Headspace and Essential Oil Analysis of Apple Flowers

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The headspace, the essential oil, and a pentane extract of domestic apple flowers (*Malus silvestris* var. Domestica) were analyzed by GC-FID and GC-FTIR-MS. More than 40 compounds were identified in the samples. The main headspace constituents, in addition to the terpenic compounds limonene, 3-carene, α-pinene, α-humulene, terpinolene, geraniol, linalool, and (*Z*)- and (*E*)-citral, are benzaldehyde, eugenol, and benzyl alcohol accompanied by the acetates of these and other alcohols. Additional information on the odor characterization of these samples is given.

INTRODUCTION

The flavors of different varieties of apple fruits were analyzed by many authors in the past years (Bundschuh et al., 1988; Cunningham et al., 1986; De Pooter et al., 1983, 1985; De Pooter and Schamp, 1989; Golias and Novak, 1983; Iwamoto et al., 1983; Maarse and Visscher, 1989; Moore et al., 1985, Neubeller and Buchloh, 1986; Olias et al., 1992; Panasiuk et al., 1980; Petro-Turza et al., 1986; Stanley et al., 1986). Only Loughrin et al. (1990) reported on the volatiles of cut apple flowers; however, neither information on odorous headspace constituents of the flowers of the living plant nor a comparison with the constituents of the essential oil and those of a pentane extract was given. Therefore, it was our aim to analyze and identify the constituents of the three samples: headspace, essential oil, and pentane extract. GC-FID and GC-FTIR-MS methods were used in connection with the GC sniffing technique for these studies.

EXPERIMENTAL PROCEDURES

Materials. Apple flowers [*Malus silvestris* (L.) Mill. var. Domestica (Borkh.), Rosaceae] were collected in the author's (L.J.) garden in the northern part of Lower Austria from the end of April to May 1992. The headspace was trapped by dynamic method according to the method of Bicchi et al. (1990), Brunke et al. (1992), and Nikiforov et al. (1992) for 12 h in charcoal tubes (SKC Inc.) using a commercial pumping-trapping system (Brey Co.) and analyzed immediately after extraction from the charcoal tubes with dichloromethane (200 μL). The essential oil was obtained by exhaustive steam distillation of 8.7 g of flowers (yield of oil, 0.3%), and the pentane extract was obtained by the Soxhlet method of 9.8 g of flowers (60 mL of pentane, 150-min extraction time; yield, 0.1%).

Organoleptic Testings. Ten microliters of each sample was placed on an odor strip and the odor characterized by perfumers.

GC Sniffing Technique. A Fractovap 2101 GC with split system and the LT Programmer 230, the Electrometer 160 (Carlo Erba Co.) and the Kompensograph III printer (Siemens Co.) were used. The GC column was a 30-m FSOT-RSL-200 fused silica column (0.32-mm i.d., 0.2-μm film thickness, Bio-Rad Co.). Conditions: detector temperature (FID), 320 °C; injector temperature, 250 °C; sniffing capillary heating, 250 °C; temperature program, 40 °C/5 min at a rate of 10 °C/min to 200 °C/20 min; H_2 carrier gas (2 mL/min); splitless mode, sniffing split ratio, 1:50 (FID detector: nose, two perfumers, four fragrance chemists).

Gas Chromatography. The volatiles were separated in a GC 14A with the integrator C-R6A (Shimadzu Co.). For other parameters, see GC Sniffing Technique.

Table I. Volatiles in the Headspace (HS), Essential Oil (EO), and Pentane Extract (PE) of *M. silvestris* Variety Domestica Flowers

compound	% peak area GC-FID HS	EO	PE
hydrocarbons			
3-carene	4.20	2.11	1.23
p-cymene	2.16	1.83	3.17
n-decane[a]	tr	0.18	2.71
dodecane[a]	tr	1.04	1.89
hexadecane[a]	tr	2.07	3.48
α-humulene	3.12	2.81	2.31
limonene	5.17	6.13	4.31
myrcene	1.03	0.98	0.42
perillene	1.19	1.11	tr
α-pinene	4.15	3.22	0.40
terpinolene	4.33	1.18	2.11
alcohols/phenols/ethers			
benzyl alcohol	4.12	8.13	1.92
borneol	0.93	1.27	0.78
cinnamic alcohol	0.17	2.13	2.11
eugenol	1.12	2.39	4.11
geraniol	4.11	3.17	2.22
hexanol[a]	0.13	0.39	1.33
3-hexen-1-ol[a]	0.47	0.71	4.14
linalool	2.03	1.78	0.83
menthol	1.30	2.39	1.00
2-phenylethyl alcohol	3.17	2.71	1.09
thymol	0.39	0.42	1.21
vanillin	tr	1.24	3.27
aldehydes/ketones			
benzaldehyde	13.35	4.20	1.71
cinnamaldehyde	4.71	5.11	4.13
(*E*)-citral	7.11	3.00	1.47
(*Z*)-citral	7.39	2.11	2.00
decanal[a]	0.72	1.13	1.88
β-ionone	0.61	1.22	2.01
acids/esters			
benzyl acetate[a]	3.17	1.08	0.91
bornyl acetate	1.13	6.12	1.08
butyl acetate[a]	1.01	2.09	3.17
cyclohexyl acetate[a]	tr	0.49	2.24
geranyl acetate	2.63	1.79	0.91
3-hexen-1-yl acetate[a]	0.78	0.92	0.82
hexyl acetate[a]	1.79	2.11	1.39
linoleic acid		1.17	3.47
methyl butyrate	0.31	0.91	4.11
palmitic acid	1.29	5.79	8.33
1,5-pentadiyl acetate	1.17	tr	tr
2-phenylethyl acetate	3.18	3.09	2.29
propyl palmitate			2.28
unidentified compounds	6.36	8.28	9.86

