Characterization of the Key Aroma Compounds in Dried Fruits of the West African Peppertree *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) Using Aroma Extract Dilution Analysis

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Application of aroma extract dilution analysis on an extract of the dried fruits of the West African peppertree *Xylopia aethiopica* obtained by extraction with diethyl ether followed by sublimation in vacuo revealed 28 odor-active compounds in the flavor dilution (FD) factor range of 4−8192, all of which could be identified. The highest FD factor was found for linalol (floral), followed by (*E*)-β-ocimene (flowery), α-farnesene (sweet, flowery), β-pinene (terpeny), α-pinene (pine needle-like), myrtenol (flowery), and β-phellandrene (terpeny). Vanillin (vanilla-like) and 3-ethylphenol (smoky, phenolic) showing somewhat lower FD factors (FD = 128) were detected for the first time as constituents of the dried fruit.

Keywords: *Xylopia aethiopica; West African peppertree; aroma extract dilution analysis; linalol; α-farnesene; (E)-β-ocimene*

INTRODUCTION

The West African Peppertree *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae), which is widely distributed in the humid forest zones of West Africa, is a slim, tall tree of ∼60 cm in diameter and up to 30 m high with a straight stem having a slightly stripped or smooth bark. It bears odoriferous fruits, which are slender pods slightly curved with ∼15 carpels and are arranged in capitula to form bouquets of 12−20 bacciferous-like capsules. The fruits are commonly used in traditional folk medicine, against cough, bronchitis, dysentery, and female sterility (Faulkner et al., 1985). Due to their attractive aroma, the crushed powdered fruits are also used in mixtures with shea butter fat and coconut oil in creams, cosmetic products, and perfumes (Burkill, 1985) as well as in the preparation of two traditional West African soups named "obe ata" and "isi-ewu".

A number of diterpenes have been identified in different parts of the tree (Ekong and Ogan, 1968; Faulkner et al., 1985; Rabunmi and Pieeru, 1992; Harrigan et al., 1994a), and also several bioactive compounds have been found in its fruit (Ekong et al., 1969; Boakye-Yiadom et al., 1977; Hasan et al., 1982; Sanni et al., 1990; Harrigan et al., 1994b; Rabunmi and Pieeru, 1992). Ogan (1971) and Ekundayo (1989) reported that the volatile fraction of the fruits mainly consists of mono- and sesquiterpenes, among which α- and β-pinene, myrcene, *p*-cymene, limonene, linalol, and 1,8-cineole were the most predominant. Additional compounds identified in the volatile oil of the fruit include β-phellandrene, (*E*)-β-ocimene, α-terpineol, bisabolene, and cuminal (Ogan, 1971). Recently, sabinene and terpinen-4-ol were found in the volatile oil of *Xylopia* fruits of a Nigerian variety (Onayade-Sontan, 1991), and two novel sesquiterpenes, namely, elemol and guaiol, were identified in the essential oil of the fruits grown in the Republic of Benin (Ayedoun et al., 1996) as well as in Cameroon (Jirovetz et al., 1997).

It is obvious from the literature review that a great number of volatiles occur in the fruits of *X. aethiopica*; however, it is as yet not clear which of these compounds are the key aroma contributors evoking the attractive overall odor of the dried fruits.

The purpose of the present investigation was, therefore, to identify the most odor-active compounds in the dried fruits of *X. aethiopica* and to rank them in their aroma contribution by means of an aroma extract dilution analysis (AEDA).

EXPERIMENTAL PROCEDURES

Chemicals. The reference compounds of the odorants listed in Table 1 were obtained from the various suppliers given in parentheses: compounds **2**, **3**, **6**, **8**, **11**−**13**, **16**, **17**, **22**, **24**, and **27** (Aldrich, Steinheim, Germany); compounds **7**, **10**, **19**, **20**, and **23** (Merck, Darmstadt, Germany); compounds **9**, **26**, and **28** (Lancaster, Mühlheim, Germany); compounds **4**, **5**, **18**, and **25** (Fluka, Neu-Ulm, Germany); and compounds **1**, **14**, and **15** (Alfa Products, Karlsruhe, Germany). α-Farnesene (**21**) was a gift from Haarmann & Reimer (Holzminden, Germany).

