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Chemical composition of absolute and supercritical carbon dioxide extract of *Aframomum melegueta*

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ABSTRACT: The compositions of absolute and supercritical CO_2 extract of *Aframomum melegueta* were analysed by GC and GC–MS; 33 components, representing more than 98.3% of the absolute, and 43 components, representing more than 98.2% of the supercritical fluid extraction (SFE) product, were identified. The major components were, in the absolute and SFE product, respectively, [6]-paradol (35.1 and 13.3%), [6]-shogaol (21.5 and 6.1%), [6]-gingerdione (9.8 and 28.0%), α-humulene (10.5 and 7.2%) and [6]-gingerol (1.3 and 10.0%). However gingerols are thermally unstable and can decompose under high temperature during GC analysis, therefore HPLC was used for oleoresins analysis and phenyl alkenones quantification. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: *Aframomum melegueta*; Zingiberaceae; supercritical fluid extraction (SFE); CO_2; β-caryophyllene; α-humulene; zingerone; paradol; shogaol; gingerdione

Introduction

The genus *Aframomum* (ginger family, Zingiberaceae) comprises more than 10 species endemic to the tropical regions of Africa. The usual distinguishing feature of *Aframomum* plants is the possession of strongly aromatic and pungent seeds. The most widely distributed and commercially important of these species is *A. melegueta* (Roscoe) K. Schum (known as maniguette). This is a perennial ginger-like herb which grows to about 1 m high. The reddish-brown seeds, commonly referred to as 'alligator pepper', 'guinea grains' or 'grains of paradise', have a strong aromatic flavour and a pungent taste.[1] These seeds are widely employed as spices, and are also ingredients in numerous local medicines. Their oil appears to have antioxidant, antimicrobial and cytoprotective properties.

The volatile constituents, which are responsible for the characteristic pleasant smell, are present in the steam volatile oil. However, volatile compounds contribute only partially to the flavour impact. The pungency of the seeds is mainly due to non-volatile phenolic alkenones known as gingerols, shogaols and paradols. These compounds possess various biological properties, in particular antiinflammatory, antioxidative and antitumour effects.[2–4]

The oleoresin obtained by solvent extraction embodies the pungent characteristics of the product. The supercritical fluid extract combines both of the important characteristics of *A. melegueta* by combining aroma and pungency compounds in the same extract.

There are few studies on the chemical composition of essential oil or extract of *A. melegueta*. In 1991, Menut *et al.*[1] identified nine compounds in essential oil of *A. meleguata* from Cameroon, in particular β-caryophyllene, α-humulene and their oxides. In 1999, Ajaiyeoba and Ekundayo[5] identified 27 compounds (98.6% of the oil) in the volatile oil from Nigerian maniguette. Solvent extracts have been studied for biological and antifeedant activity studies. In 1995, Escoubas *et al.*[6] isolated and identified four aryldecanones from *n*-hexane and methanolic seed extracts.

To the best of our knowledge, there are no reports on the chemical composition of absolute and CO_2 supercritical fluid extract of *A. melegeta*. In the present study we analysed the chemical composition of the absolute and SFE product (using carbon dioxide) obtained from *A. melegeta* from Côte d'Ivoire by GC, GC–MS, HPLC and HPLC–MS analysis.

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Experimental

Materials

A. melegueta certified as natural was supplied by Danisco Co. (Seillans, France). It was purchased in the Côte d'Ivoire in early 2002.

Isolation of Extracts

Commercial absolute and SFE product (using CO_2) of maniguette were supplied by Danisco Co. (Seillans, France). For the absolute, seeds were extracted with hexane:ethyl acetate (70:30) and the solvent mixture was evaporated to give the concrete. Treatment of this concrete by ethanol and evaporation of the solvent gave the absolute, in 4.9% yield. The SFE product was obtained in 3.5% yield.

Solvents and Chemicals

Methanol, acetonitrile and water were HPLC grade and purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). N-Vanillylnonamide was obtained from Fluka (Saint-Quentin Fallavier, France).

Analytical GC

GC analysis was carried out using a Agilent technologies 6890N gas chromatograph under the following operation conditions: vector gas, He; injector and detector temperatures, 250 °C; injected volume, 0.4 µl; split 1/10; HP1 column (J&W Scientific), polydimethylsiloxane (50 m × 0.20 mm i.d., film thickness, 0.33 µm; constant flow, 1 ml/min); temperature programme, 60–250 °C at 2 °C/min, then held at 250 °C for 60 min. Retention indices were determined with C_5–C_{28} alkane standards as reference. Relative amounts of individual components are based on peak areas obtained without FID response factor correction. Three replicates were performed for each sample. The average of these three values and the standard deviation were determined for each component identified.

