Maritime Influence on the Volatile Terpenes in the Berries of Different Ecotypes of Juniper (*Juniperus communis* L.) in Finland

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The effect of maritime locality and growth form on the volatiles of the green and blue berries of juniper, *Juniperus communis* L., was studied by gas chromatography–mass spectrometry. The compounds were isolated by a combined steam distillation–solvent extraction procedure. The berries contained volatile compounds of at least 0.4–2.5% calculated on a fresh-weight basis and 0.5–4% on a dry-weight basis. The contents (from 15 to 5.4 mg/100 g fw) and relative percentages (from 2.4 to 1.1%) of humulene and of the sum of β-elemene, caryophyllene, and terpinen-4-ol (from 28 to 9.0 mg/100 g fw and from 4.7 to 1.9%) decreased gradually from the furthest maritime growth site toward the coast. The relative percentage of α-terpinolene also displayed the same trend (from 0.84 to 0.53%).

The berries of juniper (*Juniperus communis* L.) are widely used in compounding spices, perfumes, and pharmaceutical products. The alcohol and beverage industry is one of the main users of the berry destillates.

The aroma and flavor fractions of both unripe green and ripe blue berries consist of mono- and sesquiterpene hydrocarbons. The main component is α-pinene (Hörster, 1974; Taskinen and Nykänen, 1976; Formacek and Kubeczka, 1982), but oxygenated terpenes, though existing in minor quantities, are important for the fine juniper aroma. The pharmacological use of juniper extracts is based on the diuretic effect of terpinen-4-ol (Janku et al., 1957).

The commonly known qualitative differences of the juniper berries and isolates on the market are partially due to the high biological variation of the raw material. The aim of this work was to investigate the effect of the three growth forms of *J. communis* existing in Finland and the influence of maritime environment on the composition of the berry oil.

MATERIALS AND METHODS

Juniper Berries. The juniper berries (*J. communis* L.) were collected in August 1984 on the islands of Jurmo (59°50′ N, 21°35′ E) and Berghamn (60°03′ N, 21°48′ E) in the Baltic Sea and on the mainland site of Raisio (60°29′ N, 22°08′ E) about 5 km from the coast (Figure 1). Berghamn is located in the inner and Jurmo in the outer archipelago of Turku. The berries of the second (green) and third (blue) year were picked from two prostrate, two ascending, and two pyramidal junipers at those growth sites where they existed. The prostate bushes did not grow at the Raisio site nor did the pyramidal growth forms at the Jurmo island. The material was frozen and stored at −18 °C until analysis in fall 1984.

Isolation of Volatiles. The volatiles were isolated with the combined distillation–extraction method of Maarse and Kepner (1970). A 10-g portion of thawed berries was crushed in a mortar and distilled with 100 mL of water. The solvent (13 mL) was pentane–diethyl ether (1:2, v/v). The sample bottle was maintained at boiling point and the receiving flask with the solvent at 50 °C. After 3-h isolation the extract was dried with annealed Na_2SO_4 at 8 °C for 12 h. The dried extract was evaporated to a final volume of 4 mL and stored at −70 °C until analysis. The yield of the aroma compounds to be studied was investigated by distilling one sample three times for 3 h with separate solvent portions followed by subsequent GC analysis and determination of the total content of the volatiles.

GC and GC–MS Analysis. The analyses were carried out on a Varian 3700 gas chromatograph equipped with FID (Walnut Creek, CA) and a Hewlett-Packard 3388A integrator (Palo Alto, CA). The fused silica OV-351 capillary column (25 m × 0.32 mm (i.d.), d_f 0.20 μm; Orion Analytica, Espoo, Finland) was programmed after a 2-min isothermal period from 60 to 220 °C at a rate of 4 °C/min. The split injector temperature was 240 °C and that of the detector 250 °C. The flow rate of the He carrier was 1.4 mL/min measured at 60 °C and the split ratio 1:6. The different distribution of single compounds in the injector was taken into account by using the correction factors when compared to the on-column injection analysis of the same sample. 2-Nonanone was used as the internal standard. All analyses were duplicated.

