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# Flavor Composition of Cashew (*Anacardium occidentale*) and Marmeleiro (*Croton* Species) Honeys

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The aim of this work was to characterize the volatile fractions of two Brazilian honeys known as caju and marmeleiro. The volatile components were isolated by a column extraction technique using acetone as the extraction solvent. Totals of 59 and 36 volatile compounds were definitely or tentatively identified in the caju and marmeleiro honeys, respectively, using reference substances, mass spectral libraries, and the odor qualities of the compounds eluted from the GC column. Aroma extraction dilution analysis allowed the tentative identification of furfuryl mercaptan, benzyl alcohol, $\delta$-octalactone, $\gamma$-decalactone, eugenol, benzoic acid, isovaleric acid, phenylethyl alcohol, and 2-methoxyphenol as impact volatile compounds in the caju honey. In the marmeleiro honey, only isovaleric acid, $\gamma$-decalactone, benzoic acid, and vanillin were considered to be potent odorants. This study showed that the medium- to high-boiling volatile compounds are important contributors to the characteristic aroma of these honeys.

**KEYWORDS: Caju and marmeleiro honeys; aroma extraction dilution analysis; powerful odorants**

## INTRODUCTION

Honey aroma has been a matter of study for years. The composition of the volatile fraction is directed by floral origin, the foraging habits and physiology of the bees, and the postcollection processing and storage conditions of the product. Nowadays, >300 compounds have been identified and described as volatiles in honeys of different floral types. However, a large number of new volatile compounds are expected to be identified, because there are still many honey types not yet studied. In this context, the great variety of climate and the extremely rich flora of Brazil make possible the production of honeys (e.g., caju, marmeleiro, assa-peixe, and morrão-de-candeia honeys) with single properties, of which the aroma composition deserves a particular attention. The majority of studies on honey aroma employ sophisticated techniques such as gas chromatography (GC) coupled with mass spectrometry (MS). Some Australian honeys, for instance, were analyzed by using these techniques during a study of the storage influence on its aroma (*1*). Fifteen compounds were identified in the ethyl acetate extract of these samples. These authors reported that most of the components possessing honey-like aroma had high retention times relative to methyl anthranilate, as previously reported (*2*). More recently, the GC-MS analysis of the aroma fraction isolated from several strawberry tree blossom honey samples showed a marked predominance of norisoprenoids (*3*).

In the study of honey aroma, researchers have directed their efforts not only to determine the volatile composition of honeys but also to define the odor-active compounds and to typify honeys from a specific floral and/or geographic source. Steeg and Montag concluded that benzoic acid, phenylacetic acid, phenol, *p*-cresol, 2-methoxyphenol, and eugenol were important to the honey aroma bouquet (*4*). In another work, ~100 compounds were identified in an ethyl ether extract of haze honey by adsorptive column chromatography (*5*). Among them, phenylacetaldehyde, linalool, 2-phenylethanol, and lilac aldehydes appeared to contribute to haze honey aroma. The sensory importance of the volatile compounds of linden honey was investigated employing aroma extraction dilution analysis (AEDA) (*6*). These authors showed that 21 odor compounds presented high dilution factors (DF) (e.g., phenylacetaldehyde and *cis*-oxide rose). Other important work showed that citrus honeys contain methyl anthranilate, a compound which other honeys virtually lack, at a concentration >0.5 ppm (*7*). Tan et al. (*8*−*10*) reported that manuka and kanuka honeys contain much higher aromatic acid concentrations than honeys derived from white clover. A recent work reported that English honeys could be identified if the presence of 1-penten-3-ol in honey samples could be confirmed (*11*).

The aim of the present work was to characterize for the first time the volatile fraction of two distinct honeys from north-eastern Brazil known as caju and marmeleiro.

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## MATERIALS AND METHODS

**Samples.** Two samples of Brazilian caju honey (Anacardiaceae) and two of marmeleiro honey (Rosaceae) were obtained directly from reliable beekeepers from Ceará and Piauí States, respectively. These samples were harvested between 1997 and 1998 and immediately stored under nitrogen atmosphere at −18 °C in small plastic bottles.

