Fruits, not only sweet

Comparative analysis of aroma compounds of African pears via differently coated solid-phase micro-extraction fibres (SPME) using GC-FID and GCMS

„African pears" –
Fruits of the baobab tree*

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The so-called African Pear (*Dacryodes edulis Burseraceae*) is a well known plant in West Africa, with edible fruits and bark, leaves, stem and roots which have use in local medicine against various diseases. The fruit is usually eaten raw or boiled, and the pulp is also roasted to form a type of butter. Essential oil compositions are known for parts of African pear plants growing in (Democratic Republic of) Congo and Nigeria. No information however is availabe on the composition of the aroma compounds in the fruits.

Following a series of recent publications on applications of headspace solid phase micro extraction (HS-SPME) coupled with gas chromatography-spectroscopy (GC-FID and GCMS) for extraction and identification of aroma compounds of various fruits, flowers and spices (Bonino et al., 2003; Diaz-Maroto et al., 2002; Jelen et al., 2000; Jirovetz et al., 2001; Vercammen et al., 2000), HS-SPME is of increasing importance in the aroma analysis of exotic fruits (Jirovetz et al., 2003; Shang et al., 2002). For this reason, the combined HS-SPME with GC-FID and GCMS (GC-14 and GCMS system QP-5000 from Shimadzu, both with 2 columns of different polarity) was used for the first time in the pulp aroma compound analysis of African pear fruits from Cameroon.

A decisive point of this study was the fact that already a range of different SPME fibers are commercially available. Also it is known that by using different types of SPME fibers an often dramatical change in the composition of the analysed samples is observed (e.g. aromatic and medical active plants: Bicchi et al., 2000 or fruit juices: Widder and Eggers, 2001). The aim of this study was therefore to find the right type of fiber that can extract qualitatively and quantitatively all aroma-active compounds from *D. edulis* that are responsible for the characteristic and pleasant odor impression.

Sample preparation

Dacryodes edulis fruits were bought at a local market in Ngaoundere (northern Cameroon) in September 2002, immediately after the harvest. The species identity was confirmed by a local botanist, and the control specimen was deposited at the National Herbarium of Yaoundé.

The samples investigated were prepared from a total of 5 fruits which were peeled and the pulp separated from the stone using a commercial stainless steel knife.

The pulp (300 g) was portioned in 5 x 60 g samples and each was placed into a 240 ml flask (Supelco Co., product-no. 23231), olfactorally evaluated by professional perfumers (Dragoco Co., Vienna, Austria, now Symrise) and afterwards closed with hole caps (Supelco Co., product-no. 23237) with Teflon/silicone septa (Supelco Co., product-no. 23245-U).

The pulp samples were each heated in a water bath at 40 °C for 1 hour and the volatiles extracted by solid-phase-microextraction from the headspace with the following fibers: 50/30 mm DVB/Carboxen/PDMSon a 2 cm Stable-Flex coated glass fiber (Supelco 57348-U), 50/30 mm DVB/Carboxen/PDMS-Stable-Flex fiber (Supelco 57328-U), 70 mm Carbowax/DVB-Stable-Flex fiber (Supelco 57336-U), 65 mm PDMS/DVB Stable-Flex fiber (Supelco 57326-U) and 85 mm Carboxen/PDMS Stable-Flex fiber (Supelco 57334-U).

GC-FID and GCMS analysis

Subsequent desorption of the analytes took place in the hot injector (250 °C) of the GC-14 (FID: 320 °C) or GCMS-QP5000 respectively. For the GC-FID measurements the carrier gas was hydrogen.The temperature programme was: 40 °C/5 min to 280 °C/5 min, with a heating rate of 6 °C/min. ◗

