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Fractionation of volatiles from blackcurrant (*Ribes nigrum* L.) by different extractive methods

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The volatile components of blackcurrant buds were fractionated into neutral, acidic and basic fractions. The fractions were submitted to gas chromatography (GC) and combined GC and mass spectrometry and their importance is discussed in terms of flavour impact. In this study 19 monoterpenes, 20 sesquiterpenes, 12 carbonyls, 11 esters and 34 alcohols were identified using Kovats indices and mass spectral data. From the blackcurrant cuttings volatiles were separated in which the blackcurrant berries aroma was overwhelmed by a catty note. Fractions were submitted to high performance liquid chromatography. The most polar volatile components possess a typical blackcurrant odour and contribute to the overall pleasant blackcurrant fruity aroma.

INTRODUCTION

Blackcurrant (*Ribes nigrum* L.) is an important raw material for the food industry, because it has a characteristic and excellent flavour. The most important part of the blackcurrant shrub for flavour isolation is the dormant bud, which has a very characteristic, penetrating and powerful odour (Kerslake & Menary, 1985; Le Quéré & Latrasse, 1990). The essential oil of blackcurrant buds gives off a strong terpenic flavour overwhelmed by a catty note. The sulphur-containing compound, 4-methoxy-2-methyl-2-mercaptobutane, was identified in the essential oil as the compound responsible for the catty note of the buds (Rigaud *et al.*, 1986). While the hydrocarbon fraction represents the major part of the oil, it does not explain the blackcurrant odour. However, some sesquiterpenes could be considered as flavour contributors (Latrasse *et al.*, 1982; Le Quéré & Latrasse, 1990).

The present study was undertaken in order to characterise odour quality of the blackcurrant complex aroma by means of some important constituents with typical odour qualities. The objective of the research was to fractionate the blackcurrant buds volatiles into neutral, acidic and basic fractions, in order to separate the volatile components with the blackcurrant flavour impact or to isolate the pure blackcurrant note from the blackcurrant cuttings by extraction into paraffin oil. Gas chromatography (GC), combined GC and mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) have been used to analyse the obtained fractions.

MATERIALS AND METHODS

Dormant buds (*Ribes nigrum* L. var. Eva) were harvested from cuttings in the Research Institute of Fruit and Ornamental Woody Plants in Bojnice during February 1992. The variety is descended from a crossing of the Silvergieter's Zwarte × Holland Black. The cuttings and buds were stored at -21°C before use.

Isolation of volatile components

Dormant buds (100 g) were mixed with 500 ml of distilled water and comminuted in a blender for 3 min. The volatile components were steam-distilled in a Likens-Nickerson extractor for 45 min with pentane (100 ml) as a solvent.

The blackcurrant cuttings with buds (250 g) were mixed with 1000 ml of distilled water and comminuted in a blender for 5 min. The volatile components were steam-distilled at atmospheric pressure. Ultimate volume of a distillation product was 200 ml.

Fractionation of volatile components

The pentane solution of volatile components was extracted by shaking five times with 10 ml of a 5% aqueous solution of sodium carbonate to obtain the acidic fraction. Then the pentane solution was extracted by shaking five times with 10 ml of a 5% aqueous solution of hydrochloric acid to obtain the basic fraction. The residual pentane solution represented the neutral fraction. After pH adjustment to 7, the volatile components from aqueous solutions were extracted with pentane. Three pentane fractions were obtained with different odours. After drying (Na_2SO_4) the fractions were concentrated on a water bath at 40°C under atmospheric pressure through a Vigreux column to 0·8 ml and under a stream of nitrogen to 0·5 ml for GC and GC–MS analyses.

Steam-distilled volatile components were extracted with 50 ml of purified paraffin oil for 60 min. The volatile components dissolved in this oil were extracted by shaking three times with 10 ml of a 70% aqueous solution of ethanol. Finally, paraffin oil was frozen out and an ethanolic fraction was obtained with very strong blackcurrant odour. This fraction was submitted to HPLC to compare the chromatogram with the oxygenated fraction HPLC chromatogram of the blackcurrant cuttings oil. This fraction was submitted to HPLC. A further sample of the steam-distillation extract was fractionated into hydrocarbons and oxygenated compounds by column chromatography. The HPLC chromatogram of the oxygenated compounds was compared with the steam-distillation sample.