Sample Collection and Pretreatment. Fresh fruits were picked from *X. aethiopica* trees at the Government Forest Reserve in Ogun State, Nigeria. The collected samples were botanically identified by a plant taxonomist at the Ogun State University. Voucher specimens were submitted for preservation at the Herbaria of the Department of Botany, University of Ibadan, Nigeria, and at the Forestry Research Institute of Nigeria, Ibadan, Nigeria. Following a traditional procedure,

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Table 1. Most Odor-Active Compounds (FD ≥ 4) Identified in Extracts Containing either the Neutral/Basic Volatiles (NB) or the Acidic Volatiles (AF) Isolated from the Dried Fruits of *X. aethiopica*

			retention index on		FD factor[c]	
no.	odorant[a]	odor quality[b]	FFAP	SE-54	NB	AF
1	α-tujene	sweet, terpeny	996	919	32	nd[d]
2	α-pinene	pine needle-like	1007	931	1024	nd
3	ethyl 2-methylbutanoate	fruity	1045	855	32	nd
4	camphene	terpeny	1075	949	64	nd
5	sabinene	terpeny	1095	975	32	nd
6	*β*-pinene	terpeny	1102	981	512	nd
7	myrcene	metallic, herbaceous	1158	988	16	nd
8	α-phellandrene	dill herb-like, terpeny	1167	1005	64	nd
9	α-terpinene	terpeny	1185	1025	16	nd
10	limonene	citrus-like	1190	1029	64	nd
11	1,8-cineole	peppermint-like	1194	1031	16	nd
12	(*E*)-*β*-ocimene	flowery	1250	1172	2048	nd
13	*β*-phellandrene	terpeny	1256	1033	128	nd
14	*p*-mentha-1,3,8-triene	terpeny	1375	1112	32	nd
15	3-carene	terpeny	1432	1220	8	nd
16	myrtenol	flowery	1446	1206	512	nd
17	linalol	floral	1450	1109	8192	nd
18	decanal	fatty	1497	1207	16	nd
19	α-terpineol	flowery	1582	1195	8	nd
20	terpinen-4-ol	terpeny	1630	1184	16	nd
21	α-farnesene	sweet-flowery	1750		2048	nd
22	*β*-citronellol	flowery	1762	1231	8	nd
23	hexanoic acid	sweaty	1837	1019	nd	8
24	geraniol	flowery	1840	1256	64	nd
25	4-ethyl-2-methoxyphenol	smoky, phenolic	2031	1285	nd	4
26	fenchone	camphor-like	2148		4	nd
27	3-ethylphenol	phenolic	2168	1167	nd	128
28	vanillin	vanilla-like	2568	1402	nd	128

[a] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on the two HRGC stationary phases given in the table, mass spectra obtained by MS(EI) and MS(CI), and odor quality and odor intensity perceived at the sniffing port. [b] Odor quality perceived at the sniffing port. [c] Flavor dilution (FD) factor determined in the extracts containing the acidic (AF) or the neutral/basic (NB) volatiles. Analyses were performed by two assessors in duplicates. The data differed by <2 FD factors. [d] nd, not detectable.

the fresh fruits were then carefully sun-dried and smoked before being packed into polyethylene bags under nitrogen.

Isolation of the Volatiles. Smoked, dried fruits of *X. aethiopica* (6 g) were frozen in liquid nitrogen and finely powdered by means of a commercial blender. The powder was suspended in a mixture of water (20 mL), dichloromethane (25 mL), and methanol (50 mL) and further homogenized for 1 min by using an Ultra-Turrax (Jahnke and Kunkel, Staufen, Germany). Dichloromethane (25 mL) and water (25 mL) were added, and the mixture was homogenized for 1 min. The organic layer was collected, while the aqueous layer was extracted again with dichloromethane (total volume = 200 mL). The combined organic layers were washed with brine (2 × 50 mL), then dried over Na_2SO_4, and concentrated to 100 mL using a Vigreux column (60 cm × 1 cm). The volatiles were then isolated by sublimation in vacuo as reported recently (Guth and Grosch, 1989; Schieberle, 1995). The distillate was treated three times with an aqueous sodium bicarbonate solution (0.5 mol/L; total volume 100 mL), and the ethereal solution containing the neutral/basic compounds (fraction NB) was washed twice with brine (total volume = 100 mL) and dried over Na_2SO_4. The bicarbonate solution containing the acidic compounds was adjusted to pH 3.0 with hydrochloric acid (1 mol/L), and the acidic volatiles were then extracted with diethyl ether (total volume = 150 mL; fraction AF). After washing with brine (total volume = 50 mL), the ethereal solution was dried over Na_2SO_4.

High-Resolution Gas Chromatography (HRGC)/Mass Spectrometry (MS). HRGC was performed with a Type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) by using the following capillaries: FFAP (30 m × 0.32 mm fused silica capillary; free fatty acid phase, 0.25 *μ*m; J&W Scientific, Fisons Instruments, Mainz, Germany) and SE-54 (30 m × 0.32 mm fused silica capillary DB-5; 0.25 *μ*m; J&W Scientific). The samples were applied by the cold on-column injection technique at 35 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 60 °C, held 2 min isothermally, then raised 6 °C/min to 180 °C and, finally, by 10 °C/min to 230 °C, and held for 5 min. The flow of the carrier gas, helium, was 2.3 mL/min. At the end of the capillary, the effluent was split 1:1 (by volume) into an FID and a sniffing port device using deactivated but uncoated fused silica capillaries (50 cm × 0.32 mm). The FID and the sniffing port were held at 200 °C. Linear retention indices (RI) were calculated from the retention times of *n*-alkanes as the reference. MS analysis was performed on an MD 800 (Fisons Instruments) 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 using methane as the reagent gas.