The mass spectral analyses (70 eV) were carried out on a VG 7070E mass spectrometer employed with the VG-11-250 data system (Wythenshave, Manchester, U.K.). The gas chromatograph was a Dani 3800 HR 2 ch. The same column and programming were used as in the GC analysis. Relative retention times and available mass spectra were used to verify the structures of the compounds.

Determination of the Total Volatiles. Two different methods were used to determine the total content of the volatiles. The major part of the solvent was evaporated from the extract by nitrogen stream at room temperature to the final ca. 300 μL volume of the concentrate, which was weighed. The content of the residual solvent was measured by gas chromatography using on-column injection and subtracted from the mass of the sample (method I).

Method II was a direct GC method, where 2-nonanone was used as the internal standard. All the peaks except those originating from the solvents were taken into account. No detector correction factors were used.

Dry-Weight Determination. The dry weight of the berries was determined by drying 3 g of crushed samples at 105 °C for 12 h and corrected by subtracting the contents of the volatiles determined by method I.

RESULTS AND DISCUSSION

The dry matter contents of the juniper berries of the second (unripe, green) and third (ripe, blue) year were different from each other at highly significant levels: 45.2 ± 4.5% and 69.2 ± 5.9%, respectively. Locality did not affect the dry-matter content (Figure 2). The mean values of the dry-matter contents of berries of the pyramidal junipers were somewhat lower than those of other growth forms, but the difference was not statistically significant.

A gas chromatogram of the volatiles of juniper berries is shown in Figure 3, and the means and standard deviations of the isolated fractions are presented in parts A and B of Figures 4. When calculated according to the gravimetric method, the yield of the fraction isolated in the 3-h distillation–extraction varied from 230 to 1600 mg/100 g of fresh berries. This was 65% of the amount collected with the 3 × 3 h isolation. Thus, the actual content in the berries was of the same order of magnitude as reported earlier (Analytical Methods Committee, 1984; Hörster,

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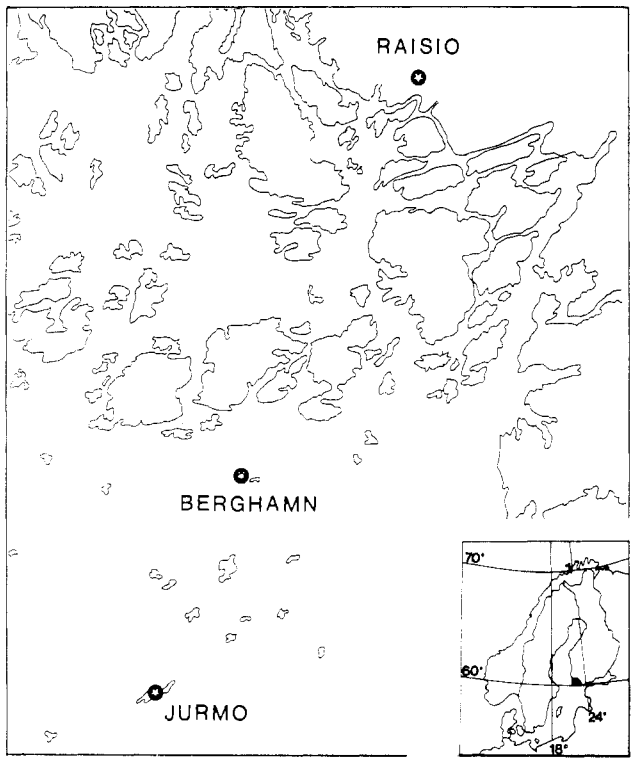

Figure 1. Map of the juniper growth area.

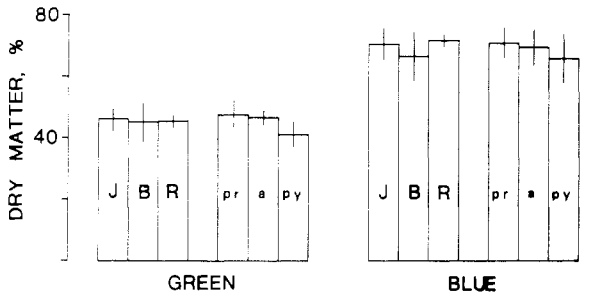

Figure 2. Dry-matter content of the green and blue juniper berries: J = Jurmo, B = Berghamn, R = Raisio, pr = prostrate, a = ascending, py = pyramidal.

