

this study.

The alkaline hydrolysis of DON may be a general reaction for trichothecenes having identical functionalities in the A ring. Under the same conditions, the related trichothecene nivalenol (1a) produced three products having HPLC and UV properties similar to those of norDON-A, norDON-B, and norDON-C. Insufficient quantities of material prevented further characterization of these products. Grove (1985) also noted the formation of a related lactone from nivalenol.

**Summary.** Other related studies (Young et al., 1986b,c) have demonstrated that bisulfite treatment shows promise for the detoxification of DON contaminated grains and that the resultant product, DON-S, appears to be nontoxic to pigs (Young et al., 1986c). In addition to DON, *Fusarium* spp. are capable of producing quite a wide variety of other trichothecenes and mycotoxins (Blackwell et al., 1985; Greenhalgh et al., 1986a,b). Some of these metabolites may be partly responsible for the observed variability in toxicity of DON-contaminated pig feeds (Foster et al., 1986). Knowledge of the scope of potential detoxification methods is of considerable practical value. In this study, trichothecenes having a conjugated 9-en-8-one group were shown to undergo facile reaction with sodium bisulfite to form sodium sulfonate salts that are stable in acid but are rapidly hydrolyzed back to the parent compound in base. In addition, these keto trichothecenes undergo rearrangement and degradation to novel compounds in the presence of alkali.

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**Registry No.** 1a, 23282-20-4; 1b, 51481-10-8; 1c, 50722-38-8; 1d, 56676-60-9; 1e, 51550-28-8; 2a, 103776-41-6; 2b, 92397-71-2; 3a, 103776-36-9; 3b, 103776-37-0; norDON-A, 103776-38-1; norDON-B, 103776-39-2; norDON-C, 103776-40-5.

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## Flavor Chemistry of Cashew Apple Juice

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The volatile constituents of commercially processed and unprocessed cashew apple juice were analyzed by headspace concentration gas chromatography-mass spectrometry. Specific analyses of sulfur-containing compounds were also performed. The major components were esters. However, alcohols, aldehydes, ketones, acids, terpenes, and sulfur-containing compounds were also identified. Except for sulfur dioxide, which had been added as a preservative to the processed juice, there were only minor differences between the two juices. Odor assessment suggested that certain esters and two aliphatic acids are important to the characteristic sickly sweet and sharp flavor of cashew apple juice. However, the overall aroma is the result of the integrated contributions of many components. Sensory evaluation revealed that the two juices were noticeably different from each other. However, they did not differ significantly in sweetness, sourness, bitterness, or fruitiness, and there was no preference for either type of juice. Slight changes in volatiles composition may be responsible for subtle changes in this complex flavor.

#### INTRODUCTION

The cashew tree (*Anacardium occidentale* L.), which originated in Brazil, is now grown in many tropical and

subtropical countries. The cashew nut is its most important product with a world production of about  $5 \times 10^5$  tons/yr (Tyman, 1980; FAO, 1983). The tree also yields an important product, the cashew "apple", to which the cashew nut is attached. Botanically, cashew apple is not a true fruit, but it has a pear shape, weighs about 10-15 g (approximately 3-6 times more than the nut, the "true fruit"), and is from 6 to 10 cm long. Its color varies from bright yellow to red, and it has a waxy skin. The cashew apple is very sour and astringent until fully ripe when it becomes edible. It is a very juicy fibrous fruit and can be consumed raw or in the form of jam, marmalade, juice, and distilled products.

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Despite the large commercial importance of the cashew nut, the cashew apple is almost neglected. Recently, in Brazil, the production of cashew apple juice is gaining much more attention. The juice is well accepted and has a vitamin C content averaging 261 mg/mL, 5–7 times that of orange juice (Lopes, 1972; Price et al., 1975; Mudambi and Rajagopal, 1977).

Several studies have been done in order to develop a suitable procedure to concentrate cashew apple juice (Vieira et al., 1982; Ramteke et al., 1984). However, during concentration processes much of the aroma is changed or lost. The first study published on flavor volatiles of cashew apple used distillation-extraction of fresh fruit from Venezuela and applied gas chromatography-mass spectrometry (GC-MS) to identify the volatiles (MacLeon and deTroconis, 1982). Those researchers found aldehydes and carene as the major cashew apple volatiles.

