

Analysis of Volatile Compounds of Taperebá (*Spondias mombin* L.) and Cajá (*Spondias mombin* L.) by Simultaneous Distillation and Extraction (SDE) and Solid Phase Microextraction (SPME)

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Volatile compounds of cajá and taperebá fruits, both classified as *Spondias mombin*, but from different geographic origins, were extracted (and analyzed) using solid phase microextraction (SPME) and simultaneous distillation and extraction (SDE). Forty-eight compounds were identified in taperebá and 47 in cajá by SPME using a DVB/CAR/PMDS fiber. (*E*)-Caryophyllene (18.7%), ethyl butyrate (10.0%), and ethyl hexanoate (7.0%) were the most abundant components in taperebá volatiles extracted by SPME, whereas myrcene (41.1%) and β -phellandrene (8.5%) were the major compounds in cajá. In the taperebá SDE extract, 46 substances were identified, and (*Z*)-caryophyllene (13.2%) and limonene (9.5%) were predominant. From the 42 substances found in the SDE extract of cajá, the major components were myrcene (38.0%) and *p*-cymene (6.2%). The two fruits showed similar chromatograms upon the use of SDE and SPME. These methods made it possible to determine 30 identical components in both fruits by using SDE and 32 by using SPME.

KEYWORDS: Taperebá; cajá; fruit flavors; volatile compounds; *Spondias mombin*; Anacardiaceae

INTRODUCTION

Tropical fruits have found a firmly growing acceptance in the markets of Europe and the United States (1). This increasing interest may be related to the public's nutritional awareness and search for a healthier lifestyle, as well as for their exotic flavors (2). They are consumed mainly as juice fruits and also for fortification of others juice fruits, such as to improve their vitamin C contents (2). In Brazil, tropical fruits are eaten fresh locally during the whole year and commercialized to others areas generally as frozen pulp. That is the case of taperebá (*Spondias mombin* L.), an ellipsoid drupe of 3–4 cm in length, having a thin and smooth yellow-orange skin, presenting a sweet–acidic pleasant flavor, which is commercialized for the production of ice cream and frozen pulp all over the country (3). Taperebá is known by different names according to the country area, being called taperebá in the north (Amazon), cajá in the northeast, and cajá-mirim in the southeast. Due to its exotic and pleasant perceived flavor, taperebá is outstanding in economic importance, likewise cupuaçu, bacuri, and açai, which also are native to the Amazon region (4, 5). It is worth pointing out that

although cajá has an aspect similar to taperebá, the perceived flavors of the two fruits are different. The volatiles of taperebá were previously studied by one of us using simultaneous distillation–extraction (SDE) two decades ago along with those of bacuri, cupuaçu, and muruci, but at that time only eight compounds were identified (6).

The volatile composition of cajá fruit was studied more recently (7, 8), but the authors claimed to have studied the fruits of *Spondia lutea* L., following the earlier work of Cruz (9). Because Cavalcante reported that *S. mombim* L. may be alternatively classified as *S. lutea* L., the two fruits can be classified as belonging to the same species (3). In addition, Maia and collaborators published a book about essential oils of the aromatic plants of the Amazon region and reported taperebá and cajá as either *Spondias mombin* L. or, alternatively, *S. lutea* L. (10).

SDE has been one of the most often cited methods for the isolation of volatiles since its first report in 1964 (11–13). Solid phase microextraction (SPME), on the other hand, is a relatively new methodology. It was introduced in 1990 and, in comparison with SDE, is simple, rapid, solvent-free, and inexpensive (14, 15).

