Volatile Compounds of Cashew Apple (*Anacardium occidentale* L.)

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The volatile compounds of a largely consumed Brazilian cashew apple variety (*Anacardium occidentale* L. var. *nanum*, Anacardiaceae) were recovered by headspace extraction or simultaneous distillation-extraction. Several compounds including esters (29), terpenes (16), hydrocarbons (9), carboxylic acids (7), aldehydes (7), alcohols (3), ketones (2), lactones (2) and norisoprenoids (1) were characterized and quantified by gas chromatography–mass spectrometry analyses.

Introduction

The *Anacardium occidentale* L. (Anacardiaceae) is a native of Brazil now cultivated throughout the tropics (Shobha *et al.*, 1992; Lima *et al.*, 1988). The species has been economically explored for decades, becoming better known for the popular cashew nut, of which approximately a quarter (30.000 t) of the best Brazilian production have been annually exported for about US$ 150 million (Secex, 1999; Pessoa *et al.*, 1995).

The rivalry in cashew nut market, nowadays mainly held by India and Brazil, has been emphasizing needs for a strategic management of cashew agriculture which, in Brazil, has been estimated to comprise an area of 6.300 km^2, greatly concentrated at Ceará State (Pimentel, 1996). In this sense, special attention has been drawn to cashew apple market as it is considered fundamental for cashew agribusiness sustainability (Barros *et al.*, 1999; Pessoa *et al.*, 1995).

Although a number of processes have already been developed for converting cashew apple into edible derivatives, little chemical data have been reported about cashew apple volatile compounds in spite of their relevance as flavorings. Previous studies concerning this subject have shown monoterpene 3-carene as the predominant constituent of a Venezuelan cashew apple (*A. occidentale* L.) extract obtained by simultaneous distillation-extraction (MacLeod and Troconis, 1982), and esters of 3-methylbutanoic acid as major constituents of a Brazilian cashew apple (*A. occidentale* L.) extract obtained by headspace extraction (Maciel *et al.*, 1986). Such controversy, interesting from the natural product chemistry point of view, was considered difficult to argue because authors used distinct methods for volatile sampling. Thus, in order to provide more facts for discussion, complementary data about Brazilian cashew apple is presented in the following report.

Experimental

Plant material

Ripe cashew apples (*A. occidentale* L. var. *nanum*) from Ceará State, Brazil, were purchased from a local market (CEASA – RJ). They were used fresh, right after detachment of cashew nuts under running tap water.

Simultaneous distillation-extraction (SDE)

Fresh cashew apples (300 g) were cut (4 pieces) and transferred into a 500 ml round-bottom flask to which distilled water (200 ml) was added. Dichloromethane (30 ml) was transferred into a 50 ml pear-shaped flask and used as extracting solvent. The SDE apparatus head containing a water-cooled condenser (~2 °C) was attached, connecting both distillation and extraction flasks. Independent SDE processes lasting either 20 min or 4 h were carried out in parallel under atmospheric pressure. A previously chosen internal standard (1 μl of 2.0% linalool in dichloromethane) was added to the extracts obtained, which were then dried with anhydrous sodium sulfate, filtered, concentrated to 200 μl under a gentle N_2 stream, and subjected to GC, GC-MS analyses.

Headspace extraction (HE)

Fresh cashew apples (1,5 kg) were cut (4 pieces) and transferred into an Erlenmeyer flask (4 l), which was then closed by a cold finger (25 × 5 cm) filled with dry ice (CO_2). Volatile compounds were collected every 30 min, during 5 h, by carefully removing the cold finger from the flask and rinsing its surface with dichloromethane. After addition of internal standard (1 μl of 2.0% linalool in dichloromethane) the extract was treated as described above.