We report here the use of headspace concentration and GC-MS to ascertain the flavor profile of the cashew apple juice from Brazil. A commercial sample stabilized with preservatives was compared with freshly prepared juice on the basis of both headspace profiles and sensory analysis.

## EXPERIMENTAL SECTION

**Samples.** Processed juice was purchased at a retail store in North Philadelphia. This juice, imported from Brazil, was bottled and pasteurized and had sulfur dioxide, benzoic acid, and sorbic acid added as preservatives. Unprocessed juice was prepared from fresh fruits at the University of Pernambuco, Brazil, frozen, and sent to us by air.

**Gas Chromatography-Mass Spectrometry.** A Finnigan (San Jose, CA) 4500 GC-MS system equipped with a Data General (Westboro, MA) Nova computer system was used to identify the headspace volatile components. A CPWAX-57CB (Chrompack, Bridgewater, NJ) capillary column (25 m  $\times$  0.33 mm with a 1.3- $\mu$ m film thickness) programmed at 50 °C (4 min) to 220 °C (4 °C/min) was used for the analyses. Helium was the carrier gas at a flow rate of 2 mL/min. A specially designed thermal tube desorber (Envirochem Inc., Kemblesville, PA) was interfaced to the injector of the GC to provide rapid heating and ease of tube exchange. Volatiles were collected by sweeping the headspace over 75 mL of juice in a flask with nitrogen at a flow rate of 80 mL/min for 40 min at room temperature, with magnetic stirring. Volatiles were collected in a 4-mm-i.d. glass tube packed with approximately 250 mg of 60/80 mesh Tenax GC (Applied Science, State College, PA). The tube was placed in the desorber and heated to 270 °C with carrier flow, and the volatiles were transferred to the GC column, where they were condensed with cooling obtained by passing nitrogen through a copper coil immersed in liquid nitrogen and through a metal sleeve which enclosed the first 24 cm of the column. Both electron impact (EI) and chemical ionization (CI) spectra were performed. Conditions for EI spectra runs were as follows: ionization potential 70 eV; ionization current 30 mA; ion source temperature 150 °C; resolution 600; scan rate 1 scan/s. A series of C<sub>2</sub>-C<sub>18</sub> fatty acid ethyl esters was injected for comparison of retention times. Standard mass spectra were obtained from the National Bureau of Standards (NBS) computerized library, except for ethyl 2-mercaptopropionate, which was purchased from Ivolin Enterprises (New York, NY).

**Gas Chromatography. Sulfur Compounds.** Specific analysis for sulfur compounds was performed on a Per-

kin-Elmer (Norwalk, CT) 3920B gas chromatograph equipped with a flame photometric detector (FPD). A 36 ft  $\times$  1/8 in. o.d. Teflon-FEP column packed with 5% polyphenyl ether and 0.5% H<sub>3</sub>PO<sub>4</sub> on 40–60-mesh Chromosorb T was employed for the analysis and programmed at 60 °C to 165 °C (32 °C/min). Ultrapure breathing air was the carrier gas at a flow rate of 25 mL/min. The peaks were identified by comparison of retention times with peaks from individual standards.

**Odor Assessment.** For concentration and GC analysis of flavor compounds, 75 mL of juice was placed in a flask fitted to the sparging unit of the UNACON (Envirochem, Inc., Kemblesville, PA) 810B automated headspace concentrator-gas chromatograph incorporating wide- and narrow-bore absorption traps in series and a flame ionization detector, as previously described (Zechman and Labows, 1985). The headspace was swept with nitrogen at a flow rate of 60 mL/min for 30 min at 37 °C. The volatiles were collected on Tenax and then automatically thermally (270 °C) desorbed and back-flushed to the GC capillary column. The oven was programmed at 60 °C (4 min) and 60 °C to 180 °C (6 °C/min) with hydrogen as the carrier gas at a flow rate of 21 cm/s. Output from the flame ionization detector was recorded on a SP-4100 computing integrator (Spectra Physcs, Santa Clara, CA) that automatically recorded retention times, relative retention times, and integrated areas.

Odor of the separated components of the concentrate was assessed at an odor port following GC separation. Of the eluent 90% was diverted through a heated line to the outside of the GC oven for odor assessment by up to three subjects. M.I.M. was particularly familiar with the juice, but the other two had sampled the juice.

