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Characterization of Steam Volatiles in the Essential Oil of Black Currant Buds and the Antioxidant Properties of Different Bud Extracts

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Currently essential oil from black currant (*Ribes nigrum* L.) buds is mainly used as a valuable perfumery ingredient. This study reports more comprehensive characterization of dormant buds of various black currant (*Ribes nigrum* L.) cultivars which are grown in Northern European countries. Essential oils were isolated from the buds by hydrodistillation and analyzed by gas chromatography−mass spectrometry (GC-MS), GC−flame ionization detection (GC-FID), and GC−olfactometry (GC-O). The most abundant compounds in black currant bud essential oil were sabinene, δ-3-carene, and terpinolene. The most frequent descriptors of the essential oil components assessed by GC-O were "woody", "terpene", "fruity", "sweet", "citrus", "herbaceous", "pine", "green", "oily", "herbal", and "musty". The residues obtained after hydrodistillation were separated into liquid and solid fractions. The solid fraction was dried and extracted with acetone (AE), while the liquid fraction (water extract) was divided in two parts, one of which was spray-dried (SDWF extracts) and the other freeze-dried (FDWF extract). In addition, a portion of whole frozen buds was extracted with methanol (ME). The radical scavenging capacity (RSC) of black currant bud extracts varied in a wide range; in the $DPPH^{\bullet}$ reaction system FDWF at the applied concentration scavenged 43–79%; SDWF, 54–80%; AE, 16–36%; ME, 42–60% of radicals; while in the $ABTS^{\bullet+}$ reaction system the RSC was 39–72, 38–53, 1–5, and 30–49%, respectively. The total amount of phenolic compounds expressed in gallic acid equivalents in FDWF varied in the range of 132–192 mg/g; in SDWF, 140–209 mg/g; in AE, 49–107 mg/g; and in ME extracts, 111–180 mg/g.

KEYWORDS: Black currant buds; *Ribes nigrum* L.; essential oil; aroma; extracts; radical scavenging; total phenolics

INTRODUCTION

Plants are important resources for the preparation of natural remedies, food additives, and other ingredients. The assessment of chemical composition and bioactivities of various plant-derived products is an interesting and useful task, particularly in searching for new sources of natural antioxidants and other health-promoting compounds, which particularly could be used as natural remedies, food additives, and functional food and nutraceutical ingredients (*1*).

Black currant (*Ribes nigrum* L.) is a shrub spontaneously growing in the cold and temperate climatic zones. The most important industrial product of black currant is berries; however, leaves and buds due to their characteristic color and excellent flavor have also found some applications as a raw material for the food and cosmetic industries (*4–6*).

The volatile fraction of berries consists of more than 150 aroma compounds, including terpenes, esters, and alcohols (*7*); however, the most important part of the black currant used for aroma isolation are dormant buds, which are harvested from the canes during the dormancy period (*5, 6*). Essential oil obtained by steam distillation of dormant buds is a mobile, pale green liquid with a cymene-like odor (*8*). It is used as a flavoring or flavor enhancer in cosmetic and food industries. Oil of buds possesses a very characteristic, penetrating, and powerful odor; the buds can accumulate remarkable amounts of essential oil exerting a strong terpenic aroma which is overwhelmed by a "catty note" (*2, 3, 6*). Latrasse et al. (*9*) subjected black currant essential oil to the gas chromatography (GC-O) (see Abbreviations Used) analysis and were the first who reported the "catty note"; they concluded that such note is important in the formation of the overall aroma of oil. The sulfur-containing

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compound 4-methoxy-2-methyl-2-mercaptobutane was later identified in the essential oil as the component responsible for the catty note with a perception threshold in water of 10^{-6} ppm (*10*).

Black currants biosynthesize an array of phenolic compounds. Phenols are generally stable compounds and have been extracted from fresh, dried, or freeze-dried plant material by using aqueous mixtures of methanol, ethanol, and acetone (*11*). Agricultural and industrial residues, such as byproduct obtained during processing of some raw materials, are promising sources of natural antioxidants. It was reported that the extracts from berries processing byproduct, particularly black currant marc contained a high amount of phenolic compounds and possessed remarkable antioxidant activity (*12*). The information on antioxidant properties of black currant buds is rather scarce. Recently it was reported that buds (opened at the end of March) and leaves (in June) had a higher content in phenolics and antioxidants than fully ripened berries (in July) (*13*). However, to the best of our knowledge, the residues of black currant buds obtained after removal of essential oils have not been analyzed for their antioxidant properties and phenolic content. Considering the high costs of buds, it is reasonable to search for the ways of a most effective use of the whole processed plant material. In our previous studies we found that the extracts isolated from the byproduct remaining after plant hydrodistillation (so-called deodorized extracts) contain remarkable amounts of valuable phenolic compounds exerting antioxidant and other activities, in some cases higher than the extracts isolated from the whole plant material (*14–16*).

It is well-known that chemical composition of secondary plant metabolites highly depends on such factors as climatic conditions, harvesting time, and plant chemotype. The essential oil of black currant buds was studied previously, and remarkable differences in the composition of the mono- and sesquiterpene hydrocarbons were reported (*2, 26–29*). These studies revealed remarkable chemical polymorphism in terpene composition in buds of different cultivars from various locations. Therefore, comprehensive evaluation of poorly characterized cultivars is an important task in optimizing the production of high added value products, such as aroma and fragrance components and functional ingredients. Taking into account the above-mentioned considerations, the main task of our study was to characterize both volatile and nonvolatile products from six black currant cultivars grown in Lithuania and to assess the possibilities of a more complex, actually wasteless use of bud raw material in the production of valuable aroma substances and antioxidatively active components.

