocyclic compound	21.4	0.31	3.83	raw nuts
36	ethyl oct-2-enoate	22.4	0.15	1.91	floral
37	methyl furoate	23.4	0.41	5.10	floral, fruity
38	unknown	25.7	0.006	0.08	roasted nuts
39	*trans*-β-farnesene	27.2	6.45	79.69	floral
40	unknown	28.0	0.26	3.19	cashew nuts
41	a sesquiterpene	32.1	0.93	11.48	floral
42	unknown	34.0	0.002	0.03	floral
43	methyl nicotinate	38.4	1.86	22.95	fragrant, faint peppermint
44	unknown	46.4	0.04	0.53	acidic

Table II. Summaries of the Mass Spectra (Eight Most Intense Peaks) of Some of the Esters Identified in Soursop Aroma Volatiles [See Also MacLeod and Pieris (1981)]

	m/e (% rel intensity)[a]							
methyl but-2-enoate	69 (100)	39 (49)	41 (48)	85 (22)	100 (18)	29 (8)	59 (7)	70 (5)
methyl hex-2-enoate	55 (100)	41 (75)	39 (60)	68 (54)	87 (52)	97 (46)	29 (30)	128 (24)
methyl octanoate	74 (100)	87 (42)	43 (32)	55 (27)	41 (21)	59 (19)	29 (17)	57 (9)
methyl oct-2-enoate	55 (10)	41 (80)	87 (72)	29 (32)	39 (27)	59 (18)	68 (12)	113 (10)
ethyl oct-2-enoate	55 (100)	41 (61)	29 (47)	73 (44)	39 (30)	99 (28)	68 (22)	125 (16)

[a] Values are *m/e*. Numbers in parentheses are percent relative intensity.

solvents were assessed, and it was found that the most representative and stable flavor extract of soursop fruit was obtained by using a Likens and Nickerson (1964) apparatus as modified by MacLeod and Cave (1975). 2-Methylbutane(isopentane) was the best solvent of those tested in this instance. The extract was concentrated by using the low-temperature–high-vacuum distillation procedure previously described (MacLeod and Cave, 1975), and the resultant essence retained the genuine aroma qualities of the original extract. The essence was examined by temperature-programmed gas chromatography using mainly an 18-ft packed column containing 10% PEG 20M as the stationary phase. Constituents of the essence were identified as far as possible by GC–MS. Chemical ionization mass spectrometry (generally using methane as the reagent gas) was particulary useful in determining the molecular weights of most components, hence rendering interpretation of conventional electron impact spectra somewhat easier.

Table I lists the volatile flavor components of soursop, together with GC retention data, quantitative data, and odor qualities of the various GC peaks. In all instances where positive identities are quoted, mass spectra of sample components either agreed with those of literature spectra, within instrumental variability, or were interpreted by application of basic mass spectrometry mechanisms and by comparisons with literature spectra of closely related compounds (i.e., those within an homologous series). Where no odor quality is given in Table I, this was due to a minor peak being incompletely resolved from an adjacent major peak, and thus no distinct odor could be recognized.

It can be seen that the soursop essence contained 44 main components of which 24 (comprising nearly 96% of the sample) have been positively identified with a further

5 (~2%) partially characterized. The majority of the identified compounds are esters (nearly 80% of the sample) with the major constituents being methyl hexanoate (~31%) and methyl hex-2-enoate (~27%). The esters comprise an interesting series of chemically related compounds in that the methyl and ethyl esters of the C_4,C_6, and C_8 saturated straight chain carboxylic acids were all present in the sample, together with the six corresponding 2-enoates. A similar, but slightly less complete, series was also obtained with another tropical fruit, wood apple, although in that case the corresponding 3-hydroxy esters were also generally detected (MacLeod and Pieris, 1981). These hydroxy derivatives were definitely not present in soursop essences, since specific searches were made for these compounds. The aforementioned previous publication (MacLeod and Pieris, 1981) includes summaries of the mass spectra of the less common esters of wood apple, since these are not widely published, if at all. Here, Table II provides similar summaries of the spectra of the additional less common esters detected in soursop.

The previous paper (MacLeod and Pieris, 1981) discussed briefly the biosynthetic relationships of these esters and also summarized previous reports of their detection as aroma components. This will not be reiterated here except to emphasize that the 2-enoates are relatively rare aroma constituents and generally they have only been located in tropical or subtropical fruits and products such as passion fruit (Murray et al., 1972; Winter and Kloti, 1972), grapes (Stern et al., 1967), and wood apple (MacLeod and Pieris, 1981). The detection of these esters in soursop further supports the contention that they might be a characteristic of tropical fruits.

Generally, the determined odor qualities (Table I) were undistinguished, and no GC peak seemed to represent any specific element of the characteristic soursop flavor. The obvious, if facile, deduction must be drawn that soursop flavor is basically a blend of the 15 esters, together with at least β-farnesene, in the correct proportions. It would certainly be important in any processed product to retain as far as possible all these components to maintain the characteristic fresh fruit flavor.

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Composition of Rough Lemon Leaf Oil

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Twenty-five of ninety-two components isolated by gas chromatography from steam-distilled rough lemon (*Citrus jambhiri* Lush.) leaf oil and aqueous distillate were identified. They were identified by gas chromatographic retention times, infrared spectroscopy, and mass spectroscopy. Quantities of the major identified components were as follows (peak area percent): limonene, 33.7; sabinene, 7.8; γ-terpinene, 7.4; β-ocimene, 7.3; linalool, 5.3; isopulegol, 4.6; geranial, 3.9; neral, 3.6; *p*-cymene, 3.3; geranyl acetate, 1.1; neryl acetate, 0.8; terpinen-4-ol, 1.0. A number of compounds not previously identified in rough lemon leaf oil were isolated. Some of these may be associated with the host preference of citrus blackfly (*Aleurocanthus woglumi*) for this citrus species. The data could be useful in taxonomic studies, for identification of new aroma compounds, or in evaluation of compounds affecting citrus blackfly.

The composition of citrus leaf oils has been investigated for determination of taxonomic relationships and identification of unique fragrance components. Recently, lemon leaves from several varieties, especially rough lemon (*Citrus jambhiri* Lush.), were found to be preferred hosts for citrus blackfly, *Aleurocanthus woglumi* (Howard, 1979; Dowell et al., 1978). Because of the potential damaging effects of this insect, compounds with attractant or repellant properties or other characteristics that affect its behavior are of interest. For these reasons, we decided to investigate in detail the leaf oil composition of rough lemon.

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A detailed study of true lemon leaf oil [*Citrus limon* (L.) Burm. f.] has been reported by Kamiyama (1967). In the Kamiyama study, gas chromatography (GC), thin-layer chromatography (TLC), and infrared spectroscopy (IR) were used to identify 25 compounds; 7 unidentified compounds were also isolated.

Attaway et al. (1966) positively identified a number of rough lemon leaf components. Kesterson et al. (1964) and Scora et al. (1969) identified rough lemon leaf components by retention times only.

Scora et al. (1969) examined nine varieties of rough lemon, two true lemons, and a hybrid and reported differences and similarities between rough and true lemon leaf oils. Rough lemon is a common rootstock in Florida commercial groves. Taxonomically, it is considered to be in a distinctly different group from the true lemon.

In our study, the oil was prepared by steam distillation. The compositions of both aqueous and oil layers were