**Sensory Analysis.** The juices were prepared according to the label on the bottle of the processed juice: 1 part of juice to 8 parts of water plus sugar equivalent sweetness. A total of 26 volunteers evaluated the juices for overall acceptability and also for sweet, sour, bitter, and fruity tastes. These ratings were on a 9-point scale, with 5 being neutral. A triangle test was also performed by six tasters, four times each, to test the ability to detect a difference between processed and unprocessed juices.

## RESULTS AND DISCUSSION

The volatiles were collected on Tenax and desorbed directly to the capillary column of the GC-MS. Both retention time and mass spectral data were used to identify 52 volatile components plus a partial identification of eight additional compounds. Table I shows those compounds identified or tentatively characterized, with their retention times. Data are given for both processed and unprocessed juice. As shown in Table I, cashew apple juice flavor is comprised of a range of volatile esters, 12 alcohols, 8 terpenes, 3 ketones, 2 aldehydes, and 2 acids. Clearly, the majority of components were common to both types of juice (Figures 1 and 2). Sulfur-containing compounds, sulfur dioxide (SO<sub>2</sub>), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS), are also present, as seen by GC-FPD.

The esters seem to be qualitatively and quantitatively the most important class of volatiles in cashew apple juice flavor. The esters comprise an interesting series of chemically related compounds, since ethyl esters of the C<sub>2</sub>-C<sub>8</sub> saturated straight-chain carboxylic acids were all present in the samples together with methyl esters of the C<sub>4</sub>-C<sub>6</sub> and the methyl and ethyl 2-enoates of C<sub>5</sub> and C<sub>6</sub>, respectively. The 2-enoates are relatively rare aroma constituents, and generally they have only been detected in tropical

Table I. Compounds Identified in Cashew Apple Juice

compd	peak <sup>a</sup>	ret time, min	present in <sup>b</sup>	
			proc	unproc
acetic acid, ethyl ester	1	4.7	+	+
butanoic acid, methyl ester	2	5.5	+	+
2-pentanone	3	6.0	+	-
butanoic acid, ethyl ester	4	7.0	+	+
2-methyl butanoic acid, methyl ester	5	7.6	+	+
dimethyl disulfide	6	7.8	+	+
3-methylbutanoic acid, ethyl ester	7	8.2	+	+
acetic acid, 3-methylbutyl ester	8	9.4	+	-
pentanoic acid, ethyl ester	9	9.7	+	+
pentanoic acid, isopropyl ester	9a	9.9	-	+
carene	10	10.0	+	+
3-methylbutanoic acid, propyl ester	11	10.2	+	-
2-butenic acid, ethyl ester	12	10.5	+	+
3-methylpentanoic acid, ethyl ester	13	10.8	+	+
hexanoic acid, methyl ester	14	11.0	+	+
2-methylenebutanoic acid, methyl ester	15	11.1	+	-
limonene	16	11.3	+	+
pyridine	17	11.7	+	-
3-methylbutanol	17a	11.9	-	+
acetic acid, pentyl ester	18	12.1	+	-
hexanoic acid, ethyl ester	19	12.5	+	+
3-methyl-2-butenic acid, ethyl ester	20	12.6	+	+
butanoic acid, 3-methylbutyl ester	20a	12.7	-	+
$\beta$ -phellandrene	21	12.8	+	+
$\gamma$ -terpinene	22	13.9	+	+
pentanoic acid, 2-methylpropyl ester	22a	13.9	-	+
2-methylpentanoic acid, butyl ester	23	14.2	+	-
2-pentenoic acid, methyl ester	23a	14.4	-	+
3-hydroxy-2-butanone	24	15.5	+	+
heptanoic acid, ethyl ester	25	15.9	+	+
2-hexenoic acid, ethyl ester	26	16.5	+	+
4-methylpentanol	27	16.8	+	+
dimethyl trisulfide	28	17.3	+	+
3-methylbutanoic acid, 3-methyl-3-butenyl ester	29	17.5	+	-
3-hexenol	30	17.5	+	+
unknown alcohol	31	18.4	+	+
octanoic acid, ethyl ester	32	18.7	+	+
2-mercaptopropionic acid, ethyl ester (tentative)	33	19.2	+	+
furfural	34	19.7	+	-
unknown alcohol	35	19.9	+	+
unknown sesquiterpene	35a	20.3	-	+
2-methylpropanoic acid (isobutyric)	36	20.4	+	+
3-ethyl-3-pentanol	37	21.4	+	+
benzaldehyde	37a	21.7	-	+
4-methyloctanoic acid, ethyl ester	38	21.8	+	-
unknown alcohol	39	22.2	+	+
isooctanol	39a	22.8	-	+
octanol	40	22.9	+	-
unknown sesquiterpene	41	23.0	+	-
caryophyllene	42	23.8	+	+
unknown sesquiterpene	42a	24.6	-	+
benzoic acid, methyl ester	43	24.6	+	+
acetophenone	44	25.6	+	+
unknown alcohol	44a	25.8	-	+
benzoic acid, ethyl ester	45	25.9	+	+
3-methylbutanoic acid (isovaleric)	46	27.0	+	+
$\gamma$ -hexalactone	47	29.0	+	-
benzyl alcohol	48	31.9	+	+
2-phenyl ethanol	49	32.9	+	+

