AROMA COMPOSITION OF BLACKCURRANT BUD EXTRACTS ISOLATED BY SIMULTANEOUS DISTILLATION/EXTRACTION

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Abstract

The aim of this study was to compare chemical composition of aroma extracts of blackcurrant buds. Dormant buds of six blackcurrant (*Ribes nigrum* L.) cultivars grown in Lithuania were collected in the experimental field of Lithuanian Institute of Horticulture in February 2006. Aroma extracts were isolated from the frozen buds in a Likens-Nickerson micro-steam distillation/extraction apparatus using cosmetic fluid CF-61 as an extraction solvent. The extracts of volatile compounds were analysed by gas chromatography with flame ionisation and mass spectrometry detectors. The aroma extracts were mainly constituted of aliphatic and oxygenated terpenes 39-46 % and 35-38 %, respectively. Blackcurrant cultivars according to the main essential oil compounds, namely sabinene, δ -3-carene and terpinolene were classified into the three chemotypes. Other quantitatively important compounds detected in aroma extracts were β -caryophyllene, β -phellandrene, *cis*- β -ocimene, γ -terpinene, terpinen-4-ol and limonene. (+)-2-Carene, β -2,3-epoxycarene, 1,9-decadiyne, cyclohexylethylacetate, *cis*- and *trans*-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol, *p*-mentha-1,4-dien-8-ol, β -selinene, 4-phenyl-1,3-thiazole, germacrene D-4-ol and aromadendrene oxide were reported in blackcurrant buds for the first time.

Key words: Ribes nigrum L., volatile compounds, cosmetic fluid

Introduction

Blackcurrant (*Ribes nigrum* L.) is a shrub growing wild in the cold and temperate climatic zones in Asia, Australia and Europe. The most important industrial product of blackcurrant are berries, however leaves and buds have also found some applications. They are used as a raw material for the food and cosmetic industries due to the characteristic colour and excellent flavour (Del Castillo *et al.*, 2002; Piry *et al.*, 1995).

Berries are the most important products of blackcurrant shrub. They are used to prepare juice, jams, liquors, sorbets, ice cream, etc. (Del Castillo *et al.*, 2002; Le Quere *et al.*, 1990). The volatile fraction of berries consists of more than 150 aroma compounds (Varming *et al.*, 2004); therefore berries have also been used as a perfume enhancer (Le Quere *et al.*, 1990; Griffiths *et al.*, 1999). However, the most important raw materials of blackcurrant for the isolation of flavour substances are dormant buds, which are harvested from blackcurrant canes during the dormancy period (Piry *et al.*, 1995; De Toro, 1994).

The aim of this study was to compare chemical composition of aroma extracts of blackcurrant buds isolated from six plant cultivars in Lithuania grown by Likens-Nickerson micro-steam distillation/extraction apparatus. Simultaneous steam distillation/extraction method has been applied for the isolation of aroma compounds since 1964 (Likens and Nickerson, 1964), by using various solvents, particularly such organic low boiling chemicals as diethyl ether and pentane. In our study we selected quite new solvent, so-called cosmetic fluid CF-61 (methoxynonafluorobutane), which is clear, colourless, fast drying substance with boiling point of 61 °C. Cosmetic fluids possess weak odour, they are environmentally favourable fluids offering a unique balance of properties.

Materials and Methods

RAW MATERIAL. Dormant buds of six blackcurrant (*Ribus nigrum* L.) cultivars (*Joniniai*, *Almiai*, *Gagatai*, *Ben Alder*, *Ben Nevis* and *Ben Lomond*) were collected in the experimental field of Lithuanian Institute of Horticulture (LIH) on 21st February, 2006. The buds were

stored in a freezer before extraction. Cosmetic fluid CF-61 (methoxynonafluorobutane) (3M, Saint Paul, Minnesota, USA) was used as an extraction solvent.

STEAM DISTILLATION-SOLVENT EXTRACTION (LIKENS-NICKERSON). Approx. 5 g of dormant buds were placed in a Likens–Nickerson micro-steam distillation/extraction apparatus together with 500 ml of glass-distilled water; 60 ml of CF-61 were used as the extraction solvent. The samples were extracted for 2 h. Volatile compounds were concentrated to 0.2 ml in a Vigreux column by purging gentle nitrogen steam. Two replicate samples were extracted from each cultivar ant the extracts were stored in a freezer before a further analysis.

GAS CHROMATOGRAPHY ANALYSIS

Gas chromatography and mass spectrometry (GC-MS). The GC-MS system consisted of a Clarus 500 gas chromatograph (PerkinElmer, USA) equipped with a mass selective detector Clarus 500 (PerkinElmer, USA) and automatic injector. The separation was performed using a non-polar fused silica capillary column Elite–5 (30 m×0.25 mm i.d. 1.0 μ m film thickness). Mass spectra were obtained by EI at 70 eV. Oven temperature was programmed from 60 °C to 250 °C (5.0 min hold) at 3 °C/min. Injection volume was 0.5 μ l at 1:100 split. The temperatures of the injector and detector were 250 °C. The samples were analyzed in duplicate.

