

## Volatile compounds of copoazú (*Theobroma grandiflorum* Schumann) fruit

Clara E. Quijano<sup>a,\*</sup>, Jorge A. Pino<sup>b</sup>

<sup>a</sup> Universidad de los Andes, Facultad de Ciencias, Dpto. de Química, Cra. 1a Este No. 18-A-10 Edif. (Q-826), Bogotá, Colombia

<sup>b</sup> Instituto de Investigaciones para la Industria Alimenticia, Carretera al Guatao km 31/2, La Habana 19200, Cuba

Received 26 September 2006; received in revised form 3 January 2007; accepted 8 January 2007

### Abstract

Volatile compounds were isolated from copoazú (*Theobroma grandiflorum* Schumann) fruit by liquid–liquid extraction at two different pH values of 3.3 (natural) and 7. The volatile extracts were analyzed by capillary GC–FID and GC–MS. Among the 56 compounds identified at two pH values, many of them were only present in the lower pH extract, confirming the presence of bound compounds in copoazú. Ethyl butanoate, ethyl hexanoate and linalool were the major constituents found in copoazú fruit.

© 2007 Elsevier Ltd. All rights reserved.

**Keywords:** Copoazú; *Theobroma grandiflorum*; Sterculiaceae; Volatile compounds; GC–MS

### 1. Introduction

The copoazú (*Theobroma grandiflorum* Schumann) from the Sterculiaceae family is an arboreal fruit species considered to be a pre-Colombian crop plant, which is still found wild in the eastern subregion of Brazilian Amazonia. It reaches a height of 15–20 m, but only less than 8 m when cultivated. Copoazú cultivation is concentrated in Perú (Brazil) where it continues to expand, although it is grown in other Brazilian regions too, mostly in small domestic and commercial gardens. It has also been introduced into the humid tropics of Colombia, Costa Rica, Ecuador, Peru and Venezuela. Several authors have rated it as one of the most promising fruits for commercialization among the rich Amazonian flora. The fruit appears in the form of a drupe and has a strong and pleasant smell and creamy exotic taste. It is smooth on the outside, ellipsoidal, 25 cm long by 12 cm wide and weighs up to 1.5 kg. The endocarp is white, soft and sour-tasting, containing 25–50 seeds in five rows. The pulp occupies approximately one-third of the fruit and is usually used to make fresh juice, ice cream,

jam and tarts (Balee, 1994; Giacometti, 1994; Moraes, Muller, DeSouza, & Antonio, 1994).

The volatile composition of Brazilian copoazú fruit has been studied by using different isolation techniques including simultaneous distillation–extraction (Alves & Jennings, 1979), headspace technique (Franco & Shibamoto, 2000) and solid-phase extraction (Boulanger & Crouzet, 2000; Fischer, Hammerschmidt, & Brunhe, 1995). In these papers different results had been reported about major constituents, e.g. ethyl butanoate and ethyl hexanoate (Alves & Jennings, 1979; Franco & Shibamoto, 2000), methyl (*E*)-2-hexenoate, methyl crotonate and hexanoic acid (Fischer et al., 1995), and 3-methylbutanol, 2-phenylethanol, linalool, (*Z*)-2,6-dimethylocta-2,7-dien-1,6-diol, 1-butanol and 1-hexanol (Boulanger & Crouzet, 2000).

Free volatile compounds, are also present in plants as glycosidically bound components. Aroma compounds can be released from these non-volatile precursors by enzymatic or acid hydrolysis during industrial processing (Winterhalter & Skouromounis, 1997). There has been much research on glycosidically bound compounds present in tropical fruits such as acerola (Boulanger & Crouzet, 2001), cashew apple (Bicalho, Pereira, Aquino Neto, Pinto, & Rezende, 2000), mango (Herianus, Lalel, & Soon, 2003),

\* Corresponding author.

E-mail address: [cquijano@uniandes.edu.co](mailto:cquijano@uniandes.edu.co) (C.E. Quijano).

litchi (Chyaua, Kob, Changa, & Maub, 2003), melón de olor (Parada, Duque, & Fujimoto, 2000), mango (Sakho, Chassagne, & Crouzet, 1997) and copoazú (Boulanger & Crouzet, 2000).