<sup>a</sup> Peak numbers correspond to numbers in Figures 1-3. <sup>b</sup> Key: + = present; - = not detected.

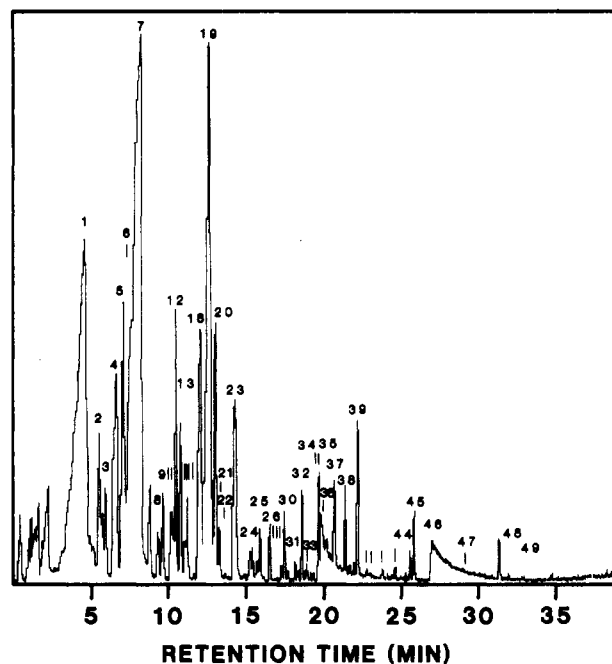


Figure 1. GC-MS total ion chromatogram of the headspace from the processed juice. Peaks are numbered according to Table I.

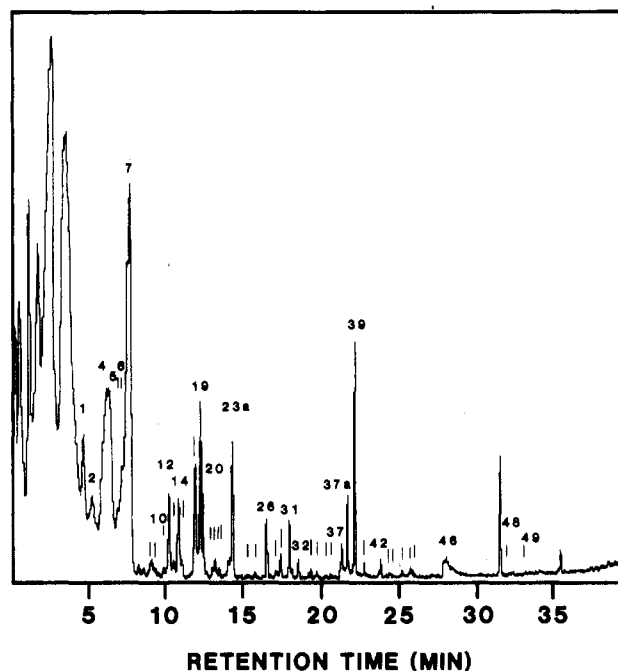
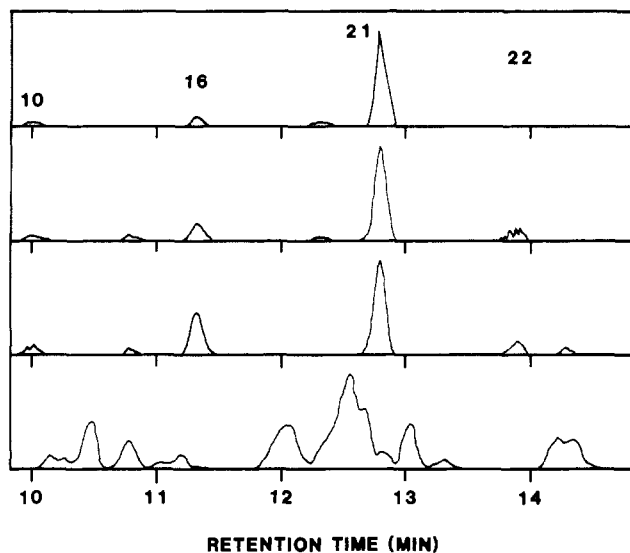


