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Analysis of volatile secondary metabolites from Colombian *Xylopia aromatica* (Lamarck) by different extraction and headspace methods and gas chromatography

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Abstract

Hydrodistillation (HD), simultaneous distillation-solvent extraction (SDE), microwave-assisted hydrodistillation (MWHD), and supercritical fluid (CO_2) extraction (SFE), were employed to isolate volatile secondary metabolites from Colombian *Xylopia aromatica* (Lamarck) fruits. Static headspace (S-HS), simultaneous purge and trap (P&T) in solvent (CH_2Cl_2), and headspace (HS) solid-phase microextraction (SPME) were utilised to obtain volatile fractions from fruits of *X. aromatica* trees, which grow wild in Central and South America, and are abundant in Colombia. Kováts indices, mass spectra or standard compounds, were used to identify more than 50 individual components in the various volatile fractions. β-Phellandrene was the main component found in the HD and MWHD essential oils, SDE and SFE extracts (61, 65, 57, and ca. 40%, respectively), followed by β-myrcene (9.1, 9.3, 8.2 and 5.1%), and α-pinene (8.1, 7.3, 8.1 and 5.9%). The main components present in the volatile fractions of the *X. aromatica* fruits, isolated by S-HS, P&T and HS-SPME were β-phellandrene (53.8, 35.7 and 39%), β-myrcene (13.3, 12.3 and 10.1%), *p*-mentha-1(7),8-diene (7.1, 10.6 and 10.4%), α-phellandrene (2.2, 5.0 and 6.4%), and *p*-cymene (2.2, 4.7 and 4.4%), respectively.

Keywords: Extraction methods; Headspace analysis; *Xylopia aromatica*; Microwave-assisted hydrodistillation; Volatile organic compounds; Phellandrene

1. Introduction

Xylopia aromatica (Lamarck) Martius (*Annonaceae* family) is a small tree (4–5 m tall) commonly found in open savannas in Central and South America [1,2], which produces white-yellowish flowers and small red cylindrical fruits along its long hanging branches. Due to its charming scent, the ground fruit from this *Annonaceae* is used in food products, perfumes and cosmetics [3]. It is reported, that there are between 100 and 150 species of *Xylopia* distributed throughout the tropical regions of the world, particularly Africa, among them, *X. aethiopica*, *X. brasiliensis*, *X. frutenscens*, *X. grandiflora*, which have been studied more completely, than *X. aromatica* [4]. The various extracts from *Xylopia* spp. have been shown to possess antiseptic and analgesic properties, and insecticidal activity against adult mosquitoes, several leaf-eating insects and houseflies [4,5]. In 1981, the essential oils of the leaf and fruit of *X. aromatica* were examined chromatographically [6], but their composition was remarkably different from that determined in this study for Colombian *X. aromatica* fruit essences. Dry ripe *X. aromatica* fruits were ground and subjected to various extraction procedures in order to completely characterise their volatile secondary metabolites, and to study the effect of isolation method upon the final volatile fraction composition. Capillary GC with different detection systems were employed to analyse both essential oils obtained by hydrodistillation (HD) and microwave-assisted hydrodistillation (MWHD), and extracts isolated by simultaneous distillation-solvent extraction (SDE) and supercritical fluid (SFE, CO_2) extractions. Different headspace techniques, such as static headspace (S-HS), simultaneous purging with N_2 and trapping in solvent (P&T), and headspace solid-phase microextraction (HS-SPME) were used to isolate the volatile fractions from dry ground fruits. Individual components were identified by Kováts indices, mass spectra (electron-impact ionization (EI)), 70 eV), and standard compounds.

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2. Experimental

2.1. Plant material and reagents

Ripe, red and undamaged fruits were collected from *X. aromatica* trees (No. COL NHC-480748, Dr. J. Murillo, National Herbarium, UN, Bogotá, Colombia) in the same garden of the small village of Lebrija, Santander (Colombia) during June–November 2001. They were oven-dried (60° C, 48 h), ground to a grain size ca. 100 μm and stored at room temperature in closed glass vials. *n*-Tetradecane and dichloromethane were purchased from Merck (Darmstadt, Germany). Poly(dimethylsiloxane) (PDMS, 100 μm) SPME fibres were acquired from Supelco. (Bellefonte, PA, USA). High-purity gases for chromatography were obtained from AGA-Fano (Bucaramanga, Colombia).

2.2. Extractive techniques

Hydrodistillation: HD was performed in a 5 l-round flask with 500 g of plant material and 4 l of water, using an electric heater (boiling water) for 2 h, and after that, the oil was decanted from the condensate, previously saturated with NaCl, and dried with anhydrous sodium sulfate.

Microwave-assisted hydrodistillation: For MWHD, the hydrodistillation apparatus was placed inside a domestic microwave oven (Kendo, 2.45 GHz, 800 W) with a side orifice through which an external glass condenser joined the round flask with the plant material (100 g) and water (2 l), inside the oven. The oven was operated for 30 min at full power, which caused water to boil vigorously and reflux. Essential oil was decanted from the condensate and dried with anhydrous sodium sulfate.

Simultaneous distillation-solvent extraction: Already-published procedures were employed to perform SDE (12 g ground dry fruit, 2 h distillation-extraction, 50 ml of dichloromethane) [7,8].

Supercritical fluid (CO_2) *extraction*: SFE used a J&W Scientific high-pressure Soxhlet extractor (Folsom, CA, USA), following a procedure described elsewhere (12 g ground dry fruit, 45° C, 1100 psi (1 psi = 6894.76 pa)) [8,9].

For chromatographic analysis, 30 μl of fruit essential oil were added to 1.0 ml of dichloromethane and 0.5 μl of *n*-tetradecane, used as I.S. Each type of fruit extraction was repeated five times.