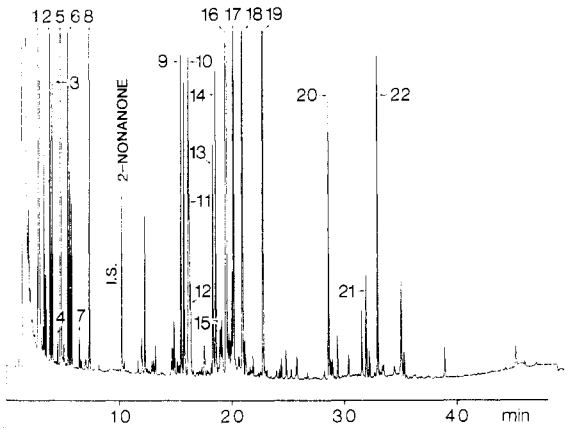

Figure 3. Gas chromatogram of the volatiles of juniper berries (Raisio, pyramidal, green berries) isolated by distillation–extraction. Programmed from 60 °C (2 min isothermal) to 220 °C at 4 °C/min on a 28 m × 0.32 mm, d_f 0.20 μm OV-351 column. The peak numbering corresponds to Table I.

1974). The corrected contents of the main components (exceeding 1% level in at least one of the samples studied), their split injector correction factors, and the isolation yeilds (%) when compared to the 3 × 3 h distillation are presented in Table I. The standard deviation of the

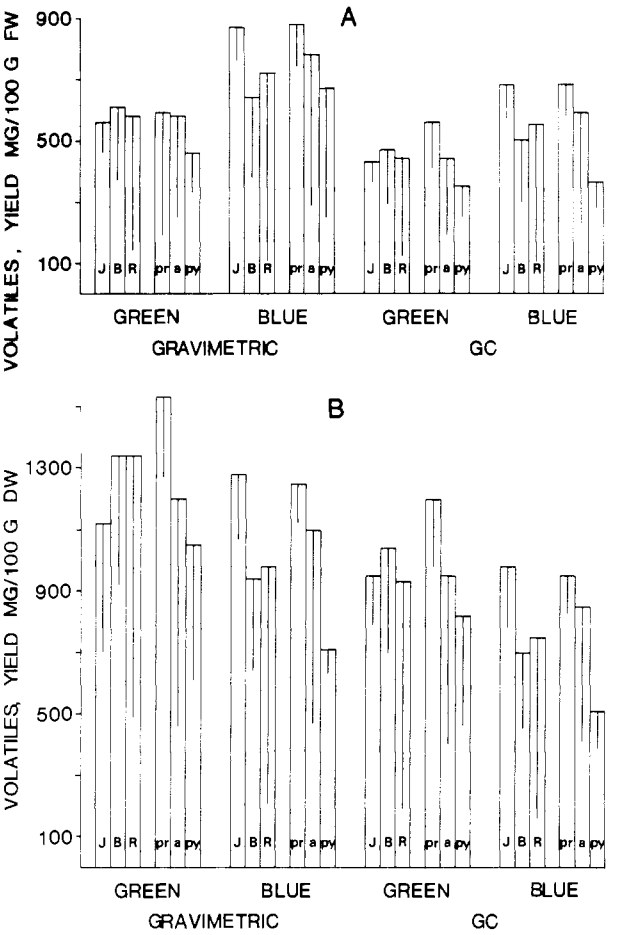

Figure 4. Yield of volatiles (3-h isolation) of green and blue juniper berries calculated on fresh-weight (A) and dry-weight (B) basis: J = Jurmo, B = Berghamn, R = Raisio, pr = prostrate, a = ascending, py = pyramidal.

contents of the individual compounds tested with seven identical berry samples was on average 5.4% (isolation and GC analysis), and that of seven repeated analyses of the same concentrate was 1.9%. In spite of the low yield of several compounds, the accuracy of the method used was sufficient and the data could be used for statistical comparison of the berries of different origins.