**Materials.** Methanol and acetonitrile were of HPLC grade (Merck). Standards of fructose (99.95%), glucose (99.5%), sucrose (99.5%), turanose (98%), maltose (99%), isomaltose (98%), gentibiose (95%), melibiose (98%), erlose (97%), melezitose (99%), maltotriose (95%), raffinose (99%), panose (98%), and isomaltotriose (95%) were from Sigma (St. Louis, MO). Acetone (99.5%) and proline (>99%) were obtained from Merck (Darmstadt, Germany). Volatile standards of 1-hydroxy-2-propanone (90%), tetradecane (>99%), 2-cyclohexen-1-one (>95%), 2-furfural (99%), pentadecane (>99%), 2-methylpropanoic acid (99%), hexadecane (99%), heptadecane (99%), 2-(2-butoxyethoxy)-ethanol (>99%), octadecane (99%), 2-butyloctanol (95%), benzyl alcohol (99%), nonadecane (99%), phenylethyl alcohol (99%), eicosane (99%), heneicosane (98%), docosane (99%), tricosane (99%), tetracosane (99%), pentacosane (99%), 5-(hydroxymethyl)furfural (HMF; 99%), vanillin (99%), 1-octadecanol (99%), hexacosane (99%), 1-eicosanol (98%), hexadecanoic acid (98%), oleic acid (>99%), isovaleric acid (99%), 2,3-butanedione (97%), butyl butanoate (98%), menthol (99%), furfuryl mercaptan (98%), linalool (97%), 2-methoxyphenol (98%), and eugenol (99%) were purchased from Aldrich (Milwaukee, WI). $\delta$-Octalactone (>95%) and $\gamma$-decalactone (>95%) were generously supplied by IFF Essências e Fragrâncias Ltda (Rio de Janeiro, Brazil). The volatile standard benzoic acid (99.5%) was obtained from Carlo Erba (Milan, Italy). Porapak Q (50−80 mesh) was from Millipore Corp. (Bedford, MA). All other reagents were of analytical grade.

**Nonvolatile Fraction Analysis.** The floral origin of the honeys was monitored by pollen analysis (*12*). Free acidity, lactone acidity, total acidity, and pH were measured according to the AOAC method 31.160 (*13*). The moisture content of honey was determined by refractometry following AOAC method 31.119 (*14*) using an Abbé refractometer (Carl Zeiss) and the Chataway table. Photometrical analysis of free proline was carried out according to AOAC method 31.126 (*15*). Results were expressed as milligrams of proline per kilogram of honey. Diastase number was measured photometrically in a Titertek Multiskan plus instrument (Eflab) according to AOAC method 31.166 (*16*). Results were calculated and expressed in Gothe units (°*G*). The determination of 5-HMF was based on a previous HPLC method (*17*). The mono- and oligosaccharides were analyzed by another HPLC method (*18*). In both methods, quantification was achieved by peak height or area comparison with standards. In the first case, results were expressed as milligrams of HMF per kilogram of honey and, in the second case, as grams per 100 g of honey for each sugar.

**Isolation of Volatile Flavor Compounds.** *Column Extraction Method.* The isolation of the volatile components of both honeys was developed using a column extraction method modified from the method proposed by Shimoda et al. (*5*). In general, the column extraction technique is a very interesting method to isolate volatile compounds from a labile matrix such as honey, because no heating is involved in this process. The major changes between Shimoda's method and the current procedure were the reduced scale of the process and the employment of acetone as the extraction solvent instead of diethyl ether. Although the chromatographic profile of diethyl ether extract was somewhat richer than the acetone extract, the last one had a stronger honey-like odor. Another advantage of acetone is that there is no formation of reactive peroxides.

First of all, a small glass column packed with 750 mg of Porapak Q porous polymer beads (50−80 mesh) (Supelco, Bellefonte, PA) was activated by heating at 225 °C during 3 h under an $N_2$ flow of 0.9−1.0 $L \cdot min^{-1}$. Then, 100 mL of an aqueous honey solution (0.20 g/mL) was passed through the column. After that, the column was inverted and washed with 20 mL of deionized water. This last procedure was done to remove water-soluble constituents (sugars) that might generate volatile artifacts in contact with the injector of the GC-FID or GC-MS (230 °C). This volume was settled by monitoring 5 mL aliquots of the washing water until no more fructose and glucose could be detected using the HPLC method mentioned above (*18*). Adsorbed volatiles were then eluted with 100 mL of acetone, and the eluate was rota-evaporated (20 °C) until dryness and then taken up in 200 mL of acetone. Two different replicates were prepared from each of the samples.