MATERIALS AND METHODS

Plant Material. Dormant buds of six black currant (*Ribes nigrum* L.) cultivars (Joniniai, Almiai, Gagatai, Ben Alder, Ben Nevis, and Ben Lomond) were harvested from cuttings in the experimental field of Lithuanian Institute of Horticulture (LIH) in January 2005. Joniniai is an early-bearing season cultivar; Almiai and Gagatai belong to midseason-bearing cultivars; while Ben Alder, Ben Nevis, and Ben Lomond are the later-bearing cultivars. The number of black currant buds from one shoot donated approximately 23 units for all examined plants. The weight of one bud varied from 0.018 to 0.040 g. The black currant buds were stored in a freezer before hydrodistillation.

Chemicals and Reagents. 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH$^{\bullet}$, 95%), anhydrous sodium carbonate, and gallic acid were from Sigma–Aldrich Chemie (Steinheim, Germany); 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS), standard Folin−Ciocalteu's phenol reagent, KCl, NaCl, Na_2HPO_4, and $K_2S_2O_8$ were from Merck (Darmstadt, Germany); KH_2PO_4 was from Jansen Chimica (Beerse, Belgium); acetone was from Standard (Lublin, Poland); and methanol was from Lachema (Brno, Czech Republic). The reference compounds for α-pinene (≥99.0%), sabinene (≥98.5%), β-pinene (≥98.5%), myrcene (≥95.0%), α-phellandrene (≥95.0%), δ-3-carene (≥98.5%), limonene (≥99.0%), γ-terpinene (≥98.5%), terpinolene (≥97.0%), terpinen-4-ol (≥99.0%), α-terpineol (≥97.0%), β-caryophyllene (≥98.5%), α-terpinene (≥95.0%), bornyl acetate (≥99.0%), terpinyl acetate (≥90.0%), and α-humulene (≥98.0%) were obtained from Fluka (Steinheim, Switzerland).

Sample Preparation. *Isolation of Essential Oil.* Frozen dormant buds (40 or 60 g) were mixed with 500 mL of distilled water, and the essential oil was isolated by hydrodistillation in a modified Clevenger-type apparatus during 3 h. The obtained essential oil was separated from the water and dried over anhydrous sodium sulfate; the yields varied from 0.8 to 1.7%. It was reported previously that the yield of oil obtained by steam distillation from flower buds was approximately 0.75% (*9*). Isolation of essential oils was performed in duplicate, and samples of essential oil were stored in a freezer prior to further analysis.

Preparation of Extracts. The residues (byproduct) of hydrodistillation were separated into solid and liquid fractions. The solid residue was dried at 30 °C and extracted with acetone, while the liquid fraction (water extract) was divided in two parts, one of which was spray-dried and the other freeze-dried. In addition, a portion of whole frozen buds was extracted with methanol.

Acetone Extracts (AE). The solid bud residue fraction was dried in a flake "abc Bio Dörrer" (Gengenbach, Germany), and 7 g of dry buds were extracted with 120 mL of acetone with automatic extractor IKA-WERKE RET control visc (Staufen, Germany). The extracts were dried in a rotary evaporator Büchi R-114 (Donau, Switzerland). The yield of extracts varied from 11.0 to 17.1%. The extractions were performed in triplicate, and the dry extracts were stored in a freezer prior to further analysis.

Spray- and Freeze-Dried Water Extracts (SDWF and FDWF). The liquid watery fraction was divided into two parts, one of which was spray-dried using a Büchi 190 minispray dryer (Donau, Switzerland) and the other freeze-dried using Maxi-Dry Lyo (Heta, Germany). Powdered extracts were stored in a freezer prior to further analysis.

Methanol Extracts (ME). A 5 g amount of frozen buds was extracted with 120 mL of methanol with automatic extractor IKA-WERKE RET control visc (Staufen, Germany). The extracts were dried in a rotary evaporator Büchi R-114 (Donau, Switzerland) and stored in a freezer prior to further analysis. The yield of extracts varied from 14.0 to 18.5%. The extractions were performed in triplicate.

Gas Chromatography. *Gas Chromatography (GC) and Gas Chromatography-Olfactometry (GC-O).* These systems consisted of a Varian 3900 gas chromatograph (Palo Alto, CA) 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 to 250 °C (5.0 min hold) at 2 °C/min. The GC-O system was equipped with a sniffing port. Column effluent was split between FID and sniffing port at a ratio of 1:1. Injection volumes were 0.1 μL at 1:100 split. The temperatures of the injector and detector were 250 °C. GC-O effluents were assessed by one assessor, who was sniffing the oils three times. The assessor traced the time when he detected the odor during the GC-O run.

The amount of the individual compounds was expressed as a peak area percentage and in arbitrary units (au) representing the concentration of the separate components in 1 kg of frozen buds. An arbitrary unit is approximately equal to 1 μg; however, response factors for each compound were not measured in this study, and therefore au cannot be directly converted in to μg.

