

## Composition of blackcurrant aroma isolated from leaves, buds, and berries of *Ribes nigrum* L.

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**Abstract.** Volatile aroma compounds of simultaneous distillation and extraction (SDE) from blackcurrant (*Ribes nigrum* L.) leaves, buds, and berries were analysed by capillary gas chromatography with OV-101, PEG 20M, and CYDEX B stationary phases, and by gas chromatography–mass spectrometry. A total of 63 compounds were identified. The monoterpenes 3-carene (20–27%), (Z)- $\beta$ -ocimene (2–11%),  $\beta$ -phellandrene (3–11%), and terpinolene (7–10%) and the sesquiterpenoids (E)- $\beta$ -caryophyllene (5–9%) and caryophyllene oxide (0.5–10%) were identified in high quantities in blackcurrant oils. The oil yields from blackcurrant buds were 0.21%, from leaves 0.04%, and from berries up to 0.003%. Variation in the concentrations of the oil components during the ripening process was studied and the enantiomeric ratio of some monoterpenoids was determined.

**Key words:** *Ribes nigrum* L., Saxifragaceae, blackcurrant, volatile aroma extract, enantiomeric ratio, GC/FID, GC/MS.

### INTRODUCTION

Blackcurrant (*Ribes nigrum* L. (Saxifragaceae)) is cultivated extensively in central and northern Europe. Its buds, berries, and also leaves have a very characteristic odour. Blackcurrant berries are used in many flavour applications of food and beverages, for jamming, and juice production. Blackcurrant buds are applied as a starting material for the preparation of the essential oil and absolutes, which are used as flavouring in cosmetics and food products. Leaves are used in canning some vegetables (cucumbers, tomatoes).

Numerous studies of the blackcurrant aroma were carried out by Anderson & von Sydow [1–4], Nursten & Williams [5], Latrasse and co-workers [6–9], Nijssen & Maarse [10], Kerlake & Menary [11], Marriott [12], Nishimura & Mihara [13],

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and Píry et al. [14]. A large number of components, of which the terpenoid hydrocarbons are quantitatively the most important, have been identified in the blackcurrant aroma. The compounds found in significant amounts are  $\alpha$ - and  $\beta$ -pinene, sabinene, 3-carene,  $\beta$ -phellandrene, and terpinolene.

Different constituents, responsible for the characteristic “catty note” of blackcurrant, have been identified. Nishimura & Mihara [13] reported isolation of 3-hydroxy-2-methyl-butyronitriles and (Z)- and (E)-2-hydroxymethyl-2-butene-nitriles from blackcurrant bud absolute as odour compounds. Latrasse and co-workers [6–8] identified 4-methoxy-2-methyl-2-butanethiol as a component with the characteristic catty note in blackcurrant buds. Non-sulphur containing aroma chemicals with blackcurrant odour (spiro-ethers) were studied by Van der Weerd [15].

The aroma from *R. nigrum* growing in Estonia has not been studied earlier by capillary GC and we did not find any literature data about the use of the chiral capillary column for blackcurrant aroma analysis.

The aim of this work was to compare the composition of blackcurrant aroma extracts isolated by the simultaneous distillation/extraction (SDE) micromethod from berries, leaves, and buds of *R. nigrum* using nonpolar (OV-101), polar (PEG 20M), and chiral (CYDEX B) capillary columns and the GC/MS method. The blackcurrant oil yields from different materials and changes in the concentrations of aroma compounds during the ripening process were studied, and the enantiomeric ratio of some monoterpenoid compounds was determined.

## EXPERIMENTAL

### Materials

Buds, leaves, and berries were harvested from one blackcurrant bush growing in Estonia (near Rapla). A total of five samples were collected on different dates (Table 1).

### Sample preparation

Aroma compounds were isolated from fresh blackcurrant material. SDE was performed in the micro-apparatus of Marcusson’s type during 2 h using *n*-hexane as

**Table 1.** Time of collection and characterization of blackcurrant samples

Sample No.	Material	Date of harvest	Ripening stage	Oil yield, %
1	Buds	May 2000		0.21
2	Berries	4 July 2000	Unripe	0.002
3	Berries	17 July 2000	Fully ripe	0.003
4	Berries	16 August 2000	Overripe	0.001
5	Leaves	August 2000		0.04

the solvent and *n*-tetradecane as the internal standard for yield determination. For the isolation procedure 100–140 g of fruits and about 20 g of leaves and buds were used.

