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Identification of the Key Aroma Compounds in Dried Fruits of *Xylopia aethiopica*

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West African "Peppertree" [*Xylopia aethiopica* (Dunal) A. Rich, Annonaceae] is a slim, tall tree of about 60–70 cm in diameter and up to 15–30 m high with straight stem and a slightly stripped or smooth bark. It is widely distributed in the humid forest zones of West Africa especially along rivers in the dry country sides (Irvine 1961). *Xylopia aethiopica* has a wide variety of application, the very odorous roots of the plant are employed in West Africa in tinctures, administered orally to expel worms and other parasitic animals from the intestines, or in teeth-rinsing and mouth-wash extracts against toothaches. The fruits are also used in various forms and exhibit revulsive properties, especially when mashed with grains. These properties are used advantageously in the external treatment of rheumatism. Crushed powdered fruits can also be mixed with shea butter fat and coconut oil and used as creams, cosmetic products, and perfumes (Burkill 1985), and the dried fruits are also used as spices in the preparation of two special local soups named "obe ata" and "isi-ewu" taken widely in the southwest and southeastern parts of Nigeria.

Ekong and Ogan (1968), were the first to report on the chemical composition of *Xylopia aethiopica*, and several publications have appeared subsequently on this subject. A number of diterpenes from the bark, fruits, and pericarp of the plant have been reported, Faulkner et al. (1985); Rabunmi and Pieeru (1992); Harrigan et al. (1994). Ekundayo (1989) published a review of the volatiles in a number of Annonaceae species among which includes *Xylopia aethiopica* and, reported that they consist mainly of mono and sesquiterpenoids with typical constituents being α- and β-pinene, myrcene, p-cymene, limonene, linalool, and 1,8-cineole. Recently, two new sesquiterpenes, elemol and guaiol (among other terpenes) were found in the essential oil of the fruit from the Republic of Benin (Ayedoun et al. 1996) while Jirovetz et al. (1997) gave a semblance of the aroma note from the essential oil in the fruit of *Xylopia aethiopica* from Cameroon.

No attempt had been undertaken to rank the volatiles in their flavor contribution. In order to detect these compounds, volatiles isolated from the crushed dried fruits of *Xylopia aethiopica* from Nigeria have been analyzed by High-Resolution Gas Chromatography (HRGC) and eluate sniffing.

EXPERIMENTAL PROCEDURES

Chemicals

The reference compounds of the odorants listed in the tables were obtained from the various suppliers given in parentheses: no. 2, 6, 8, 11, 16, 17, 22, and 24, (Aldrich, Steinheim, Germany); no. 7, 10, 19, 20, and 23 (Merck, Darmstadt, Germany); no. 9, 26, and 27 (Lancaster, Mühlheim, Germany); no. 4, 5, 18, and 25 (Fulka, Neu-Ulm, Germany); no. 1, 14, and 15 (Alfa Products, Karlsruhe, Germany). α-Farnesene was a gift.

Isolation of the Volatile Oil

The smoked, dried fruits of *Xylopia aethiopica* (6 g) were immediately frozen in liquid nitrogen and finely powdered by means of a commercial blendor (Janke & Kunkel, Stanfen). The powder was extracted with solvent mixture of methanol, water, and dichloromethane. The extract (organic phase) was dried over sodium sulfate and concentrated on a Vigreux column (50 cm × 1 cm internal diameter) and the volatiles isolated by sublimation in vacuo using the equipment described by Guth and Grosch (1989).

Separation of Volatiles into Acidic and Neutral/Basic Fractions

By treatment of the distillate with aqueous sodium bicarbonate (Hofmann and Schieberle 1995), a fraction of the acidic volatiles (fraction AV) and of the neutral/basic volatiles (fraction N/B) were obtained. After

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drying over anhydrous sodium sulfate, both fractions were concentrated to 200 ml (Schieberle 1991) and the odor-active compounds evaluated by aroma extract dilution analysis (AEDA).

Capillary Gas Chromatography (HRGC)-Mass Spectrometry (MS)

HRGC was performed with a Carlo Erba gas chromatograph, (Type 5160, MEGA SERIES) using fused silica thin-film capillaries: capillary FFAP, capillary SE54 (DB-5) (each 30 m × 0.32 mm; J and W. Scientific, Fisons, Mainz, Germany; film thickness, 0.25 μm). Samples were applied by the "cold on-column" injection technique at 35°C. After 2 min, the temperature of the oven was raised by 40°C/min to 60°C, held 2 min isothermally, then raised by 6°C to 180°C and, finally, by 10°C/min to 230°C. The retention indices were calculated by using n-alkanes as the reference (Schieberle 1991). Mass spectrometry was performed on an MD800 (Fisons Instruments, Mainz, Germany) in tandem with the capillaries described above. Mass spectra in the electron impact mode (MS/EI) were generated at 70 eV and in the chemical ionization mode (MS/CI) at 110 eV with isobutane as the reagent gas.

