Analysis of the Volatiles in the Seed Oil of *Hibiscus sabdariffa* (Malvaceae) by Means of GC–MS and GC-FTIR

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The low concentrated volatiles of the seed oil of *Hibiscus sabdariffa* (Malvaceae, Engl. Roselle), eluting ahead of the fatty acids (main constituents of this oil; ca. 90%) in gas chromatographic measurements, were analyzed by combined GC–MS and GC-FTIR. More than 25 volatiles, mainly unsaturated (one or two double bonds) hydrocarbons, alcohols, and aldehydes predominating from C_8 to C_{13}, were identified.

The calyx and leaves of *Hibiscus sabdariffa* are very important for curries, sauces, jellies, and pickles (Mohiuddin and Zaidi, 1975). The high contents of proteins (Al-Wandawi et al., 1984), amino acids (Singh, 1988), and fatty acids (Ahmed and Hudson, 1982; Cornelius et al., 1970; Ahmed et al., 1979; Sarojini et al., 1984) are characteristic for the seed oil of Roselle. Most of these less volatile compounds were analyzed by TLC and GLC with various detection methods. The volatiles of *H. sabdariffa* oil with low retention index, eluting ahead of the bulk of fatty acids, are unknown until today. The aim of this work was the identification of these low concentrated volatiles (total amount ca. 7%) by the use of efficient gas chromatographic–spectroscopic systems.

MATERIALS AND METHODS

H. sabdariffa plants (Malvaceae, Engl. Roselle) from Divisa (Herrera, Panama) were dried in the air (27–37 °C; humidity of the atmosphere 80%) for 4 days and the sperm–nucleus separated afterward. The seed oil was extracted by using the Soxhlet method (4 h) after the seeds were partially crushed in a domestic electric grinder and the solvent (*n*-hexane) was removed under reduced pressure. Total yield of seed oil was 23%.

The investigations were performed in two steps: First, the genuine seed oil was analyzed by the mentioned gas chromatographic–spectroscopic systems (sample 1). In the second step, the oil was treated with diazomethane for the preparation of the corresponding methyl esters of the free acids (FAMES) and investigated in the same way (sample 2). Injected volume for gas chromatographic measurements was 1.0 μL of a 0.1% solution (dichloromethane).

GC-FID. A HRGC Mega Series GC with integrator system (Carlo Erba) was used. The column was a 30-m bonded RSL-200-FSOT (PDDS) fused silica column with 0.32 mm i.d. and 0.17-μm film thickness (Bio-Rad). The carrier gas was hydrogen (35 kPa). The temperature was programmed from 60 to 300 °C with a heating rate of 10 °C/min. The injector temperature was 250 °C, and the detector (FID) temperature was 350 °C. The split ratio was 1:20.

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Table I. Volatile Constituents of *H. sabdariffa* Seed Oil

compound	%[a]	ID[b]
2,2-dimethyl-3-propyloxirane	0.5	1, 2
2-methyl-5-methoxypentan-2-ol	0.2	1, 2
5-methyltetrahydro-2-furanmethanol	1.3	1, 2
2-octen-1-ol	0.3	1, 2, 3
octanoic acid	0.2	1, 2, 3
2,4-dimethylheptan-1-ol	0.7	1, 2, 3
1,4-nonadiene	0.2	1, 2
2-nonen-1-ol	0.3	1, 2, 3
nonanal	0.4	1, 2, 3
nonanoic acid	0.1	1, 2, 3
camphor	0.2	1, 2, 3
linalool	0.1	1, 2, 3
decanol	0.3	1, 2, 3
2,4-decadienal	0.3	1, 2
2,4-dimethyloctanol	0.1	1, 2
undecane	0.2	1, 2, 3
2,4-undecadienal	0.4	1, 2
2,5-dimethylnonane	0.2	1, 2, 3
dodecane	0.1	1, 2, 3
dodecanol	0.3	1, 2, 3
2-ethyl-1-decanol	0.1	1, 2
1,13-tetradecadiene	0.1	1, 2
2-(1-methylheptyl)cyclohexanone	0.2	1, 2
7-hexadecene	0.1	1, 2
heptadecane	0.2	1, 2
1-heptadecene	0.1	1, 2

[a] Concentrations by GC-FID analysis (sample 2). [b] Identification by 1, mass spectra (GC–MS); 2, IR spectra (GC-FTIR; 3, coinjection of pure compound (GC-FID, GC–MS, GC-FTIR).