The analysis of variance of the total volatiles showed no differences arising from the locality, the growth form, or even the age of the berries (Figure 4A,B). The result was the same on both dry-weight- and fresh-weight-based calculations. This was due to the extremely high deviation of the aroma compounds between individual junipers. However, in just two of the cases studied, a single juniper contained more volatile compounds in the second-year berries than in the third-year berries calculated on fresh-weight basis and vice versa when calculated on dry-weight basis.

When the gravimetric method (method I) was used, the yield of the volatiles from unripe berries was 590 ± 260 mg/100 g on fresh-weight basis and that of the ripe berries 740 ± 360 mg/100 g. The corresponding values obtained with the GC method were 450 ± 190 mg/100 g and 560 ± 270 mg/100 g (Figure 4A,B). The values obtained by method II are lower mainly due to the split ratios and the detector responses of the analytes differing from that of the internal standard; thus, the gravimetric method (method I) gives results closer to the corrected values. The ripe juniper berries are known to contain more volatiles than the unripe berries (Mihaijlov, 1968; Günther, 1952; Gelsomini et al., 1988). According to Hörster (1974), the

Table I. Correction Factors and the Corrected Contents of the Volatile Terpenes in the Juniper Berry Oil (*J. communis*)

compound[a] (no.)	GC corrn factor[b]	isolation yield[c]	content,[d] mg/100 g dw	content,[d] mg/100 g fw
α-pinene (1)	0.88	67	270–1180	130–600
β-pinene (2)	0.97	72	8–51	5–24
sabinene (3)	0.53	87	1–130	tr[e]–86
Δ^3-carene (4)	0.62	100	tr–19	tr–10
myrcene (5)	0.99	72	25–610	18–360
limonene (6)	0.88	70	3–77	2–29
γ-terpinene (7)	1.00	100	tr–16	tr–11
α-terpinolene (8)	1.03	67	1–22	1–10
unknown (9)	1.10	69	1–23	tr–10
β-elemene (10)				
caryophyllene (11)	1.04	64	7–160	3–110
terpinen-4-ol (12)				
humulene (13)	1.12	65	5–58	3–43
unknown[f] (14)	1.23	72	1–50	1–26
germacrene D (15)	1.15	52	3–15	1–12
β-selinene[g] (15)				
γ-cadinene (16)	1.23	76	22–260	16–180
unknown[f] (17)	1.19	70	5–44	3–30
δ-cadinene (18)	1.48	56	21–290	10–200
γ-muurolene[g] (18)				
α-muurolene[g] (19)	1.37	59	tr–83	tr–63
unknown[f] (20)	1.50	58	1–130	tr–60
unknown[f] (21)	1.86	26	6–110	4–81
α-cadinol (22)	1.34	25	12–230	7–168

[a] Compounds listed exceeded the 1% level of uncorrected peak areas in at least one of the trial members. [b] Correction factor when compared to the on-column injection response. [c] Yield of 1 × 3-h extraction (%) compared to the yield of 3 × 3-h extraction. [d] Corrected according to GC correction factors and isolation yields. [e] <0.5 mg/100 g. [f] Sesquiterpene hydrocarbon, MW 204. [g] Tentative identification.

differences between the berries of different ages were not statistically significant.

The highest total yields of volatiles in different growth forms were obtained in the blue berries of the prostate junipers, 880 ± 140 mg/100 g calculated on fresh-weight basis (Figure 4A), and in the green berries of the prostate-type bushes, 1530 ± 260 mg/100 g calculated on dry-weight basis (Figure 4B). The pyramidal junipers had on average the lowest content of the isolated fraction. All these values have to be regarded as minimum values because of low yield of several compounds in the isolation procedure (Table I).

The biological deviation of the terpene composition in the berries was extremely high (Table I) as stated earlier by Hörster (1974). Genetic differences in individual junipers could not be eliminated from the maritime effect on the phenotype of the berries. In many cases the difference between the lowest and highest value in the content of one compound is greater than 10-fold.