[a] Compounds identified also by coinjection.

Gas Chromatography-Fourier Transform Infrared Spectroscopy-Mass Spectrometry. A HP-5890A GC in connection

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Table II. Odor Characterization of *M. silvestris* Variety Domestica Flower Samples

headspace	beautiful, highly volatile, living apple flower like, flowery, mild fruity; at the beginning light coumarinic, benzaldehyde, and heliotropine note; later sweet, caramel and powdery notes
essential oil (steam distillate)	fatty, unclean-gas-like, not characteristic in any way, mild green, weak flowery, greaves-like
pentane extract	flowery, sweet, peeled-potato-like; at the beginning cinnamic note, and later reminiscent of vanilla

with the HP-5965B IRD (MCT detector) and the HP-5970C MSD was used. This system was under control of the data systems HP-9000/340 (IRD) and HP-9000/300 (MSD). Operation parameters for spectra registration were as follows: IR range from 4000 to 850 cm^{-1}; mass range from 35 to 450 amu (EI mode, 70 eV; acquisition cyclus times, IRD 0.15s, MSD 0.35 s); carrier gas, helium; interface heating, 220 °C. For other parameters, see GC Sniffing Technique. IR spectra and MS spectra of the detected compounds were identified by correlation with EPA and ROBERTET (on-line) IR library and NBS and WILEY (on-line) or FOOD and NIST (off-line) MS library. Many nonterpenic constituents could further be identified by GC-FID by coinjection of pure substances and correlation of their Kovats indices (labeled in Table I).

RESULTS AND DISCUSSION

More than 50 compounds in the headspace of apple flowers were detected by GC-FID and thereof the structures of 40 constituents identified by GC–FTIR–MS (Table I). The main components (higher than 4%) in the headspace sample are benzaldehyde, (*Z*)-citral, (*E*)-citral, limonene, cinnamaldehyde, terpinolene, 3-carene, α-pinene, benzyl alcohol, and geraniol. This result is somewhat surprising because Loughrin et al. (1990) found benzyl alcohol as the most prominent compound and only eight volatiles in the headspace of apple flowers. We explain this difference in results not only by the fact that another variety of apple flowers (*Malus domestica* cv. Red Delicious) was investigated but moreover that the headspace of detached flowers was analyzed, whereas our findings were obtained from living apple flowers. In this context it is noteworthy to observe that the concentration of the so-called "leaf alcohol", 3-hexen-1-ol—a catabolism product of various unsaturated fatty acids—is in the pentane extract the highest and in the essential oil still higher than in the headspace.

In the essential oil 57 compounds were detected by GC-FID and the structures of 41 of them identified by means of GC–FTIR–MS (Table I). The main constituents of the *M. domestica* flower oil (higher than 4%) thus are benzyl alcohol, limonene, bornyl acetate, palmitic acid, and cinnamaldehyde (Table I).

The structure of 42 compounds of 63 constituents of the pentane extract (detected by GC-FID) could be identified by means of GC–FTIR–MS, but the structure determination of a greater number of higher hydrocarbons ($C_n > 16$) seemed to be of lower importance for the aim of this study. The main constituents of the pentane extract (higher than 4%) are palmitic acid, limonene, 3-hexen-1-ol, cinnamaldehyde, eugenol, and methyl butyrate (Table I).

The olfactoric characterization by perfumers showed that only the headspace sample possesses the typical fragrance of living apple flowers, while the odor of the essential oil was not characteristic in any way (Table II). The odor of the pentane extract was reminiscent of the odor impression of cinnamic compounds and vanillin, although a weak note of apple flowers was perceptible. Nevertheless, all compounds typical of the *Rosaceae* species could be found, such as benzyl alcohol, a product from the phenylpropanoid metabolism, or 2-phenylethyl alcohol and also their acetates. These constituents partly cause the sweet, flowery notes observed in the headspace as well as in the pentane extract and to a minor extent also in the essential oil, where minor components overtop this pleasant odor impression.

Using the GC sniffing technique no single constituent of each sample could be found as dominant and characteristic enough to be recognized as typical of apple flowers. Therefore, we concluded that the typical odor impression of the flowers of *M. silvestris* var. Domestica is a mixture of a great number of these identified single compounds, especially of those in the headspace of the living plant.

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