Gas chromatography and combined gas chromatography and mass spectrometry

The obtained fractions were subjected to a Hewlett-Packard 5890 gas chromatograph (columns purchased from Germany) equipped with a flame ionization detector (FID), mass selective detector HP 5970 and 5971 A with a NIST data system. A non-polar capillary column was used under the following conditions: 25 m × 0·2 mm i.d. fused silica capillary column coated with dimethylpolysiloxane bonded phase (0·3 μm film), helium carrier gas 0·5 ml/min; oven temperature was programmed from 40 to 200°C at 2°/min. A polar capillary column was used under the following conditions: 50 m × 0·2 mm i.d. fused silica capillary column coated with Carbowax 20M bonded phase (0·1 μm film), helium carrier gas 0·5 ml/min; oven temperature was programmed for 2 min initial hold at 30°C, from 30 to 170°C at 4°/min and for 20 min hold at 170°C. Injection volume for each fraction was 0·5 μl at 1:40 split. The split injection port was maintained at 240°C, detector (FID) temperature 240°C. Electron impact mass spectra were recorded at 70 eV with a source temperature of 170°C. Measurements of peak areas were achieved by using an electronic integrator (HP 3370 B). Retention indices for the linear temperature-programmed section of the chromatograms were determined as described by Van den Dool and Kratz (1963).

High-performance liquid chromatography

A high-pressure pump HPP 4001 equipped with a UV–Vis Detector LCD 2563 and a Line recorder TZ 4620 were used. Separations were performed using the Separon-SGX-C18 column (150 × 3 mm), diameter of particles 5 μm, reversed-phase HPLC column. Operating conditions were as follows: mobile phase methanol/water 1/1 (v/v), flow velocity 0·3 ml/min, sensitivity 4, UV detection at 254 nm, injection volume 0·5 μl (LCI-30 injector), chart speed 0·6 cm/min.

RESULTS AND DISCUSSION

Fractionation of essential oil by column chromatography on a silica gel is effective in separating hydrocarbons from oxygenated compounds, but the catty or blackcurrant notes are not eluted. For this reason, a separation into neutral, acidic and basic fractions was chosen. The fractions were analysed by capillary GC and GC–MS, and the results are given in Table 1. Identification of all the compounds was based on their mass spectra and further supported by comparison of their Kovats retention indices, which were calculated using *n*-alkanes C_7–C_{22} as external references. Calculated indices confirm the general elution sequence and the mass spectra obtained on GC–MS agreed with those in the literature (Jennings & Shibamoto, 1980; Engel & Tressl, 1983; Koyasako & Bernhard, 1983; Tressl *et al.*, 1983; Le Quéré & Latrasse, 1990). Kovats retention indices on polar and non-polar columns represent the average of retention indices from the neutral, acidic and basic fractions. Relative percentage composition of identified compounds is determined by separating on the polar column. Quantitation of peaks was expressed as a percentage of the total peak area.

In this study, 19 monoterpenes, 20 sesquiterpenes, 12 carbonyls, 11 esters and 34 alcohols were identified using Kovats indices and mass spectral data. As shown in Table 1, sabinene, Δ^3-carene, α-phellandrene, β-phellandrene, γ-terpinene, terpinolene, β-caryophyllene, terpinen-4-ol, germacrene D, bicyclogermacrene and spathulenol were recognised as the major components. Hydrocarbons comprise 78·4% of the neutral fraction, 77·9% of the acidic fraction and 82·5% of the basic fraction. Oxygenated compounds comprise 21·6% of the neutral fraction, 21·4% of the acidic fraction and

Table 1. Compounds identified in the fractionated essential oil of blackcurrant buds and their Kovats indices