The objective of the present study is to investigate the volatile composition of taperebá and cajá fruits collected in their native regions of Brazil, Pará State and Ceará State, respectively, using

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Table 1. Volatile Composition Identified for Cajá and Taperebá by SDE and SPME

substance	RI _{exptl} ^a	RI _{ref} ^b	SPME		SDE ^c		
			Taperebá ^d (%)	Cajá ^d (%)	Taperebá ^d (%)	Cajá ^d (%)	
esters							
ethyl butyrate ^g	801	800	10.0	0.6	6.1	0.9	
butyl acetate	820	812	3.4	1.6	1.3	1.4	
isopentyl acetate	875	876	0.3	tr ^e	— ^f	—	
propyl butyrate ^g	895	896	tr	—	—	—	
ethyl pentanoate ^g	898	898	0.1	—	—	—	
pentyl acetate	914	915	tr	—	—	—	
isobutyl butyrate	961	961	—	0.6	—	—	
butyl butyrate ^g	999	993	2.8	3.9	3.8	4.1	
ethyl hexanoate ^g	1001	1002	7.0	4.9	6.2	3.3	
hexyl acetate ^g	1010	1008	0.3	0.8	0.5	—	
butyl (<i>E</i>)-2-butenolate	1045	1045	0.1	tr	0.1	0.1	
methyl benzoate	1092	1091	0.3	—	0.6	1.3	
pentyl butyrate	1096	1093	0.1	—	—	—	
ethyl 3-hydroxyhexanoate ^g	1126	1134	0.4	0.1	0.2	1.1	
butyl 3-hydroxybutanoate	1133	1133	—	—	—	0.1	
ethyl benzoate	1171	1170	0.2	0.1	0.1	0.1	
butyl hexanoate	1188	1188	1.2	2.0	3.7	0.3	
ethyl octanoate	1194	1195	0.9	0.2	1.7	0.2	
octyl acetate	1208	1211	0.1	0.1	0.2	—	
butyl (<i>E</i>)-2-hexenoate	1243	1243	—	tr	—	—	
benzyl butyrate ^g	1339	1345	—	—	—	0.9	
butyl octanoate	1389	1389	—	0.4	—	—	
ethyl cinnamate	1464	1462	0.3	0.1	1.4	0.6	
aldehydes and carboxylic acids							
(<i>E</i>)-2-hexenal	857	854	—	0.1	0.1	tr	
benzaldehyde ^g	955	961	—	—	—	0.2	
5-methylfurfural	963	963	—	0.9	—	—	
octanoic acid ^g	1179	1179	—	—	0.3	1.2	
simple alcohols and ketones							
(<i>Z</i>)-3-hexenol	861	857	—	0.1	0.3	—	
hexanol ^g	873	867	—	0.5	0.3	0.3	
5-cepranone	1618	1618	—	—	0.4	—	
lactones							
γ -hexalactone	1056	1059	—	—	—	0.1	
monoterpene hydrocarbons							
α -pinene ^g	932	939	0.2	0.4	—	0.2	
camphene	948	953	—	2.5	—	0.3	
sabinene	973	976	—	1.4	—	0.4	
β -pinene	979	980	—	0.1	—	—	
myrcene	991	991	0.1	41.1	0.7	38.0	
α -phellandrene	1005	1005	—	0.6	—	0.1	
α -terpinene	1018	1021	—	—	—	0.4	
<i>p</i> -cymene	1025	1026	—	0.4	—	6.2	
limonene ^g	1029	1031	0.5	—	9.5	—	
β -phellandrene	1031	1031	—	8.5	—	—	
<i>cis</i> - β -ocimene	1039	1040	0.1	0.2	2.6	0.1	
<i>trans</i> - β -ocimene	1049	1050	tr	1.1	1.5	1.1	
γ -terpinene	1060	1053	—	0.1	—	—	
terpinolene	1087	1088	tr	0.2	0.5	0.5	
<i>allo</i> -ocimene	1129	1129	0.3	0.2	0.9	—	
monoterpene alcohols							
<i>cis</i> -linalool oxide	1070	1074	tr	0.1	0.2	0.3	
linalool ^g	1093	1098	0.4	1.1	0.4	0.8	
myrcenol	1117	1118	tr	—	0.1	0.4	
<i>cis</i> - β -terpineol	1150	1144	—	—	0.2	—	
α -terpineol ^g	1180	1189	0.1	0.2	2.0	5.1	
sesquiterpene hydrocarbons							
α -cubebene	1349	1351	—	tr	—	—	
α -longipinene	1351	1351	0.1	—	0.3	—	
cyclosativene	1358	1368	0.2	—	0.2	—	
α -copaene	1366	1376	7.2	0.7	4.4	—	
(<i>Z</i>)-caryophyllene ^g	1406	1404	1.7	0.1	13.2	4.9	
α -gurjunene	1409	1409	2.5	0.1	0.6	—	
(<i>E</i>)-caryophyllene	1419	1418	18.7	0.7	0.1	0.1	
<i>trans</i> - α -bergamotene	1438	1436	1.1	0.1	0.4	0.1	
α -humulene	1450	1454	0.4	0.2	0.2	1.8	
(<i>E</i>)- β -farnesene ^g	1455	1458	0.4	0.1	0.7	0.1	
<i>allo</i> -aromadendrene	1460	1461	0.2	—	—	—	
α -amorphene	1482	1482	1.2	—	2.1	—	
β -selinene	1489	1485	0.2	0.1	0.7	0.1	
α -selinene	1493	1494	1.1	0.1	0.1	0.1	
α -muurolene	1499	1499	0.8	—	0.1	0.1	

