Studies on the Key Odorants Formed by Roasting of Wild Mango Seeds (*Irvingia gabonensis*)

A. O. Tairu,[†] T. Hofmann,[‡] and P. Schieberle*[,§]

Department of Chemical Sciences, University of Agriculture, Abeokuta, Ogun State, Nigeria, Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, 85748 Garching, Germany, and Institut für Lebensmittelchemie der Technischen Universität München, Lichtenbergstrasse 4, 85748 Garching, Germany

Application of the aroma extract dilution analysis on a concentrate of volatiles obtained by solvent extraction and high vacuum distillation from roasted seeds (180 °C; 15 min) of wild mango (*Irvingia gabonensis*) revealed 32 odor-active compounds with flavor dilution (FD) factors ranging from 8 (low odor activity) to 2048 (high odor activity). The identification experiments based on the use of reference odorants revealed methional (cooked potato-like) followed by 2-acetyl-1-pyrroline (roasty, popcorn-like), butan-2,3-dione, pentan-2,3-dione, 2-ethyl-3,5-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine as the key aroma compounds among the 27 odorants identified. All odorants are reported for the first time as components of roasted wild mango seeds.

Keywords: *Wild mango; Irvingia gabonensis; aroma extract dilution analysis; methional; 2-acetyl-1-pyrroline*

INTRODUCTION

Wild mango (*Irvingia gabonensis*, Aubry Lecomte ex O'Rork-Baill, family: Simarubaceae) grows naturally in parts of Africa extending from Senegal to the Sudan and to the South of Angola. The fruit, which is not related botanically to the well-known, cultivated mango *Mangifera indica* L. (Okafor, 1981), is available between July and October. Due to the pleasant roasty-nutty aroma generated upon thermal processing, the roasted seeds are commonly used in Africa as a flavoring in traditional dishes.

Numerous data are reported in the literature on nutritional and dietary aspects of the seeds (Eka, 1980; Okafor, 1975a,b; Okingbo, 1977; Abaelu and Akinrimisi, 1980; Aina, 1990; Giami et al., 1994). However, no information is available on the volatile compounds evoking the attractive nutty overall aroma of the roasted kernels.

For several reasons, there is growing interest in the food industry to make use of plant materials generating roast flavors upon heating. To find novel sources of roast aromas, knowledge on key aroma compounds which can be generated from certain raw materials has to be extended. The purpose of the present investigation was, therefore, to characterize the key odorants generated by roasting wild mango seeds using the aroma extract dilution analysis.

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially and used as received (numbers refer to Table 1): nos. **1**, **6**, **12**, **17**, **24**, **25**, and **31** (Aldrich, Steinheim, Germany); nos. **2**, **4**, **14**, and **27** (Merck, Darmstadt, Germany); nos. **11** and **32** (Lancaster, Mühlheim, Germany); nos. **3** and **28**−**30** (Fluka, Neu-Ulm, Germany); and nos. **19**−**21** (Alfa Products, Karlsruhe, Germany). Other compounds were synthesized and purified according to the literature cited: nos. **18**, **22**, and **23** (Cerny and Grosch, 1993), no. **9** (Buttery et al., 1982), no. **13** (Schieberle, 1991), and no. **26** (Czerny et al., 1996).

Samples. Ripe fruits were picked by one of the authors (A. O. Tairu) from wild mango trees grown on a farm in Fiditi near Oyo, Oyo State, Nigeria, in 1997. The samples were botanically identified by a plant taxonomist at the Ogun State University. Voucher specimens were submitted for preservation at Herbaria of the Department of Botany, University of Ibadan, Nigeria and at the Forestry Research Institute of Nigeria, Ibadan, Nigeria. The flesh was removed from the fruits, and the seeds were carefully sun-dried (about 4 days) to obtain a moisture content of 12−14% before sealing in polyethylene bags under nitrogen. The material was transported to Germany and stored in a refrigerator at −18 °C before use.