### Capillary gas chromatography

GC analyses were performed on a Chrom-5 gas chromatograph equipped with a flame ionization detector. Helium was used as the carrier gas with a splitting ratio 1:150. Fused silica capillary columns with stationary phases of different polarity were used. Table 2 specifies the columns used and the conditions of the analysis.

A Hewlett-Packard Model 3390A integrator was applied for data processing. Quantitation of peaks was expressed as a percentage of the total peak area. The yields (%) of aroma compounds were determined using the internal standard according to the formula

$$X = K_{st} (A_x/A_{st})\%$$

where  $A_x$ ,  $A_{st}$  – the total peak area of the aroma substances and the peak area of the internal standard, respectively

$K_{st}$  – % of the internal standard in the sample material

Component identification was based on the comparison of the temperature programming retention indices (RI) determined on different columns with authentic RI data either from our data bank or obtained from the literature [16–19]. The results obtained were confirmed by GC/MS.

### Gas chromatography/mass spectrometry

Mass spectrometric analyses were carried out on a Hitachi M-80B gas chromatograph double-focusing mass spectrometer using a SPB-1 (30 m × 0.32 mm) fused silica capillary column. The temperature program was 3 min at 50°C, then 50–120°C at 5°C/min and 120–290°C at 8°C/min.

**Table 2.** Capillary columns and operating conditions

Parameter	Stationary phase		
	OV-101	SW-10	CYDEX B
Column length, m	50	60	50
Column internal diameter, mm	0.20	0.32	0.22
Stationary phase film thickness, μm	0.50	0.25	0.25
Plate number for <i>n</i> -decane at 90°C	145 000	300 000	170 000
Helium flow rate, cm/sec	17–25	17–25	30–35
Injector temperature, °C	160	260	160
Column temperature, °C	50–250	70–220	60–220
Programming rate, °C/min	2	2	2

## RESULTS AND DISCUSSION

The recoveries of essential oils from different fresh blackcurrant materials obtained applying the SDE micromethod with *n*-hexane as the solvent varied in the range 0.001–0.21% (Table 1). The yields of oil from blackcurrant berries from the three samples of different ripening stage were quite similar (0.001–0.003%) being the highest for fully ripe berries. A much higher yield of oil was obtained from blackcurrant leaves (0.04%) and especially from blackcurrant buds (0.21%). According to the literature data the oil yield from blackcurrant leaves is 0.08–0.74 mg/g [12] depending on the isolation method (distillation or extraction), from buds 2–5 mg/g [8], and from berries 10–13 ppm [3].

The complex nature of the blackcurrant aroma is demonstrated in the chromatogram (Fig. 1). Table 3 lists the compounds identified in the blackcurrant oils and their relative amounts (%) in the oils isolated from berries, buds, and leaves. The RI data in three stationary phases are also reported.

Altogether 63 compounds were identified in the blackcurrant oils studied. Perillaldehyde, decanoic acid, and palmitic acid have not been found in blackcurrant oil before. The monoterpene hydrocarbon fraction was the main part of the oil for all the parts of blackcurrant with its relative amounts varying from 55% to 67% of the oils (Table 3). The composition of the major monoterpenes was found to be in good agreement with the previous studies [1–14]. 3-Carene,  $\beta$ -phellandrene, (*Z*)- and (*E*)- $\beta$ -ocimene, limonene, and terpinolene were identified in high quantities (0.9–26.9%).

Sesquiterpenes made up 7.5–15.7% of the blackcurrant oils. The major sesquiterpene in the oils was (*E*)- $\beta$ -caryophyllene (4.6–9.3%). The other sesquiterpenes made up less than 4.1% in all samples. Oxygenated terpenes were found in quantities 5.7–14.2% of oils. From the 20 oxygenated monoterpenes identified in the blackcurrant oils only  $\alpha$ -terpineol and citronellyl acetate were found to form over 1%. The main oxygenated sesquiterpene was caryophyllene oxide (0.5–9.8%). The other groups of compounds in the oils were aromatic compounds, aliphatic aldehydes, alcohols and acids, and *n*-alkanes C<sub>16</sub>–C<sub>21</sub>.