As a part of our continued studies on the flavour of Colombian fruits this paper describes the volatile composition of the fruit pulp of copoazú (*T. grandiflorum* Schumann) fruit, which were isolated at two pH values.

## 2. Materials and methods

### 2.1. Fruits

Fresh mature copoazú fruits were picked from bushes grown in Caquetá, Colombia, and transported by airplane to the laboratory within 24 h after harvest. The fruits were allowed to ripen at room temperature. Two groups of six fruits each one were used for analyses. After separation of the skin and seeds the pulp was gently mixed in a commercial blender. The pulps for each group were immediately subjected to extraction.

### 2.2. Isolation of volatile compounds

One kilogram of each maturation stage fruit (peeled and without seeds) and 1 L of distilled water (natural pH 3.3) or 0.2 M phosphate buffer (pH 7) was blended in a commercial blender for 10 min and then centrifuged at 2400 g for 20 min. Internal standard (0.25 mg of *n*-decanol) was added to the supernatant and it was immediately subjected to the isolation of volatiles. The method used was continuous liquid–liquid extraction with 250 ml of pentane/dichloromethane (1:1, v/v) for 8 h (Drawert & Rapp, 1968). The extracts were dried over anhydrous sodium sulphate and concentrated with a Kuderna–Danish apparatus with a Vigreux column to 0.2 ml.

### 2.3. Gas chromatography (GC–FID)

An HP 6890 GC with FID equipped with a fused silica HP-Innowax capillary column (60 m × 0.25 mm × 0.25 μm film thickness) was used. The temperature was 4 min isothermal at 50 °C and then 40–220 °C at a rate of 4 °C/min. The carrier flow rate (nitrogen) was 1 ml/min. Injector and detector temperatures were kept at 220 °C. Volumes of 1 μl were injected with a split ratio of 1:10. Retention indices were calculated for separated compounds relative to a C<sub>8</sub>–C<sub>25</sub> *n*-alkanes mixture (Majlat, Erdos, & Tacas, 1974).

### 2.4. Gas chromatography–mass spectrometry (GC–MS)

An HP 6890 Series II coupled to AT-5973 N Agilent Technologies (Palo Alto CA, USA) mass detector was used. A similar column and temperature program as GC–FID were used. Mass spectra were recorded in the electron-impact mode at 70 eV by 1.8 scans/s. Detection was performed in the scan mode between 30 and 400 Da.

### 2.5. Qualitative and quantitative analysis of volatile compounds

Constituents were identified by comparison of their mass spectra with those in NBS, NIST or our FLAVOR-LIB data base created with authentic standards, and confirmed in many compounds by relative retention indices of authentic standards. Mass spectra from the literature (Adams, 2001; Jennings & Shibamoto, 1980; MacLafferty & Staffer, 1989) were also compared.

Quantitative determinations were carried out by internal standard method from GC–FID peak areas, without the use of calibration curves. Concentrations are uncorrected and are based on the ratio of the area of a compound compared to the internal standard. Results of the analyses for both samples were averaged.

## 3. Results and discussion

The volatile compounds of copoazú fruit were obtained by liquid–liquid extraction and analyzed by GC and GC–MS. A valid aroma concentrates were prepared by using the established procedures with an acceptable extraction efficiency and relatively low danger of artefact formation (Drawert & Rapp, 1968; Parada et al., 2000). The concentrated extracts showed aroma notes resembling the flavour of fresh fruit, described as sweet–grassy–fruity. Each one of the aroma concentrates showed different aroma notes, those obtained at natural (pH 3.3) had higher notes resembling the flavour of fresh fruit, whereas the other two concentrates obtained at pH 7 had aroma notes while were less sweet and fruity.

Table 1 lists the identified compounds along with their concentrations. Quantifications were based upon GC–FID peak integration data, so accuracy in potentially limited by a number of factors, including coelution of two or more components and differences in FID response factors among compounds. The quantitative data in Table 1 show that the total amount of volatile compounds per kilogram of fresh fruit was 6.68 and 1.34 mg for extractions at natural (pH 3.3) and neutral (pH 7), respectively.