2.3. Headspace methods

Purge and trap extraction: A laboratory-made dynamic purge and trap apparatus, similar to that described by Umano and Shibamoto [10] was used to collect volatile compounds from ground dry *X. aromatica* fruits. Nitrogen (99.995% purity) was used as purging gas and dichloromethane as trapping solvent in 2 h-extractions from the headspace of ground dry fruits (100 g, 40° C). The dichloromethane solution (ca. 50 ml) was concentrated in a Kuderna-Danish apparatus, followed by dry N_2 evaporation to 1.0 ml. *n*-Tetradecane (0.5 μl) was added as I.S. to the final extract.

Static headspace: The S-HS procedure was carried out on a headspace sampler (Hewlett-Packard (HP) 7694E, Palo Alto, CA, USA), connected to a gas chromatograph (HP 5890A Series II), to analyse the vapour phase above 5 g of ground dry fruits contained in a 20 ml vial at 35° C. The sample loop and transfer line temperatures were 100 and 110° C, respectively. The experimentally determined equilibration time was 30 min.

Headspace solid-phase microextraction: For the HS-SPME procedure, a PDMS-coated (100 μm) SPME-fibre was exposed for 60 min at 22 ± 1 °C to the vapour phase above 10 g of chopped dry fruits contained in a 50 ml vial. Preliminary experiments using fibre exposition times between 5 and 120 min were used to set this parameter at 60 min in all experiments. The collected substances were thermally desorbed (260° C, 5 min) from the SPME fibre into a gas chromatograph (HP 5890A Series II), using in the injection port a SPME liner and splitless mode. All procedures were repeated five times.

2.4. Chromatographic analysis

Compound identification was based on mass spectra (EI, 70 eV) obtained with a gas chromatograph (Agilent Technologies 6890 Plus, Palo Alto, CA, USA), equipped with a mass selective detector (Agilent Technologies 5973), split/splitless injector (1:30 split ratio), and a data system (HP ChemStation 1.05), with NBS 75K, Wiley 138K and NIST 98 mass spectra libraries. A capillary column 50 m × 0.25 mm (i.d.) coated with 5% phenyl poly(methylsiloxane) (0.25 μm film thickness) (HP-5MS) was used for GC/MS analysis. The GC oven temperature was programmed from 45 (15 min) to 250 °C (15 min) at 5° C min^{-1} for the analysis of the essential oils, S-HS and P&T volatile fractions, as well as for SDE and SFE extracts. For the analysis of volatile compounds isolated by HS-SPME, the GC oven was programmed from 40 (5 min) to 220 °C (5 min) at 4 °C min^{-1}. The temperatures of the ionisation chamber and of the transfer line were set at 185 and 285° C, respectively. Mass spectra and reconstructed ion currents (chromatograms) were obtained by automatic scanning at 5.19 scan s^{-1}, in the mass range *m/z* 30–300. Chromatographic peaks were checked for their homogeneity with the aid of the mass chromatograms for the characteristic fragment ions.

A gas chromatograph (HP 5890 A Series II), equipped with flame ionisation detection (FID), split/splitless injector (1:30 split ratio), and a data system (HP ChemStation HP Rev. A.06.03 [509]) was used for GC-FID analysis of essential oils. The detector and injector temperatures were set at 250° C. A capillary column 50 m × 0.20 mm i.d., coated with 5%-phenyl poly(methylsiloxane) (0.20 μm film thickness) (HP-5) was used. The oven temperature was programmed from 40 (15 min) to 250 °C (40 min) at 3 °C min^{-1}. Helium was used as carrier gas, with 152 kPa column head

pressure and 35.7 cm s^{-1} linear velocity. Hydrogen and air at 30 and 300 ml min^{-1}, respectively, were utilised in FID, with nitrogen (30 ml min^{-1}) as a make-up gas. The various compounds were identified by comparison of their Kovàts retention indices [11], determined utilising a linear scale on the HP-5 column, and of the mass spectra of each GC component with those of standard substances and reported data [12–14].

3. Results and discussion

Fig. 1 presents two typical chromatographic profiles of *X. aromatica* (Lamarck) fruit volatile fractions, obtained by S-HS and MWHD methods. Peak identification and relative amounts of the various compounds present in the volatile fractions obtained by seven different isolation techniques, appear in Table 1. Unfortunately, it was not possible to achieve, on the capillary column (50 m × 0.25 mm i.d., 0.25 μm, HP-5MS), the complete separation of closely-eluted monoterpenes, limonene (peak no. 11) and β-phellandrene (peak no. 12), as it appears on Fig. 2A. In order to obtain a better "separation" of these compounds and, hence, to be able to quantify them, we used two different approaches. One consisted of the high dilution of the essential oil (the separation of these compounds was improved, but it never was base-line resolution); and another one, of obtaining extracted ion chromatograms, based on the "diagnostic" fragments, i.e. *m/z* 67 and 68, typically formed during limonene EI (70 eV) dissociation, and not present in the mass spectrum of β-phellandrene (Fig. 2B). The contribution of these fragments to the reconstructed ion current (area) of the chromatographic peak with $t_R = 20.74$ min, was used to calculate approximately the fraction corresponding in this peak to limonene.

The HD- and MWHD-essential oils, and SDE-extracts were rather similar in their composition, but differed from the SFE-extracts, which contained higher amounts of sesquiterpenoids and heavier hydrocarbons ($C_n > 18$). More than 35 volatile secondary metabolites were found at concentrations above 0.01% in the essential oils and extracts. β-Phellandrene was the main component (40−65%), followed by β-myrcene (5–9%), α-pinene (6–8%), cryptone (1–3%), α-phellandrene (2–4%), *p*-cymene (1–8%), methyl salicylate (1–2%), and *p*-mentha-1(7),8-diene (<4%). Monoterpene hydrocarbons, $C_{10}H_{16}$, represented the main compound family in these oils and extracts (64–94%).