The main terpene in all the berry samples was α-pinene (18–58%). Also, myrcene (7–23%) and γ-cadinene (5–13%) were in abundance (Figure 3; Table I). The total content of β-elemene, terpinen-4-ol, and caryophyllene, which did not separate completely in the GC runs, was on average 3% and that of sabinene over 1%. According to Formacek and Kubeczka (1982), the main components of a commercial juniper oil were α-pinene (70.8%), β-pinene (13.7%), myrcene (2.7%), and limonene (2.6%). The major compounds of the Hungarian juniper berries were shown to be α-pinene (35%), terpinen-4-ol (9.5%), and myrcene (9.5%). The content of γ-cardinene was 2.9% (Analytical Methods Committee, 1984). Taskinen and Nykänen showed (1976) that the pentane extract from Italian juniper berries contained 46% α-pinene, 18% myrcene and α-terpinene, and 8.6% sabinene. The share of γ-cadinene was shown to be only 0.79%.

Table II. Significant Differences in the Contents of the Terpenes Listed in Table I Resulting from the Locality (J = Jurmo, B = Berghamn, R = Raisio), Growth Form (pr = Prostrate, a = Ascending, py = Pyramidal), and Age of Berries (III = Ripe, Third Year, II = Unripe, Second Year)

variable	compound	signif level, %	mean of contents,[a] mg/100 g fw
locality	β-elemene, caryophyllene, terpinen-4-ol	5	J = 28, B = 15, R = 9.0
	humulene	5	J = 15, B = 8.0, R = 5.4
growth form	α-pinene	5	pr = 280, a = 190, py = 160
age	germacrene D, β-selinene	0.1	III = 3.3, II = 1.3

[a] Yields of the 3-h isolation procedure.

Table III. Significant Differences in the Relative Shares of the Terpenes Listed in Table I Resulting from the Locality (J = Jurmo, B = Berghamn, R = Raisio), Growth Form (pr = Prostrate, a = Ascending, py = Pyramidal), and Age of Berries (III = Ripe, Third Year, II = Unripe, Second Year)

variable	compound	signif level, %	mean of shares, %
locality	α-terpinolene	5	J = 0.84, B = 0.68, R = 0.53
	β-elemene, caryophyllene, terpinen-4-ol	1	J = 4.7, B = 2.9, R = 1.9
	humulene	1	J = 2.4, B = 1.6, R = 1.1
	unknown (21)	5	R = 0.92, B = 0.61, J = 0.50
growth form	γ-cadinene	5	a = 10, pr = 9.5, py = 6.8
	δ-cadinene, γ-muurolene	5	a = 6.1, pr = 3.6, py = 3.5
	α-muurolene	1	py = 3.2, pr = 1.7, a = 0.77
	unknown (21)	1	a = 0.88, pr = 0.58, py = 0.45
	α-cadinol	0.1	a = 1.9, py = 0.98, pr = 0.94
age	γ-cadinene	5	III = 10, II = 8.1

The effects of the studied biological and environmental factors on the volatile compounds were tested with analysis of variance (Tables II and III). The contents and relative shares of the total content of β-elemene, caryophyllene and terpinen-4-ol, and humulene decreased gradually from the furthest maritime growth site, Jurmo, toward the coast. The relative share of α-terpinolene also displayed the same trend, whereas that of the unknown compound 21 increased toward the coast.

The content of α-pinene was highest in the low bushes (prostate) and lowest in the pyramidal junipers (Table II). The percentage shares of the sesquiterpene hydrocarbons γ-cadinene, δ-cadinene and/or γ-muurolene, and compound 21 decreased following the ecotype of the order ascending–prostrate–pyramidal. α-Muurolene on the other hand was fairly abundant in the berries of the pyramidal form. α-Cadinol decreased in the order ascending–pyramidal–prostrate (Table III).

Surprisingly, only the content of the mixture peak of germacrene D and β-selinene and the share of γ-cadinene were statistically higher in the ripe blue berries than in the green berries. However, the blue berries of each individual juniper studied contained more β-elemene/caryophyllene/terpinen-4-ol, humulene, germacrene D/β-selinene, and α-muurolene than the green berries from the same bush. The share of α-pinene, again, was in all but one case higher in the unripe than in ripe berries. Hörster investigated (Hörster et al., 1975; Hörster, 1974) the unripe and ripe juniper berries and found no statistically signif-

icant differences in the contents of α-pinene, β-pinene, sabinene, myrsene, and 1,4-cineol.