Table 1 (Continued)

substance	RI _{exptl} ^a	RI _{ref} ^b	SPME		SDE ^c	
			Taperebá ^d (%)	Cajá ^d (%)	Taperebá ^d (%)	Cajá ^d (%)
<i>trans</i> -β-guaiene	1503	1500	—	—	—	0.1
β-bisabolene	1508	1509	—	—	—	0.1
γ-cadinene	1512	1513	0.1	—	3.1	—
δ-cadinene	1524	1524	2.7	0.1	0.2	—
α-cadinene	1541	1538	0.1	—	—	—
cadina-1,4-diene	1532	1532	0.2	—	0.3	—
caryophyllene alcohol	1570	1568	tr	—	0.2	—

^a Retention indices obtained using a series of *n*-alkanes (C₇–C₂₆) on an Ultra-2 (5% phenyl-polymethylsiloxane) column (25 m × 0.25 mm i.d.). ^b Literature data (12).

^c Peak area percentage of total FID area excluding the solvent peak. ^d Percent must be considered approximations. ^e tr: trace (concentration < 0.1%). ^f —, not detected.

^g Also identified by co-injection of reference standards.

these two methods and to compare qualitatively the results in order to try to explain the differences found in the perceived flavors of the two fruits.

MATERIALS AND METHODS

Plant Material. Ripe taperebá fruits were manually collected in February 2002 in the northern part of Brazil, municipality of Salinópolis (south latitude 0° 39' 00", Greenwich west longitude 47° 19' 30"), Pará state. The species were identified as *Spondias mombin* L. by a local botanist and deposited at the herbarium of Embrapa Amazonia Oriental, voucher no. IAN 175165. Taperebá is an ellipsoid drupe of 4 cm in length having a thin and smooth orange skin, presenting a sweet–acidic flavor, and weighing between 14 and 16 g.

Ripe cajá fruits were manually collected in April 2001 in the northeastern part of Brazil, municipality of Maranguape (south latitude 3° 53' 00", Greenwich west longitude 38° 41' 00"), Ceará state. The species was identified by a local botanist as *Spondias mombin* L. and deposited at the Prisco Bezerra Herbarium in Universidade Federal do Ceará, under no. 18437. Cajá has an aspect similar to that of taperebá, an ellipsoid drupe of 3 cm in length having a thin and smooth orange skin presenting a sweeter flavor. The fruits are not as heavy as taperebá, weighing between 6 and 9 g. In both cases, fruits were frozen immediately after ripening and sent by plane to Rio de Janeiro. The fruits were received in two different packages. Each batch to be analyzed consisted of the homogenized mixture of the pulp of ~10 fruits (~40 g for cajá and 100 g for taperebá), which were frozen until analysis.

Simultaneous Distillation and Extraction. The extraction was carried out in a modified Likens–Nickerson apparatus during 2 h (16). Forty grams of the pulp puree and 50 mL of distilled water were added to a 100 mL round-bottom flask. Dichloromethane (1.5 mL) was added to a 4 mL conical flask. Both flasks were connected to the apparatus, and more dichloromethane and distilled water were added in the central arm. The flask containing dichloromethane was heated by using a water bath at 60 °C (bath temperature), and the flask with the water was heated by using an oil bath at 120 °C (bath temperature).