Gas Chromatography–Mass Spectrometry (GC-MS). The GC-MS system consisted of a Clarus 500 gas chromatograph (Perkin Elmer, Shelton, Connecticut) equipped with mass selective detector Clarus 500 (Perkin Elmer) and automatic injector. The separation was performed using a nonpolar fused silica capillary column Elite-5 (30 m × 0.25 mm i.d.; 0.25 μm film thickness). Mass spectra were obtained by EI at 70 eV. Oven temperature was programmed from 60 to 250 °C (5.0 min hold) at 3 °C/min. The injection volume was 0.5 μL at 1:200 split; the temperatures of the injector and detector were 250 °C. The compounds were identified by comparison of their KI relative to C_5−C_{18}

n-alkanes obtained on a nonpolar DB-5 column with those provided in the literature (*20*), by comparison of their mass spectra with those recorded in NIST and WILEY 275 libraries and reported in published articles (*2, 6*) and by co-injection of available reference compounds. The samples were analyzed in duplicate.

Evaluation of Antioxidant Activity. AE, SDWF, FDWF, and ME extracts were tested for their radical scavenging capacity (RSC) by using $DPPH^{\bullet}$ radical scavenging and $ABTS^{\bullet+}$ radical cation decolorization reaction systems; in addition the concentration of phenolic compounds was measured in the extracts. All determinations were performed in triplicate.

$DPPH^{\bullet}$ Radical Scavenging Assay. The RSC of black currant bud extracts against stable $DPPH^{\bullet}$ was determined by a slightly modified DPPH radical scavenging assay (*17*). The solution of $DPPH^{\bullet}$ (6×10^{-5} M) was prepared daily, before measurements on a UV/vis spectrophotometer Perkin Elmer (Waltham, Massachusetts) at 515 nm. The extracts were dissolved before the reaction in methanol to obtain 0.15% solution. A 2 mL aliquot of $DPPH^{\bullet}$ solution was mixed with a 50 μL of extract solution in 1 cm path length disposable microcuvette. The decreasing absorbance was read during 16 min reaction time at 1 min intervals until the absorbance curve reached the plateau phase. Simultaneously the absorption of blank sample containing the same amount of methanol and $DPPH^{\bullet}$ solution was prepared and measured daily.

$ABTS^{\bullet+}$ Radical Cation Decolorization Assay. $ABTS^{\bullet+}$ was produced by reacting ABTS radical cation with potassium persulphate ($K_2S_2O_8$) (*18*). Stock solution of ABTS (2 mM) was prepared by dissolving in 50 mL of phosphate buffered saline (PBS) obtained by dissolving 8.18 g of NaCl, 0.27 g of KH_2PO_4, 1.42 g of Na_2HPO_4, and 0.15 g of KCl in 1 L of water. $ABTS^{\bullet+}$ was produced by reacting 50 mL of ABTS stock solution with 200 μL of $K_2S_2O_8$ solution and allowing the mixture to stand in the dark at room temperature for 15–16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the assessment of extracts, the $ABTS^{\bullet+}$ solution was diluted with PBS to obtain the absorbance of 0.800 ± 0.030 at 734 nm. The extracts were dissolved before the reaction in PBS to obtain 0.1% solution. A 3 mL aliquot of $ABTS^{\bullet+}$ solution was mixed with 30 μL of extract solution in the 1 cm path length microcuvette. The absorbance was read at ambient temperature after 1, 4, 6 and 10 min. PBS solution was used as a blank sample.

Total Amount of Phenolic Compounds. The concentration of phenolic compounds in extracts of black currant buds was determined by Folin–Ciocalteu method (*19*). For the preparation of a calibration curve 1 mL reference gallic acid solutions in ethanol (aliquots of 0.025, 0.075, 0.100, 0.175, and 0.350 mg/mL) were mixed with 5 mL of standard Folin−Ciocalteu reagent and diluted with distilled water (1:10) and 4 mL of 7.5% sodium carbonate solution in distilled water. The absorption was read after 30 min at 765 nm, and the calibration curve was drawn, which showed very good linear dependence between absorption and gallic acid concentration ($y = 9.699x + 0.104$; $R^2 = 0.997$). For determination of phenolics 1 mL of extract to obtain 0.05% solution was mixed with 5 mL of Folin–Ciocalteu reagent and 4 mL of 7.5% sodium carbonate solution; the absorption was read after 30 min. The concentration of phenolics compounds (*C*) was expressed in milligrams of gallic acid equivalents (GAE) per gram of plant extract.

Statistical Analysis. In most cases the results are provided as a mean of the three measurements. Standard deviations (SD) did not exceed 3% for the majority of the values obtained. Analyses of variance were performed at a level of $P < 0.05$ to evaluate the significance of differences between mean values. Correlation coefficients (*R*) to determine the relationship between antioxidant activities obtained in two different RSA reaction systems and between RSA and the concentration of total phenolic compounds in the extracts were calculated using MS Excel software (CORREL statistical function).

RESULTS AND DISCUSSION

Chemical Composition of Essential Oils. Dormant bud essential oils from six *Ribes nigrum* cultivars were analyzed for chemical composition by using GC-FID and GC-MS. In total, 48 compounds were detected; the identified compounds constituted more than 92% of the total integrated GC peak area of each cultivar analyzed.

The essential oils were mainly constituted of aliphatic and oxygenated terpenes and some other compounds. Hydrocarbon terpenes were the most abundant components constituting from 40 (Ben Alder) to 51% (Joniniai) in the total oil, while oxygenated terpenes constituted approximately 31% in all six cultivars. Hydrocarbon and oxygenated terpene fractions were also found to be the major ones in the previously published article (*2*), which reported the percentage of hydrocarbon fraction as high as 90−95%; the content of oxygenated fraction varied from 5 to 10%. The ratio of these two main fractions in bud oils analyzed in our study was quite different, in favor of oxygenated components. However, it should be noted that we used hydrodistillation for the isolation of oil, while in the previously preformed study (*2*) the authors used simultaneous distillation−extraction in a Likens−Nickerson apparatus, i.e. the method which is not applicable on a commercial scale. Monoterpenes and sesquiterpenes were identified among the hydrocarbon and oxygenated fractions; however, monoterpenes were prevailing in both these groups.

