Identifying Volatiles in Soursop and Comparing Their Changing Profiles during Ripening

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Abstract. The volatile compounds in soursop (*Annona muricata* L.) were obtained by a liquid-liquid continuous extraction procedure from the aqueous solution of blended soursop pulp and analyzed by gas chromatography (GC) and GC-mass spectrometry (MS). Twelve volatiles were identified by comparing their mass spectra and Kovats retention indexes with those of standard compounds: five were identified tentatively from MS data only, eight are being reported for the first time. (Z) -3-hexen-1-ol was the main volatile present in mature-green fruit, while methyl (E) -2-hexenoate, methyl (E) -2-butenate, methyl butanoate, and methyl hexanoate were the four main volatiles present in ripe fruit. Concentrations of these five volatiles decreased and several other unidentified volatiles appeared when the fruit became overripe.

There has been an increased number of exotic tropical fruit, such as papaya, mango, and carambola, appearing on supermarket shelves in the United States and western Europe. As familiarity with the novel taste of these fruit increases, demand for them is likely to increase in the future for fresh and processed products. Soursop has a unique, pleasant aroma and an intriguing sweet-sour flavor (Morton, 1966). The fruit have been used in products such as tropical fruit drinks, sherbert, ice cream, jams, and jellies. Most research on soursop has dealt with nutritional composition and physical and chemical changes during ripening (Gebhardt et al., 1982; Paull, 1982; Wenkam and Miller, 1965). MacLeod and Pieris (1981a) and Paull et al. (1983) have analyzed the aroma components of ripe soursop, but there has been little work on the changes in volatiles in soursop fruit after har-

vest (Paull et al., 1983). More information on the chemical changes occurring during soursop ripening would be valuable to determine optimal harvest time, storage conditions, and processing procedures for the fruit. The objectives of this study were to identify the main volatiles in ripe soursop and compare their profiles at various ripening stages (mature-green, ripe, and overripe).

Firm, mature-green soursop fruit were obtained from the Univ. of Hawaii's Poamoho Agricultural Experiment Station on the island of Oahu and the Waiakea Experimental Farm on the island of Hawaii. Only those fruit that were graded at the same ripeness stage were used for this study. These fruit were held at room temperature (25C) for ripening. The day that mature-green fruit were harvested was arbitrarily recorded as day 0. Single fruit were taken for analysis on days 3,4,5,6,7, and 10. By smelling the fruit and measuring their pH, texture, and soluble solids concentrations, we determined that fruit held for 5 and 6 days were ripe and ready to eat.

Sample preparation. The procedure used to extract the volatiles from soursop was similar to that used by Nishimura et al. (1989). The sample was prepared by removing peel, core, and seeds from the fruit and obtaining 500 g of soursop pulp. The pulp was blended with 500 ml of distilled water, the slurry was centrifuged at 3000 rpm for 10 min, and the super-

natant was decanted. The extraction procedure was repeated with 300 ml of distilled water and the supernatants were combined. The volatiles were extracted for 24 h with methylene chloride in a liquid-liquid continuous extractor. The methylene chloride fraction was dried with sodium sulfate and the volatiles were concentrated to 2 ml on a Kuderna-Danish concentrator (Kontes, Vineland, N.J.) with a Snyder column. The extract was concentrated further to 1 ml by slowly flushing it with purified N at 20C. The essence concentrate was stored at -40C.

Gas chromatography (GC). A Hewlett Packard (HP, Avondale, Pa.) 5890 gas chromatography containing a flame ionization detector (FID) and a nitrogen-phosphorus detector (NPD) was used with an HP Ultra-1 capillary column (50m×0.31 mm×0.17 μm). The GC conditions for FID analysis were detector, 250C; injector, 250C; oven, isothermal for 4 min at 30C, then programmed to 2 10C at 2 C/min, helium carrier gas flow rate, 0.91 ml·min⁻¹ with a column head pressure of 110 kPa. The GC conditions for NPD were the same as for FID, except that the detector temperature was set at 275C. An IBM (Armonk, N. Y.) PS/2 model 50Z computer and a Perkin Elmer Nelson Systems (Cupertino, Calif.) PC integrator software (revision 5.0) were used to process the GC data.

GC-mass spectrometry (GC-MS). An HP 5890 gas chromatography coupled to an HP 5970 mass selective detector (MSD) with an HP 5970A GC/MS/data system and a DB-1 capillary column (J&W Scientific, Folsom, Calif.) (30 m × 0.25 mm × 0.25 μm) were used for the GC-MS analysis. The GC conditions were the same as above, except that the flow rate of the carrier gas was 1.23 ml·min⁻¹ with a column head pressure of 69 kPa. The ionization voltage (electron impact mode) was 70 eV.