Roasting Process. Samples (10 g) were thermally treated at 180 °C for 15 min in a closed vessel using a thermostated alumina bloc (Schieberle, 1992). In preliminary sensory experiments (10 panelists), these conditions were found to give the most attractive roast aroma. After roasting, the seeds were immediately frozen in liquid nitrogen prior to isolation of the volatiles.

Isolation of the Volatiles. The frozen, roasted material (10 g) was ground by means of a commercial blendor, sieved (particle size: 60 μm), then suspended in tap water (10 mL), and extracted with diethyl ether (70 mL) by vigorous stirring for 15 min. The mixture was filtered over filter paper, and the residue was

* Author to whom correspondence should be addressed (phone ++49-(0)89-289 13265; fax ++49-(0)89-289 14183; e-mail Peter.Schieberle@Lrz.tum.de).

[†] University of Agriculture.

[‡] Deutsche Forschungsanstalt für Lebensmittelchemie.

[§] Institut für Lebensmittelchemie der Technischen Universität München.

10.1021/jf990765u CCC: $19.00 © 2000 American Chemical Society
Published on Web 05/27/2000

Table 1. Key Odorants (FD ≥ 8) Identified in Roasted Seeds of Wild Mango

			RI[a] on		FD-factor[c] in	
no.	odorant[a]	odor quality[b]	FFAP	SE-54	NB	AF
1	3-methylbutanal	malty	920	650	512	<1
2	butan-2,3-dione	buttery	979	596	1024	<1
3	methyl 3-methylbutanoate	sweet, fruity	1017		64	<1
4	pentan-2,3-dione	buttery	1054	696	1024	<1
5	unknown	sweet	1153		32	<1
6	hexanal	green	1082	803	64	<1
7	unknown	sweet	1225		32	<1
8	unknown	earthy	1295		64	<1
9	2-acetyl-1-pyrroline	roasty, popcorn-like	1330	906	1024	<1
10	unknown	sweet	1348	1154	16	<1
11	dimethyltrisulfide	sulfurous	1375	971	64	<1
12	nonanal	fatty	1397	1105	8	<1
13	2-propionyl-1-pyrroline	roasty, popcorn-like	1400		128	<1
14	trimethylpyrazine	earthy	1406	1000	32	<1
15	unknown	sweet	1412	1193	32	<1
16	unknown	roasty	1423	1193	32	<1
17	3-isopropyl-2-methoxypyrazine	earthy	1427	1097	256	<1
18	2-ethyl-3,6-dimethylpyrazine	potato-like	1430	1079	128	<1
19	(*E*)-2-octenal	fatty	1434	1060	8	<1
20	acetic acid	sour	1439	605	<1	128
21	methional	cooked potato-like	1450	905	2048	<1
22	2-ethyl-3,5-dimethylpyrazine	potato-like	1455	1083	1024	<1
23	2,3-diethyl-5-methylpyrazine	potato-like	1485	1158	1024	<1
24	3-isobutyl-2-methoxypyrazine	earthy	1514	1184	128	<1
25	(*E*)-2-nonenal	fatty	1529		64	<1
26	2-ethenyl-3,5-dimethylpyrazine	potato-like	1552	1102	64	<1
27	butanoic acid	sweaty	1612	821	<1	64
28	phenylacetaldehyde	flowery, honey-like	1638	1047	32	<1
29	(*E*)-2-decenal	fatty	1648		16	<1
30	2- and 3-methylbutanoic acid	sweaty	1653	878	<1	128
31	(*E*,*E*)-2,4-decadienal	fatty, waxy	1812	1319	128	<1
32	vanillin	vanilla-like	2573	1402	<1	128

[a] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on 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. Flavor dilution (FD) <1 means that the compound was not detected by sniffing at the given concentration in the original extract (FD = 1 by definition). Analyses were performed by two assessors in duplicates. The data differed by fewer than two FD factors.

extracted again with diethyl ether (two extractions, total volume: 150 mL). The combined organic layers were washed with aqueous sodium chloride (300 g/L; 2 × 50 mL), then dried over Na_2SO_4 (5 g), and finally concentrated at 40 °C to about 80 mL using a Vigreux column (60 × 1 cm). The volatiles were then isolated by solvent assisted flavor evaporation (SAFE; Engel et al., 1999). The distillate was washed three times with an aqueous sodium bicarbonate solution (0.5 mol per L; total volume 100 mL), and the combined ethereal solution containing the neutral/basic compounds (fraction NB) was twice washed 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. The fractions were then concentrated to about 200 μL by microdistillation as described recently (Schieberle, 1991).