α-Thujene, α- and β-pinenes, myrcene, α- and β-phellandrenes, α- and γ-terpinenes, *cis*- and *trans*-sabinene hydrates, terpinen-4-ol, α-terpineol, bornyl acetate, terpinyl acetate, citronellyl acetate, germacrenes B and D, β-caryophyllene, α-humulene, and several other compounds were identified in the analyzed black currant bud essential oils (**Table 1**). The most abundant volatile compounds were sabinene (0.24–64.10%), δ-3-carene (0.36–47.42%), and terpinolene (0.38–20.33%) (**Figure 1**). Three chemotypes according to the main essential oil components can be observed: first, with sabinene as the major compound (Joniniai, Almiai, and Gagatai); second, with high amounts of δ-3-carene and terpinolene (Ben Alder and Ben Nevis); and the third one, with a remarkable amount of all three above-mentioned hydrocarbon monoterpenes (Ben Lomond). Sabinene, δ-3-carene, and terpinolene were distinguishing components in previously reported chemotypes of the 11 black currant cultivars; however, the percentage distribution of these components in the oils from this study was different (*29*). δ-3-Carene, terpinolene, and *cis*-β-ocimene were reported previously as a major hydrocarbon monoterpenes in Ben Alder leaf oil; they accounted for over 80% of the total monoterpenes. The levels of sabinene in Ben Alder cultivar analyzed in our study were considerably lower than those reported for the leaf oils isolated from Ben Lomond cultivar (*3*). The other major quantitative compounds in bud oils were β-ocimenes. Higher concentration of *cis*-β-ocimene was in Almiai (1158 au/kg; 8.0%) and Gagatai (1645 au/kg; 9.3%), while the amount of *trans*-β-ocimene was higher in Gagatai (1421 au/kg; 8.1%). Sabinene, δ-3-carene, terpinolene, α- and β-phellandrenes, γ-terpinene, β-caryophyllene, terpinen-4-ol, germacrene D, bicyclogermacrene, and spathulenol were reported previously as important components in the dormant black currant buds; moreover, several sesquiterpene chemotypes were distinguished (*6, 29*). All these compounds were found in the buds analyzed in our study, except for bicyclogermacrene and spathulenol. α-Humulene was the most abundant sesquiterpene in the bud oils analyzed in our study; it constituted 1.9–3.9%, however, β-caryophyllene was a major sesquiterpene in Joniniai cultivar, 8.3%.

The concentrations of some other compounds were remarkably lower, e.g., δ-2-carene (33−134 au/kg; 0.2–0.8%) and 6,10,14-trimethyl-2-pentadecanone (10−20 au/kg; 0.1%) were found in Almiai, Gagatai, and Ben Lomond cultivars; limonene

Table 1. Composition and Odor Descriptors of Black Currant Buds of Six Different Cultivars, au/(kg of frozen buds)