Table 2 contains the relative amounts of different compound families, found in the Colombian *X. aromatica* fruit essential oils, extracts, and HS fractions, and grouped as monoterpene and sesquiterpene hydrocarbons, their oxygenated derivatives, and benzenoids. Among the four extractive techniques employed, SFE isolated a larger amount (ca. 30%) of heavier compounds (sesquiterpenoids, benzenoids and hydrocarbons), while with HD, MWHD and SDE methods only ca. 4% of these compounds were extracted. Basically, the same trend was observed in our previous studies on volatiles of *Cananga odorata* (flowers) [8] and *Lippia alba* (leaves) [15], where different extraction techniques were compared, and it was shown that SDE was particularly effective in the isolation of the most volatile metabolites, i.e. monoterpene hydrocarbons, and the SFE method permitted to isolate sesquiterpenoids and heavier oxygenated compounds (benzenoids) preferencially.

Almost the same number of components at concentrations above 0.01% was found in the HD (2 h extraction) and MWHD (30 min extraction) essential oils, with very similar yields (ca. 1.5%), a phenomenon which was already described elsewhere [16]. It is interesting to note, that the essential oil composition of Colombian *X. aromatica* fruits differs substantially from that reported for the fruit oil from Brazilian *X. aromatica*, in which the main component was limonene (23%), followed by citronellol (12%), β-pinene (11%), α-pinene (10%), carvone (5%), ocimene (4%), and myrcene (3%) [6].

X. aromatica fruit volatile fractions were isolated, using S-HS, P&T and HS-SPME techniques. The volatiles present in the vapour phase surrounding *X. aromatica* fruits consisted basically (ca. 99%) of monoterpene hydrocarbons (Fig. 1, Table 1). P&T and HS-SPME are methods with a simultaneous concentration step involved. The composition of volatile fractions obtained by these techniques would depend strongly on purging time or on fibre exposure period. The longer this time, the higher the relative amounts of sesquiterpenoids and other less volatile oxygenated compounds that could be isolated. In this study, for HS-SPME analysis of *X. aromatica* fruits we used 60 min for fibre exposition time. The SPME process in the headspace is controlled both by molecular diffusion rates and distribution coefficients, K_D, which are very different for highly-volatile monoterpenes (diffusion rates are high, and K_D are low) and low-volatile sesquiterpenoids (low diffusion rates with relatively high K_D). So, a "compromise" was necessary; which is why we chose 60 min for fibre exposure time, just as we had carried out elsewhere [15]. Seventeen, thirty-eight and fifty volatile secondary metabolites were detected at concentrations above 0.01% in the S-HS, P&T and HS-SPME volatile fractions, respectively. The main components in these fractions were β-phellandrene (36–54%), followed by β-myrcene (10–13%), *p*-mentha-1(7),8-diene (7–11%), *p*-cymene (2–5%), and α-phellandrene (2–6%).

In order to obtain a condensed representation of the compositional differences of the various volatile fractions obtained from *X. aromatica* fruits, the Table 1 data were subjected to principal component analysis (PCA) (STATISTICA, Version 6.0, StatSoft, Tulsa, OK, USA). Most (ca. 99%) of the information content of Table 1 can be represented by two principal components, the first one of which corresponds to 96% of the variance and is mainly