The results display that the prostrate juniper bushes in particular, existing typically on the rocky islands in the Finnish archipelago, give good-quality berries with appropriate content of volatiles (International Standard, 1984).

Registry No. α-Pinene, 80-56-8; β-pinene, 127-91-3; sabinene, 3387-41-5; 3-carene, 74806-04-5; myrcene, 123-35-3; limonene, 138-86-3; γ-terpinene, 99-85-4; α-terpinolene, 586-62-9; β-elemene, 33880-83-0; caryophyllene, 87-44-5; terpinen-4-ol, 562-74-3; humulene, 6753-98-6; germacrene D, 23986-74-5; β-selinene, 17066-67-0; γ-cadinene, 39029-41-9; δ-cadinene, 483-76-1; γ-munrolene, 30021-74-0; α-munrolene, 10208-80-7; α-cadinol, 481-34-5.

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Volatile Compounds Formed from Thermal Interaction of 2,4-Decadienal with Cysteine and Glutathione

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Mixtures of 2,4-decadienal with either cysteine or glutathione were reacted in a closed sample cylinder in an aqueous medium. Each solution was adjusted to pH 7.5 and heated for 1 h at 180 °C, a representative frying temperature. The volatiles produced by degradations and interactions were isolated by simultaneous solvent–steam distillation and analyzed by gas chromatography and coupled gas chromatography–mass spectrometry. A total of 45 compounds was identified from the thermal interaction of 2,4-decadienal and cysteine with 2,4,6-trimethylperhydro-1,3,5-dithiazine as the major component. A total of 42 volatiles was determined from the thermal interaction of 2,4-decadienal and glutathione, and 2-pentylpyridine was the major component. Most of the identified products can be accounted for by well-known chemical pathways.

Volatiles generated from lipid–protein interaction in deep-fat fried foods have been reviewed by Ho et al. (1987). Due to the complexity of proteins and lipids found in foods, model systems were used for the study of these interactions. Amino acids, such as cysteine, valine, and lysine, were used in model systems by a few investigators (Lien and Nawar, 1974; Sims and Fioriti, 1975; Breitbart and Nawar, 1981; Henderson and Nawar, 1981). N-Substituted amides and nitriles were reported as the major products when amino acids were heated with short-chain triglycerides, while 2-pentylpyridine became the major product when valine was heated with linoleic acid and its esters.

In the present study, the thermal interaction between either 2,4-decadienal and cysteine or 2,4-decadienal and glutathione was examined. In an earlier publication, we reported the decomposition of cysteine and that of glutathione when heated separately under the same conditions (Zhang et al., 1988). 2,4-Decadienal was chosen because it is the major degradation product of linoleic acid, which is the main component of vegetable oils such as soybean oil and corn oil (Snyder et al., 1985; Patton et al., 1959). Both cysteine and glutathione are sulfur-containing components found in natural food materials. They were selected as models to simplify the lipid–protein interactions and to study the flavor component formations in deep-fat fried foods.

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EXPERIMENTAL SECTION

Sample Preparation. A total of 600 mg (0.005 mol) of cysteine (98% free base, crystalline; Sigma Chemical Co., St. Louis, MO) or 1500 mg (0.005 mol) of glutathione (98–100%, reduced form, crystalline; Sigma) was dissolved in 100 mL of distilled water, the solution was adjusted to pH 7.5 with 1 N sodium hydroxide or 1 N hydrochloric acid, and 200 mg of 2,4-decadienal (0.001 mol) (reagent grade; Aldrich Chemical Co., Milwaukee, WI) was added. The mixture was transferred into a 0.3-L Hoke SS-DOT sample cylinder and sealed, followed by heating the cylinder at 180 °C in an oil bath for 1 h. The heated mixture of 2,4-decadienal and cysteine possessed a fresh onionlike sulfur note and a bloody and burnt beef aroma. The other heated mixture possessed a garliclike sulfur note. Next, the reaction mass was simultaneously solvent steam-distilled with use of diethyl ether in a Nickerson-Likens

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