Solid Phase Microextraction. The SPME apparatus was purchased from Supelco Inc. (Bellefonte, PA). A 50/30 μm divinylbenzene–carboxen–poly(dimethylsiloxane) fiber and a 100 μm poly(dimethylsiloxane) (PDMS) fiber were used. The fibers were activated according to the manufacturer's instructions. For each extraction, 2.0 g of the pulp puree and 0.5 g of NaCl were placed in a 4 mL vial. In each extraction the sample was kept for 1 h in reposal to achieve the partition equilibration between the sample and headspace. After this time, the SPME fiber was exposed to the headspace of the solution to adsorb the analytes. After 3 min, the fiber was withdrawn into the needle and then introduced into a heated chromatograph injector for desorption and analysis.

Gas Chromatography. The analysis was performed in an HP 5890 series II gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with a 25 m × 0.25 mm Ultra 2 (5% phenyl-polymethylsiloxane) capillary column (film thickness = 0.33 μm) and a flame ionization detector (FID). The injector temperature was kept at 220 °C

and the detector at 280 °C. Hydrogen was used as the carrier gas at a linear velocity of 31.5 cm/s (measured at 100 °C). The oven temperature was programmed for 40 °C (2 min), increased at 3 °C/min to 280 °C, and held for 10 min. Injections were made in splitless mode. For SPME, the fiber was exposed inside the injector for 2 min (splitless time = 2 min). For SDE, 0.5 μL of the extract was injected (splitless time = 0.5 min).

Gas Chromatography—Mass Spectrometry. The volatile constituents were analyzed using an Agilent (Palo Alto, CA) 5973 mass selective detector coupled to an Agilent 6890 gas chromatograph, equipped with a 30 m × 0.25 mm HP-5 MS (5% phenyl-polymethylsiloxane) capillary column (film thickness = 0.25 μm). Helium was used as the carrier gas at a linear velocity of 31.5 cm/s (measured at 100 °C). The oven temperature program was the same as described under Gas Chromatography. Injections were made in splitless mode as described above. The quadrupole mass detector was operated at 150 °C in electron impact mode at 70 eV. The ion source temperature was 230 °C, and the transfer line was at 280 °C.

Identification of the Volatiles. As previously reported (17) in the literature and largely used (14, 15, 18), peaks were identified according to the linear retention indices (Kovats indices) and mass spectra. Linear retention indices were calculated by using a series of *n*-paraffins (C₇–C₂₆), and mass spectra were compared with either data compiled in a Wiley 6th edition library or published mass spectral data (19). However, to ensure a more positive identification, reference standards (Aldrich Chemical Co., St. Louis, MO) of ethyl butyrate, propyl butyrate, ethyl pentanoate, butyl butyrate, ethyl hexanoate, hexyl acetate, ethyl 3-hydroxyhexanoate, benzyl butyrate, benzaldehyde, octanoic acid, hexanol, α-pinene, limonene, linalool, α-terpineol, (*Z*)-caryophyllene, and (*E*)-β-farnesene were used in the present work.

No external or internal standards were used in the present work. Therefore, results were described in terms of relative (percent) areas and were visually reproducible. At least two parallel chromatographic analyses were made for each SDE extract.

Over five SDE and SPME experiments were carried out for each fruit sample.

Because relative area was used, no attempt to use any statistical method was made. Therefore, the results described in the present work are representative of a characteristic run of each sample; therefore, chromatographic data must be regarded as approximations.

RESULTS AND DISCUSSION

Forty-eight volatile substances were identified in the SPME of taperebá and 47 when this technique was applied to cajá. Terpenic hydrocarbons predominated in both fruits; (*E*)-caryophyllene (18.7%) was the major compound in taperebá, and myrcene (41.1%) was the major compound in cajá (Table 1). Similarly high terpene contents in tropical fruits have already been reported (18). Among the esters, the most abundant were ethyl butyrate (10.0%) and ethyl hexanoate (7.0%) in taperebá and ethyl hexanoate (4.9%) and butyl butyrate (3.9%) in cajá.