Compound	Kovats indices		Fractions (peak area%)[a]		
	Carbowax 20M	Dimethylpolysiloxane	N	A	B
Methyl butyrate	980	708	0·02	0·01	0·00
α-Thujene	1035	934	1·11	1·12	0·13
α-Pinene	1041	941	0·64	0·75	0·00
Camphene	1077	952	0·14	0·16	0·00
β-Pinene	1105	980	0·25	0·35	0·00
Butanol	1110	653	0·03	0·05	0·04
Sabinene	1127	972	11·1	10·8	10·2
4-Carene	1144	998	0·55	0·24	0·00
Propyl isovalerate	1148	928	0·03	0·02	0·01
Δ^3-Carene	1169	1016	20·4	17·6	17·3
Myrcene	1173	992	0·48	2·34	2·06
α-Phellandrene	1179	1004	3·21	1·14	1·44
α-Terpinene	1184	1020	1·33	1·25	1·19
1,4-Cineole	1192	1011	0·05	0·07	0·07
Limonene	1202	1032	1·71	2·07	1·54
2-Hexenal	1209	826	0·03	0·04	0·03
β-Phellandrene	1217	1029	1·88	4·02	3·65
1,8-Cineole	1223	1024	0·22	0·28	0·27
γ-Terpinene	1246	1061	2·90	2·51	2·45
(*Z*)-Ocimene	1251	1039	1·03	1·16	1·01
(*E*)-Ocimene	1255	1052	0·49	0·64	0·77
m-Cymene	1267	1023	0·09	0·07	0·07
p-Cymene	1279	1026	0·34	0·38	0·29
Terpinolene	1293	1092	11·5	11·4	11·3
allo-Ocimene	1368	1133	0·12	0·13	0·15
Nonanal	1382	1085	0·06	0·11	0·03
2-Octanol	1394	985	0·15	0·28	0·16
1-Octene-3-ol	1417	970	0·13	0·32	0·13
cis-Linalool oxide	1425	1067	0·32	0·26	0·09
Dihydromyrcenol	1443	1063	0·14	0·27	0·13
trans-Linalool oxide	1450	1080	0·13	0·10	0·08
α-Cubebene	1456	1346	0·21	0·09	0·00
trans-Sabinene-hydrate	1461	1350	0·56	0·69	0·21
δ-Elemene	1470	1352	0·08	0·00	0·00
2-Nonanol	1483	1087	0·49	0·73	0·23
α-Copaene	1490	1397	0·07	0·00	0·00
Linalool	1502	1094	0·15	0·22	0·19
Dihydrolinalool	1509	1124	0·17	0·23	0·19
α-Gurjunene	1523	1425	0·10	0·04	0·00
2-Nonenal	1534	1144	0·24	0·19	0·07
β-Cubebene	1547	1417	0·06	0·00	0·00
cis-Sabinene-hydrate	1550	1096	0·41	0·52	0·18
β-Elemene	1562	1408	0·22	0·16	0·04
cis-*p*-Menth-2-en-1-ol	1568	1142	0·11	0·13	0·00
Isopulegol	1575	1147	0·08	0·09	0·03
Isobornyl acetate	1582	1278	0·15	0·09	0·00
Myrcenol	1584	1104	0·24	0·30	0·17
Citronellyl formate	1588	1261	0·23	0·10	0·05
β-Caryophyllene	1606	1431	4·98	5·79	12·2
Menthol	1615	1167	0·49	0·73	0·65
Aromadendrene	1618	1452	0·23	0·14	0·00
Terpinen-4-ol	1626	1178	4·67	4·13	4·05
β-Copaene	1631	1446	0·02	0·00	0·00
trans-*p*-Menth-2-en-1-ol	1634	1128	0·11	0·13	0·00
γ-Elemene	1639	1441	0·85	0·74	0·39
Citronellyl acetate	1643	1338	0·46	0·25	0·12
trans-Piperitol	1648	1203	0·27	0·24	0·11
Ethyl benzoate	1650	1157	0·15	0·04	0·00
Alloaromadendrene	1656	1473	0·57	0·62	0·89
Isoborneol	1660	1159	0·19	0·20	0·05
α-Terpineol	1662	1191	0·24	0·26	0·13
Pulegone	1665	1230	0·15	0·11	0·07
Neryl formate	1668	1267	0·39	0·17	0·08
Sabinol	1678	1134	0·20	0·26	0·24