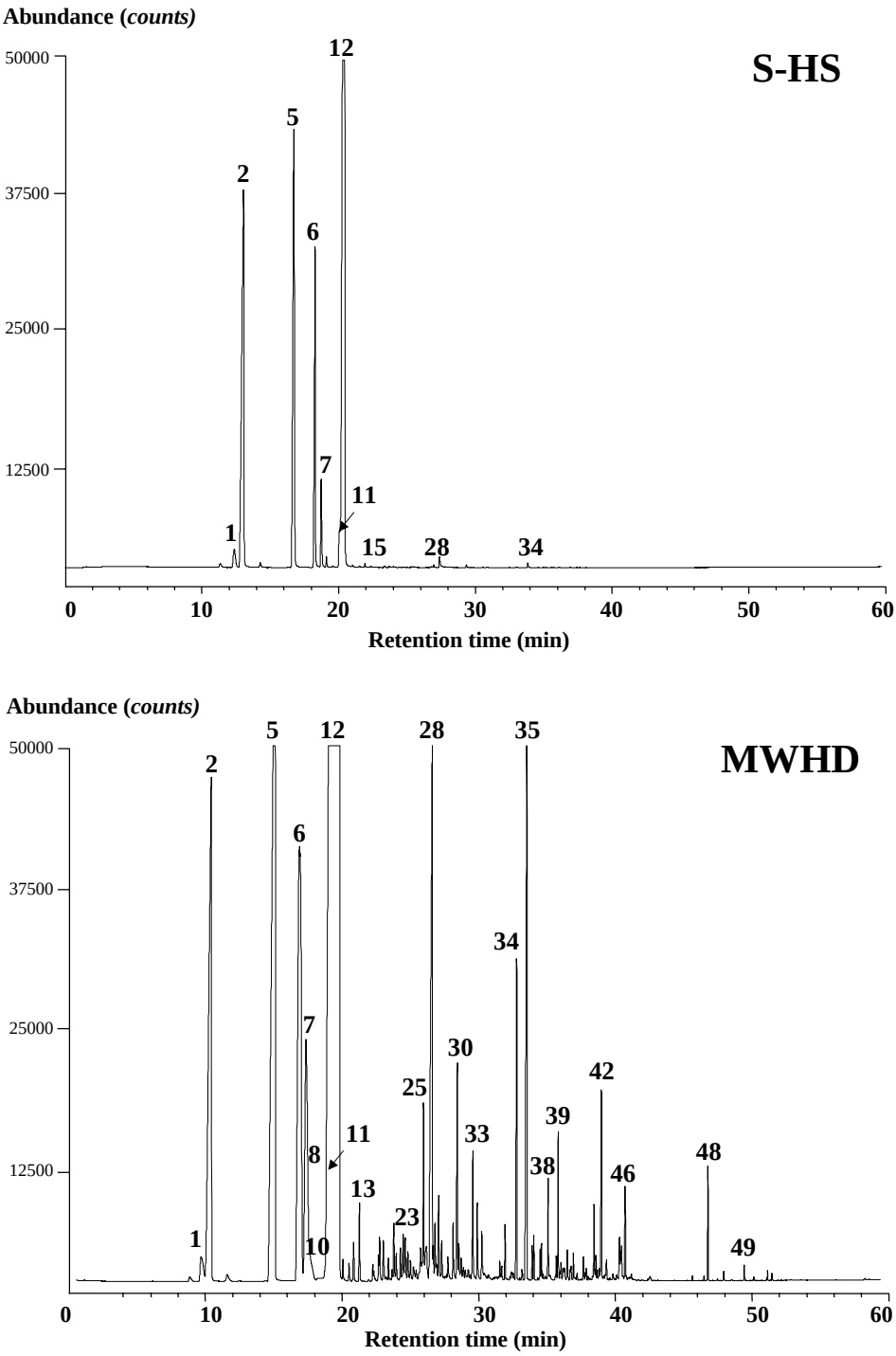

Fig. 1. Typical chromatograms of the *Xylopia aromatica* (Lamarck) fruit volatile fractions obtained by different extractive (MWHD) and headspace (S-HS) techniques. Peak assignments and identification appear in Table 1. FID. HP-5 [50 m × 0.20 mm i.d., 0.25 μm (d_f)].

related to β-phellandrene relative amount (Table 3). Fig. 3 contains the representation of these volatile fractions in the subspace formed by the first two principal components. The location of each fraction in this subspace is determined by its chemical composition. The close proximity of the MWHD, HD and SDE volatile fractions confirms that, as mentioned above, they have similar compositions. On the other hand, the HS techniques afforded volatile fractions with compositional differences along both principal components. These differences corresponded mostly to variations in β-phellandrene, β-myrcene, and α-pinene contents (Table 1). PCA thus showed that the HD, SDE and MWHD

Table 1
Chemical composition of the volatile secondary metabolites obtained from *Xylopia aromatica* (Lamarck) fruits by different extractive and headspace methods