Analysis of the aroma using SDE led to the identification of 46 compounds in taperebá and 42 in cajá (Table 1). Myrcene

Table 2. Volatile Composition Identified for Cajá and Taperebá by SPME Using PDMS and DVB/CAR/PDMS Fibers

substance	Taperebá ^a (%)		Cajá ^a (%)	
	DVB/CAR/PDMS	PDMS	DVB/CAR/PDMS	PDMS
esters				
ethyl butyrate	10	1.7	0.6	0.5
butyl acetate	3.4	0.3	1.6	1.4
isopentyl acetate	0.3	tr ^b	tr	0.2
propyl butyrate	tr	— ^c	—	—
ethyl pentanoate	0.1	—	—	—
pentyl acetate	tr	—	—	—
isobutyl butyrate	—	—	0.6	—
butyl butyrate	2.8	2.3	3.9	4.8
ethyl hexanoate	7.0	7.0	4.9	1.5
hexyl acetate	0.3	0.3	0.8	0.1
butyl (<i>E</i>)-2-butenolate	0.1	0.1	tr	—
methyl benzoate	0.3	0.2	—	—
pentyl butyrate	0.1	0.1	—	—
ethyl 3-hydroxyhexanoate	0.4	—	0.1	—
ethyl benzoate	0.2	0.2	0.1	—
butyl hexanoate	1.2	2.1	2.0	—
ethyl octanoate	0.9	1.7	0.2	—
octyl acetate	0.1	—	0.1	2.4
butyl (<i>E</i>)-2-hexenoate	—	—	tr	—
benzyl butyrate	—	—	—	—
butyl octanoate	—	—	0.4	0.4
ethyl cinnamate	0.3	0.1	0.1	—
aldehydes and carboxylic acids				
(<i>E</i>)-2-hexenal	—	—	0.1	—
5-methylfurfural	—	—	0.9	—
simple alcohols and ketones				
(<i>Z</i>)-3-hexenol	—	—	0.1	—
hexanol	—	—	0.5	0.2
monoterpene hydrocarbons				
α -pinene	0.2	0.1	0.4	0.5
camphene	—	—	2.5	—
sabinene	—	—	1.4	1.3
β -pinene	—	—	0.1	—
myrcene	0.1	0.1	41.1	66
α -phellandrene	—	—	0.6	—
<i>p</i> -cymene	—	—	0.4	—
limonene	0.5	5.0	—	—
β -phellandrene	—	—	8.5	12
<i>cis</i> - β -ocimene	0.1	2.0	0.2	0.1
<i>trans</i> - β -ocimene	tr	2.0	1.1	1.4
γ -terpinene	—	—	0.1	0.1
terpinolene	tr	—	0.2	0.3
<i>allo</i> -ocimene	0.3	—	0.2	0.1
monoterpene alcohols				
<i>cis</i> -linalool oxide	tr	—	0.1	tr
linalool	0.4	0.1	1.1	1.5
myrcenol	tr	0.1	—	—
α -terpineol	0.1	0.1	0.2	—
sesquiterpene hydrocarbons				
α -cubebene	—	—	tr	—
α -longipinene	0.1	0.2	—	—
cyclosativene	0.2	tr	—	—
α -copaene	7.2	11.2	0.7	0.4
(<i>Z</i>)-caryophyllene	1.7	1.0	0.1	—
α -gurjunene	2.5	0.4	0.1	—
(<i>E</i>)-caryophyllene	18.7	32.8	0.7	0.8
<i>trans</i> - α -bergamotene	1.1	0.7	0.1	—
α -humulene	0.4	0.2	0.2	0.3
(<i>E</i>)- β -farnesene	0.4	0.5	0.1	0.3
<i>allo</i> -aromadendrene	0.2	0.2	—	—
α -amorphene	1.2	—	—	—
β -selinene	0.2	—	0.1	0.1
α -selinene	1.1	2.0	0.1	0.1
α -muurolene	0.8	0.5	—	—
γ -cadinene	0.1	0.2	—	—
δ -cadinene	2.7	3.5	0.1	0.1
α -cadinene	0.1	tr	—	—
cadina-1,4-diene	0.2	0.2	—	—
caryophyllene alcohol	tr	tr	—	—

^a Percent must be considered approximations. ^b tr, trace (concentration < 0.1%). ^c —, not detect.