Standard compounds. Standards were obtained from commercial sources. Volatiles were identified by comparing their mass spectra and retention indexes (RIs) with those of standard compounds. The Kovats RI (Ettre, 1964) also was used for identification.

The essence concentrate obtained from the methylene chloride extraction of ripe soursop (on day 5) had a strong aroma characteristic of soursop. Seventeen compounds were identified or tentatively identified with their GC retention data, Kovats RI, and relative peak areas (Table 1). Twelve volatiles were identified by GC-MS and the Kovats RI. Five volatiles were tentatively identified based on comparisons of mass spectra with literature spectra (Stein, 1987).

Of the volatiles identified, three are being reported for the first time for soursop: methyl pentanoate, linalool, and methyl 2,4-hexadienoate. The others were identified by MacLeod and Pieris (1981a) and Paull et al. (1983). Volatiles tentatively identified for the first time in soursop included 2-methyl-2-butanol, 1-methylethyl 2-butenate, methyl 2-hydroxy-3-methylpentanoate, 4-methylbenzaldehyde, and methyl 3-phenyl-2-propenoate.

The occurrence of some of these volatiles

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Table 1. Lemon juice composition at harvest and after 8 weeks of storage under various conditions.

Treatment ^a	Chemical constituents						
	Extractable juice ^c	SSC ^a (%juice)	pH	Acidity (% juice)	Ascorbic acid (mg/100 ml juice)	Reducing sugars (% juice)	Nonreducing sugars (% juice)
At harvest	44.5 c ^a	9.1 a-c	2.3 b	6.7 c-e	45.9 c	1.4 de	1.1 c
Cold storage							
A (2C)	45.3 c	8.6 c	2.3 b	6.6 de	49.2 a-c	1.2e	0.8 e
B (8C)	49.2 ab	8.9 a-c	2.4 ab	6.2 g	49.5 a-c	1.5 de	0.9 d
C (13C)	49.5 a	9.2 a-c	2.3 b	6.3 fg	49.0 a-c	1.5 de	1.0d
Intermittent warming							
D	47.6 a-c	8.8 be	2.3 b	6.9 a-c	47.9 a-c	1.2e	0.9 d
E	46.3 be	9.3 ab	2.3 b	7.0 ab	50.1 a-c	1.7 cd	1.0d
F	49.9 a	9.4 a	2.4 ab	6.7 c-e	52.4 a	1.5 de	0.9 d
G	47.6 a-c	8.6 c	2.5 a	6.5 ef	50.1 abc	1.4 de	1.0d
H	46.4 bc	9.2 a-c	2.4 ab	7.1 a	49.5 a-c	1.7 cd	1.1 c
CO ₂ for 24 h/week at 13C							
I (10%)	47.6 a-c	9.0 a-c	2.3 b	6.8 b-d	51.3 ab	3.4 a	1.4a
J (20%)	48.9 ab	8.9 a-c	2.4 ab	6.6 de	47.9 a-c	2.3 b	1.2b
K (30%)	46.4 bc	8.9 a-c	2.5 a	6.8 b-d	47.4 bc	2.0 be	1.1 c

^aSSC = soluble solids concentration.

^bD = 2 cycles, 1 week 2C, 3 weeks 13C; E = 2 cycles, 1 week 8C, 3 weeks 13C; F = 2 cycles, 2 weeks 2C, 2 weeks 13C; G = 2 cycles, 3 weeks 2C, 1 week 13C; H = 2 cycles, 3 weeks 8C, 1 week 13C.

^cPercentage (w/w) of juice to fresh weight.

^aMean separation in columns by Duncan's multiple range test, $P < 0.05$.

Spain (40 km from Crevillente), where they were kept at 13C. The next day, the fruit were washed with water and dipped in thiabendazole at 2 g-liter⁻¹ at 20C for 3 min. Fruit of uniform size and appearance (120 g, 60 mm in diameter, green-yellow, and free of defects) were randomized into 150-fruit lots as three replicates of 50 fruit each per treatment. The plastic boxes containing the fruit were placed inside 360-liter gas-tight chambers in cold storage rooms at 2, 8, or 13C with an RH >95% in each. The respective vapor pressure differences were ≤ 30, 60, and 80 Pa.