In total, 24 esters, 13 terpenes, 8 alcohols, 4 carbonyls, 4 acids, 2 lactones and 1 phenol were identified. Of the 56 components identified, 34 are reported for the first time in this fruit. Major components in all aroma concentrates were ethyl butanoate, linalool and ethyl hexanoate. These two esters had been reported in higher amounts by Alves and Jennings (1979) and Franco and Shibamoto (2000), whereas linalool was found as major compound in the study of Boulanger and Crouzet (2000).

The acid hydrolysis of glycosides in fruits usually occurred at ca. pH 3.0–3.5, close to the normal pH values of the fruits (Buttery, Takeoka, Teranishi, & Ling, 1990). The data in Table 1 show that aroma concentrates obtained at natural (pH 3.3), have many more volatile compounds and often a higher amount than in those obtained at pH 7. These results suggest the presence of gly-

Table 1  
Volatile compounds of copoazú fruit ( $\mu\text{g}/\text{kg}$ )

Compound	RI <sub>exp</sub>	RI <sub>ref</sub>	pH 3.3	pH 7
Ethyl acetate*	821	825	250	102
2-Propanol*	873	884	–	4
Ethanol*	889	900	77	29
Ethyl propanoate*	921	925	52	–
3-Methylbutyl propanoate*	935	938	20	–
Ethyl butanoate	1021	1025	389	381
Ethyl 2-methylbutanoate	1037	1045	162	–
Butyl acetate	1043	1050	440	7
3-Methylbutyl acetate*	1005	1107	43	–
1-Butanol	1108	1113	129	13
1-Penten-3-ol*	1125	1130	24	–
2-Methyl-2-pentenal*	1148	1154	–	5
Myrcene*	1151	1156	12	–
3-Methylbutanol	1175	1183	3	2
Butyl butanoate	1205	1207	–	14
Ethyl hexanoate	1223	1225	1253	359
(E)- $\beta$ -Ocimene*	1238	1244	37	28
3-Methyl-3-buten-1-yl acetate*	1251	–	62	3
Hexyl acetate*	1263	1265	201	4
Terpinolene*	1272	1274	15	–
3-Hydroxy-2-butanone	1273	1273	102	–
3-Methyl-2-buten-1-ol	1285	–	278	–
Ethyl 2-methylpentanoate*	1297	1302	40	12
1-Hexanol	1335	1343	30	7
3Z-Hexenol	1361	1368	18	6
Nonanal*	1378	1383	19	–
Butyl hexanoate	1405	1412	79	5
Ethyl octanoate*	1412	1420	52	10
(Z)-Linalool oxide (furanoid)	1422	1425	27	–
(E)-Linalool oxide (furanoid)	1435	1449	31	8
Acetic acid	1439	1443	47	–
(E,E)-2,4-Heptadienal*	1443	–	5	–
Ethyl sorbate*	1446	1448	6	–
Methyl 3-hydroxybutanoate*	1453	1460	44	–
Linalool	1496	1500	986	104
Ethyl 3-hydroxybutanoate	1497	1501	86	–
$\beta$ -Caryophyllene*	1573	1576	23	5
Ethyl decanoate*	1621	1624	37	6
Butanoic acid	1625	1629	117	29
Propyl butanoate*	1654	1658	48	5
Ethyl 3-hydroxyhexanoate	1661	1660	520	–
$\alpha$ -Terpineol	1662	1661	440	89
Linalyl propanoate*	1682	1683	111	51
Germacrene D*	1695	1703	37	5
Nerol*	1754	1757	12	–
Ethyl dodecanoate*	1821	1824	27	–
Geraniol	1841	1848	42	–
Hexanoic acid*	1831	1834	21	4
2-Phenylpropyl acetate*	1925	1929	37	2
$\gamma$ -Octalactone*	1965	1968	8	–
Octanoic acid*	2039	2035	70	–
trans-Cinnamyl acetate*	2098	2102	32	21
Eugenol*	2099	2103	35	15
$\delta$ -Decalactone	2148	2150	9	–
Cedryl acetate*	2182	2186	29	6
(E,E)-Farnesyl acetate*	2278	2283	10	–

– Not detected.