Gas chromatography with a flame ionization detector (GC–FID). GC analysis was carried out on a VARIAN 3900 gas chromatograph (Palo Alto, California, USA) equipped with a flame ionization detector (FID) and automatic injector. The separation was performed using a nonpolar fused silica capillary column DB-5 (50 m×0.32 mm i.d. 0.52 µm film thickness). Oven temperature was programmed from 100 °C to 250 °C (5.0 min hold) at 2 °C/min. Injection volumes were 1.0 µl at 1:10 split. The temperatures of the injector and detector were 250 °C. The samples were analyzed in triplicate. The amount of the individual compounds was expressed as a GC peak area percentage.

Results and Discussion

The compounds were identified by using GC–MS, while the content of separated components was measured by GC–FID. The identified in aroma extracts compounds constituted more than 93 % of the total integrated GC peak area of each extract. The compounds were identified by comparison of their KI relative to C_5 - C_{18} n-alkanes, obtained on a non-polar DB-5 column with those provided in the literature (Adams, 2001); by comparison of their mass spectra with the data provided by NIST data system and literature sources (Le Quere *et al.*, 1990, Piry *et al.*, 1995).

In general, it was found that aliphatic (39-46 %) and oxygenated terpenes (35-38 %) were major fractions in blackcurrant bud aromatic extracts. The content of aliphatic mono and sesquiterpenes was 26–30% and 11–17%, respectively; while the amount of oxygenated mono and sesquiterpenes was 25–28% and 9–11%, respectively.

The most abundant volatile compounds in blackcurrant buds were monoterpenes sabinene, δ -3-carene and terpinolene. It was observed that blackcurrant cultivars analyzed in this study according to the main compounds in aroma extracts may be classified into the three chemotypes. *Joniniai, Almiai* and *Gagatai* were defined as cultivars biosynthesising sabinene (34–48%) as a major essential oil constituent; *Ben Alder* and *Ben Nevis* were assigned to a second group, which is characterised by a high amount of δ -3-carene (approx. 35%) and terpinolene (21–23%); while *Ben Lomond* contained remarkable amount of the all three major terpenes, sabinene, δ -3-carene and terpinolene (35%, 16% and 12%, respectively); therefore this cultivar was attributed to a separate chemotype (Figure 1).

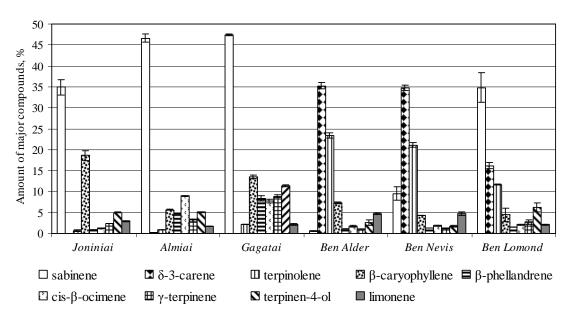


Figure 1. Major compounds of blackcurrant bud aroma extracts

Other quantitavely important compounds in aroma extracts of buds were β -caryophyllene (4–19%), β -phellandrene (1–9%), *cis*- β -ocimene (1–9%), γ -terpinene (1–9%), terpinen-4-ol (2–12%) and limonene (1–5%). The highest amounts of all compounds listed above were detected in *Almiai* (2–6%) and *Gagatai* (2–14%) cultivars (Figure 1).

α-Thujene, α- and β-pinene, myrcene, (+)-2-carene, α-terpinene, *trans*-β-ocimene, α-terpineol, α-humulene, germacrene D and several other compounds were identified in the blackcurrant bud aromatic extracts (Table 1). Sabinene, δ-3-carene, terpinolene, α- and β-phellandrene, γ-terpinene, β-caryophyllene, terpinen-4-ol, germacrene D, bicyclogermacrene and spathulenol were reported previously as the major components in dormant buds of blackcurrant (Piry *et al.*, 1995). All these compounds were found in the buds analysed in our study, except for bicyclogermacrene. To the best of our knowledge, (+)-2-carene, β-2,3-epoxycarene, *cis*- and *trans*-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol, 1,9-decadiyne, cyclohexylethylacetate, p-mentha-1,4-dien-8-ol, β-selinene, 4-phenyl-1,3-thiazole, germacrene D-4-ol and aromadendrene oxide were not previously reported in blackcurrant buds (Le Quere *et al.*, 1990, Piry *et al.*, 1995).