GC–MS

GC–MS analysis was accomplished by using a Hewlett-Packard 5890/5971A system, with an HP1 column (50 m × 0.20 mm; film thickness, 0.33 µm). Temperature programme was the same as above; gas vector, He; constant pressure, 25 p.s.i. Retention indices were determined with C_5–C_{28} alkane standards as reference; mass spectra were performed at 70 eV; mass range, 35–400 amu. Identification of the constituents was based on comparison of the retention times with those of pure references and on computer matching against a commercial library (Wiley, MassFinder 2.1 Library) and a home made mass spectra library built up from pure substances and MS literature data.[7–12]

HPLC–Electrospray MS

An HPLC 1100 instrument (Agilent technologies) with a photodiode array detector set at 280 nm was coupled with a MSD quadrupole mass spectrometer. Chromatographic conditions were as follows: column, Microsorb-MV 100 C_{18}, 250 mm × 4.6 mm, 5 µm particle size (Varian Analytical Instrument, Les Ulis, France); eluent, water with 1% acetic acid and acetonitrile (35/65); flow rate, 1 ml/min; mass range measured, 100–500 amu; Quadrupole temperature; 150 °C. The spectra were acquired in the positive mode. Drying N_2 temperature, 350 °C; 12 ml/min; nebulizing N_2, 60 p.s.i. The HPLC was directly connected to the mass spectrometer without stream splitting.

HPLC

Phenyl alkenones separation was performed using an HPLC Varian ProStar with a photodiode-array detector set at 280 nm (Varian Analytical Instruments, Les Ulis, France). Chromatographic conditions were the same as above for HPLC–MS. Phenyl alkenone quantification was carried out using capsaicin (N-vanillylnonamide) as external standard (AFNOR NF ISO 13685).[13] For each extract, a 500 mg sample was dissolved in 100 ml methanol. Three replicates were performed for each sample. The average of these three values and the standard deviation were determined for each component quantified. A 20 µl volume of the sample solution was injected into the HPLC column.

Results and discussion

The components of the two extracts, the percentage of each constituent (by direct integration of FID responses) and the retention indices are listed in Table 1 according to their elution order on the HP-1 column (mass spectra of unknown compounds are presented in Table 2). Chromatographic profiles of the extracts reveal 33 identified constituents that represented 98.3% of the total GC area for absolute, and 43 identified constituents that represented 98.2% of the total GC area for supercritical fluid (SF) extract. The analysis revealed that absolute contained four oxygenated monoterpenes (0.2%), 11 sesquiterpene hydrocarbons (15.9%), six oxygenated sesquiterpenes (1.6%) and five phenolic alkenones (75.7%). For the SF

Table 1. Chemical composition of absolute and SF extract of *A. melegeta*

Compounds[a]	RI[b]	Absolute (% ± SD)[c]	SFE (% ± SD)[c]
n-Hexanal[#]	777	4.6 ± 0.5	7.6 ± 0.4
Heptan-2-one[#]	866	tr	tr
Heptan-2-ol[#]	885	—	0.2
α-Pinene[#]	929	—	0.5
Sabinene[#]	963	—	tr
β-Pinene[#]	968	—	tr
n-Octanal[#]	979	0.2 ± 0.1	0.2 ± 0.1
α-Phellandrene[#]	996	—	1.3 ± 0.2
Δ-3-Carene[#]	1003	—	0.3
p-Cymene[#]	1009	—	0.2 ± 0.1
2-Heptyl acetate[#]	1019	0.1	1.1 ± 0.1
β-Phellandrene[#]	1021	—	0.4
(*E*)-β-Ocimene[#]	1033	tr	0.2 ± 0.1
Nonan-2-one[#]	1068	tr	—
trans-Linalool oxide	1070	tr	0.1
Linalool[#]	1082	0.2	0.6
4,8 Dimethylnona-1,3,7-triene	1104	—	tr
α-Terpineol[#]	1176	—	tr
α-Phellandrene epoxide	1179	—	tr
1-Octyl acetate[#]	1189	tr	—
Linalyl acetate[#]	1237	tr	—
Isopulegyl acetate	1267	tr	—
Terpenyl acetate[#]	1327	—	0.1
Unknown (1)	1355	0.1	tr
α-Copaene	1384	tr	tr
β-Elemene	1394	tr	tr
β-Caryophyllene[#]	1415	5.2	3.2
trans-β-Farnesene	1443	tr	tr
α-Humulene	1451	10.5 ± 0.2	7.2 ± 0.1
Germacrene D	1470	tr	1.1
γ-Muurolene	1488	tr	0.2
β-Selinene	1490	0.1	0.3 ± 0.1
α-Selinene	1494	tr	0.1
γ-Cadinene	1505	0.1	0.1
Δ-Cadinene	1521	tr	tr
Elemol	1528	—	0.6
(*E*)-Nerolidol	1550	0.3 ± 0.1	0.2
Caryophyllene oxide[#]	1562	0.3	0.5
Humulene oxide	1575	0.1	0.1
Humulene epoxide	1586	0.6	1.1 ± 0.1
Zingerone[#]	1607	8.0 ± 0.9	13.1 ± 0.1
Unknown (2)	1611	0.2	—
Humulene epoxide I	1614	0.1	tr
α-Cadinol	1635	0.2	0.2 ± 0.1
Palmitic acid	1937	tr	tr
[6]-Paradol	2218	35.1 ± 0.3	13.3 ± 2
Phenolic alkanone (3)	2222	0.3	0.1
[6]-Shogaol	2273	21.5 ± 0.4	6.1 ± 0.3
Phenolic alkanone (4)	2278	0.5 ± 0.1	tr
[6]-Gingerdione	2306	9.8 ± 0.4	28.0 ± 1.9
[6]-Gingerol	2345	1.3 ± 0.4	10.0 ± 0.9
Phenolic alkanone (5)	2390	0.2 ± 0.1	tr