Aroma Extract Dilution Analysis (AEDA)

The flavor dilution (FD) factors of the odorants in the fractions AV and N/B of *Xylopia aethiopica* were determined by AEDA (Schieberle 1995). An aliquot of the respective distillate (0.5 μL of 200 μL) was separated on capillary FFAP, the efluent ratio was split to an FID and a sniffing port (1 + 1 by vol.), and the odor-active regions and the odor qualities were assigned by three assessors (GC/O). The extract was stepwise diluted with diethyl ether (1 + 1 by vol.), and aliquots of the dilutions were evaluated by two assessors. The FD chromatogram (FD versus retention indices) was plotted.

RESULTS

From the FD chromatogram obtained by applying the AEDA on an extract containing the neutral/basic volatiles of *Xylopia aethiopica*, 24 odor-active compounds were sensorilly detected in the flavor range of 4-8196. Among these compounds, 6 odorants showed very high FD factors of greater than or equal to 512 (nos. 2, 6, 12, 16, 17, and 21; Fig. 1). All of the key odorants could be identified on the basis of the criteria given in footnote b of Table 1.

In the fraction of the acidic volatiles, four odorants were sensorially detected (Table 2) with all of them fully identified. Among them, 3 odorants 3-ethylphenol, 4-ethy-2-methoxyphenol and hexanoic acid have not been reported as aroma compounds found in *Xylopia aethiopica*. Vanilline and 3-ethylphenol gave a reasonably high FD-factors of 128 and are considered as being important odorant in the fruit.

All the odor-active compounds detected were terpenes eliciting the characteristic flowery and terpeny notes except 3-ethylphenol, (phenollic); vanilline, (vanilla-like); hexanoic acid, (acidic); and 4-ethyl-2-methoxyphenol, (smoky). Linalool (no. 17; Table 1), with pepperish-flowery odor showed the highest FD factor while trans-β-ocimene (flowery note) and α-farnesene (sweet-flowery note) had the same FD factor

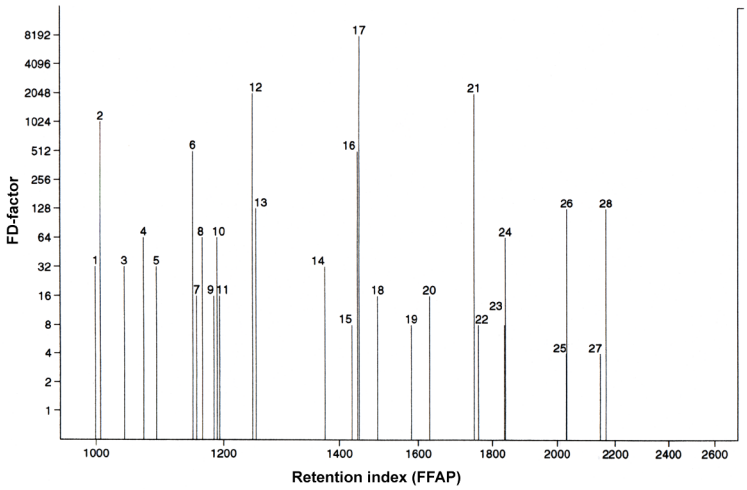

Fig. 1. Flavor dilution chromatogram of the dried fruit of *Xylopia aethiopica* fruits.

Table 1. Most odor-active compounds in the neutral/basic volatiles (FD ³ 4) in *Xylopia aethiopica*.

No.[z]	Aroma quality[y]	Retention index[x] FFAP	Retention index[x] SE-54	FD factor	Aroma compound[w]
1	Sweet, terpeny	996	919	32	α-Thujene
2	Terpeny	1007	931	1024	α-Pinene
3	Fruity	1045	855	32	Ethyl-2-methylbutanoate
4	Spicy, fruity	1075	949	64	Camphene
5	Terpeny	1095	975	32	Sabinene
6	Terpeny	1152	981	512	β-Pinene
7	Metallic	1158	988	16	Myrcene
8	Light minty	1167	1005	64	α-Phellandrene
9	Terpeny	1185	1025	16	α-Terpinene
10	Light, lemon-like	1190	1029	64	Limonene
11	Light, pepperment-like	1194	1031	16	1,8-Cineole
12	Flowery	1250	1172	2048	trans-β-Ocimene
13	Terpeny	1256	1033	128	β-Phellandrene
14	Fruity, terpeny	1375	1112	32	p-Mentha-3,8-triene
15	Terpeny	1432	1220	8	3-Carene
16	Flowery	1446	1206	512	Myrtenol
17	Flowery, pepperish	1450	1109	8192	Linalool
18	Flowery, fatty	1497	1207	16	Decanal
19	Flowery, lilac-like	1582	1195	8	α-Terpeniol
20	Light mint, terpeny	1630	1184	16	Terpinen-4-ol
21	Sweet, flowery	1750	--	2048	α-Farnesene
22	Flowery	1762	1231	8	b-Citronellol
24	Flowery, rose-like	1840	1256	64	Geraniol
27	Sweet, camphoracious	2148	--	4	Fenchone

[z]Numbers refer to Fig. 1.