compounds	KI	Joniniai (au/kg)	Almiai (au/kg)	Gagati (au/kg)	Ben Alder (au/kg)	Ben Nevis (au/kg)	Ben Lomond (au/kg)	odor note[a]
α-thujene,[b−e] t[f]	930	179	130	165	tr[g]	78	129	woody (*23, 25*); green, herbal (*25*)
α-pinene[b−e,h]	939	188	583	747	tr	294	247	pine, woody (*8, 23, 24*); terpene, camphoraceous (*8, 23*); fruity, sweet, green, citrus, lime (*23*); pine, turpentine (*25*)
camphene,[b−e] t	955	nd[l]	63	88	tr	tr	15	camphoraceous (*8, 23−25*); terpene (*8, 23,*); sweet, fruity, oily, herbal (*23*)
sabinene[b−d,h]	985	5165	9294	10070	25	39	7409	woody (*23, 25*); citrus, spicy (*23*); pepper, turpentine (*25*)
β-pinene[b−d,h]	987	199	429	533	nd	863	465	woody, pine (*8, 23−25*); terpene, resinous (*8, 23*); musty, green, sweet (*23*); terpentine, resinous (*25*)
myrcene[b−d,h]	994	156	413	449	120	601	111	sweet (*8, 23, 24*); balsamic, plastic (*8, 24*); metallic, musty, geranium, fruity, ethereal, soapy, citrus, spicy, woody (*23*); balsamic, musty, spicy (*25*)
δ-2-carene,[b,c] t	1008	nd	105	134	nd	nd	33	na[j]
α-phellandrene[b−d,h]	1010	17	110	tr	25	149	51	mint, herbaceous (*8, 23, 24*); citrus, pepper (*8, 23*); fruity, lime, juniper (*23*); turpentine, mint, spicy (*25*)
δ-3-carene[b−d,h]	1013	88	52	72	4979	7052	3141	sweet, terpene (*8*); lemon, resin (*25*)
α-terpinene[b−d,h]	1021	46	58	75	276	119	109	citrus (*8, 23, 25*); woody, terpene (*8*); fuel, ethereal, fruity (*23*)
p-cymene,[b−d] t	1026	98	219	59	9	17	9	weak citrus, spicy (*23, 24*); fruity, fuel, sweet, herbal (*23*); solvent, gasoline, citrus (*25*)
limonene[b−d,h]	1033	tr	nd	nd	96	650	91	citrus, camphoraceous, terpene (*8*); citrus, licorice, green, ethereal, fruity (*23*); citrus, mild, sweet, orange (*24*); lemon, orange (*25*)
β-phellandrene,[b−d] t	1036	257	559	253	78	201	249	terpene, mint (*23, 25*); fruity, herbal (*23*)
cis-β-ocimene,[b−d] t	1041	237	1158	1645	59	283	214	citrus, herbaceous (*23, 25*)
trans-β-ocimene,[b−d] t	1051	95	131	1421	13	361	313	herbaceous, sweet (*23, 25*)
γ-terpinene[b−e,h]	1062	87	99	180	323	63	23	herbaceous, citrus (*8, 23, 24*); slightly bitter, woody, terpene, sweet (*8, 23*); gasoline, turpentine (*25*)
cis-sabinene hydrate,[b−d] t	1074	63	43	144	270	86	151	balsamic (*23, 25*); warm, woody, mild (*23*)
terpinolene[b−e,h]	1087	122	71	67	2047	3170	1316	sweet, pine (*8, 23*); woody, fruity, slightly anisic (*23*); plastic, petroleum (*24*); pine, plastic (*25*)
trans-sabinene hydrate,[b−d] t	1102	24	37	93	85	20	57	balsamic, woody (*23, 25*); warm, mild (*23*)
trans-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol,[b,e] t	1126	14	17	63	nd	nd	27	na
cis-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol,[b,e] t	1144	tr	10	30	30	99	17	na
thujol,[b] t	1177	nd	nd	nd	13	21	22	na
terpinen-4-ol[b−e,h]	1184	346	241	18	50	30	492	pepper, woody, musty (*23, 24*); herbaceous, terpene, sweet, pine, fruity, licorices, moldy (*23*); turpentine, nutmeg, musty (*25*)
p-cymen-8-ol,[b−e] t	1186	nd	tr	tr	7	14	9	floral, sweet, citrus (*23*); citrus, musty (*25*)
α-terpineol[b−d,h]	1194	31	17	421	28	222	24	peach (*8, 23*); lilac (*8, 24*); floral (*23, 24*); anise, oily, mint (*23, 25*); fruity, toothpaste (*23*); fragrant (*24*)
cis-piperitol,[b−d] t	1195	nd	tr	tr	144	23	nd	herbaceous (*23*)
trans-piperitol,[b−e] t	1208	8	tr	36	23	14	9	herbaceous (*25*)
cyclohexylethylacetate,[b] t	1249	nd	tr	nd	8	27	15	powerful, sweet, fruity (*8, 24*)
bornyl acetate[b−e,h]	1283	17	67	95	9	37	32	fresh, strong, pine (*8*); pine, camphoraceous, herbal, balsamic (*23*)
terpinyl acetate[b−e,h]	1346	20	15	15	6	12	8	sweet, refreshing, herbaceous (*8*); waxy (*23, 25*)
citronellyl acetate,[b−d] t	1351	5	9	10	47	18	9	rose (*8, 23, 25*); fresh, fruity (*8*); citrus, oily, berry, fragrant, musty, dusty (*23*)
β-elemene,[b−e] t	1386	36	tr	tr	5	9	9	waxy, herbaceous, fresh (*23, 25*)
β-caryophyllene[b−e,h]	1418	691	13	10	22	27	12	woody, spicy (*23, 25*); terpene, musty, green, fruity, sweet (*23*)
γ-elemene,[b−d] t	1426	26	nd	nd	nd	nd	nd	green, woody, oily (*25*)
α-humulene[b−e,h]	1452	169	370	330	415	622	352	woody (*25*)
germacrene D,[b−d] t	1477	9	182	94	134	182	129	oily, green, woody (*23*); woody, spicy (*25*)
β-selinene,[b,c] t	1482	143	nd	nd	nd	nd	nd	herbaceous (*23, 25*)
α-selinene,[b,c] t	1499	9	92	tr	9	tr	105	na
β-guaiene,[b−d] t	1506	20	nd	nd	nd	nd	nd	woody (*8, 25*); oily (*23*); spicy (*25*)
δ-cadinene,[b−e] t	1513	92	10	105	194	189	34	pungent (*8*); herbaceous (*23*); thyme, medicine, woody (*25*)
germacrene B,[b−e] t	1554	70	nd	nd	nd	nd	nd	na
germacrene D-4-ol,[b,c] t	1570	15	36	37	7	87	11	na
caryophyllene oxide,[b−e] t	1574	72	tr	9	183	62	49	herbaceous, sweet, fruity, sawdust (*23*); herbaceous, sweet, spicy (*25*)
τ-cadinol,[b,c] t	1633	25	10	nd	18	103	nd	na
α-cadinol,[b−e] t	1647	19	11	10	79	50	22	herbaceous, woody (*25*)
6,10,14-trimethyl-2-pentadecanone,[b,d] t		nd	20	tr	nd	nd	10	na

[a] Odor note was described as indicated in listed references (in parentheses). [b] Identification supported by good match of MS. [c] Identification supported by KI (*20*). [d] Identification supported by agreement with published data (*2, 6*). [e] Detected by olfactometry. [f] t, tentative identification. [g] tr, trace (<5 au/kg). [h] Identification supported by co-injection of reference compounds. [i] nd, not detected. [j] na, not available.