**Table 1.** Free Acidity, Lactone Acidity, Total Acidity, pH, Moisture, Diastase Activity, Proline, HMF, and Sugar Contents of Honey Samples

| parameter | Caju honey | Marmeleiro honey |
|---|---|---|
| free acidity (mequiv/kg of honey) | 44.0/47.0 | 23.2/24.0 |
| lactone acidity (mequiv/kg of honey) | 7.0/6.8 | 5.1/7.0 |
| total acidity (mequiv/kg of honey) | 51.0/53.8 | 29.1/30.3 |
| pH | 3.9/3.7 | 3.7/3.7 |
| moisture (g/100 g of honey) | 17.1/17.1 | 20.5/20.5 |
| diastase number (°*G*) | 11.5/13.3 | 9.6/11.5 |
| 5-HMF content (mg/kg of honey) | 7.7/7.1 | 2.6/3.0 |
| proline content (mg/kg of honey) | 1323.9/1481.4 | 437.8/449.8 |
| fructose (g/100 g of honey) | 32.8/33.8 | 38.2/39.0 |
| glucose (g/100 g of honey) | 20.6/21.4 | 25.8/26.9 |
| sucrose (g/100 g of honey) | 0.48/0.52 | 0.06/0.18 |
| turanose (g/100 g of honey) | 0.78/0.90 | 1.10/1.18 |
| maltose (g/100 g of honey) | 1.10/1.50 | 1.80/2.40 |
| isomaltose (g/100 g of honey) | 0.50/0.70 | 1.60/2.20 |
| gentibiose (g/100 g of honey) | 0.15/0.25 | 0.42/0.58 |
| melibiose (g/100 g of honey) | 0.14/0.26 | 0.06/0.14 |
| erlose (g/100 g of honey) | 0.05/0.15 | 0.05/0.11 |
| melezitose (g/100 g of honey) | 0.23/0.37 | 0.03/0.10 |
| maltotriose (g/100 g of honey) | 0.15/0.25 | 0.5/0.7 |
| raffinose (g/100 g of honey) | 0.18/0.22 | 0.16/0.22 |
| panose (g/100 g of honey) | ND[b] | 0.26/0.34 |
| isomaltotriose (g/100 g of honey) | ND | 0.13/0.27 |

[a] According to the European Codex Honey Standards and Brazilian legal regulations (*21*, *25*) a well-processed and ready to be consumed honey must have the following characteristics: water maximum level, 20–21 g/100 g of honey; reducing sugars, ≥65 g/100 g; apparent sucrose content, ≤5 g/100 g; free acidity, ≤40 mequiv/kg; diastase no., ≥8 °*G*; 5-HMF content, ≤40 mg/kg of honey. [b] ND, not detected.

**Capillary Gas Chromatography.** Capillary GC analysis was carried out on a Carlo Erba gas chromatograph model FTV 4300 equipped with a flame ionization detector (FID). The chromatograms were obtained using a Shimadzu Chromatopak C-R6A integrator. Separation was achieved on a 30 m × 0.25 mm i.d. fused silica capillary column, coated with cross-linked poly(ethylene glycol) 20 M, with a film thickness of 0.25 mm (Supelcowax-10, Supelco). The oven temperature was programmed to rise from 50 to 230 °C at 3 °C/min. The last temperature was maintained for 30 min. The injector temperature was 230 °C, and the detector was held at 240 °C. Helium was employed as the carrier gas at an optimum linear speed of 28 cm/s (50 °C). An injection splitter was used at a split ratio of 20:1. Retention indices were estimated by using a modified Kovats method (*19*). The extract volume injected in the GC system was 2.0 $\mu$L.

**Capillary GC-MS.** Electron-impact mass spectrometric analyses were developed on a gas chromatograph−mass spectrometer system GC-17A/QP5050 from Shimadzu. The column and chromatographic conditions were the same as described for GC analysis. The mass spectrometer was operated at an ionization voltage of 70 eV and an ion source temperature of 240 °C. The MS identification was on the basis of comparison with the NIST12.lib and NIST62.lib mass spectral libraries.