Peak no.[a]	Compound	Kovàts indices[b]	Relative peak area (%) ± ts/$\sqrt{n}$ (n = 5, 95% confidence)						
			Extractive techniques				HS methods		
			HD	MWHD	SDE	SFE	S-HS	P&T	HS-SPME
1	α-Thujene	930	0.35 ± 0.037	0.30 ± 0.012	3.7 ± 0.68	0.2 ± 0.17	0.99 ± 0.025	1.2 ± 0.52	0.23 ± 0.025
2	α-Pinene[c]	939	8.1 ± 0.35	7.3 ± 0.20	8.1 ± 0.25	5.9 ± 0.35	14.3 ± 0.12	15.3 ± 0.52	10.31 ± 0.012
3	Camphene[c]	953	1.7 ± 0.19	1.4 ± 0.17	1.37 ± 0.037	tr	0.86 ± 0.012	0.5 ± 0.52	0.20 ± 0.025
4	β-Pinene[c]	980	0.04 ± 0.012	tr	0.11 ± 0.037	tr	0.10 ± 0.012	0.62 ± 0.050	0.02 ± 0.012
5	β-Myrcene[c]	991	9 ± 2.6	9.3 ± 0.32	8.2 ± 0.31	5.1 ± 0.36	13.3 ± 0.25	12.30 ± 0.012	10.1 ± 0.92
6	*p*-Mentha-1(7),8-diene[c]	1004	3.65 ± 0.062	3.1 ± 0.24	2.31 ± 0.037	0.05 ± 0.012	7.11 ± 0.012	10.6 ± 0.47	10.4 ± 0.24
7	α−Phellandrene	1005	3.44 ± 0.037	2.3 ± 0.72	3.8 ± 0.32	2.6 ± 0.39	2.21 ± 0.025	4.95 ± 0.012	6.4 ± 0.30
8	Δ^3-Carene[c]	1009	0.02 ± 0.012	0.58 ± 0.099	0.17 ± 0.025	tr	0.29 ± 0.074	0.2 ± 0.27	3.5 ± 0.58
9	α-Terpinene	1020	0.10 ± 0.012	0.01 ± 0.012	0.52 ± 0.012	tr	0.20 ± 0.062	0.3 ± 0.42	0.43 ± 0.012
10	*p*-Cymene[c]	1028	1.1 ± 0.16	1.31 ± 0.025	2.3 ± 0.46	8.4 ± 0.35	2.18 ± 0.025	4.7 ± 0.61	4.4 ± 0.12
11	Limonene[c]	1031	2.7 ± 0.12	2.8 ± 0.12	2.8 ± 0.12	1.9 ± 0.12	2.3 ± 0.12	1.1 ± 0.12	2.2 ± 0.87
12	β-Phellandrene[c]	1031	61 ± 1.9	64.8 ± 0.37	57.2 ± 0.19	39.5 ± 0.25	53.8 ± 0.273	35.7 ± 0.12	39 ± 2.1
13	*cis*-β-Ocimene	1040	0.19 ± 0.025	0.17 ± 0.025	1.07 ± 0.025	tr	1.1 ± 0.26	1.2 ± 0.31	0.10 ± 0.025
14	*trans*-β-Ocimene	1051	0.04 ± 0.012	0.06 ± 0.012	1.2 ± 0.40	tr	tr 0.000	0.3 ± 0.34	0.96 ± 0.025
15	γ-Terpinene	1062	0.02 ± 0.012	0.05 ± 0.012	0.83 ± 0.037	tr	0.13 ± 0.025	1.06 ± 0.062	0.10 ± 0.012
16	*cis*-Sabinene hydrate	1068	0.06 ± 0.012	0.01 ± 0.012	0.19 ± 0.037	tr	0.02 ± 0.012	0.28 ± 0.050	0.10 ± 0.025
17	Fenchone	1087	0.02 ± 0.012	0.02 ± 0.012	0.1 ± 0.36	0.95 ± 0.012	tr	0.30 ± 0.025	0.11 ± 0.012
18	α-Terpinolene	1088	0.06 ± 0.012	0.03 ± 0.012	0.14 ± 0.037	tr	tr	0.24 ± 0.050	0.15 ± 0.012
19	*trans*-Sabinene hydrate	1100	0.02 ± 0.012	0.02 ± 0.012	0.30 ± 0.025	tr	tr	0.45 ± 0.050	0.36 ± 0.037
20	Fenchol	1112	0.02 ± 0.012	tr	0.5 ± 0.39	tr	tr	0.3 ± 0.16	0.26 ± 0.025
21	α-Campholenal	1130	0.16 ± 0.012	0.10 ± 0.025	0.14 ± 0.025	tr	tr	0.62 ± 0.037	0.15 ± 0.087
22	Camphene hydrate	1149	0.02 ± 0.012	0.05 ± 0.012	0.11 ± 0.037	tr	tr	0.4 ± 0.19	0.10 ± 0.099
23	Citronellal[c]	1152	0.06 ± 0.012	0.2 ± 0.15	0.2 ± 0.14	tr	tr	tr	0.16 ± 0.025
24	Pinene oxide[c]	1156	0.06 ± 0.012	tr	0.1 ± 0.34	tr	tr	tr	0.10 ± 0.012
25	Phellandral	1159	0.25 ± 0.037	0.21 ± 0.050	0.81 ± 0.037	0.80 ± 0.050	tr	tr	0.10 ± 0.012
26	Thujanol	1165	0.02 ± 0.012	0.06 ± 0.025	0.07 ± 0.062	tr	tr	0.10 ± 0.037	0.59 ± 0.012
27	Terpinen-4-ol[c]	1176	0.06 ± 0.012	0.04 ± 0.012	0.10 ± 0.012	1.0 ± 0.12	tr	tr	0.30 ± 0.012
28	Cryptone	1177	2.7 ± 0.88	1.27 ± 0.012	1.3 ± 0.35	1.9 ± 0.30	0.29 ± 0.012	1.71 ± 0.062	0.83 ± 0.025
29	Methyl benzeneacetate	1178	0.14 ± 0.025	0.07 ± 0.025	0.11 ± 0.099	1.55 ± 0.087	tr	tr	0.10 ± 0.012
30	α-Terpineol[c]	1189	0.05 ± 0.012	0.17 ± 0.012	0.80 ± 0.025	0.86 ± 0.025	tr	0.18 ± 0.012	0.24 ± 0.012
31	Phellandrene epoxide	1190	0.03 ± 0.012	0.06 ± 0.012	0.03 ± 0.012	0.08 ± 0.012	tr	0.25 ± 0.012	0.19 ± 0.087
32	*cis*-Piperitol	1193	0.11 ± 0.025	0.05 ± 0.012	0.1 ± 0.11	0.10 ± 0.050	tr	0.12 ± 0.012	0.28 ± 0.037
33	Myrtenol	1194	0.02 ± 0.012	0.15 ± 0.012	0.20 ± 0.099	0.64 ± 0.062	tr	0.28 ± 0.025	0.10 ± 0.062
34	Methyl salicylate[c]	1195	1.8 ± 0.11	1.6 ± 0.11	1.1 ± 0.20	2.08 ± 0.050	0.10 ± 0.012	0.49 ± 0.062	1.11 ± 0.012
35	4-iPr-Benzaldehyde	1205	1.20 ± 0.025	1.38 ± 0.025	1.48 ± 0.050	1.07 ± 0.062	tr	1.31 ± 0.037	0.33 ± 0.012
36	*trans*-Piperitol	1209	0.03 ± 0.012	0.06 ± 0.012	0.14 ± 0.050	0.09 ± 0.025	tr	tr	0.10 ± 0.012
37	Citronellol[c]	1224	0.13 ± 0.025	0.06 ± 0.025	0.11 ± 0.037	0.1 ± 0.26	tr	tr	0.11 ± 0.050
38	iPr-Benzenemethanol	1271	0.16 ± 0.025	0.12 ± 0.037	0.30 ± 0.087	2.03 ± 0.062	tr	0.32 ± 0.037	0.10 ± 0.012
39	α-Cubebene	1351	0.27 ± 0.037	0.38 ± 0.012	0.48 ± 0.050	1.88 ± 0.074	tr	0.35 ± 0.099	1.10 ± 0.062
40	α-Copaene	1376	0.05 ± 0.074	0.04 ± 0.062	0.06 ± 0.025	1.6 ± 0.25	tr	0.28 ± 0.012	0.32 ± 0.037
41	α-*cis*-Bergamotene	1415	0.04 ± 0.012	0.06 ± 0.099	0.05 ± 0.025	1.81 ± 0.037	tr	0.11 ± 0.050	0.20 ± 0.099

Table 1 (*Continued*)