Generally the same substances although with quantitative differences were present in the oils of the different blackcurrant materials. Camphene and linalool were found in leaves and buds but not in berries. Some aliphatic oxygenated compounds (heptanal, decanal, 2-decanol, ethyl decanoate, palmitic acid, but also 1,8-cineole, myrtenal, 1-*p*-menthen-9-al, and perillaldehyde), not found in leaves, were identified in small quantities in buds and in the highest quantities in blackcurrant berries. The aromatic hydrocarbon *p*-cymenene was also found in small quantities in buds and leaves, but occurred in quantities up to 5% in berries. Compared with the other parts the blackcurrant buds contained more monoterpenes ( $\beta$ -phellandrene, terpinolene,  $\alpha$ -pinene, limonene) and *n*-alkanes. The aroma from blackcurrant leaves contained more  $\beta$ -ocimene isomers and caryophyllene oxide than the oils from the other parts. The blackcurrant aroma isolated from berries was rich in aromatic and aliphatic compounds and oxygenated monoterpenes.



**Table 3.** Retention indices (RI) and composition of the essential oils of blackcurrant buds, berries, and leaves. The components identified in the highest yields are in bold

Compound	RI			Concentration*, %					
	OV-101	SW-10	CYDEX B	Buds	Berries			Leaves	
				1	2	3	4	5	
$\alpha$ -Thujene <sup>MS</sup>	923	1029	954	0.2	tr.	0.1	tr.	0.1	
(-)- $\alpha$ -Pinene <sup>MS</sup>	930	1029	985	0.6	0.3	0.4	0.4	0.3	
(+)- $\alpha$ -Pinene <sup>MS</sup>	930	1029	990	2.3	1.2	1.8	1.6	1.2	
<i>n</i> -Heptanal	879		1000	tr.	0.3	0.4	0.7	–	
(+)-Camphene	940	1074	1010	0.2	–	–	–	0.1	
Sabinene <sup>MS</sup>	963	1125	1024	2.4	1.3	1.6	1.3	1.9	
Myrcene <sup>MS</sup>	980	1161	1026	0.8	0.3	0.4	0.4	0.6	
(+)- $\beta$ -Pinene <sup>MS</sup>	967	1116	1035	0.3	0.3	0.4	0.2	0.8	
(-)- $\beta$ -Pinene <sup>MS</sup>	967	1116	1039	0.1	0.1	0.2	0.1	0.2	
(+)(-)- <b>3-Carene</b> <sup>MS</sup>	1002	1148	1045	<b>20.2</b>	<b>21.2</b>	<b>26.9</b>	<b>20.2</b>	<b>25.4</b>	
$\alpha$ -Phellandrene <sup>MS</sup>	993	1167	1046	0.7	0.7	0.8	0.8	0.3	
$\alpha$ -Terpinene <sup>MS</sup>	1007	1180	1055	1.1	2.2	2.5	1.9	0.7	
<b>(Z)-<math>\beta</math>-Ocimene</b> <sup>MS</sup>	1026	1232	1069	}	<b>6.7</b>	<b>2.7</b>	<b>5.5</b>	<b>2.1</b>	<b>11.3</b>
(-)-Limonene <sup>MS</sup>	1020	1204	1069						
<b>(+)-Limonene</b> <sup>MS</sup>	1020	1204	1072		<b>4.9</b>	<b>3.4</b>	<b>3.2</b>	<b>3.9</b>	<b>2.3</b>
<i>p</i> -Cymene <sup>MS</sup>	1010	1273	1079	0.1	0.9	0.8	0.4	0.6	
<b>(E)-<math>\beta</math>-Ocimene</b> <sup>MS</sup>	1037	1250	1083	}	<b>2.4</b>	<b>2.0</b>	<b>1.5</b>	<b>0.9</b>	<b>7.7</b>
(-)- $\beta$ -Phellandrene	1019	1213	1083						
<b>(+)-<math>\beta</math>-Phellandrene</b> <sup>MS</sup>	1019	1213	1086		<b>11.3</b>	<b>5.4</b>	<b>6.7</b>	<b>6.1</b>	<b>2.6</b>
$\gamma$ -Terpinene <sup>MS</sup>	1047	1246	1100	0.3	0.8	0.7	0.4	0.3	
1,8-Cineole <sup>MS</sup>	1020	1211	1114	tr.	0.6	0.3	0.4	–	
<b>Terpinolene</b> <sup>MS</sup>	1078	1282	1124	<b>10.2</b>	<b>8.7</b>	<b>8.7</b>	<b>6.8</b>	<b>7.7</b>	
<b><i>p</i>-Cymenene</b> <sup>MS</sup>	1072	1428	1157	0.2	<b>3.5</b>	<b>4.8</b>	<b>4.0</b>	0.3	
1-Octen-3-ol <sup>MS</sup>	970	1450	1142	0.1	0.2	0.5	0.1	0.2	
Oxygenated mono-terpene C <sub>10</sub> H <sub>18</sub> O	1055	1431	1167	0.1	0.4	0.5	1.0	–	
(-)-Fenchone	1066	1397	1205	0.1	0.4	0.3	0.4	0.2	
Methyl benzoate	1068		1205						
<i>trans</i> -Linalool oxide	1072	1451	1210	0.1	0.3	0.3	0.2	–	
(-)-Linalool <sup>MS</sup>	1085	1547	1238	0.1	–	–	–	0.3	
(+)-Linalool <sup>MS</sup>	1085	1547	1240	0.1	–	–	–	0.3	
Methyl chavicol	1175	1660	1300	–	0.7	0.3	1.2	–	
<i>n</i> -Decanal	1185		1311	–	0.3	0.4	0.3	0.1	
(-)-Terpinen-4-ol <sup>MS</sup>	1160	1593	1321	0.2	0.5	0.2	0.6	0.2	
(+)-Terpinen-4-ol <sup>MS</sup>	1160	1593	1324	0.1	0.2	0.2	0.4	0.1	
2-Decanol	1197		1335	0.1	0.4	0.4	0.6	–	
(+)-Myrtenal	1166		1348	tr.	0.5	0.4	0.3	–	
1- <i>p</i> -Menthen-9-ol <sup>MS</sup>	1166		1352	0.1	1.4	0.7	0.6	–	
(+)- $\alpha$ -Terpineol <sup>MS</sup>	1172	1690	1360	0.1	1.0	0.7	1.0	0.3	
(-)- $\alpha$ -Terpineol <sup>MS</sup>	1172	1690	1363	0.1	1.6	1.1	1.6	0.3	
Isoborneol	1146		1370	0.1	0.6	0.8	0.6	0.2	
Borneol	1150	1693	1376	0.2	0.2	0.4	0.4	0.3	
Bornyl acetate	1268	1574	1381	0.4	0.2	0.2	0.3	0.5	
Nerol	1213	1800	1389	0.1	0.6	0.7	0.9	0.3	
Citronellyl acetate <sup>MS</sup>	1334	1671	1432	0.7	1.0	1.1	0.8	0.4	
(-)-Perillaldehyde	1243	1774	1441	0.1	0.5	0.5	0.4	–	