**Gas Chromatography—Olfactometry (GC-O) and Aroma Extraction Dilution Analysis (AEDA).** The odor profile of each honey was assessed by direct sniffing of the GC eluate as it flowed from the chromatograph. The chromatographic conditions were the same as reported previously, excluding the use of a flow splitter to divide the GC effluent between the chemical detector and the sniffing port (1:10 FID/sniffing port). Five trained panelists (20−40 years old) performed the sensory analysis both on standard solutions and on the samples. Volatile compounds with a detectable odor were characterized by a description of their odors, and their retention times were recorded. To

**Table 2.** Volatile Compounds of Caju and Marmeleiro Honeys

| compound | KI[e] | RT[e] | odor note | Caju | | Marmeleiro | |
|---|---|---|---|---|---|---|---|
| | | | | FD[e] | ID[e] | FD[e] | ID[e] |
| 2,3-butanedione[a,d] | | 3.54 | rancid, buttery, cabbage-like | 0 | + | 2 | + |
| butyl butanoate[a,d] | 1224 | 10.33 | perfume, flowery, delightful | 16 | + | | – |
| 1-hydroxy-2-propanone* | 1296 | 13.11 | | | + | | + |
| tetradecane* | 1400 | 17.27 | | | + | | – |
| furfuryl mercaptan[a,d] | 1424 | 18.50 | coffee-like, sulfurous | 16 | + | | – |
| 2-cyclohexen-1-one* | 1425 | 18.51 | | | + | | – |
| linalool oxide (*trans*-furanoid)[b,c] | 1437 | 19.15 | | | + | | + |
| 1-hydroxy-2-pentanone[b] | 1445 | 19.51 | | | – | | + |
| 2,6,11-trimethyldodecane[b] | 1454 | 20.00 | | | + | | – |
| 2-furfural* | 1456 | 20.07 | | | + | | – |
| 2-hexyl acetate[b] | 1459 | 20.26 | | | + | | + |
| linalool oxide (*cis*-furanoid)[b–d] | 1474 | 21.00 | | | – | | + |
| 1,2-ethanediol diacetate[b] | 1477 | 21.15 | | | + | | + |
| 2-ethylhexanol[b,c] | 1479 | 21.23 | | | + | | + |
| 2-methyltetradecane[b] | 1483 | 21.42 | | | + | | – |
| pentadecane* | 1500 | 22.30 | | | + | | – |
| 4-methyl-3-heptanol[b] | 1505 | 22.47 | | | + | | + |
| linalool[a,d] | 1548 | 23.46 | green, refreshing, rain | | – | 0 | + |
| propanoic acid[b] | 1555 | 24.04 | | | + | | + |
| 2-methylpropanoic acid* | 1571 | 24.52 | isovaleric acid derivative, rank smell of perspiring feet | 4 | + | 8 | + |
| hexadecane* | 1600 | 25.44 | | | + | | – |
| 2,4-dimethyl-1,3-dioxolane-2-methanol[b] | 1624 | 26.41 | | | – | | + |
| menthol[a,d] | 1630 | 26.56 | rain, grass, lemon, mint | 0 | + | 2 | + |
| 2-methylhexadecane[b] | 1654 | 27.56 | | | + | | – |
| isovaleric acid[a,d] | 1674 | 28.38 | isovaleric acid like, cheese, rank smell of perspiring feet | 32 | + | 16 | + |
| 4-hydroxy-4-methyl-2-pentanone[b] | 1691 | 29.05 | | | – | | + |
| hexadecene[b] | 1699 | 29.33 | | | + | | – |
| heptadecane* | 1700 | 29.40 | | | + | | – |
| isomer of linalool oxide (epoxylinalool)[b–d] | 1744 | 31.11 | pleasant, popcorn-like, roast | | – | 8 | + |
| linalool oxide (*trans*-pyranoid)[b,c] | 1770 | 32.08 | | | – | | + |
| 2-(2-butoxyethoxy)ethanol* | 1796 | 33.09 | | | + | | – |
| octadecane* | 1800 | 33.25 | | | + | | – |
| 2-butyloctanol* | 1848 | 35.06 | | | + | | – |
| 2-methoxyphenol[a,d] | 1849 | 35.07 | burned thing, smoky odor | 1024 | + | | – |
| benzenemethanol (benzyl alcohol)* | 1877 | 36.15 | green, grass, Paraguay tea | 16 | + | | – |
| nonadecane* | 1900 | 37.04 | | | + | | – |
| benzene ethanol (phenylethyl alcohol)* | 1929 | 38.01 | floral, herb-like, spicy | 128 | + | 0 | + |
| (*Z*)-3-hexenyl hexanoate[b,c] | 1945 | 38.51 | | | + | | + |
| $\delta$-octalactone[a,d] | 1970 | 39.34 | very sweet, sucrose, honey, delightful, vanillin-like, wood-like | 16 | + | 0 | + |
| diethylene glycol[b] | 1975 | 39.51 | | | + | | – |
| eicosane* | 2000 | 40.33 | | | + | | – |
| 2-hexyl-1-octanol[b] | 2069 | 42.56 | | | + | | – |
| heneicosane* | 2100 | 43.57 | | | + | | – |
| linalool acetate[b,c] | 2117 | 44.16 | | | – | | + |
| hexadecanal[b] | 2124 | 44.41 | | | + | | – |
| 2,6-dimethyl-?,?-octadiene-?,?-diol (linalool hydroxy)[b,c] | 2145 | 45.17 | | | – | | + |
| $\gamma$-decalactone[a,d] | 2153 | 45.45 | sweet and sour sugar with herb, hard candy, rubber | 16 | + | 16 | + |
| eugenol[a,d] | 2173 | 46.17 | spicy, meat spice, vanilla, sweet, honey-like | 16 | + | 2 | + |
| nonanoic acid[b,c] | 2174 | 46.19 | | | + | | + |
| docosane* | 2200 | 47.13 | | | + | | – |
| tricosane* | 2300 | 50.23 | | | + | | – |
| tetracosane* | 2400 | 53.22 | | | + | | – |
| benzoic acid* | 2444 | 54.55 | pleasant odor, sweet | 16 | + | 16 | + |
| pentacosane* | 2500 | 56.22 | | | + | | + |
| 5-(hydroxymethyl)furfural* | 2513 | 56.58 | | | + | | + |
| vanillin* | 2577 | 58.42 | sweet, vanilla-like, delightful, hard candy, sucrose | | – | 16 | + |
| benzeneacetic acid[b–d] | 2578 | 58.44 | light smell of honey | 0 | + | | – |
| 1-octadecanol* | | 58.50 | | | + | | + |
| hexacosane* | | 59.09 | | | + | | – |
| benzenepropanoic acid[b,c] | | 60.12 | | | + | | – |
| isovanillin[b] | | 62.48 | | | – | | + |
| 1-eicosanol* | | 65.10 | | | + | | – |
| nonacosane[b] | | 69.43 | | | + | | – |