* By courtesy of Dr. Honoré Tabuna, France

Compound	RI	S-1[1]	S-2[2]	S-3[3]	S-4[4]	S-5[5]	Aroma impressions
Dimethyl sulphide	309	0.3	0.1	0.4	0.4	0.2	sharp, Allium-like
Ethanol	503	tr[6]	nd[7]	0.5	0.2	0.1	etheral, alcohol-like
2-Butanol	590	0.2	0.1	0.7	0.2	0.1	medicinal, etheral
Hexanal	801	tr	0.1	0.1	0.2	0.2	fatty, grassy, green
2-Methyl butanoic acid	837	0.8	0.7	1.3	0.9	0.6	fruity-fatty, spicy
(Z)-3-Hexen-1-ol	861	tr	tr	0.7	0.5	0.2	green („leaf alcohol"), fresh
Hexanol	865	0.1	tr	0.6	0.4	0.2	alcoholic, ethereal, medicinal
Heptanal	899	tr	0.1	0.2	0.1	tr	fatty, sweet, woody, nutty, fruity
α-Thujene	925	0.1	0.1	0.3	0.2	tr	herbal, green
α-Pinene	934	59.8	59.1	47.1	55.6	60.5	woody, pine-like
Camphene	946	1.4	1.6	2.1	1.7	1.6	fresh, camphoraceous
Isoamyl propionate	952	0.6	0.5	0.8	0.3	0.2	fruity, pineapple-like
Sabinene	974	1.4	1.6	2.1	1.5	1.5	spicy, warm-woody
β-Pinene	981	8.0	7.9	6.7	7.7	8.2	woody, pine-like
Myrcene	989	14.2	13.9	12.9	14.0	14.8	sweet-balsamic, plastic-side-note
α-Phellandrene	1004	0.2	0.5	0.4	0.3	0.3	minty, herbal, spicy
δ-3-Carene	1011	0.3	0.2	0.1	0.2	0.2	sweet, refined limonene-note
π-Cymene	1027	0.5	0.7	0.5	0.4	0.2	weak citrus-note
Limonene	1031	3.8	4.0	3.4	6.4	4.3	citrus-, lemon- and orange-note
α-Terpinene	1034	0.1	0.2	0.1	0.2	tr	terpene-like
β-Phellandrene	1036	0.1	0.1	tr	0.1	tr	herbal, spicy
1,8-Cineole	1038	tr	0.1	tr	0.6	tr	fresh, eucalyptus-like
(Z)-β-Ocimene	1040	tr	nd	nd	0.1	nd	spicy (estragon- and basil-notes)
(E)-β-Ocimene	1048	tr	nd	nd	0.1	tr	spicy (estragon- and basil-notes)
γ-Terpinene	1061	tr	0.1	0.1	tr	tr	herbal, citrus-note
Terpinolene	1090	tr	0.1	0.2	tr	tr	sweet-piney, slightly sweet-anisic
Linalool	1101	0.1	0.1	1.3	tr	nd	floral, citrus-lemon-orange notes
Nonanal	1104	nd	0.1	0.8	nd	nd	fatty, waxy
2-Phenyl ethyl alcohol	1116	1.9	1.8	2.3	1.7	1.9	floral, rose-note
(Z)-Pinocarveol	1139	1.2	1.2	2.5	1.0	1.0	camphoraceous
Verbenol	1178	1.8	1.7	3.1	1.6	1.9	minty, spicy
Terpinene-4-ol	1183	0.4	0.5	2.4	0.1	0.9	spicy, woody-earthy, liliac-notes
α-Terpineol	1198	0.4	0.3	1.1	0.2	0.8	liliac odor, floral, fruity
Decanal	1204	tr	0.1	0.6	tr	tr	sweet-waxy, floral, citrus-note
Verbenone	1215	tr	0.1	0.4	0.1	tr	minty, spicy
Carvone	1255	tr	nd	0.2	tr	nd	spicy, fresh, herbal
α-Copaene	1391	0.2	0.2	0.1	0.3	tr	woody, spicy
β-Caryophyllene	1437	1.1	1.1	0.7	0.6	0.4	terpene-odor, woody, spicy
Aromadendrene	1459	0.1	0.3	0.1	0.2	tr	woody, spicy
α-Humulene	1472	tr	0.1	0.1	0.1	tr	weak woody
Nerolidol	1565	tr	nd	0.9	tr	0.1	rose-, apple-, citrus-like green
δ-Cadinol	1658	tr	tr	0.6	0.1	nd	spicy
α-Cadinol	1675	tr	0.1	0.5	0.1	nd	spicy
Farnesol	1834	tr	nd	0.3	0.1	nd	floral-oily

S-1: 50/30 µm DVB/Carboxen/PDMS-2cm-StableFlex – **S-2:** 50/30 µm DVB/Carboxen/PDMS-StableFlex
S-3: 70 µm Carbowax/DVB-StableFlex – **S-4:** 65 µm PDMS/DVB-StableFlex – **S-5:** 85 µm Carboxen/PDMS-StableFlex
tr: 6trace compound (less than 0.1 %) – **nd:** not detected

Table 1: Headspace aroma compounds from the pulp of Dacryodes edulis fruits from Cameroon by using differently coated SPME fibers in order of their retention indices (RI) on a carbowax column in percentage (%-peak area, calculated from GC/FID analysis). Aroma impressions of identified headspace SPME pulp constituents from published data elsewhere (Arctander, 1969; Bauer et al., 1997; Fazzalari, 1978; Furia & Bellanca, 1975; Ohloff, 1994; Sigma-Aldrich, 2001).

The columns were 30 m x 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 mm (BioRad, Germany) and 60 m x 0.32 mm bonded Stabilwax, with a film thickness of 0.50 mm (Restek, USA). Quantification was achieved using peak area calculations in %, and compound identification was carried out partly using correlations between retention times (Retention indices according to Adams, 2001; Davies, 1990; Jennings & Shibamoto, 1980; Kondioya & Berdaque, 1996; Tudor, 1997).

For the GCMS experiments the carrier gas was helium; injector temperature 250 °C; interface-heating at 300 °C, EI-mode was 70 eV, and the scan-range was 41 - 450 amu. All other parameters were the same as for the GC/FID analysis. Mass spectra correlations were performed using Wiley, NBS, NIST and our own library as well as published data (Adams, 2001; Jennings & Shibamoto, 1980; Joulain & König, 1998).