Table 3. Continued

Compound	RI			Concentration*, %				
	OV-101	SW-10	CYDEX B	Buds	Berries			Leaves
				1	2	3	4	5
Ethyl decanoate	1375		1457	0.2	0.7	0.4	0.6	–
Geranyl acetate <sup>MS</sup>	1365	1755	1474	0.4	0.5	0.7	0.7	0.3
<b>(+)(-)-(E)-<math>\beta</math>-Caryophyllene<sup>MS</sup></b>	1412	1586	1476	<b>8.3</b>	<b>9.3</b>	<b>5.2</b>	<b>4.6</b>	<b>4.7</b>
$\gamma$ -Elemene <sup>MS</sup>	1423		1494	0.2	1.4	1.1	3.8	0.4
$\alpha$ -Humulene <sup>MS</sup>	1443	1653	1511	0.7	0.8	0.3	1.0	0.8
Germacrene D	1470	1690	1541	0.2	0.2	0.2	0.2	0.2
Bicyclogermacrene <sup>MS</sup>	1482	1716	1563	0.1	0.8	0.4	2.0	0.1
$\delta$ -Cadinene <sup>MS</sup>	1509	1749	1572	0.2	0.2	0.2	0.1	0.5
<i>n</i> -Hexadecane	1600	1600	1600	0.1	0.5	0.2	0.7	0.1
<b>Germacrene B<sup>MS</sup></b>	1547	1805	1615	<b>4.1</b>	<b>3.0</b>	<b>1.6</b>	<b>1.6</b>	<b>0.8</b>
Decanoic acid	1368	2270	1652	0.2	0.5	0.3	0.2	0.1
<i>n</i> -Heptadecane	1700	1700	1700	0.2	1.0	0.5	1.9	0.3
<b>(+)(-)-Caryophyllene oxide<sup>MS</sup></b>	1564	1960	1728	<b>2.4</b>	<b>1.0</b>	<b>0.5</b>	<b>0.6</b>	<b>9.8</b>
<i>n</i> -Octadecane	1800	1800	1800	0.2	0.8	0.5	tr.	0.2
<i>n</i> -Butyl cinnamate <sup>MS</sup>			1850	0.2	1.0	0.8	0.9	0.7
<i>n</i> -Nonadecane	1900	1900	1900	0.3	1.0	0.8	1.1	0.5
<i>n</i> -Eicosane <sup>MS</sup>	2000	2000	2000	tr.	0.4	tr.	0.3	0.1
<i>n</i> -Heneicosane <sup>MS</sup>	2100	2100	2100	–	0.5	0.2	1.8	–
Palmitic acid	1945		2217	0.1	2.1	2.2	2.9	0.4
COMPONENT GROUPS:								
Monoterpenes				65.0	55.0	67.0	51.4	64.4
Oxygenated monoterpenes				3.3	12.2	10.3	12.7	4.4
Sesquiterpenes				13.8	15.7	9.0	13.3	7.5
Oxygenated sesquiterpenes				2.4	1.0	0.5	0.6	9.8
Aliphatic compounds				1.5	8.7	6.8	11.2	1.8
Total %				86.0	92.6	93.6	89.2	87.9