**Table 2.** (Continued)

| compound | KI[e] | RT[e] | odor note | Caju FD[e] | Caju ID[e] | Marmeleiro FD[e] | Marmeleiro ID[e] |
|---|---|---|---|---|---|---|---|
| hexadecanoic acid* | | 70.08 | | | + | | + |
| 1-heneicosanol[b] | | 71.07 | | | + | | + |
| 1-docosanol[b] | | 74.47 | | | + | | + |
| oleic acid* | | 85.53 | rancid, sour | 8 | + | 0 | + |
| 1-hexacosanol[b] | | 88.33 | | | + | | – |
| 17-pentatriacontene[b] | | 98.48 | | | + | | – |
| 1-heptacosanol[b] | | 99.28 | | | + | | – |

[a] Identified by coelution with standard volatile compounds. [b] Identified by the mass spectra data. [c] Identified by comparing the calculated KI with the theoretical KI (literature). [d] Identified by sensory analysis (sniffing port); (–) not detected; (+) detected; (*) compound considered definitely identified (identified at least by coelution with standard volatile compounds and mass spectra data). [e] KI, modified Kovats index (*19*); RT, retention time (minutes) in the GC/FID; FD, dilution factor obtained by AEDA; ID, identification.

identify the impact odor compounds with the overall aroma of the samples, AEDA was carried out. The aroma extracts of the samples were diluted by a factor of 2 several times to form a series in which each member was 2 times as concentrated as the next most diluted sample (*20*). The concentrations of the impact volatile compounds were evaluated by external standardization. The injected volume of each extract in the GC-O system was also 2.0 $\mu$L.