(*continued*)

Table 1—*contd.*

Compound	Kovats indices		Fractions (peak area%)[a]		
	Carbowax 20M	Dimethylpolysiloxane	N	A	B
α-Humulene	1681	1462	1·38	1·39	1·67
γ-Muurolene	1688	1479	0·16	0·07	0·00
4-Terpinyl acetate	1690	1331	0·39	0·24	0·02
Viridiflorene	1694	1485	0·04	0·00	0·00
Borneol	1696	1164	0·42	0·51	0·22
Neryl acetate	1703	1352	0·30	0·14	0·09
Germacrene D	1708	1493	1·73	1·80	2·05
Citronellol	1719	1215	0·34	0·35	0·21
α-Muurolene	1726	1498	0·23	0·19	0·08
Verbenone	1729	1194	0·27	0·17	0·09
Geranyl acetate	1733	1365	0·27	0·10	0·06
Bicyclogermacrene	1741	1507	6·43	7·56	9·88
cis-Piperitol	1750	1208	0·28	0·27	0·12
Nerol	1755	1219	0·58	0·60	0·53
δ-Cadinene	1761	1529	0·16	0·08	0·04
γ-Cadinene	1765	1522	0·13	0·00	0·00
Carvone	1789	1227	0·18	0·04	0·00
Geraniol	1795	1244	0·39	0·38	0·11
Carveol	1816	1222	0·14	0·13	0·04
Germacrene B	1828	1582	1·13	1·77	1·79
α-Ionone	1839	1410	0·42	0·37	0·34
m-Cymen-8-ol	1843	1185	0·31	0·29	0·21
p-Cymen-8-ol	1849	1187	0·66	0·54	0·35
Geranyl isovalerate	1898	1605	0·13	0·05	0·01
β-Ionone	1926	1479	0·38	0·27	0·22
α-Nerolidol	1965	1520	0·37	0·41	0·30
Caryophyllene oxide	1981	1602	0·34	0·25	0·11
β-Nerolidol	2003	1552	0·29	0·34	0·27
Humulene oxide	2016	1628	0·20	0·10	0·04
Cuminalcohol	2041	1279	0·18	0·19	0·07
Spathulenol	2103	1596	3·88	4·62	5·87
Isospathulenol	2215	1664	0·15	0·16	0·10

[a] Fractions: N, neutral; A, acidic; B, basic.

17·5% of the basic fraction. The relative percentages of monoterpenes and sesquiterpenes among hydrocarbons and the relative percentages of carbonyls, esters and alcohols among oxygenated compounds are given in Table 2. These relative proportions are very different. The diversity in the relative percentage compositions is responsible for different aroma impacts. It should be emphasised that the quantitative ratios among the volatile components are often more important than the effects of individual ones. The aroma impact of individual components in the volatile mixture is also a problem of olfactory perception (Píry, 1993).

The neutral fraction is characterised by a typical lemon or citrus-fruity odour. The acidic fraction is characterised by a catty odour. As a consequence of acid hydrogen of its mercapto group, 4-methoxy-2-methyl-2-mercaptobutane was quantitatively extracted into the acidic fraction:

$$Na_2CO_3 + 2\ H_2O = 2\ NaOH + H_2CO_3$$

$$R\text{–}SH + NaOH = R\text{–}SNa + H_2O$$

As a consequence of monoterpenes overlapping, this compound was not registered. The monoterpenes present in the obtained fractions do not explain the blackcurrant odour. However, some sesquiterpenes could be considered as blackcurrant flavour contributors. The basic fraction possesses a blackcurrant berries odour and contains more sesquiterpenes and less monoterpenes than the neutral and acidic fraction (Table 2).