The treatments were A) 8 weeks at 2C, B) 8 weeks at 8C, C) 8 weeks at 13C, D) 1 week at 2C + 3 weeks at 13C + 1 week at 2C + 3 weeks at 13C, E) 1 week at 8C + 3 weeks at 13C + 1 week at 8C + 3 weeks at 13C, F) 2 weeks at 2C + 2 weeks at 13C + 2 weeks at 2C + 2 weeks at 13C, G) 3 weeks at 2C + 1 week at 13C + 3 weeks at 2C + 1 week at 13C, H) 3 weeks at 8C + 1 week at 13C + 3 weeks at 8C + 1 week at 13C, I) 10% CO₂ for 24 h weekly during storage at 13C, J) 20% CO₂ for 24 h

weekly during storage at 13C, and K) 30% CO₂ for 24 h weekly during storage at 13C.

Physical and chemical analyses were performed on three 10-fruit samples at harvest and after each storage trial. Fruit were weighed with a precision of ±0.01 g and weight loss was calculated as the percentage of initial fresh weight. Extractable juice obtained by squeezing was calculated as the percentage (w/w) of juice to fresh weight. Juice was used to determine total soluble solids concentration (SSC), pH, titratable acidity (TA), reducing and nonreducing sugars, and ascorbic acid. SSC was determined with an Abbé refractometer (readings at 20C). TA, calculated as the percentage of citric acid, was determined by titrating 1 ml of juice with 0.1 N NaOH. Reducing and nonreducing sugars were determined using the method of Ting (1956). Ascorbic acid was determined by the Association of Official Analytical Chemists (AOAC, 1984) calorimetric method.

Carbon dioxide concentrations were obtained using calibrated capillaries, differential

manometers, and gas mixers enriched with a known flow of compressed air. The gas flow was humidified in a mixer before entering the gas-tight chambers containing the fruit.

After they were removed from storage, all fruit were examined for decay and disorders.

Data were subjected to analysis of variance and means were separated by Duncan's multiple range test, where appropriate.

Juice composition of lemons stored for 8 weeks at 2C did not show any significant difference in constituents, except for a slightly lower nonreducing sugar content compared to fruit at harvest (Table 1). TA and nonreducing sugars decreased in lemons stored at 8 or 13C compared to fruit at harvest. No significant differences were evident between the latter two temperatures. Chemical composition of juice from lemons under intermittent warming was not different or only slightly different from fruit at harvest. Carbon dioxide promoted higher levels of reducing sugars, but nonreducing sugars were higher only in lemons treated with 10% and 20% CO₂ relative to

Table 2. Incidence of weight loss, decay, and physiological disorders of lemons after 8 weeks of storage under various conditions.

Treatment ^a	Wt loss (%)	Decay and disorders (% fruit affected)					
		<i>Alternaria</i>	Red blotch	Peteca	Membranosis	Pitting	Oleocellosis
Cold storage							
A (2C)	2.5 ab ^a	0 c	0 d	0 b	32.6 C	4.0 a	31.3a
B (8C)	3.4 ab	0 c	9.3 c	0 b	79.3 a	0.7 b	0.7 b
C (13C)	4.8 a	3.3 a	44.0 b	0.7 b	1.3 e	0 b	0 b
Intermittent warming							
D	3.1 ab	2.0 ab	35.3 b	0 b	12.7 de	2.7 b	4.0 b
E	4.4 a	0.7 be	44.0 b	0 b	51.9b	0 b	2.7 b
F	4.1 ab	0 c	7.3 c	0 b	25.3 cd	0 b	0 b
G	1.4b	0 c	0 d	4.0 a	52.6 b	0.7 b	3.3 b
H	1.6b	0 c	9.3 c	1.3b	81.0a	2.7 b	4.0 b
CO ₂ at 13C							
I (10%)	3.7 ab	2.7 a	70.1 a	0 b	0 e	0 b	0 b
J (20%)	1.6b	2.7 a	73.3 a	0 b	0 e	0 b	0 b
K (30%)	3.3 ab	1.3 a-c	84.0 a	0 b	0 e	0 b	0 b

^aD = 2 cycles, 1 week 2C, 3 weeks 13C; E = 2 cycles, 1 week 8C, 3 weeks 13C; F = 2 cycles, 2 weeks 2C, 2 weeks 13C; G = 2 cycles, 3 weeks 2C, 1 week 13C; H = 2 cycles, 3 weeks 8C, 1 week 13C.

^aMean separation in columns by Duncan's multiple range test, $P \leq 0.05$.

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