Gas chromatography (GC)

High resolution gas chromatography (HRGC) analyses were performed with a HP 5890-II gas chromatograph (Hewlett Packard, Palo Alto, USA) equipped with a flame ionization detector (FID) and a HP-5 fused silica capillary column (30 m × 0.25 mm d.i., d.f. = 0.25 μm, 5% phenylmethylsilicone). Hydrogen was used as carrier gas at a linear velocity of 50 cm/s. Injector and detector temperatures were 250 °C and 280 °C, respectively, and the following oven temperature program was applied: 40 °C (10 min) to 280 °C (10 min), at an increasing rate of 3 °C/min. Sample volumes were 1 μl (splitless mode), and data were recorded on an HP 3396-II integrator.

Quantitative analyses were based on GC peak area ratios to internal standard, and retention indices (RI) were determined according to E. Kovats (Grob, 1995), using a mixture of linear hydrocarbons (C_8-C_{21}, Aldrich, St. Louis, USA) as reference.

Mass spectrometry (MS)

HRGC-MS analyses were performed under electron impact ionization (70 eV), on a HP 5972 MSD (Hewlett Packard, Palo Alto, USA) coupled to a HP 5890-II GC. Column and temperature program were the same as for CG analyses. The GC-MS interface was at 280 °C and the MS scan range was m/z 40 to 400.

Results and Discussion

The yields of HE, SDE/20 min, and SDE/4 h of cashew apple are presented in Table I. Though quantitatively superior in comparison to the HE procedure, recovering of cashew apple volatiles by SDE resulted in extracts of decreased qualitative complexity. This is due to several reasons since uncontrolled heat-promoted changes in molecular interactions were likely to occur during SDE affecting volatile release from cashew apple. Decomposition of compounds during SDE (Shimoda *et al.*, 1995) and decrease in solute transfer to the vapor phase due to increase in medium viscosity (Roberts *et al.*, 1996) and/or flavor binding to denatured proteins/enzymes (Lubbers *et al.*, 1998; Druaux *et al.*, 1995) could all have been occurred simultaneously.

Table I. Total recovery of cashew apple volatile compounds by headspace extraction (HE) and simultaneous distillation-extraction (SDE) procedures.

	HE/5 h	**SDE/20 min**	**SDE/4 h**
Yield [μg/kg]*	305	445	1000
Compounds characterized [μg]	99	227	880

* Mass per kilogram of fresh cashew apple.

The partial composition of the extracts analyzed are shown in Table II. Seventy six compounds comprising esters (29), terpenes (16), hydrocarbons (9), carboxylic acids (7), aldehydes (7), alcohols (3), ketones (2), lactones (2), and norisoprenoids (1) were characterized by GC-MS and RI constituting, as calculated from GC peak area ratios to internal standard, approximately 32%, 51%, and 88% of cashew apple HE, SDE/20 min and SDE/4 h extracts, respectively.

Remarkably, compound ethyl 2-hydroxy-4-methylpentanoate was exclusively detected in the SDE/4 h extract accounting for 70% (702 μg) of its total mass. Though now considered a thermal artefact, this hydroxy ester indicated a high concentration of leucine in cashew apples analyzed, since 2-hydroxy-4-methylpentanoic acid has been identified as a leucine plant metabolite (Yu and

Table II. Compounds characterized in cashew apple extracts obtained by headspace extraction (HE) and simultaneous distillation-extraction (SDE).