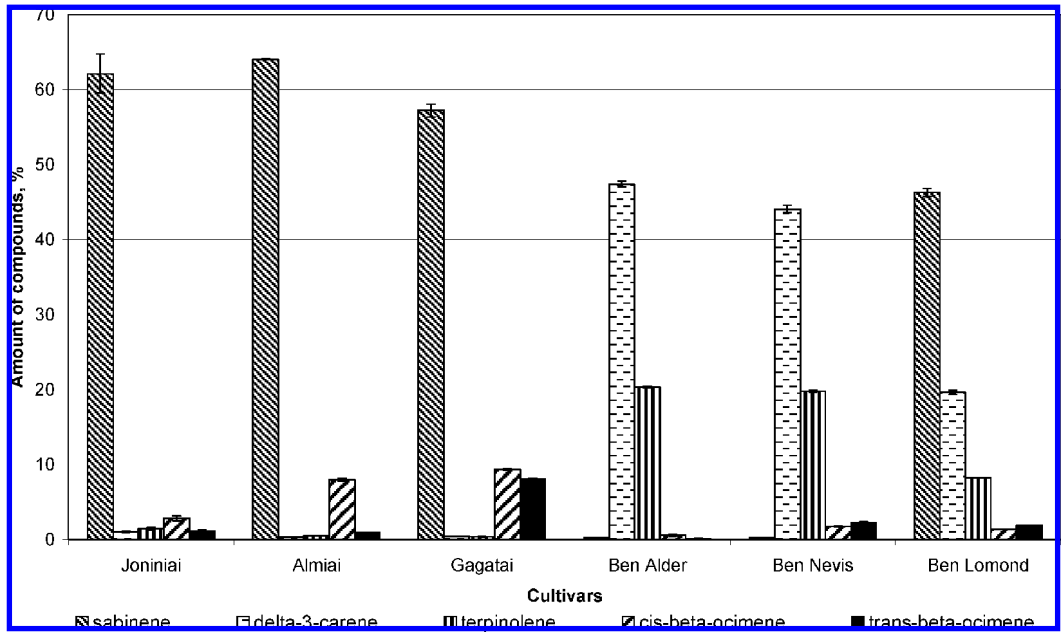

Figure 1. Major compounds of black currant buds essential oil.

(91−650 au/kg; 0.1–4.1%), thujol (13−22 au/kg; 0.1%), *p*-cymen-8-ol (7−14 au/kg; 0.1%), and cyclohexylethylacetate (8−27 au/kg; 0.0–0.10%) were identified in Ben Alder, Ben Nevis, and Ben Lomond cultivars. Some compounds, namely, γ-elemene, β-selinene, β-guaiene, and germacrene B were detected solely in Joniniai cultivar (**Table 1**). To the best of our knowledge, tentatively identified δ-2-carene [3,7,7-trimethyl-bicyclo(4.1.0)hept-2-ene], α-selinene [(naphthalene, 1,2,3,4,4a,5,-6,8*a*-octahydro-4*a*,8-dimethyl-2-(1-methylethenyl)-, 2*R*-(2α,4*a*α,-8*aβ*))-], β-selinene [(naphthalene, decahydro-4*a*-methyl-1-methylene-7-(methylethenyl)-, 4*aR*-(4*a*α,7α,8*aβ*))-], germacrene D-4-ol [2,7-cyclodecadien-1-ol, 1,7-dimethyl-4-(1-methylethyl)-, (1*S*-(1*R**,2*E*,4*R**,7*E*))-], and τ-cadinol [1-naphtalenol, 1,2,3,-4,4*a*,7,8,8*a*-octahydro-1,6-dimethyl-4-(1-methylethyl)-, (1*R*-(1α,-4*β*,4*aβ*,8*a*α))-] were not previously reported in black currant bud essential oil (*2, 6, 27, 29*). These components are frequently detected in various essential oils.

Odor Active Compounds of Black Currant Bud Essential Oil. The essential oil obtained by hydrodistillation of black currant buds is used as flavoring and fragrance material. Terpenes constitute the majority of the compounds identified in buds (*2, 21, 22, 27, 29*).

Black currant buds possess a very characteristic, penetrating, and powerful aroma. It was reported that the overall odor of their oil can be characterized as a strong terpenic overwhelmed by a "catty" note (*6*). It was also reported that the most important compounds for the aroma of oils were present in the polar fractions, even though they were found in very small amounts. 4-Methoxy-2-methyl-2-mercaptobutane, which is responsible for the characteristic "catty" note was present in oil in small amounts, varying from trace to 0.04% (*2*). This sulfur-containing compound was not detected in our study by GC-MS and GC-FID as a separate constituent in the chromatogram; however it was detected by GC-O as the constituent eluting from the column before α-thujene.

Although a catty note is a typical odor characteristic of black currant buds, other volatile compounds present in the oil also possess typical odor characteristics. Therefore, it is likely that the overall aroma of black currant buds depends on a complex mixture of odor active compounds (**Table 1**). The differences in the composition are responsible for different aroma impacts. It should be emphasized that the ratios of the contents of volatile components are often more important than the effects of individual compounds. The aroma impact of individual components in the volatile mixture is also associated with certain problems of olfactory perception (*6*). In general, the aroma of the mixture depends on the composition of volatile constituents, which is defined by the amount of the compounds in the matrix and their properties. Also, the odor threshold of a particular compound plays an important role in the aroma perception.