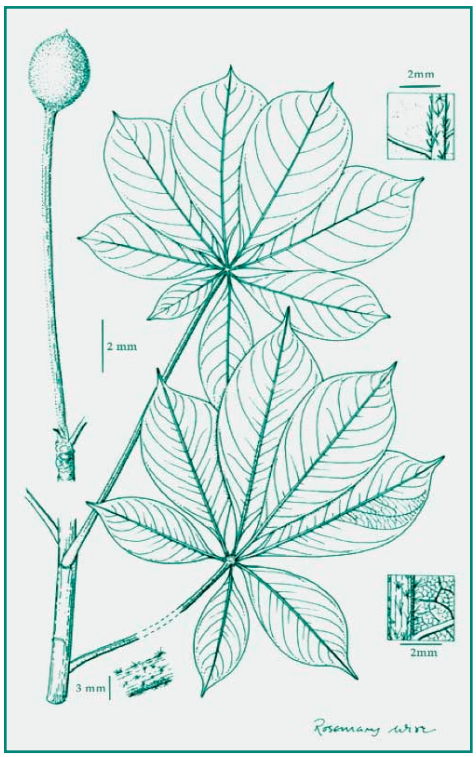
Results and discussion

The pulp samples of ripe African pear fruits from Cameroon were olfactorally evaluated by professional perfumers as follows:

pleasant warm-woody-balsamic (pinene-like), fresh-fruity (citrus-like), sweet-fruity (direction of ripe plum), weak minty-floral and in the background fatty and spicy aroma;

About 50 volatiles could be detected and more than 40 of them identified in the pulp headspace of D. edulis. Especially monoterpenes, such as α-pinene (47.1 % - 60.5 %) myrcene (12.9 % - 14.8%), β-pinene (6.7 % - 8.2 %) and limonene (3.4 % - 6.4 %) were found to be main compounds in the pulp headspace of *D. edulis* fruits (see Table 1)

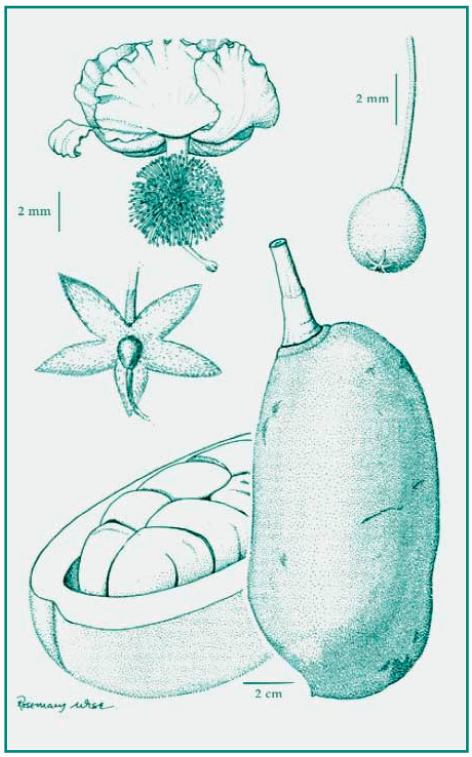
Using correlations of analytical data with odor-notes of identified essential oil compounds published elsewhere (Arctander, 1969; Bauer et al., 1997; Fazzalari, 1978; Furia & Bellanca, 1975; Ohloff, 1994; Sigma-Aldrich, 2001; see Table 1), we can state the following:



Leave and fruit of the baobab tree

- dominating sweet-woody-balsamic odor impressions can be attributed to the main compounds α- and β-pinene as well as myrcene
- fresh-fruity (citrus-like) and sweet-fruity (direction of ripe plum) aroma notes are known from limonene, p-cymene, and some terpinene derivatives
- spicy odor possesses some mono-terpenes, such as sabinene, phellandrene and ocimene derivatives, and sesquiterpenes , such as aromadendrene and cadinols
- green and fresh (camphoraceous- and minty-note) odor impressions are characteristic for hexane derivatives (green-grassy),camphene, pinocarveol, verbenol, verbenone (fresh-minty) as well as linalool, Terpinen-4-ol and α-Terpineol (floral)

Comparison of the analytical results from the extraction with the different SPME fibers clearly shows that by using the DBB/Carboxen/PDMS/Stable-Flex Fibers all aroma active compounds in the headspace of African Pears can be indentified and detected using GC-FID and GCMS analysis. Thus also all olfactoric impressions of the identified compounds can be correlated with the overall aroma of *D. edulis*. Using the other 3 fibers some compounds which add a considerable amount to the characteristic flavour of the pulp of African Pears could not be detected.



Summary

To summarize the investigation of pulp HS-SPME aroma compounds of Dacryodes edulis fruits from Cameroon with GC-FID and GCMS, we can report that α-pinene, β-pinene, myrcene and limonene are the main components among more than 40 compounds identified and account also mainly for the aroma impression of the African Pear. Other Headspace compounds are present in medium or low concentrations and also have some importance to the overall aroma. These main and minor compounds could only be extracted in detectable concentrations from the headspace of the African Pear by using the DVB/Carboxen/PDMS-Stable-Flex fibers.

Thus this fiber was found to be optimal for the aroma compounds analysis of exotic fruits.

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