AEDA. The flavor dilution (FD) factors of the odor-active compounds were determined by AEDA (Schmid and Grosch, 1986; Schieberle and Grosch, 1987) of the following dilution series: Either fraction NB or fraction AF (each concentrated to 200 *μ*L) was stepwise diluted with diethyl ether (1+1). HRGC/olfactometry was then performed with aliquots (0.5 *μ*L) of the original distillate and the diluted extracts.

RESULTS

Upon grinding of dried fruits of *X. aethiopica* an intense overall aroma with floral, smoky, and terpene- and pepper-like odor notes could be detected. An aroma concentrate was prepared from the ground material by solvent extraction and high-vacuum distillation. To avoid interferences with the GC separations, the acidic volatiles (fraction AF) were separated from the extract by treating the flavor distillate with sodium bicarbonate.

Application of the AEDA on the neutral/basic volatile fraction (fraction NB) exhibiting an intense flowery-terpeny overall odor revealed 24 odor-active compounds in the FD factor range of 4−8192 (column NB in Table 1), among which compounds **2**, **12**, **17**, and **21** showed by far the highest FD factors. The identification experiments revealed linalol (**17**), α-farnesene (**21**), (*E*)-*β*-ocimene (**12**), and α-pinene (**2**) to be responsible for the

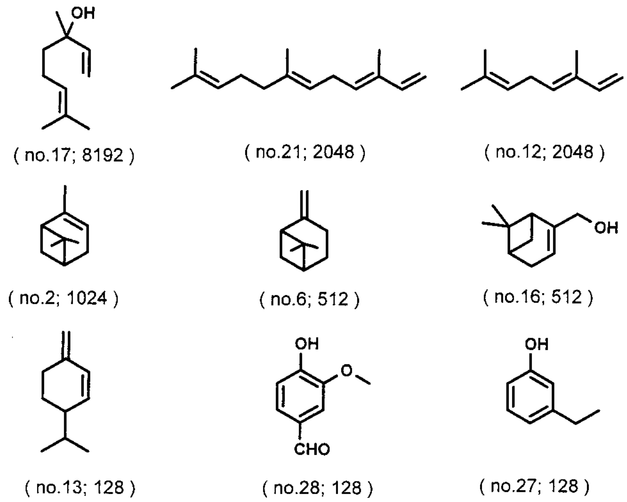

Figure 1. Structures of the key odorants (FD ≥ 128) identified in dried fruits of *X. aethiopica*. Numbering refers to Table 1. FD factors are given in parentheses.

flowery and terpeny odor notes detected in the corresponding odor-active regions. In addition, β-pinene (**6**), myrtenol (**16**), and β-phellandrene (**13**) were found as important contributors to the overall odor of the *Xylopia* fruit. Camphene (**4**), α-phellandrene (**8**), limonene (**10**), and geraniol (**24**) (Figure 1) showed lower odor activities.

Application of the AEDA on fraction AF, eliciting an intense sweet-phenolic odor, revealed four odor-active compounds in the FD factor range of 4−128 (column AF in Table 1). On the basis of their high FD factors, vanillin (**28**) and 3-ethylphenol (**27**) were identified as main contributors to the overall odor of the acidic volatile fraction. Both odorants could unequivocally be identified by comparison of their mass spectra (EI, CI), their retention indices on two capillaries (FFAP, SE-54), and the odor quality with those of the reference compounds. Hexanoic acid (**23**) and 4-ethyl-2-methoxyphenol (**25**) were identified with somewhat lower odor impacts.

DISCUSSION

The results of the AEDA indicated the nine odorants shown in Figure 1, namely, linalol, (*E*)-β-ocimene, α-farnesene, α-pinene, β-pinene, myrthenol, β-phellandrene, vanillin, and 3-ethylphenol, to be the main contributors to the overall floral terpeny, smoky odor of the dried fruits of the West African peppertree *X. aethiopica*. Being well in line with the pepper-like odor quality, linalol was very recently also identified as the most odor-active compound of black pepper (Jagella and Grosch, 1999). The terpenes characterized in the present investigation have earlier been reported as constituents of the volatile oil of *X. aethiopica* (Ayedoun et al., 1996; Jirovetz et al., 1997); however, this is the first time that these compounds were successfully ranked with respect to their flavor contribution. The phenolic compounds vanillin and 3-ethylphenol were identified for the first time in the fruits of *X. aethiopica*. It is very likely that these phenolics evoke the sweet-smoky odor note present in the overall aroma of the dried fruits.

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