Compound	Relative concentration [µg/kg][a]			RI[b]
	HE/5 h	SDE/20 min	SDE/4 h	
Esters				
Methyl 3-methylbutanoate	nd	nd	nd	861
Ethyl 2-butenoate[c]	nd	nd	nd	863
Ethyl 2-methylbutanoate	nd	nd	nd	864
Ethyl 3-methylbutanoate[c]	nd	nd	nd	867
Methyl 2-methyl-2-butenoate	nd	nd	nd	873
Ethyl pentanoate[c]	<0.1	–	–	902
Methyl hexanoate[c]	<0.1	0.6	0.2	927
Ethyl 2-methyl-2-butenoate[c]	9.9	65.2	11.7	948
Ethyl hexanoate[c]	20.7	26.3	34.5	1002
Z-Ethyl 3-hexenoate	0.8	1.4	–	1008
E-Ethyl 3-hexenoate	0.2	0.1	0.1	1012
Ethyl 2-hexenoate[c]	3.5	3.5	2.1	1046
Ethyl 2-hydroxy-4-methylpentanoate	–	–	702.0	1078
Methyl benzoate[c]	–	0.2	1.1	1095
Pentyl isopentanoate	<0.1	0.9	1.3	1106
Butyl 2-methyl-2-butenoate[c]	<0.1	0.1	<0.1	1136
Ethyl benzoate[c]	0.3	3.7	2.1	1170
Ethyl octanoate[c, d]	9.8	15.8	5.8	1190
Ethyl benzenoacetate	<0.1	0.7	0.6	1243
Ethyl 2-octenoate	0.2	0.2	0.3	1246
Hexyl 2-methyl-2-butenoate	–	0.1	<0.1	1330
Z-Ethyl cinnamate	–	–	1.5	1373
E-Methyl cinnamate	<0.1	2.2	1.1	1378
Ethyl decanoate	1.6	1.4	0.1	1396
E-Ethyl cinnamate	0.3	9.9	13.9	1463
Hexyl benzoate	–	–	0.9	1577
Ethyl dodecanoate	0.3	0.3	<0.1	1595
Ethyl tetradecanoate	<0.1	0.4	<0.1	1794
Ethyl hexadecanoate	<0.1	1.3	<0.1	1995
Lactones				
γ-Nonalactone	<0.1	0.1	0.5	1359
γ-Dodecalactone	0.6	33.3	50.8	1659
Carboxylic acids				
Octanoic acid	0.4	0.3	0.3	1186
Nonanoic acid	0.4	0.6	1.0	1282
Decanoic acid	0.4	0.5	<0.1	1379
Dodecanoic acid	1.6	1.2	1.1	1569
Tetradecanoic acid	3.6	0.1	1.0	1776
Hexadecanoic acid	18.6	8.7	22.3	1986
Octadecanoic acid	7.6	–	–	2177
Aldehydes and Ketones				
Furfural[c, d]	–	–	nd	nd
Hexanal[d]	nd	nd	nd	799
Benzaldehyde[c, d]	<0.1	0.9	1.1	956
Phenylacetaldehyde[d]	<0.1	0.8	3.3	1037
Nonanal[d]	0.5	24.5	0.4	1101
Decanal	0.3	1.2	0.4	1205
E-2-Decenal	0.2	3.0	0.6	1260
6-Methyl-5-hepten-2-one	<0.1	0.9	0.1	988
Acetophenone[c, d]	0.3	4.7	–	1060
Alcohols				
1-Octanol[c]	<0.1	0.2	–	1072
Hexadecanol	1.6	1.2	1.4	1880
Octadecanol	8.2	0.5	3.8	2084
Terpenes				
α-Tujene	0.2	–	–	930
o-Cymene	0.2	1.3	<0.1	1022
Limonene[c, d]	0.4	0.5	0.4	1027
α-Cubebene	0.3	0.1	<0.1	1348
α-Copaene	0.3	0.1	–	1374
α-*cis*-Bergamotene	–	0.1	0.6	1414
β-Caryophyllene[c, d]	0.2	0.9	0.6	1417
α-*trans*-Bergamotene	0.2	1.3	1.6	1435
γ-Muurulene	<0.1	0.1	<0.1	1474
β-Bisabolene	nd	0.7	1.2	1507
γ-Cadinene	<0.1	0.1	<0.1	1513
δ-Cadinene	0.3	0.6	0.5	1523
α-Calacorene	<0.1	0.1	0.1	1540
α-Muurulol	<0.1	<0.1	<0.1	1643
α-Cadinol	–	–	1.1	1656
Curcufenol	0.4	–	–	1715
Norisoprenoids (C_{13})				
Geranylketone	0.7	<0.1	<0.1	1452
Hydrocarbons				
Naphthalene	0.2	0.1	<0.1	1175
Tridecane	0.1	0.2	0.3	1299
Tetradecane	0.2	0.3	0.3	1399
Pentadecane	0.3	1.0	1.4	1500
Hexadecane	0.3	0.1	0.6	1600
Heptadecane	0.3	0.2	0.7	1700
Octadecane	<0.1	<0.1	0.2	1800
Nonadecane	<0.1	<0.1	0.5	1900
Eicosane	0.2	0.6	0.7	2000