Figure 2. GC-MS total ion chromatogram of the headspace from the unprocessed juice. Peaks are numbered according to Table I.

or subtropical fruits such as wood apple (MacLeod and Pieris, 1981a), soursop (MacLeod and Pieris, 1981b), and passion fruit (Murray et al., 1972). It is suggested that these particular esters might be a characteristic of tropical fruits (MacLeod and Pieris, 1981b). The methyl and ethyl esters of benzoic acid are also present. Especially interesting is the wide variety of acids from which the esters were derived. The presence of the corresponding acids and alcohols (e.g., isovaleric acid and the ethyl and propyl isovalerates) helps to confirm the identity of the esters.

The presence of isobutyric and isovaleric acids in the juice contributes to the bite and pungent/sour odor characteristics of the cashew apple juice. When the pH, frequently between 4.4-6.0, is elevated to approximately



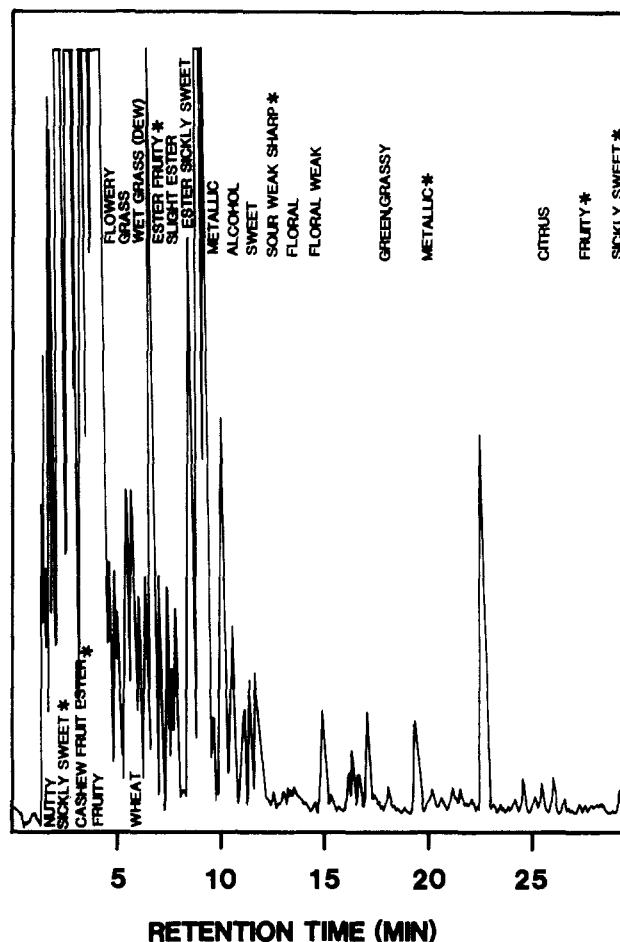
**Figure 3.** Computer-reconstructed mass chromatograms for terpene hydrocarbons in the processed juice. Top to bottom:  $m/z$  93, 121, 136, total ions. Terpene peaks are numbered according to Table I.

pH 9–10, the aroma of the juice changes, losing some sharpness. Aliphatic acids have also been recently reported as major volatile components in African mango (Sakho et al., 1985).

Some of the alcohols could not be completely identified. The spectra obtained were composites of two or more individual spectra, since the peaks were only partially resolved by the gas chromatographic system. However, those identified in Table I matched the spectra in the NBS library and also had the expected retention times. Benzyl alcohol, 2-phenylethanol, and 3-hexen-1-ol are well-known as aroma constituents in fruits in general.