GC–MS. A varian 3400 GC with a Finnigan MAT INCOS 50 mass spectrometer and a Data General MicroECLIPSE data system were used. Ionization was EI (70 eV), and scan time cyclus was 0.73 s. Mass range was 35–450 amu. The carrier gas was helium (50 kPa). Ion source temperature was 160 °C. Interface heating was at 280 °C. For other parameters, see GC-FID.

GC-FTIR. For this analysis a HP-5890A GC in connection with a HP-5965B IRD (MCT detector) and the data system HP-9000/340C ChemStation were used. The carrier gas was helium (50 kPa). Detector cooling was by liquid nitrogen. Cell purge was by nitrogen. The wavelength range was 4000 to 800 cm^{-1}. Interface heating was at 250 °C. For other parameters, see GC-FID.

The mass spectra of the compounds were correlated with published data (see literature cited) or with library mass spectra [NBS and Wiley (on-line) and NIST and FOOD (off-line)] and the IR spectra with cited literature or library IR spectra [EPA and ROBERTET (on-line)].

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DISCUSSION

The genuine seed oil of Roselle was analyzed in two steps (see Materials and Methods). In the first sample (genuine oil) more than 25 volatile constituents could be identified (see Table I) by GC–MS and GC-FTIR measurements. The MS and IR spectra of these analyses were correlated with library spectra or with the spectra of coinjected pure compounds, always under the control of the retention times (GC-FID).

The described fatty acids were found in this sample (high retention time range).

In the second sample the fatty acids (as FAMES) and the mentioned volatiles were identified (GC–MS and GC-FTIR) and quantified by GC-FID integration data. The total amount of fatty acids was higher than 90%. [Concentration data of fatty acid of sample 2 (GC-FID peak area): linolic acid, 43.2%; oleic acid, 24.7%; palmitic acid, 17.3%; sterculic acid, 2.1%; and linoleic acid, 1.2%. Concentration less than 1%: myristic acid, palmitoleic acid, epoxyoleic acid, stearic acid, malvalic acid, dihydrosterculic acid, and eicosenoic acid.] The amount of volatiles was less than 8%.

In conclusion we report that, aside from the well-known fatty acids, more than 25 volatiles of low concentration (less than 8% of the total amount of the seed oil), mainly lower (predominantly C_8 to C_{13}) unsaturated hydrocarbons, alcohols, and aldehydes, were identified in the seed oil of *H. sabdariffa* by the use of GC–MS, GC-FTIR, and GC-FID. These compounds may be important for the aroma of this valuable oil in the food industry and useful for the identification of seed oils from different geographic origins.

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Registry No. Linoleic acid, 60-33-3; oleic acid, 112-80-1; palmitic acid, 57-10-3; sterculic acid, 738-87-4; linolenic acid, 463-40-1; myristic acid, 544-63-8; palmitoleic acid, 373-49-9; epoxyoleic acid, 24560-98-3; stearic acid, 57-11-4; malvalic acid, 503-05-9; dihydrosterculic acid, 5711-28-4; eicosenoic acid, 28933-89-3; 2,2-dimethyl-3-propyloxirane, 17612-35-0; 2-methyl-5-methoxypentan-2-ol, 55724-04-4; 5-methyltetrahydro-2-furanmethanol, 6126-49-4; 2-octen-1-ol, 22104-78-5; octanoic acid, 124-07-2; 2,4-dimethylheptan-1-ol, 98982-97-9; 1,4-nonadiene, 58688-15-6; 2-nonen-1-ol, 22104-79-6; nonanal, 124-19-6; nonanoic acid, 112-05-0; camphor, 76-22-2; linalool, 78-70-6; decanol, 112-30-1; 2,4-dicadienal, 2363-88-4; 2,4-dimethyloctanol, 141063-73-2; undecane, 1120-21-4; 2,4-undecadienal, 13162-46-4; 2,5-dimethylnonane, 17302-27-1; dodecane, 112-40-3; dodecanol, 112-53-8; 2-ethyl-1-decanol, 21078-65-9; 1,13-tetradecadiene, 21964-49-8; 2-(1-methylheptyl)cyclohexanone, 54549-90-5; 7-hexadecene, 18899-19-9; heptadecane, 629-78-7; 1-heptadecene, 6765-39-5.