## RESULTS AND DISCUSSION

**Nonvolatile Fraction Analyses.** Results of the nonvolatile fraction of the honeys are presented in **Table 1**. The mean moisture content (20.5 g/100 g of honey) of the marmeleiro samples was close to the recommended value. However, no fermentation was observed in the samples, and it was confirmed by the low mean free acidity (23.6 mequiv/kg) found. On the other hand, the mean free acidity of the caju honeys was somewhat higher than the established limit. Nevertheless, after an extensive revision of the methods used in routine honey control, the European Honey Commission of Apimondia proposed a change in this parameter for future European Union (EU) and Codex Alimentarius standards (*21*). According to this commission, the free acidity of a fresh and not fermented honey must be $\leq$50 mequiv/kg. Thus, from this point of view, the mean free acidity value of the caju samples could be considered to be normal. The reducing sugar content of the caju honey was <65 g/100 g of honey. Normally, a low content of reducing sugars in honeys is associated with samples diluted with water. However, the low mean moisture content of these samples (17.1 g/100 g of honey) excluded this possibility. Thus, these caju honey samples could be produced by the bees using the nectar of the caju flowers and the exudates of the caju fruit. This possibility is based on the European Honey Commission proposals for future EU and Codex Alimentarius standards that established a reducing sugar content of at least 45 g/100 g of honey for blends of honeydew and blossom honeys (*21*). Indeed, sugar analysis is an important complementary parameter to authenticate honey origin and must be used together with pollen analysis (*22*−*24*). The contents of the individual oligosaccharides in both honeys were in agreement with previous reports (*18*), and the remainder of the data obtained were within the ranges normally found for ripe and not excessively heated honeys (*21*, *25*).

**Volatile Compounds.** From a comparison of both honeys, it is apparent that the volatile fraction of caju honey (59 components) was richer than that of marmeleiro honey (36 compounds). The identification was carried out using reference substances, mass spectral libraries, and the odor qualities of the compounds eluted from the GC column. Only the compounds identified using at least reference compounds and mass spectral data were considered to be definitely identified (see **Table 2**). The volatile fraction of the caju honey was divided into the following groups: hydrocarbons (19), alcohols (14), acids (9), esters (4), aldehydes (3), ketones (5), and miscellaneous compounds (5). On the other hand, the volatile fraction of the marmeleiro samples was divided as follows: linalool-related compounds (7), alcohols (9), acids (7), esters (3), aldehydes (3), ketones (6), and hydrocarbons (1). As can be observed, the major group of volatile compounds found in caju honeys was the hydrocarbon group, which was rich in high molecular weight compounds (e.g., nonacosane). In contrast, only one hydrocarbon (pentacosane) was detected in the marmeleiro honey. The linalool-related compounds (e.g., linalool and linalool acetate) represented one of the greatest groups of this last honey. On the contrary, only one component of this group (*trans*-linalool oxide) was found in the caju honey samples. These two groups could be used to make a distinction between these two types of honeys.

**GC-Sniffing and AEDA Analysis.** The sensory analysis, developed using a sniffing-port apparatus coupled to a GC instrument, showed that the medium- to high-boiling compounds are important contributors to the characteristic aroma of these honeys. The chromatographic regions between 30:36 and 76:00 min and between 30:36 and 61:06 min were especially rich in compounds with sweet and honey-like aroma in the caju and marmeleiro samples, respectively. These results are in agreement with data obtained in previous works (*2*, *5*). This sensory analysis also allowed the tentative identification of some compounds not detected by the GC-FID and GC-MS techniques (**Table 2**). The following additional compounds were tentatively identified in such a way: butyl butanoate, furfuryl mercaptan, $\delta$-octalactone, and 2-methoxyphenol (caju honey); linalool (marmeleiro honey); menthol, $\gamma$-decalactone, eugenol, 2,3-butanedione, and isovaleric acid (both honeys). The presence of these compounds was strengthened by the use of standard volatile compounds. AEDA analysis associated with the data from GC-MS and/or reference substances allowed the tentative identification of some impact volatile compounds in both honeys. As can be observed in **Table 2**, nine volatile compounds with high dilution factors (DF $\geq$ 16) were considered to be relevant to the overall aroma of caju honey. These compounds were furfuryl mercaptan (<1 ppb), benzyl alcohol (3 ppb), $\delta$-octalactone (<1 ppb), $\gamma$-decalactone (<1 ppb), eugenol (<1 ppb), and benzoic acid (4 ppb) (all with DF = 16); isovaleric acid (<1 ppb; DF = 32); phenylethyl alcohol (16 ppb; FD = 128), and 2-methoxyphenol (<1 ppb; DF = 1024). In the marmeleiro honey, only four impact volatile compounds, all of them with dilution factors of 16, were disclosed. These compounds were isovaleric acid (<1 ppb), $\gamma$-decalactone (<1