Peak no.[a]	Compound	Kovàts indices[b]	Relative peak area (%) ± ts/$\sqrt{n}$ ($n = 5$, 95% confidence)						
			Extractive techniques				HS methods		
			HD	MWHD	SDE	SFE	S-HS	P&T	HS-SPME
42	*trans*-Caryophyllene[c]	1418	0.03 ± 0.012	0.20 ± 0.025	0.09 ± 0.037	2.48 ± 0.099	tr	0.23 ± 0.012	0.51 ± 0.050
43	α-*trans*-Bergamotene	1436	0.03 ± 0.012	0.03 ± 0.012	0.08 ± 0.062	2.15 ± 0.062	tr	0.12 ± 0.012	0.31 ± 0.11
44	β-*cis*-Farnesene[c]	1442	0.03 ± 0.012	0.03 ± 0.012	0.07 ± 0.087	1.90 ± 0.062	tr	0.05 ± 0.012	0.21 ± 0.012
45	Germacrene D	1482	0.05 ± 0.012	0.06 ± 0.012	0.12 ± 0.062	1.10 ± 0.037	tr	tr	tr
46	δ-Guaiene	1490	0.06 ± 0.025	0.06 ± 0.050	0.13 ± 0.025	1.7 ± 0.91	tr	0.10 ± 0.037	1.02 ± 0.087
47	Spathulenol	1537	0.04 ± 0.012	0.09 ± 0.012	0.10 ± 0.050	1.56 ± 0.037	–	0.1 ± 0.14	
48	Guaiol	1586	0.12 ± 0.037	0.16 ± 0.012	tr	1.34 ± 0.012	–	0.3 ± 0.26	
49	Sesquiterpenol	1598	0.06 ± 0.025	0.06 ± 0.012	tr	1.46 ± 0.087	–	–	0.51 ± 0.099
50	β-Eudesmol	1622	tr	tr	tr	1.58 ± 0.087	–	–	0.4 ± 0.16
51	Sesquiterpenol	1661	tr	tr	tr	2.38 ± 0.062			0.31 ± 0.112
52	Hydrocarbons, $C_n > 18$	1840	–	–	–	0.99 ± 0.012	–	–	tr

[a] Peak number in Fig. 1.

[b] I_K were determined on the HP-5 column.

[c] Peak identity was also confirmed using a standard compound. tr: traces and iPr: isopropyl.

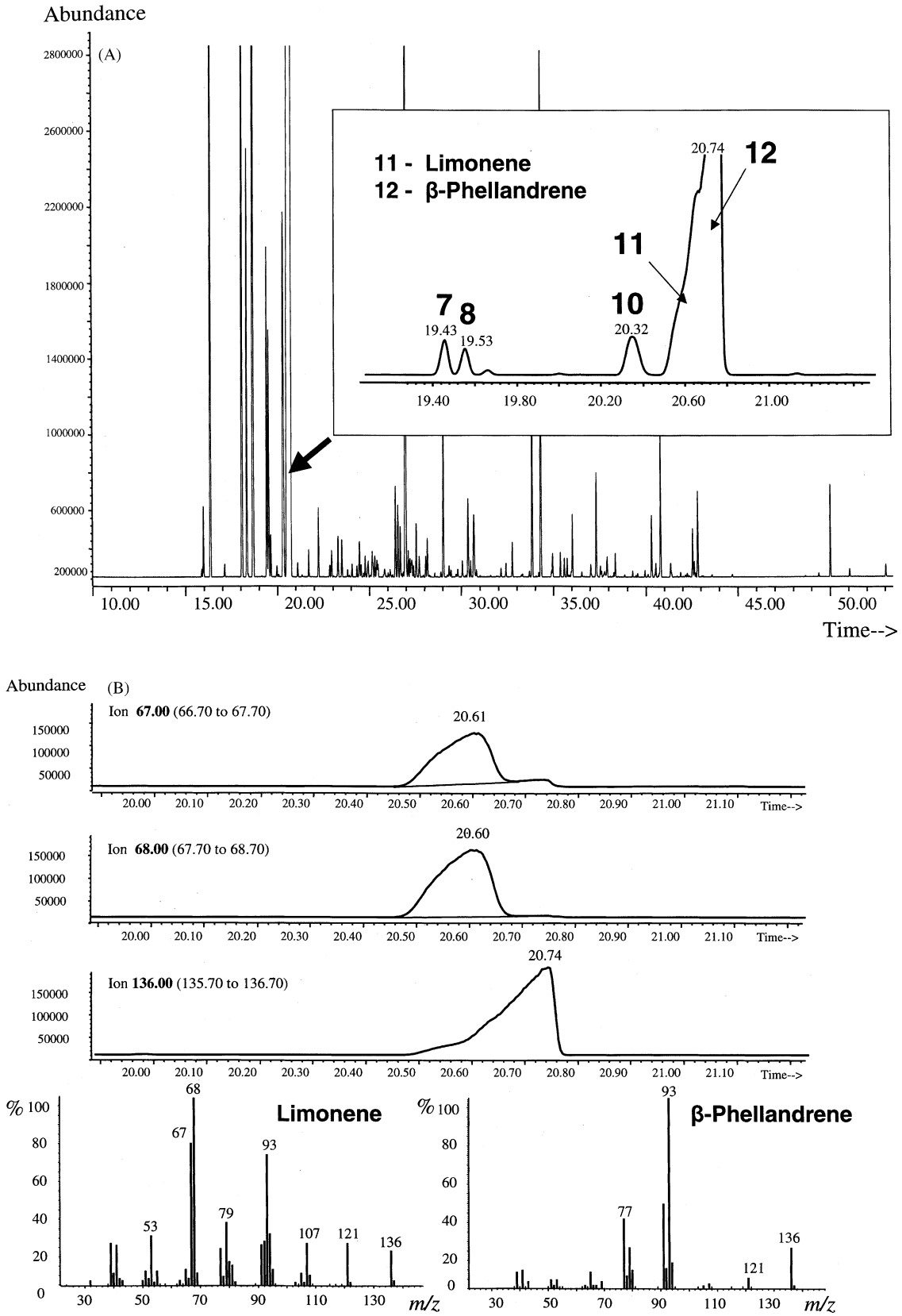

Fig. 2. (A) Total ion current of *Xylopia aromatica* (Lamarck) fruit essential oil (MSD, EI, 70 eV, HP-5 MS, 50 m) with the chromatogram enlargement ($t_R = 19 - 22$ min). (B) Extracted ion chromatograms for *m*/*z* 67, 68 and 136 ions and mass spectra of limonene and β-phellandrene.