[a] Compounds are listed in order of their elution from a HP1 column.
[b] RI, retention indices as determined on HP1 column using the homologous series of *n*-alkanes.
[c] SD, standard deviation.
tr = trace (<0.1%).
[#] structure confirmed by standard compound injection.

extract, we observed eight monoterpene hydrocarbons (2.9%), five oxygenated monoterpenes (0.8%), 11 sesquiterpene hydrocarbons (12.2%), seven oxygenated sesquiterpenes and five phenolic alkenones (70.5%). The major components were phenolic alkenones: zingerone (8.0% and 13.1%, respectively), [6]-paradol (35.1% and 13.3%), [6]-shogaol (21.5% and 6.1%), [6]-gingerdione (9.8% and 28.0%), [6]-gingerol (1.3% and 10.0%) and two sesquiterpene hydrocarbons: β-caryophyllene (5.2% and 3.2%), α-humulene (10.5% and 7.2%) for the two extracts. However, there are several difficulties in the identification and quantification of the phenolic alkenones using gas chromatography. These difficulties are due to the partial thermal degradation of the gingerols (4-, 6-,

Table 2. Mass spectra of unknown compounds

Unknown compound	RI*	Mass spectrum**
1	1355	204(M^+, 1.7), 173(28.2), 125(24.6), 124(25.4), 103(31.2), 99(26), 96(20.1), 74(24.7)
2	1611	220(M^+, 13.7), 138(26.0), 137(28.8), 119(80.7), 109(80.7), 107(52.0), 105(39.16), 95(100), 93(71.7), 91(60.9), 85(94.5), 83(30.6), 82(93.9), 81(67.5), 77(37.7), 69(67.2), 55(54.9), 43(44.6), 41(81.2)
3	2100	248(M^+, 11.9), 149(29.9), 120(15.7), 107(100), 77, 57, 40
4	2222	293(9.3), 292(43.2), 193(12.8), 165(40.5), 164(3.8), 152(10.9), 151(100), 107(5.5), 57(7.4), 41(5.1)
5	2278	290(M^+, 28.1), 219(22.9), 165(29.3), 164(6.4), 152(7.9), 151(100), 107(7.7), 55(14.9)
6	2390	179(19.7), 151(18.8), 150(7.2), 138(9.1), 137(100), 124(6.2), 122(5.1), 119(9.1), 91(6.0), 55(5.2), 43(15.4), 43(10.1), 41(6.9), 40(14.2)

* Retention indices on HP1 column.
** Only the most abundant ions are given.

Table 3. Quantification of the main pungent constituents by HPLC (280 nm)

Compounds	Concentration (mg/ml) ± SD	
	Absolute	SFE
[6]-Gingerol	0.61 (24.5%)	1.05 ± 0.03 (42.0%)
[6]-Shogaol	0.09 (3.6%)	0.5 (10.0%)
[6]-Paradol	0.58 (23.3%)	0.28 ± 0.03 (11.2%)

8-, 10-, 12-) to their shogaol homologues (6-, 8- and 10-).[14,15] Despite this, gas chromatography is able to quantify the phenolic ketones in terms of total gingerols and shogaols.[16]

HPLC has been used for the study of these compounds in ginger oleoresin analysis.[17–20] However, the quantification may require authentic gingerols and shogaols. Obtaining these unstable compounds requires much time and money. An AFNOR method proposes the quantification of these compounds in ginger extracts using only one compound, capsaicin (N-vanillylnonamide) as external standard. This method was used in this study to analyse maniguette extracts. Prior to quantification, HPLC-Electrospray MS was used to identify the individual pungent constituents in the extracts. [6]-Gingerol, [6]-shogaol and [6]-paradol were positively identified in extracts depending on their $[M+H]^+$, $[M+MeOH]^+$ ions. However, we were not able to clearly identify characteristic ions of gingerdione.

Quantification of the three phenyl alkenones identified is presented in Table 3. SFE extract is rich in [6]-gingerol compared with absolute. This can be explained by the degradation of this product during the solvent evaporation cycle used for obtention of the absolute. These results show the limit of gas chromatography in quantifying certain products.

The chemical composition of the two extracts is close; however, significant differences in the relative amounts of major components were observed between the two extracts. SF extract is richer in volatile compounds than in the absolute, and sesquiterpene hydrocarbons are more abundant in the absolute. The pungent constituents can be identified by GC–MS, but this technique is not appropriate for estimating the relative quantities. HPLC using an AFNOR method led to a more precise quantification of three of these compounds: [6]-gingerol, [6]-shogaol and [6]-paradol.

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