(38.0%), α -terpineol (5.1%), (*Z*)-caryophyllene (4.9%), and butyl butyrate (4.1%) were the major compounds in cajá.

Curiously, (*Z*)-caryophyllene (13.2%), instead of the *E* isomer, was the most abundant component of taperebá when it was

analyzed by SDE. In fact, only 0.1% of the *E* isomer was detected. These results are compatible with the thermal isomerization of (*E*)-caryophyllene to the *Z* isomer via a Cope rearrangement (20). However, no other thermal isomerization process was noticed.

Hexanol, (*Z*)-3-hexenol, and (*E*)-2-hexenal were detected in low quantity. These substances, produced biosynthetically from unsaturated fatty acids, are the precursors for the straight-chain esters (21).

SPME consists of three processes: matrix desorption, adsorption of headspace analytes by the coating fiber, and thermal desorption of the adsorbed analytes into the gas chromatograph injection port. In the present work the fibers were never allowed to touch the samples.

For volatile compounds, the release of analytes into the headspace is relatively easy because analytes tend to vaporize once they are desorbed from their matrix. The most important experimental parameters, speed, sensitivity, accuracy, and precision, are determined largely during extraction. Because in the present work we were interested in the volatile composition of both fruits, we made no attempt to investigate how those parameters could influence the volatile composition. Therefore, we found that 1 h in equilibrium was sufficient to reach the partition equilibrium and 3 min was sufficient for fiber adsorption. In these conditions through visual inspection, the extraction method was reproducible. Almost identical chromatograms were observed with very small variability in relative peak area composition. Probably changes in these experimental conditions will affect desorption-adsorption and, consequently, sensitivity and reproducibility.

In SDE, distillation-extraction time and solvent choice seem to be the most important variables to affect sensibility and reproducibility. In the present work the volatile material was collected in 1.5 mL of dichloromethane. This solvent continuously extracted the water distillate, which was generated at ~100 °C (boiling point of water). External bath heating to 120 °C was necessary to keep a continuous smooth water steam distillation.

Dichloromethane was used because generally it appears to be the best solvent for extraction of a wide class of flavors. These variables, time and solvent, were considered to be important and we used the better conditions, ever trying to minimize alteration in volatile original composition (15).

After some assays, 2 h of continuous distillation-extraction with dichloromethane was found to be the best compromise. After this period of time, extensive browning started to occur and volatile composition suffered alteration, probably due to artifact formation and sugar degradation. The choice of dichloromethane was made because it appears to be the best solvent for extraction of a wide class of flavor compounds (11). However, even though the above compromise seemed to be the smoother and more reproducible, heating the fruit sample to 100 °C normally led to extensive glycoside degradation, increasing the relative yield of free terpenic alcohols such as α -terpineol (22). This phenomenon was previously investigated by Chassagne and Crouzet (22) and, although considered as a possibility, was not investigated in the present work.

SPME on the contrary is a rather mild method of analysis and normally does not lead to artifact formation. However, because fiber composition could affect the analyte's composition, we further investigated the volatile composition by using the same experimental conditions but with another fiber, polydimethylsiloxane (PMDS) (Table 2). In this case fewer compounds were extracted and the percentage of hydrocarbons in-

creased. For instance, the concentration of (*E*)-caryophyllene rose from 18.7% to 32.8% and poorer ester extraction occurred, therefore showing that fiber composition and, hence, affinity for the target analytes are crucial in SPME sampling. Nonpolar compounds are more likely to be extracted by nonpolar coatings and vice versa (15).

It is worth pointing out that due to the presence of the solvent it is impossible to detect some compounds in the SDE extracts. The presence of volatile compounds such as, for instance, some light esters was not observed, but they were, in turn, easily detected by using SPME.

The two fruits studied gave similar SDE extracts, and 30 compounds were present in both extracts. The same can be stated in relation to the data from SPME analysis, where 32 compounds were present in both extracts. The quantitative and qualitative differences in the volatile composition of the two fruits may account for the perceived flavor differences in these fruits. The differences found in the present work may be due to soil and/or climate factors of the different geographic regions of the country, as well as some diversity in the plant germplasm.

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