Ethyl 2-mercaptopropionate was tentatively identified on the basis of the presence and relative abundance of the fragment ion peaks at  $m/z$  61, 88, and 134 in the mass spectra and upon detailed comparison of spectra that showed a very good match. However, the retention time (12.0 min) for standard ethyl 2-mercaptopropionate did not agree with the retention time (19.2 min) obtained for the compound in the cashew apple juice volatiles. Thus, the identity of this component is in doubt, though it may be structurally related. Ethyl 2-mercaptopropionate has been found in trace amounts among the volatile components of natural concord grape essence (Kolor, 1983), and it has a distinct characteristic odor.

The chromatograms were searched specifically for terpenes, since MacLeod and deTroconis (1982) reported terpene hydrocarbons as the major group of volatiles of Venezuelan cashew apple fruit. Analysis of the computer-reconstructed mass chromatograms for  $m/z$  93, 121, and 136 (Figure 3) showed the presence of four monoterpenes: carene, limonene,  $\beta$ -phellandrene,  $\gamma$ -terpinene. Carene was a minor constituent. MacLeod and deTroconis (1982) reported that carene was the major component and also a major contributor to cashew apple odor quality. This is rather unusual, since carene is a relatively rare terpene aroma volatile, although these authors have confirmed this compound in three out of four fruits studied of Venezuelan origin. Relative abundances of aroma compounds can vary considerably depending on cultivar. For example, carene content in mango ranged from nondetectable in three Indian and Sri Lankan cultivars to 26.0% of total aroma volatiles in a Venezuelan cultivar to 76.4% in one from Florida (MacLeod and Snyder, 1985). Since this current



**Figure 4.** Odor characterization following GC separation of the unprocessed juice. \*Most representative of the cashew apple juice flavor.

study worked with cashew apple juice from Brazil, the fruit's origin could be an explanation for the major differences between the main groups of volatile components of cashew apple from Venezuela and from Brazil. Another possibility can be the difference in the sampling technique. The previous work obtained the essence from the whole fruit plus water by a distillation-extraction method (MacLeod and deTroconis, 1982). The present work concentrated the headspace volatiles above the fruit juice in a polymer matrix.

MacLeod and deTroconis (1982) also detected in their sample a large proportion (26%) of aldehydes that represent a relatively high concentration in fruit aroma volatiles for this class of compound. Despite a considerable search for aldehydes, only two were identified in the cashew apple juice volatiles studied here. Even alkali treatment to dissociate possible  $\text{SO}_2$  adducts did not show additional aldehydes.

Sulfur compounds are important flavor contributors in different foodstuffs, generally appearing in small concentrations. Sulfur dioxide ( $\text{SO}_2$ ) is added as a preservative to the processed juice. Both juices were examined for sulfur-containing volatiles by direct headspace GC-FPD. As was expected,  $\text{SO}_2$  is the major sulfur compound observed in the processed juice but is absent in the unprocessed juice. Other sulfur compounds, DMS, DMDS, and DMTS, were detected in both juices but in low concentrations.

In order to identify those compounds mainly contributing to the characteristic flavor of the juice, the odors of the individual components of the essence were assessed at an odor port following GC separation. Considered in-

dividually, none of the compounds identified duplicate the flavor of the juice. This indicates that the typical flavor for cashew apple juice is probably the result of an integrated response to the contribution of a wide spectrum of compounds. However, a number of GC peaks were determined to have some importance to the overall cashew apple juice (Figure 4). Those peaks corresponding to esters such as methyl and ethyl butyrate and ethyl isovalerate contribute the sickly sweet and fruity flavors characteristic of the esters. The two acids (isovaleric and isobutyric) contribute the pungent/sour odor characteristics of acids. Although odor assessment was not performed on the sulfur compounds, these might also be important for the flavor intensity.

MacLeod and deTroconis (1982) found for Venezuelan cashew apple fruit essence that hexanal, carene, limonene, *trans*-hex-2-enal, and benzaldehyde were important components for the odor quality. Again, this shows that there is probably a considerable flavor difference between cashew apple from Venezuela and Brazil. These chemical and sensory differences deserve further study.

Sensory evaluation revealed that there was no significant difference in sweetness, as expected by the sample preparation, and also no difference in sourness, fruitiness, or bitterness. There was a distinguishable difference ( $p < 0.01$ ) in the overall flavor but no preference for either type of juice over the other.