The most prevailing descriptors of black currant buds were "woody", "terpene", "fruity", "sweet", "citrus", "herbaceous", "pine", "green", "oily", "herbal", and "musty". The aroma mixture of black currant bud oil constituted the both hydrocarbon monoterpenes (α-thujene, α-pinene, camphene, γ-terpinene, and terpinolene) and sesquiterpenes (β-elemene, β-caryophyllene, α-humulene, δ-cadinene and germacrene B), and oxygenated monoterpenes (*cis*- and *trans*-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol, terpinen-4-ol, *trans*-piperitol, and bornyl and terpinyl acetate) and sesquiterpenes (caryophyllene oxide and α-cadinol) (**Table 1**). All these compounds belong to the quantitatively minor bud oil constituents except for terpinolene. Piry et al. (*6*) separated black currant bud oil into hydrocarbon and oxygenated terpene fractions; however, "catty" or "black currant" notes were not eluted. For this reason they separated essential oil into neutral, acidic, and basic fractions. The neutral fraction was characterized by a typical lemon or citrus-fruity odor, while the acidic one was characterized by a catty odor. The hydrocarbon fraction although being the major quantitatively part in the oils did not fully explain the black currant odor. Monoterpenes represent essentially green and resin-like notes. However, some sesquiterpenes could be considered as black currant flavor contributors. The content of the oxygenated compounds representing higher polarity fraction was remarkably lower; however, it contained the most odorous volatile compounds and exhibited the characteristic black currant odor. Of these, terpene alcohols can be considered to be the most important flavor impact compounds for the black currant (*2, 6*).

Evaluation of Antioxidant Activity. RSC of black currant bud extracts varied in a wide range; in the DPPH$^{\bullet}$ reaction system FDWF at the applied concentration scavenged 43–79%;

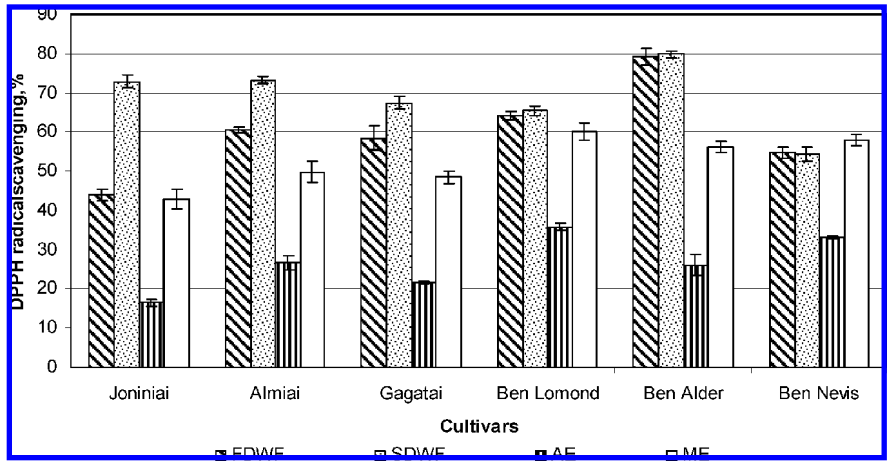

Figure 2. RSC of black currant bud extracts measured in the DPPH$^{\bullet}$ reaction system.

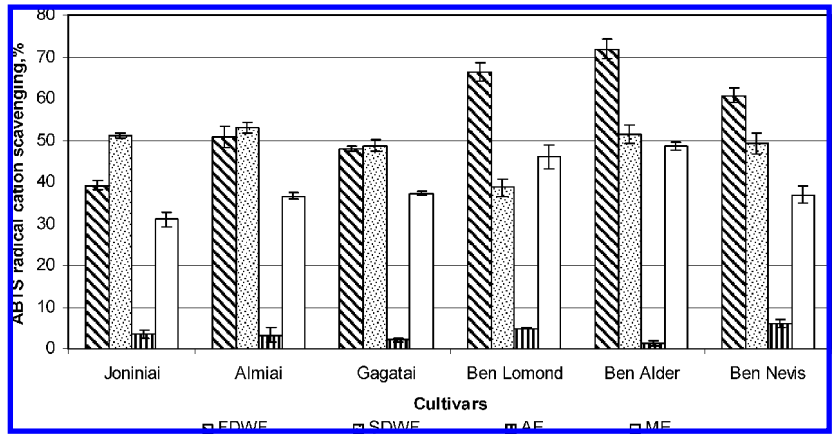

Figure 3. RSC of black currant bud extracts measured in the ABTS$^{\bullet+}$ reaction system.

SDWF, 54–80%; AE, 16–36%; ME, 42–60% of radicals (**Figure 2**), while in the ABTS$^{\bullet+}$ reaction system the RSC was 39–72, 38–53, 1–5, and 30–49%, respectively (**Figure 3**). It is obvious that antioxidant capacity depends both on the extraction method and the cultivar of black currant.