[a] Mass per kilogram of fresh cashew apple. [b] Retention indices on HP-5 calculated according to E. Kovats (Grob, 1995). [c] Previously characterized in Brazilian cashew apple HE extract (Maciel *et al.*, 1986). [d] Previously characterized in Venezuelan cashew apple SDE/4h extract (McLeod and Troconis, 1982). nd: Not determined. –: Not detected.

Spencer, 1969). However, the presence of other leucine derivatives such as 3-methylbutanoate esters (Schreier, 1984; Schwimmer, 1981), abundant in previously studied Brazilian cashew apples (Maciel *et al.*, 1986), was detected in all extracts analyzed (Table II). Whereas this result is in keeping with earlier reports concerning amino acid composition of some cashew apple (*A. occidentale*) varieties (Xavier Filho *et al.*, 1990; Nagajara and Nampoothiri, 1986) it contrasts with the scarcity of esters (0.7%) in Venezuelan cashew apple SDE/4 h extract (MacLeod and Troconis, 1982), thus emphasizing that ecological factors and/or plant cultivar rather than extraction methods could have determined the chemical differences between earlier analyzed Venezuelan (MacLeod and Troconis, 1982) and Brazilian (Maciel *et al.*, 1986) cashew apple volatile compounds.

The aldehydes characterized indicated the occurrence of oxidation reactions, which were clearly impaired during SDE procedures. Compounds such as nonanal, decanal, and 2-decenal, most concentrated in the SDE/20 min extract, have been reported as typical degradation products of oleic acid (Shipe, 1980; Tressl *et al.*, 1980). As quantitatively demonstrated recently (Rocha *et al.*, 1998), escaping of vapors from the SDE system could have led to the low concentration of these aldehydes in the SDE/4 h extract, especially if they were bulky formed during the early events of the SDE process. Compounds such as benzaldehyde and phenylacetaldehyde were, in contrast, more concentrated in the SDE/4 h, thus suggesting their release as a late SDE event. Possible precursors of these aldehydes could have been glycosides, since benzyl and phenylethyl alcohols were found as bound aglycones in cashew apples from the same batch of those presently analyzed (Bicalho *et al.*, 2000).

Based on labeling studies (Albrecht *et al.*, 1992) the increased concentration of γ-dodecalactone in the SDE extracts apparently is an indication of linolenic acid degradation. The hexadecanoic (palmitic) acid was the most abundant fatty acid characterized, possibly because it is also one of the main primary products of fatty acid biosynthesis in higher plants (Salisbury and Moss, 1991; Kolattukudy, 1969). The above interpretations are consistent with the fact that oleic, linolenic, and palmitic acids have been previously characterized as the main fatty acids of cashew apple (*A. occidentale*; Maia *et al.*, 1975).

In contrast to results concerning Venezuelan cashew apples, in which 3-carene alone accounted for 24% of a SDE/4 h extract (MacLeod and Troconis, 1982), 3-carene was not detected in any of the extracts analyzed. This again suggested that ecological factors and/or plant cultivar would have determined the chemical differences between Venezuelan (MacLeod and Troconis, 1982) and Brazilian (Maciel *et al.*, 1986) cashew apple volatiles.

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