High-Resolution Gas Chromatography (HRGC)/Mass Spectrometry (MS). HRGC was performed by means of a Type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) and by using the following capillaries: FFAP (30 m × 0.32 mm i.d. fused silica capillary; free fatty acid phase, 0.25 μm film thickness; J&W Scientific, Fisons Instruments, Mainz, Germany) and SE-54 (30 m × 0.32 i.d. mm fused silica capillary DB-5; 0.25 μm film thickness; J&W Scientific, Fisons Instruments, Mainz, Germany). The 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/min to 180 °C, held 5 min isothermally, and, finally, raised by 10 °C/min to 230 °C, and held for 5 min. The flow of the carrier gas helium was 2.3 mL/min. Linear retention indices (RI) were calculated from the retention times of *n*-alkanes as the references. MS analysis was performed by means of an MD 800 mass spectrometer (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 using methane as the reagent gas.

Aroma Extract Dilution Analysis (AEDA). At the end of each capillary, the HRGC-effluent was split 1:1 (by vol) into a flame ionization detector (FID) and a sniffing port device as described previously (Schieberle, 1991) using deactivated but uncoated fused silica capillaries (50 cm × 0.32 mm i.d.). The FID and the sniffing port were held at 200 °C. The flavor dilution (FD) factors of the odor-active compounds were then determined by AEDA (Schieberle, 1995) of the following dilution series: The original extract (200 μL) of either fraction NB or fraction AF was stepwise diluted with diethyl ether (1+1). HRGC/Olfactometry was performed with aliquots (0.5 μL) of the original extract and of the diluted samples.

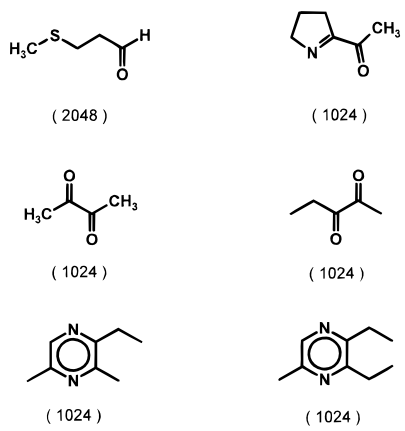

Figure 1. Structures of the most odor-active volatiles identified in roasted wild mango seeds (numbering refers to Table 1; flavor dilution (FD) factors are given in parentheses).

RESULTS

In preliminary experiments the roasting parameters (temperature, time) were optimized in order to generate an attractive overall roast aroma. The seeds were roasted at three different temperatures (150, 180, 220 °C) and for different times (5, 15, 30 min). The material was ground and evaluated by an experienced sensory panel (10 members). As a result, the most attractive roasty, nutty odor was judged for a sample obtained dry-heating of the seeds at 180 °C for 15 min. This sample was used for the identification experiments. An aroma concentrate was then prepared from the roasted material by solvent extraction and high vacuum distillation. To avoid interferences of the gas chromatographic separations, the acidic volatiles (fraction AF) were separated from the neutral/basic volatiles (fraction NB) by treatment of the flavor distillate with sodium bicarbonate. To focus the identification experiments on the odor-active volatiles, both extracts were then analyzed by the aroma extract dilution analysis (AEDA).