FDWF, SDWF, and ME extracts were remarkably stronger antioxidants than AE extracts in DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ reaction systems (**Figures 2** and **3**). SDWF extracts of Joniniai, Almiai, and Gagatai cultivars were stronger antioxidants than FDWF extracts; while both extract types from other cultivars (Ben Alder, Ben Nevis, and Ben Lomond) had similar antioxidant activity in the DPPH$^{\bullet}$ reaction. Comparing different cultivars, the RSC of FDWF and SDWF extracts was highest for Ben Alder, while AE and ME extracts were the strongest radical scavengers in the case of Ben Lomond (**Figure 2**). SDWF extracts of Joniniai, Almiai, and Gagatai cultivars were stronger antioxidants in the DPPH$^{\bullet}$ system, while FDWF extracts of Ben Alder, Ben Nevis, and Ben Lomond cultivars had the strongest RSC in the ABTS$^{\bullet+}$ reaction (**Figures 2** and **3**). Some work has been performed previously on various black currant processing byproducts. It was reported that the RSA of black currant byproduct obtained after pressing in the DPPH$^{\bullet}$ reaction system varied from 49 to 15% (*12*). It was also reported that the RSC of the black currant press residue and pomace varied in a wide range, from 0.04 to 0.45 mmol of Trolox equivalents/(g of plant material). The RSC for ABTS$^{\bullet+}$ was always higher for the black currant press residue solvent extracts compared to the black currant pomace extracts; however, after acid hydrolysis, the residues from the pomace extracts showed higher scavenging activity than those from press residue extracts (*11*). In our study

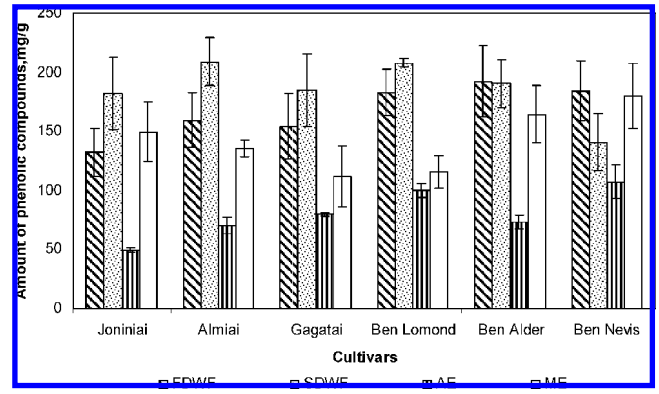

Figure 4. Total amount of phenolic compounds of black currant bud extracts (in gallic acid equivalents).

the strongest correlations between RSC in DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ reaction systems were found for FDWF and ME extracts, 0.85 and 0.78, respectively; the correlations for SDWF and AE extracts were remarkably lower, 0.34 and 0.58, respectively.

The total amount of phenolic compounds, expressed in gallic acid equivalents, in FDWF varied in the range of 132–192 mg/g; in SDWF, 140–209 mg/g; in AE, 49–107 mg/g; and in ME extracts, 111–180 mg/g (**Figure 4**). The lowest amount of phenolic compounds as well as RSC in both DDPH$^{\bullet}$ and ABTS$^{\bullet+}$ systems were found for the AE extracts (**Figures 2**−**4**); however, the concentration of phenolic compounds in AE extracts from Ben Lomond and Ben Nevis cultivars was comparatively high (approximately 103 mg/g; **Figure 4**). It was

reported previously that the total amount of phenolics in the black currant byproduct obtained after pressing varied from 2426 to 9721 mg/L (*12*). Kapasakalidis et al. (*11*) found that the level of total phenols varied depending on the type of plant tissue (press residue or pomace) and sample treatment methods (solvent extraction/acid hydrolysis, solvent type); it ranged from 11 to 87 mg/g for press residue and from 9 to 73 mg/g for pomace. Best correlations between the total content of phenolics and RSA in $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ reaction systems were found for FDWF (0.78 and 0.98) and AE (0.90 and 0.58) extracts. For comparison, Lapornik et al. (*12*) reported that the correlation between the content of total polyphenols and antioxidant activity was 0.74.

It can be concluded that the results obtained extend scientific knowledge on the variations in the composition of essential oils of black currant dormant buds which largely depend on the plant cultivar. Black currant cultivars analyzed in the present study according to the main essential oil compounds were separated into the three different chemotypes: sabinene as a major compound (Joniniai, Almiai, and Gagatai); high amounts of δ-3-carene and terpinolene (Ben Alder and Ben Nevis); a remarkable amount of all three terpenes (Ben Lomond). In addition, δ-2-carene, α- and β-selinene, germacrene D-4-ol, and τ-cadinol were reported for the first time as bud essential oil constituents. GC-O analysis of essential oil components separated by GC suggests that several terpenes and one sulfur-containing compound are likely to contribute to the overall odor of black currant buds of essential oil.

Black currant bud extracts and byproducts of the essential oil production, particularly the water fraction, can be further processed to obtain antioxidatively active substances; the radical scavenging capacity of freeze- and spray-dried liquid distillation byproducts was comparatively high. The RSC of the acetone extract isolated from the solid hydrodistillation residue was remarkably lower, indicating that antioxidatively active components are higher polarity water soluble compounds. The differences in the RSC as well as the concentration of phenolics between spray- and freeze-dried products were insignificant; therefore spray drying as cheaper techniques seems to be more feasible in the preparation of antioxidatively active components from black currant buds. Further studies should be focused on the identification and quantification of antioxidatively active compounds in the extracts as well as on the possibilities to use natural antioxidants from black currant buds for various applications, which are likely to improve commercial feasibility of bud essential oil production.

ABBREVIATIONS USED

GC, gas chromatography; GC-MS, gas chromatography–mass spectrometry; GC-O, gas chromatography–olfactometry; GC-FID, gas chromatography with flame ionization detector; au, arbitrary units; KI, Kovats retention indices; RSC, radical scavenging capacity; AE, acetone extract; ME, methanol extract; SDWF, spray-dried water extract; FDWF, freeze-dried water extract.

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