Table 2
Composition according to compound families, of the volatile secondary metabolites present in *Xylopia aromatica* fruits

Compound family	Relative amount (%) ± ts/$\sqrt{n}$ ($n = 5$, 95% confidence)						
	Extractive techniques				HS methods		
	HD	MWHD	SDE	SFE	HS	P&T	HS-SPME
Monoterpene hydrocarbons	92 ± 2.6	93.5 ± 0.72	94.0 ± 0.68	63.7 ± 0.38	98.9 ± 0.26	90.3 ± 0.61	89 ± 2.1
Oxygenated monoterpenes	3.8 ± 0.88	2.5 ± 0.15	4.4 ± 0.38	6.4 ± 0.26	0.30 ± 0.024	5.0 ± 0.19	4 ± 1.3
Sesquiterpene hydrocarbons	0.56 ± 0.074	0.82 ± 0.099	1.08 ± 0.087	14.6 ± 0.91	–	1.2 ± 0.10	3.7 ± 0.11
Oxygenated sesquiterpenes	0.22 ± 0.037	0.31 ± 0.012	0.10 ± 0.050	8.32 ± 0.087	–	–	1.5 ± 0.26
Benzenoids	3.3 ± 0.11	3.2 ± 0.11	3.0 ± 0.19	6.76 ± 0.087	0.10 ± 0.012	2.12 ± 0.062	1.64 ± 0.012

techniques could be "interchanged" affording almost the same extract composition. In contrast, HS techniques did differ in the volatile fraction composition obtained.

Up to date, there are no previous reports on *X. aromatica* fruit HS composition. β-Phellandrene and β-myrcene, which, respectively, exhibit the typical terpeny and herbaceous odour notes, were the main components of both the fruit essential oil and its volatile fraction. In a study of six different Amazonian *Annonacea* species, Jurgens et al. [17] reported the dominance of benzenoids (methyl, ethyl and benzyl benzoates, 2-phenylethyl alcohol, benzyl acetate, etc.) in *X. aromatica* flower aroma. Many of these benzenoids were also detected at high concentrations in flowers of another of the *Annonaceae* family tree, cultivated in Colombia, i.e. *C. odorata* [8]. However, in Colombian *X. aromatica* fruits we did not detect these components at high concentrations, which are typical for the *Annonaceae* family, although some other benzenoids, such as methyl salicylate, 4-isopropylbenzaldehyde and isopropylbenzenemethanol were isolated in small amounts (Table 1). The pleasant fragrance of *Xylopia* fruits originated from the complex combination of the different odour notes, i.e. terpeny (α-thujene, camphene, β-pinene, α-terpinene, β-phellandrene, Δ^3-carene and terpinen-4-ol), pine needle-like (α-pinene), flowery (myrtenol, α-terpineol, β-farnesene, *cis*-β-ocimene), herbaceous (myrcene), citrus (limonene), and camphor-like (fenchone), among others [5], composing per se an exotic perfume.

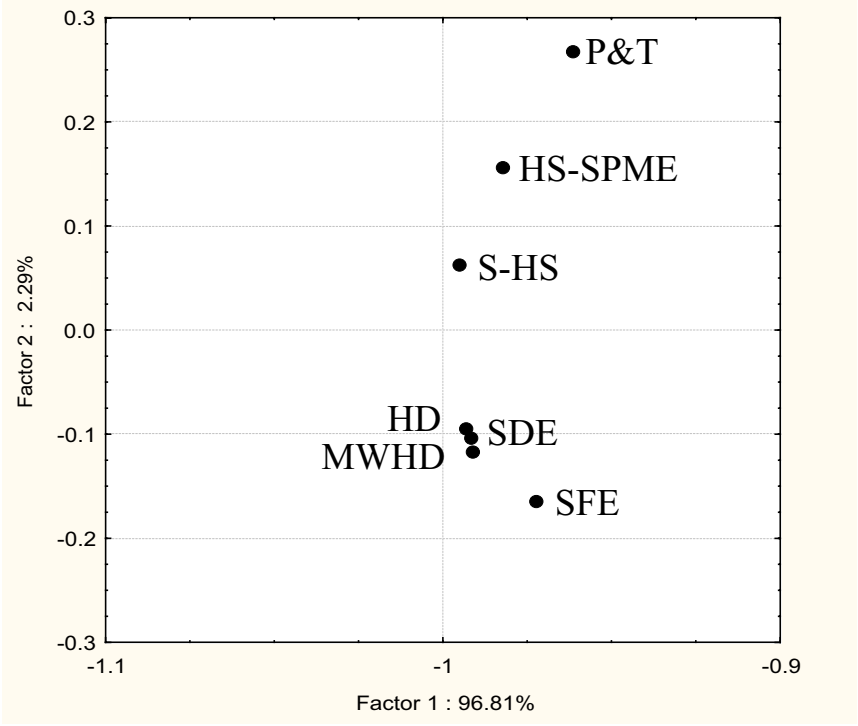

Fig. 3. Representation of the *Xylopia aromatica* (Lamarck) fruit volatile fractions in the coordinate system formed by the first two principal components (loadings are reported in Table 3).