<sup>MS</sup> – determined on GC/MS

\* – determined on CYDEX B

tr. – traces (<0.05%)

The aroma from fully ripe blackcurrant berries showed a higher yield and contained more monoterpenoic hydrocarbons and less oxygenated terpenes than the aroma of unripe and overripe berries.

The enantiomer ratios (the amount of one enantiomer expressed as percentage of the total amount of the pair of compounds) of some monoterpenoic compounds in the blackcurrant aroma samples are presented in Table 4. It was found that in the enantiomeric composition of the compounds studied the aroma from different blackcurrant parts and from berries of different ripening stage was quite similar.

**Table 4.** Enantiomeric distribution of some monoterpenic compounds in blackcurrant aroma

Compound	Enantiomeric distribution, %				
	Buds	Berries			Leaves
	1	2	3	4	5
(-)- $\alpha$ -Pinene	19.9	20.3	19.1	18.8	22.5
(+)- $\alpha$ -Pinene	80.1	79.7	80.9	81.2	77.5
(+)- $\beta$ -Pinene	75.0	65.1	67.3	69.7	81.7
(-)- $\beta$ -Pinene	25.0	34.9	32.6	30.3	18.3
(-)-Linalool	61.5	–	–	–	54.8
(+)-Linalool	38.5	–	–	–	45.2
(-)-Terpinen-4-ol	61.3	67.2	58.8	60.0	57.6
(+)-Terpinen-4-ol	38.7	32.8	41.2	40.0	42.4
(+)- $\alpha$ -Terpineol	42.8	39.5	37.2	39.0	48.3
(-)- $\alpha$ -Terpineol	57.1	60.5	62.8	61.0	51.7

– not detected

In most cases a significant (+)-enantiomer excess was observed for the monoterpenes  $\alpha$ - and  $\beta$ -pinene (65–82%), but (-)-enantiomer excess (57–67%) was observed for oxygenated compounds (linalool, terpinen-4-ol,  $\alpha$ -terpineol). In the case the content of linalool and  $\alpha$ -terpineol in the oil of blackcurrant leaves was very low (<0.1%), the enantiomeric distribution was not considerable. (-)-Limonene and (-)- $\beta$ -phellandrene were eluted with (Z)- $\beta$ -ocimene and (E)- $\beta$ -ocimene (Table 3) and therefore the enantiomeric distribution of their enantiomers could not be determined.

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## **Mustsõstra (*Ribes nigrum* L.) lehtede, pungade ja marjade aroomi keemiline koostis**

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Lenduvad aroomikomponendid eraldati mustsõstra lehtedest, pungadest ja marjadest samaaegse destillatsiooni ja ekstraktsiooni mikromeetodi abil ja analüüsiti kapillaargaasikromatograafiliselt OV-101, PEG 20M ja CYDEX B kolon-

nide abil ning kromatomassispektromeetrilisel meetodil. Aroomisegudes identifitseeriti kokku 63 komponenti, neist suurimates kogustes leiti 3-kareeni (20–27%), (*Z*)- $\beta$ -otsimeeni (2–11%),  $\beta$ -fellandreeni (3–11%), terpinoleeni (7–10%), (*E*)- $\beta$ -kariöfülleeni (5–9%) ja kariöfüllenoksiidi (0,5–10%). Õli saagised olid suurimad mustsõstra pungade puhul (0,21%), lehtedest saadi õli 0,04% ja marjadest vaid kuni 0,003%. Selgitati aroomi koostise muutus marjade valmimisel ja määrati mõnede monoterpeenide enantiomeeride jaotussuhted mustsõstra lehtedes, pungades ja marjades.