\* Reported for the first time.

cosidically bound or phosphate bound compounds like in other fruits (Luan, Hampel, Mosandl, & Wust, 2004; Olle et al., 1998; Osorio, Duque, Suárez, Salamanca, & Uruña, 2002).

It is interesting to note that two lactones were found in aroma concentrates at natural pH, one of them reported for the first time. These compounds may be present in the form of glycosidically bound hydroxy acids in copoazú. Limonoid glucosides also exist in citrus fruits as glycosidically bound  $\delta$ -hydroxy acids (Hasegawa, Bennett, Herman, Fong, & Ou, 1989), although free limonoids are known as derivatives of triterpenes  $\delta$ -lactones. A hydroxy acid might lose water spontaneously to yield a lactone under acidic conditions.

Many terpenes were detected only at higher amount in the aroma concentrates obtained at lower pH, such as linalool, linalool oxides,  $\alpha$ -terpineol, nerol and geraniol. Of them, nerol is reported for the first time in this fruit.

Eugenol was also found only in the aroma concentrates obtained at the lower pH. Some conjugated forms of phenols have also been reported in grape (Strauss, Gooley, Wilson, & Williams, 1987) and in Lamiaceae species (van de Dries & Svendsen, 1989). The role of these compounds as precursors of phenolic volatile compounds is unknown. Eugenol was found earlier as a glycosidically bound compound in copoazú (Boulanger & Crouzet, 2001). In addition, other probable glycosidically bound compounds, such as 1-butanol, 1-hexanol, butanoic acid, hexanoic acid, and the hydroxy esters were found in high amounts in the aroma concentrates obtained at the lower pH value. These results confirm the strategy approach of use low pH during isolation of volatile compounds to investigate fruit flavours.

## Acknowledgements

Grateful acknowledgements are expressed to the Faculty of Sciences of the University of the Andes for the financial support. We thank Jaime Campos for supplying the fruits used in this study.

## References

- Adams, R. P. (2001). *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. Carol Stream, IL: Allured Publishing Co.
- Alves, S., & Jennings, W. G. (1979). Volatile composition of certain Amazonian fruits. *Food Chemistry*, 4, 149–159.
- Balee, W. (1994). *Footprints of the forest*. New York: Columbia University Press.
- Bicalho, B., Pereira, A. S., Aquino Neto, F. R., Pinto, A. C., & Rezende, C. M. (2000). Application of high-temperature gas chromatography–mass spectrometry to the investigation of glycosidically bound components related to cashew apple (*Anacardium occidentale* L. var. *nanum*) volatiles. *Journal of Agriculture and Food Chemistry*, 48, 1167–1174.
- Boulanger, R., & Crouzet, J. (2000). Free and bound flavour components of Amazonian fruits: 3. Glycosidically bound components of cupuacu. *Food Chemistry*, 70, 463–470.
- Boulanger, R., & Crouzet, J. (2001). Identification of the aroma components of acerola (*Malpighia glabra* L.): free and bound flavour compounds. *Food Chemistry*, 74, 209–216.
- Buttery, R. G., Takeoka, G., Teranishi, R., & Ling, L. C. (1990). Tomato aroma components: Identification of glycoside hydrolysis volatiles. *Journal of Agricultural and Food Chemistry*, 38, 2050–2053.