In the extract containing the neutral/basic volatiles from the roasted seeds, 28 odor-active compounds were detected showing FD-factors in the range of 8−2048. Six odorants, whose structures are displayed in Figure 1, were identified as the key contributors to the intense roasty-nutty overall odor to the neutral/basic fraction. With the highest FD-factor of 2048, the cooked potato-like smelling methional was characterized among the key odorants in the roasted seed extract, followed by 2-acetyl-1-pyrroline exhibiting an intense popcorn-like odor note, 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine, both showing earthy, roasted potato-like odor qualities, and the buttery smelling diones 2,3-butandione and 2,3-pentandione. By comparing the retention indices, the mass spectral data and, most important, the odor qualities and odor intensities of the odor-active compounds detected by AEDA with the corresponding data of reference odorants 22 further odorants were identified in the neutral/basic fraction. The results of the identification experiments in combination with the FD-factors (column NB in Table 1) revealed 3-methylbutanal (no. **1**) and 3-isopropyl-2-methoxypyrazine (no. **17)** as further important odorants high aroma activities among the roasted seed volatiles, followed by 2-propionyl-1-pyrroline, 2-ethyl-3,6-dimethylpyrazine, 3-isobutyl-2-methoxypyrazine, and (*E*,*E*)-2,4-decadienal, which were judged to occur with somewhat lower FD-factors.

In the fraction of the acidic volatiles (AF) five additional odorants were identified (column AF in Table 1) namely acetic acid, 2- and 3-methylbutanoic acid, vanillin, and butanoic acid.

DISCUSSION

GC/Olfactometry of a flavor extract from roasted wild mango seeds revealed 32 odor-active compounds in the HRGC eluate, 27 of which were identified using both mass spectra and retention indices as well as odor quality and odor intensity determined with reference odorants. Application of the aroma extract dilution analysis showed that among them methional, 2-acetyl-1-pyrroline, and the earthy, potato-like smelling pyrazines 2-ethyl-3,5-dimethyl- and 2,3-diethyl-5-methylpyrazine as well as the two buttery smelling diones 2,3-butan- and 2,3-pentandione are the key contributors to the overall roasty, nutty odor developing during roasting.

Methional, odorant 21 in Table 1, has also been characterized as a key contributor to the flavors of popcorn (Schieberle, 1991), rye bread crust (Schieberle and Grosch, 1987), or cara malt (Fickert and Schieberle, 1998). 2-Acetyl-1-pyrroline, odorant 9 in Table 1, has previously been identified as a key odorant in basmati rice (Buttery et al., 1982), popcorn (Schieberle, 1991), wheat bread crust (Schieberle and Grosch, 1987), or roasted sesame seeds (Schieberle, 1992).

Methional and 3-methylbutanal are well-known to be generated by a Strecker-degradation of the amino acids methionine and leucine, respectively, and 2-acetyl-1-pyrroline has been shown to be generated from the amino acids proline and ornithine by Maillard-type reactions (Schieberle, 1990). It can, therefore, be assumed that during roasting of the mango seeds these odorants are formed by similar reactions from these amino acids. The concentrations of the free amino acids in the seeds were, however, not determined.

Based on AEDA results, the two pyrazines 2-ethyl-3,5-dimethyl- and 2,3-diethyl-5-methylpyrazine have been characterized as important flavor contributors to many processed foods, such as rye bread crust (Schieberle and Grosch, 1987), popcorn (Schieberle, 1991), roasted sesame seeds (Schieberle, 1992), or roasted beef meat (Cerny and Grosch, 1993). The formation pathway of 2-ethyl-3,5-dimethylpyrazine involving the amino acid alanine was recently clarified by Amrani-Hemaimi et al. (1995) using labeled alanine. The reason these two pyrazines are very often detected among the key odor contributors of roasted foods is their extremely low odor threshold, compared to other pyrazines (Czerny et al., 1996). Although other pyrazines such as 2-methylpyrazine are also formed during roasting, their comparative high odor thresholds make them unlikely as flavor contributors.

It is interesting to note that, with the exception of methional and dimethyl trisulfide, no sulfur-containing odorant was present among the key odorants of the roasted mango seeds indicating low amounts of the precursor amino acid cysteine.

ACKNOWLEDGMENT

This work was made possible through the privilege given to Dr. Tairu by the Institute of Food Chemistry, Technical University of Munich, the University of Agriculture, Abeokuta, Ogun State, Nigeria, and the

generous support of the Alexander von Humboldt Stiftung, Bonn, Germany.