[y]Odor quality perceived at the sniffing port.

[x]Retention index.

[w]Odorants were identified by comparing them with reference compounds on the basis of the following criteria: retention index (RI) on two stationary phases detailed in the table, mass spectra MS(CI), odor quality, and odor threshold (ratio of FID signal to FD factor) at the sniffing port.

(nos. 12 and 21; Table 1). α-Pinene having a typical terpeny odor note had an FD factor twice as much as β-pinene, another compound with terpeny odor note, myrtenol with flowery note which slightly resembles that of linalool (but lacks the pepperish note of linalool) had the same FD factor with β-pinene (no. 6; Table 1). Both β-phellandrene (terpeny note) and 3-ethylphenol (phenollic note) had the same FD factor of 128, the phenollic odor of 3-ethylphenol and the vanilla-like of vanilline are very easily noticed during the sniffing experiments even though these compounds appeared with very low chromatogram peaks. Camphene with spicy note had the same FD factor with limonene (bearing light citrus-like odor note) and α-phellandrene (terpeny note). Compounds with relatively low FD factors include α-thujene (terpeny note), sabinene (terpeny note), myrcene (metallic note), α-terpenene (terpeny note), 1,8-cineole (light pepperment-like note), p-mentha-3,8-triene (fruity, terpeny note), terpinen-4-ol (light minty, terpeny note). The results of the identification experiments are as shown in Table 1.

Table 2. Odor-active compounds in acidic fraction of dried *Xylopia aethiopica* fruits (FD ³ 4).

No.	Aroma quality[z]	Retention index[y] FFAP	SE-54	FD factor	Aroma compound[x]
23	Acidic	1837	1019	8	Hexanoic acid
25	Smoky	2031	1285	4	4-Ethyl-2-methoxyphenol
26	Vanilla-like	2033	1402	128	Vanilline
28	Phenollic	2168	1167	128	3-Ethylphenol

[z]Odor quality perceived at the sniffing port.
[y]Retention index
[x]Cf footnote w; Table 1.

DISCUSSION

The simple chromatogram of the volatile oil of *Xylopia aethiopica* gave an indication of the presence of numerous terpenoids compounds in it, however upon application of aroma extract dilution analysis on the sample it became clear that several of these compounds have little or no contribution to the overall odor quality of the fruit volatiles. We thus paid our attention on those odorants with FD factor of 4 and above, as this range was considered to be significant enough for our studies.

Results obtained showed that linalool, β-trans-ocimene, α-farnesene, α-pinene, β-pinene, myrtenol, β-phellandrene, and 3-ethylphenol were the most important odorants present in the volatile oil of the fruit with linalool being the most intense giving the pepperish note, characteristic of the ground, dried, smoked fruits of *Xylopia aethiopica*. Linalool has also been found to be an important odor-active compound present in black pepper (*Piper nigrum* L.) by Jagella (pers. commun.). All the terpenoids detected in the volatile oil of *Xylopia aethiopica* have been reported earlier by previous workers (Ayedoun et al. 1996; Jirovetz et al. 1997). However all aroma compounds found in the acidic fraction are being reported for the first time as being present in the fruits of *Xylopia aethiopica*. Ayedoun et al. (1996) reported the presence of p-mentha-3,8-diene but, its odor note was not detected during the AEDA experiments, however, p-mentha-3,8-triene with a fruity, terpene-like odor was detected instead.

The commercially exploitable features of volatile oils of *Xylopia aethiopica* have not been investigated to any extent before, however, a close examination of the compositional features of the *Xylopia aethiopica* essential oils should reveal a promising commercial potential which could be effectively exploited. As organoleptic properties of essential oils are usually due to the oxygenated components, volatile oils of *Xylopia aethiopica* in which linalool, myrtenol, and terpinen-4-ol are very important, may be ideal resource materials as odor fixtures in toiletries and perfumes and this might explain why the locals use the dried fruits in both expectorants and disinfectant applications.

Results reported here suggest that linalool is an important odor-active compound in the fruit of *Xylopia aethiopica* and also that AEDA in combination with GC-sniffing is an excellent approach for the determination of key compounds causing the aroma in the sample as well as other similar samples.

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