Since the most striking difference between the juices was the presence of sulfur dioxide in the processed juice, this might be responsible for the difference in the overall flavor. Sulfur dioxide, added as a preservative, has a disagreeable odor. Elimination or replacement of this preservative may, by itself, improve the product. Reduction in the levels of free acids by processing or by genetic selection may also improve the flavor. The flavor of cashew apple juice is chemically complex, and a wide range of constituents contributes to the overall flavor. All of them must be considered in the design and operation of commercial juice processing facilities. Such considerations may lead to a stable acceptable product and to increased use of an underutilized resource.

**Registry No.** AcOEt, 141-78-6; PrCO<sub>2</sub>Me, 623-42-7; MeCOPr, 107-87-9; PrCO<sub>2</sub>Et, 105-54-4; EtCH(CH<sub>3</sub>)CO<sub>2</sub>Me, 863-57-5; MeS<sub>2</sub>Me, 624-92-0; *i*-PrCH<sub>2</sub>CO<sub>2</sub>Et, 108-64-5; AcO(CH<sub>2</sub>)<sub>2</sub>Pr-*i*, 123-92-2; BuCO<sub>2</sub>Et, 539-82-2; BuCO<sub>2</sub>Pr-*i*, 18362-97-5; *i*-

PrCH<sub>2</sub>CO<sub>2</sub>Pr, 557-00-6; MeCH=CHCO<sub>2</sub>Et, 10544-63-5; EtCH(CH<sub>3</sub>)CH<sub>2</sub>CO<sub>2</sub>Et, 5870-68-8; BuCH<sub>2</sub>CO<sub>2</sub>Me, 106-70-7; EtC(=CH<sub>2</sub>)CO<sub>2</sub>Me, 2177-67-5; *i*-Pr(CH<sub>2</sub>)<sub>2</sub>OH, 123-51-3; AcOCH<sub>2</sub>Bu, 623-63-7; BuCH<sub>2</sub>CO<sub>2</sub>Et, 123-66-0; Me<sub>2</sub>C=CHCO<sub>2</sub>Et, 638-10-8; PrCO<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>Pr-*i*, 106-27-4; BuCO<sub>2</sub>Bu-*i*, 10588-10-0; PrCH(CH<sub>3</sub>)CO<sub>2</sub>Bu, 6297-41-2; EtCH=CHCO<sub>2</sub>Me, 818-59-7; MeCH(OH)COMe, 513-86-0; Bu(CH<sub>2</sub>)<sub>2</sub>CO<sub>2</sub>Et, 106-30-9; PrCH=CHCO<sub>2</sub>Et, 1552-67-6; *i*-Pr(CH<sub>2</sub>)<sub>3</sub>OH, 1320-98-5; MeS<sub>3</sub>Me, 3658-80-8; *i*-PrCH<sub>2</sub>CO<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C(CH<sub>3</sub>)=CH<sub>2</sub>, 54410-94-5; EtCH=CH(CH<sub>2</sub>)<sub>2</sub>OH, 544-12-7; Bu(CH<sub>2</sub>)<sub>3</sub>CO<sub>2</sub>Et, 106-32-1; MeCH(SH)CO<sub>2</sub>Et, 19788-49-9; *i*-PrCO<sub>2</sub>H, 79-31-2; Et<sub>3</sub>CHOH, 597-49-9; PhCHO, 100-52-7; BuCH(CH<sub>3</sub>)(CH<sub>2</sub>)<sub>2</sub>CO<sub>2</sub>Et, 56196-53-3; PhCO<sub>2</sub>Me, 93-58-3; AcPh, 98-86-2; PhCO<sub>2</sub>Et, 93-89-0; *i*-PrCH<sub>2</sub>CO<sub>2</sub>H, 503-74-2; PhCH<sub>2</sub>OH, 100-51-6; Ph(CH<sub>2</sub>)<sub>2</sub>OH, 60-12-8; β-phellandrene, 555-10-2; carene, 74806-04-5; limonene, 138-86-3; pyridine, 110-86-1; γ-terpinene, 99-85-4; furfural, 98-01-1; isoctanol, 26952-21-6; octanol, 29063-28-3; caryophyllene, 87-44-5; γ-hexalactone, 695-06-7.

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