ppb), benzoic acid (23 ppb), and vanillin (437 ppb). Only isovaleric acid, γ-decalactone, and benzoic acid were impact volatile compounds presented in both honeys. The disclosed impact volatile compounds could be separated into two distinct groups: the first one contains compounds with pleasant notes and the second, off-flavor compounds. In the first group, furfuryl mercaptan showed an odor resembling coffee and cooked meat. Indeed, this last compound has a very low threshold in water (0.005 mg/L) and is considered to be very important to coffee aroma (*26*). According to our knowledge, this compound has not been previously described in the literature as a honey constituent. Its origin could be associated with the interaction between the compounds formed by the degradation of sulfur-containing amino acids (e.g., methionine and cysteine) with the compounds formed by the degradation of sugars. Because honeys have generally very low amounts of sulfur-containing amino acids, it is easy to understand the difficulty of detecting these compounds in this kind of food. The benzyl alcohol exhibited a pleasant odor of Paraguay tea. This compound was detected in other honey types such as haze (*5*) and rape honeys (*11*). δ-Octalactone, with its delightful sweet odor, was considered to be a positive impact volatile compound in caju honey. GC-O analysis allowed also the tentative identification of this compound in the marmeleiro sample (<1 ppb), but in this case it was not considered a potent odorant (DF < 16; see **Table 2**) because its odor was perceived only in the nondiluted extract. Shimoda et al. (*5*) also considered this compound important to haze honey aroma. The other tentatively identified lactone (γ-decalactone), with its sweet odor containing a little sour note, contributed expressively to the overall aroma of both honey species (caju and marmeleiro). Eugenol, which has a spicy and sweet aroma, had a powerful impact only in caju honey. Although the odor of this compound had also been felt in the marmeleiro sample, its DF was recorded as only 2. This disagreement between the dilution factors could be explained by the different relative concentrations of eugenol in these samples, although this compound had been present in trace amounts (<1 ppb) in both honey types. Eugenol was previously characterized as an important odorant of linden honey (*6*). Benzoic acid, which is used as a fixer compound in some perfumes and as a component of some lavender waters (*27*), revealed itself as an important odorant in both honeys, emanating a sweet pleasant odor. This compound was previously reported in heather honeys by Speer and Montag (*28*). Another compound well-known in the perfume industry is phenylethyl alcohol, with its floral, spicy, and herb-like aroma. This alcohol was considered to be one of the most important impact volatile compounds of caju honey, due to its high dilution factor (FD = 128). Phenylethyl alcohol was also considered to be a powerful aroma compound of linden honeys (*6*). Nevertheless, in the marmeleiro sample this substance was perceived only in the nondiluted extract (**Table 2**). The last member of the first group was vanillin, with its delightfully sweet and vanilla-like aroma. The low threshold of vanillin in air (0.6−1.2 ng/L) could explain its importance to the honey aroma (*6*). Likewise, phenylethyl alcohol was also a strong volatile compound of linden honeys (*6*). The second group of impact volatile compounds was formed by two members: isovaleric acid and 2-methoxyphenol. Isovaleric acid exhibited an unpleasant odor associated with the rank smell of perspiring feet and was considered to be an important off-flavor compound in both honeys. This result was in agreement with the low thresholds exhibited by this compound: 2.45 ppb in air (v/v) (*5*) and 750 ppb in water (*29*). 2-Methoxyphenol, with no doubt, could be considered to be the most important off-flavor of caju honey (DF = 1024). According to Shimoda et al. (*5*), its threshold in air is 1 ppb (v/v). In water its threshold was reported as 3 ppb (*30*).

The information obtained in the current work showed that some medium- to high-boiling-point volatile compounds are important contributors to the caju and marmeleiro honey flavors. Additionally, we have identified a great number of volatiles (powerful odorants or not) present in the caju and marmeleiro samples and also developed an extraction procedure that reduced the artifact formation to a minimum. Some of the compounds identified may be useful for the characterization of these two honey types.

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