Table 3
Contribution of each *Xylopia aromatica* constituent to the main principal components, used to classify the volatile fractions obtained by different techniques

	Compound	Factor 1	Factor 2
1	α-Thujene	0.373	−0.040
2	α-Pinene	−3.078	1.546
3	Camphene	0.445	−0.109
4	β-Pinene	0.678	0.007
5	β-Myrcene	−2.896	1.129
6	*p*-Mentha-1(7),8-diene	−1.384	1.740
7	α-Phellandrene	−0.718	0.502
8	Δ^3-Carene	0.445	0.174
9	α-Terpinene	0.645	−0.016
10	*p*-Cymene	−0.744	0.041
11	Limonene	−0.094	−0.139
12	β-Phellandrene	−17.564	−0.747
13	*cis*-β-Ocimene	0.527	0.061
14	*trans*-β-Ocimene	0.589	−0.006
15	γ-Terpinene	0.608	0.041
16	*cis*-Sabinene hydrate	0.694	−0.032
17	Fenchone	0.635	−0.098
18	α-Terpinolene	0.696	−0.033
19	*trans*-Sabinene hydrate	0.663	0.002
20	Fenchol	0.671	−0.028
21	α-Campholenal	0.664	0.007
22	Camphene hydrate	0.691	−0.016
23	Citronellal	0.701	−0.068
24	Pinene oxide	0.718	−0.062
25	Phellandral	0.613	−0.158
26	Thujanol	0.680	−0.018
27	Terpinen-4-ol	0.636	−0.127
28	Cryptone	0.189	−0.102
29	Methyl benzeneacetate	0.607	−0.185
30	α-Terpineol	0.599	−0.125
31	Phellandrene epoxide	0.693	−0.032
32	*cis*-Piperitol	0.687	−0.047
33	Myrtenol	0.647	−0.085
34	Methyl salicylate	0.283	−0.224
35	4-iPr-Benzaldehyde	0.371	−0.093
36	*trans*-Piperitol	0.709	−0.072
37	Citronellol	0.704	−0.074
38	iPr-Benzenemethanol	0.542	−0.192
39	α-Cubebene	0.460	−0.128
40	α-Copaene	0.579	−0.136
41	α-*cis*-Bergamotene	0.583	−0.180
42	*trans*-Caryophyllene	0.503	−0.202
43	α-*trans*-Bergamotene	0.553	−0.197
44	β-*cis*-Farnesene	0.581	−0.193
45	Germacrene D	0.647	−0.155
46	δ-Guaiene	0.534	−0.122
47	Spathulenol	0.610	−0.183
48	Guaiol	0.610	−0.155
49	Sesquiterpenol	0.595	−0.145
50	β-Eudesmol	0.600	−0.158
51	Sesquiterpenol	0.552	−0.225
52	Hydrocarbons, $C_n > 18$	0.665	−0.140

4. Conclusion

Colombian *X. aromatica* fruit essential oils (HD, MWHD) and the various volatile fractions, obtained by different HS techniques (S-HS, P&T, HS-SPME), were rich in β-phellandrene (up to 65%), a component of interest to the perfume industry and a useful starting material for fine organic synthesis. The relative chemical compositions of oils and extract, obtained by HD or MWHD and SDE, were similar, but differed from volatile fractions, isolated by S-HS, P&T and HS-SPME. SFE isolated a larger amount of heavier compounds (sesquiterpenoids, benzenoids and hydrocarbons). SDE was particularly effective for monoterpene hydrocarbon isolation. HD- and MWHD-essential oils were very close in their composition, but for the same oil yield, the time required for MWHD was one-fourth of that for HD extraction. The relative amounts of volatiles from *X. aromatica* dry fruits, extracted by HS-SPME or P&T methods, depended upon fibre exposure or purging times.

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References

[1] H. Garcia Barriga, Flora Medicinal de Colombia, Tercer Mundo Editores, Bogotá, 1992.
[2] J.E. Correa, H.Y. Bernal, Especies Vegetales Promisorias de los Países del Convenio Andrés Bello, Editorial Guadalupe, Bogotá, 1989.
[3] C. Morin, S. Ngouela, B. Nyassé, E. Tsamo, M.C. Brochier, J. Nat. Prod. 61 (1998) 264.
[4] O. Ekundayo, J. Essent. Oil Res. 1 (1989) 223.
[5] A.O. Tairu, T. Hofmann, P. Schieberle, J. Agric. Food Chem. 47 (1999) 3285.
[6] J.B. da Silva, A.B. Rocha, Rev. Cienc. Farm. (Sao Paulo) 3 (1981) 33.
[7] M. Godefroot, P. Sandra, M. Verzele, J. Chromatogr. 203 (1981) 325.
[8] E.E. Stashenko, N. Quiroz, J.R. Martínez, J. High Resolut. Chromatogr. 19 (1996) 353.
[9] E.E. Stashenko, R. Acosta, J.R. Martínez, J. Biochem. Biophys. Methods 43 (2000) 379.
[10] K. Umano, T. Shibamoto, J. Agric. Food Chem. 35 (1987) 14.
[11] E. Kováts, Adv. Chromatogr. 1 (1965) 229.
[12] W. Jennings, T. Shibamoto, Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography, Academic Press, New York, 1980.
[13] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, Allured Publishers, Carol Stream, IL, 1995.
[14] D. Joulain, W.A. König, The Atlas of Specral Data of Sesquiterpene Hydrocarbons, E.B.-Verlag, Hamburg, 1998.
[15] E.E. Stashenko, B.E. Jaramillo, J.R. Martínez, J. Chromatogr. A 1025 (2004) 93.
[16] J.R.J. Paré, J.M.R. Bélanger, S.S. Stafford, Trends Anal. Chem. 13 (1994) 176.
[17] A. Jurgens, A.C. Webber, G. Gottsberger, Phytochemistry 55 (2000) 551.