Table 2. Relative percentages of monoterpenes and sesquiterpenes among hydrocarbons and of carbonyls, esters and alcohols among oxygenated compounds

Volatile components	Neutral fraction	Acidic fraction	Basic fraction
Monoterpenes	76·0	73·7	64·9
Sesquiterpenes	24·0	26·3	35·1
Carbonyls	12·6	9·40	6·69
Esters	11·7	5·65	2·52
Alcohols	75·8	85·0	90·8

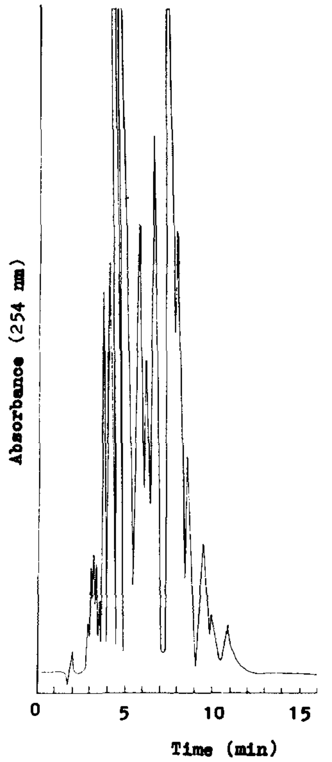

Fig. 1. HPLC analysis of the volatiles of blackcurrant cuttings dissolved in ethanolic extract.

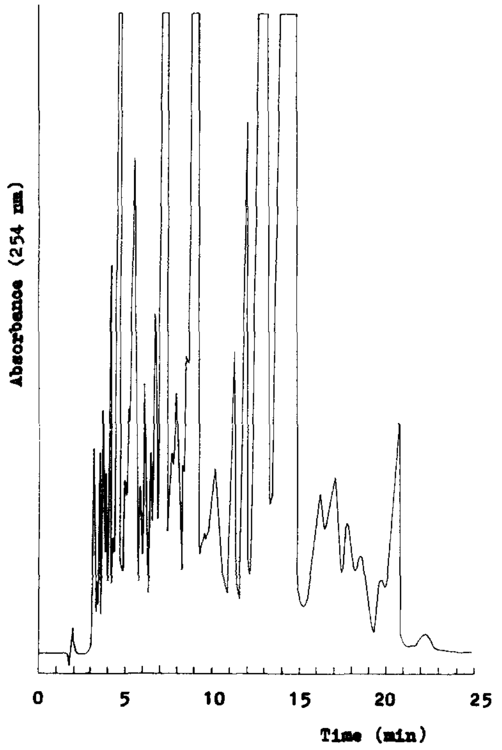

Fig. 2. HPLC analysis of the oxygenated compounds of blackcurrant cuttings volatiles.

The complexity of the blackcurrant aroma determined that the minor peaks in the chromatogram were of greatest interest. The oxygenated compounds content was from 17·5 to 21·6% of the whole oil. However, these polar volatiles contained the most odorous volatile compounds and exhibited the characteristic blackcurrant odour. Of these, alcohols can be considered to be the most important compounds for the blackcurrant flavour impact. The basic fraction contains more alcohols than the acidic and neutral fraction.

Volatile components of the blackcurrant cuttings dissolved in the ethanolic extract possess a very pleasant blackcurrant berries aroma overwhelmed by the catty note. The HPLC chromatogram of this extract is given in Fig. 1. In comparison with the HPLC chromatogram of the oxygenated compounds of blackcurrant cuttings (see Fig. 2), it follows that the ethanolic extract contains only the most polar compounds of low molecular weight (alcohols, carbonyls and sulphur-containing compounds) from the oxygenated compounds. As a consequence of the reversed-phase HPLC column, the hydrocarbons have a higher retention time under the same chromatographic conditions, and increasing numbers of carbon atoms caused an increase in retention time.

The most polar volatile compounds with low molecular weight possess a typical blackcurrant aroma and contribute most to the blackcurrant flavour impact. The sesquiterpenes could be considered as blackcurrant flavour contributors.

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