Table 1

Compounds	Jonininiai	Almiai	Gagatai	Ben Alder	Ben Nevis	Ben Lomond
α-thujene	1.34±0.08	1.08±0.06	2.82±0.24	0.13±0.01	0.34±0.02	0.71±0.20
α-pinene	0.87 ± 0.08	2.37 ± 0.02	1.18 ± 0.04	0.51 ± 0.08	0.96 ± 0.04	0.81±0.06
β-pinene	1.71±0.07	3.20±0.04	2.37 ± 0.55	2.67 ± 0.05	3.06±0.10	3.20±0.23
myrcene	0.93±0.06	2.31±0.01	1.27 ± 0.06	0.14 ± 0.01	0.89±0.03	0.55±0.06
(+)-2-carene	nd	nd	nd	0.36±0.11	0.36 ± 0.01	0.17±0.10
a-terpinene	1.22±0.03	1.68 ± 0.20	4.31±0.11	0.67 ± 0.04	0.07 ± 0.04	1.74±0.52
trans-β-ocimene	1.23±0.03	1.15 ± 0.02	1.20±0.03	2.75 ± 0.04	2.79±0.17	1.32±0.07
a-terpineol	0.13±0.01	0.10 ± 0.02	nd	0.11 ± 0.01	0.25 ± 0.02	0.08±0.02
α-humulene	4.53±0.34	2.33 ± 0.08	4.93±0.15	2.34±0.08	0.54 ± 0.07	1.96±0.85
germacrene D	3.31±0.23	1.15 ± 0.03	3.87 ± 0.15	3.27 ± 0.07	1.44 ± 0.13	1.27±0.60
spathulenol	2.17±0.00	0.48 ± 0.01	1.53±0.13	1.50 ± 0.04	0.17±0.03	0.46±0.12
camphene	0.05 ± 0.00	0.34 ± 0.00	nd	0.12±0.01	0.13±0.01	0.06±0.01
α-phellandrene	0.15 ± 0.00	0.81 ± 0.02	0.65 ± 0.02	0.45 ± 0.14	0.42 ± 0.01	0.38±0.03

Composition of blackcurrant bud aromatic extracts of 6 cultivars, %

p-cymene nd nd 0.53±0.02 0.12±0.00 nd 0.21±0.06 cis-sabinene hydrate 0.70±0.02 0.43±0.05 0.67±0.05 0.09±0.01 0.22±0.02 0.61±0.12 trans-sabinene hydrate 0.84±0.03 0.51±0.06 1.24±0.62 0.20±0.01 0.16±0.01 0.67±0.11 β-2,3-epoxycarene nd nd 0.92±0.11 nd 0.06±0.02 0.03±0.00 trans-s1-methyl-4-(1- 0.59±0.02 0.55±0.03 0.76±0.10 0.27±0.00 0.55±0.08 0.56±0.03 0.70±0.11 0.40±0.09 cyclohexen-1-ol 0.26±0.03 0.37±0.02 0.55±0.08 0.56±0.03 0.70±0.01 0.40±0.09 p-mentha-1,5-dien- 8-ol 0.07±0.02 nd nd 0.51±0.01 0.42±0.04 0.12±0.01 p-cymen-8-ol 0.28±0.01 0.34±0.01 0.51±0.01 nd 0.12±0.01 0.42±0.01 0.42±0.01 0.42±0.01 0.42±0.01 0.42±0.01 0.42±0.01 0.42±0.01 0.42±0.01 0.12±0.01 p-cedecaliyne 0.04±0.00 0.64±0.02	Compounds	Jonininiai	Almiai	Gagatai	Ben Alder	Ben Nevis	Ben Lomond
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6,10,14-trimethyl-		0.53±0.03	0.22±0.05	0.42 ± 0.08	0.52±0.12	0.36 ± 0.02	0.19±0.10
		0.47 ± 0.02	0.14±0.05	0.95±0.13	0.51±0.03	0.37±0.11	0.04±0.02
	6,10,14-trimethyl- 2-pentadecanone	nd	0.06±0.01	0.34±0.00	0.12±0.01	0.05±0.01	0.07±0.02

nd-not detected

Conclusions

Six blackcurrant bud cultivars were analyzed in the present study and identified compounds constituted more than 93 % of total integrated GC peak area of each extracts. The major compounds of aromatic extracts were sabinene, δ -3-carene, terpinolene, β -caryophyllene, β -phellandrene, *cis*- β -ocimene, γ -terpinene, terpinen-4-ol and limonene. Blackcurrant cultivars according to the main essential oil components (sabinene, δ -3-carene and terpinolene) were separated into the three chemotypes: 1st with sabinene as a major compound (*Joniniai, Almiai* and *Gagatai*); 2nd with high amounts of δ -3-carene and terpinolene (*Ben Alder* and *Ben Nevis*); and 3rd with remarkable amount of all three terpenes (*Ben Lomond*).

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