LITERATURE CITED

Abaelu, A. M.; Akinrimisi, E. O. Amino acid composition of Irvingia garbonensis (Apon) oil seed proteins. *Nig. J. Nutr. Sci.* **1980**, *1*, 133−135.

Aina, J. O. Physico-chemical changes in African Mango (*Irvingia gabonensis*) during normal storage ripening. *Food Chem.* **1990**, *36*, 205−212.

Amrani-Hemaimi, M.; Cerny, C.; Fay, L. B. Mechanisms of formation of alkylpyrazines in the Maillard reaction. *J. Agric. Food Chem.* **1995**, *43*, 2818−2822.

Buttery, R. G.; Ling, L. C. 2-Acetyl-1-pyrroline: an important component of cooked rice. *Chem. Ind.* **1982**, 958−959.

Cerny, C.; Grosch, W. Quantification of character-impact odor compounds of roasted beef. *Z. Lebensm. Unters Forsch.* **1993**, *196*, 417−422.

Czerny, M.; Wagner, R.; Grosch, W. Detection of odor-active ethenylalkylpyrazines in roasted coffee. *J. Agric. Food Chem.* **1996**, *44*, 3268−3272.

Engel, W.; Bahr, W.; Schieberle, P. Solvent Assisted Flavour Evaporation (SAFE) − a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Z. Lebensm. Unters. Forsch.* **1999**, in press.

Eka, O. U. Proximate composition of seeds of bush mango tree and some properties of dika fat. *Nig. J. Nutr. Sci.* **1980**, *1*, 33−36.

Fickert, B.; Schieberle, P. Identification of the key odorants in barley malt (caramalt) using GC/MS techniques and odour dilution analyses. *Food* (*Nahrung*) **1998**, *42*, 371−375.

Giami, S. Y.; Okonkwo, V. I.; Akusu, M. O. Chemical composition and functional properties of raw, heat-treated and partially proteolysed wild mango (*Irvingia gabonensis*) seed flour. *Food Chem.* **1994**, 237−243.

Okafor, J. C. Varietal delimitation in Irvignia gabonensis (Irvingiaceae). *Bull. Jard. Bot. Nat. Belg.: Nat. Plantentium Belg.* **1975a**, *45*, 211−221.

Okafor, J. C. The place of wild (uncultivated) fruits and vegetables in Nigerian diet. In *Proceedings of the National Seminar on Fruits and Vegetables*; Ojehomon, O. O., Town, P. A., McLean, K., Eds.; Caxton Press: Ibadan, Nigeria, 1975b; pp 153−171.

Okafor, J. C. Woody plants of nutritional importance in traditional farming systems of the Nigerian humid tropics. Ph.D. Thesis, University of Ibadan, Ibadan, Nigeria, 1981.

Okingbo, B. C. Neglected plants of horticultural and nutritional importance in traditional farming systems of tropical Africa. *Acta Horticulturae* **1977**, *53*, 1−31.

Schieberle, P.; Grosch, W. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm. Unters. Forsch.* **1987**, *185*, 111−113.

Schieberle, P. Recent developments in methods for analysis of volatile flavor compounds and their precursors. In *Characterization of Foods: Emerging Methods*; Goankar, A., Eds.; Elsevier: Amsterdam, 1995; pp 403−431.

Schieberle, P. Primary odorants of popcorn. *J. Agric. Food Chem.* **1991**, *39*, 1141−1144.

Schieberle, P. The role of free amino acids present in yeast as precursors of the odorants 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine in wheat bread crust. *Z. Lebensm. Unters. Forsch.* **1990**, *191*, 206−209.

Schieberle, P. Studies on the flavor of roasted sesame seeds. In *Progress in Flavor Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured Publ. Corp.: Carol Stream, IL, 1993; pp 343−360.

Received for review July 13, 1999. Revised manuscript received March 28, 2000. Accepted April 2, 2000.

JF990765U