- Chyaua, C.-C., Kob, P.-T., Changa, C.-H., & Maub, J.-L. (2003). Free and glycosidically bound aroma compounds in lychee (*Litchi chinensis* Sonn.). *Food Chemistry*, *80*, 387–392.
- Drawert, F., & Rapp, A. (1968). Gas-Chromatographische Untersuchung pflanzlicher Aromen. I. Anreicherung, Trennung, Identifizierung von flüchtigen Aromastoffen in Traubenmost und Wein. *Chromatographia*, *1*, 446–457.
- Fischer, N., Hammerschmidt, F. J., & Brunhe, E. J. A. (1995). Contribution to the analysis of the flavour of cupuaçu fruit (*Theobroma grandiflora*). *Fruit Processing*, *5*, 61–65.
- Franco, M. R. B., & Shibamoto, T. (2000). Volatile composition of some Brazilian fruits: umbu-caja (*Spondias citherea*), camu-camu (*Myrciaria dubia*), araca-boi (*Eugenia stipitata*), and copoazu (*Theobroma grandiflorum*). *Journal of Agricultural and Food Chemistry*, *48*, 1263–1265.
- Giacometti, D. C. (1994). Neglected crops: 1492 from a different perspective. In J. E. H. Bermejo & J. León (Eds.). *Plant Production and Protection Series No. 26* (pp. 205–209). Rome: FAO.
- Hasegawa, S., Bennett, R. D., Herman, Z., Fong, C. H., & Ou, P. (1989). Limonoid glucosides in citrus. *Phytochemistry*, *28*, 1717–1720.
- Herianus, J. D., Lalel, Z. S., & Soon, C.-T. (2003). Glycosidically-bound aroma volatile compounds in the skin and pulp of 'Kensington Pride' mango fruit at different stages of maturity. *Postharvest Biology and Technology*, *29*, 205–218.
- Jennings, W., & Shibamoto, T. (1980). *Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography*. New York: Academic Press.
- Luan, F., Hampel, D., Mosandl, A., & Wust, M. (2004). Enantioselective analysis of free and glycosidically bound monoterpene polyols in *Vitis vinifera* L. vs. Morio Muscat and Muscat Ottonel: Evidence for an oxidative monoterpene metabolism in grapes. *Journal of Agricultural and Food Chemistry*, *52*, 2036–2041.
- MacLafferty, F. W., & Staffer, D. B. (1989). *The Willey/NBS registry of mass spectral data*. New York: John Wiley and Sons.
- Majlat, P., Erdos, Z., & Tacas, J. (1974). Calculation and application of retention indices in programmed temperature–gas chromatography. *Journal of Chromatography*, *91*, 89–110.
- Moraes, V. H. D., Muller, C. H., DeSoucza, A. G. C., & Antonio, I. C. (1994). Native fruit species of economic-potential from the Brazilian Amazon. *Angewandte Botanik*, *68*(1-2), 47–52.
- Olle, D., Baumes, R. L., Bayonove, C. L., Lozano, Y. F., Sznaper, C., & Brillouet, J. M. (1998). Comparison of free and glycosidically linked volatile components from polyembryonic and monoembryonic mango (*Mangifera indica* L.) cultivars. *Journal of Agricultural and Food Chemistry*, *46*, 1094–1100.
- Orosio, C., Duque, C., Suárez, M., Salamanca, L. E., & Uruña, F. (2002). Free, glycosidically bound and phosphate bound flavors constituents of badea (*Passiflora quadrangularis*) fruits pulp. *Journal of Separation Science*, *25*, 147–154.
- Parada, F., Duque, C., & Fujimoto, Y. (2000). Free and bound volatile composition and characterization of some glucoconjugates as aroma precursors in melón de olor fruit pulp (*Sicana odorifera*). *Journal of Agricultural and Food Chemistry*, *48*, 6200–6204.
- Sakho, M., Chassagne, D., & Crouzet, J. (1997). African mango glycosidically bound volatile compounds. *Journal of Agricultural and Food Chemistry*, *45*, 883–888.
- Strauss, S. R., Gooley, P. R., Wilson, B., & Williams, P. J. (1987). Application of droplet countercurrent chromatography to the analysis of conjugated forms of terpenoids, phenols and other constituents of grape juice. *Journal of Agricultural and Food Chemistry*, *35*, 519–524.
- van de Dries, J. M., & Svendsen, A. B. (1989). A simple method for detection of glycosidic bound monoterpenes and other volatile compounds occurring in fresh plant material. *Flavour and Fragrance Journal*, *4*, 59–61.
- Winterhalter, P., & Skouromounis, G. (1997). Glycoconjugated aroma compounds: Occurrence, role and biotechnological transformation. In R. G. Berger (Ed.). *Biotechnology* (Special Vol. 55). Berlin: Springer Verlag.