

Olfactory and Quantitative Analysis of Aroma Compounds in Elder Flower (*Sambucus nigra* L.) Drink Processed from Five Cultivars

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Fresh elder flowers (*Sambucus nigra* L.) were extracted with an aqueous solution containing sucrose, peeled lemon slices, tartaric acid, and sodium benzoate to make elder flower syrup. Aroma compounds emitted from the elder flower syrup were collected by the dynamic headspace technique and analyzed by GC–FID and GC–MS. A total of 59 compounds were identified, 18 of which have not previously been detected in elder flower products. The concentrations of the identified volatiles were measured in five elder cultivars, Allesoe, Donau, Sambu, Sampo, and Samyl, and significant differences were detected among cultivars in the concentration levels of 48 compounds. The odor of the volatiles was evaluated by the GC-sniffing technique. *cis*-Rose oxide, nerol oxide, hotrienol, and nonanal contributed to the characteristic elder flower odor, whereas linalool, α -terpineol, 4-methyl-3-penten-2-one, and (*Z*)- β -ocimene contributed with floral notes. Fruity odors were associated with pentanal, heptanal, and β -damascenone. Fresh and grassy odors were primarily correlated with hexanal, hexanol, and (*Z*)-3-hexenol.

Keywords: *Sambucus nigra*; *Caprifoliaceae*; volatiles; aroma compounds; dynamic headspace; olfactometry; odor classification

INTRODUCTION

Elder flowers (*Sambucus nigra* L.) have a strong flowery and pleasant odor that may have a fetid note, depending mainly on the source of the plant material (Eberhardt and Pfannhauser, 1985a; Joulain, 1987). The odors of flower extracts are often closer to the native odor than that of the essential oil made from elder flowers (Toulemonde and Richard, 1983). Unfortunately, the characteristic odor of the flowers may be due to volatile compounds that seldom occur in flower extracts, because of losses of volatile aroma compounds during home making, industrial processing or the sampling method used for quantification of the volatiles (Toulemonde and Richard, 1983; Joulain, 1987).

The elder flower drink, which has a flowery, honey-like, and sweet taste (Gotfried, 1993) is well-known in many countries (Eberhardt and Pfannhauser, 1985a; Jacksic and Daffertshofer, 1985; Treptow, 1985; Prendergast and Dennis, 1997; Viberg and Sjöholm, 1997), and recipes are available from the literature (Schumacher, 1983; Gotfried, 1993). Minor quantities are made by industries in England, Sweden, and Denmark. The market in the U.K. in 1995 for elder flower drinks was 15 million liters to a value of 9.5 million English pounds (Prendergast and Dennis, 1997).

Collection of aroma compounds from elder flower extracts has been made by steam distillation (Richter and Willühn, 1974; Toulemonde and Richard, 1983; Eberhardt and Pfannhauser, 1985a,b), supercritical extraction (Pfannhauser, 1986), maceration and simultaneous fermentation (Farkas et al., 1995), pentane extraction (Bayonove, 1973; Toulemonde and Richard,

1983), or the enfleurage process technique (Joulain, 1987). To the best of our knowledge, the use of dynamic headspace sampling of aroma compounds emitted from extracts of elder flowers has not been published.

Separation and identification of volatile elder flower compounds has been carried out by capillary gas chromatography combined with mass spectrometry (GC–MS) and infrared spectrophotometry (Velišek et al., 1981; Toulemonde and Richard, 1983; Eberhardt and Pfannhauser, 1985a,b; Pfannhauser 1986; Joulain, 1987; Farkas et al., 1995). More than 100 volatile compounds have been identified by different authors, but they only agree on a few. The reason for this variation could be that different wild bushes grown under different environmental conditions have been analyzed by different extraction and concentration techniques (Velišek et al., 1981; Toulemonde and Richard, 1983; Míková et al., 1984; Eberhardt and Pfannhauser, 1985a,b; Pfannhauser, 1986).

The floral green and floral odor of elder flowers have been shown to be related to the occurrence of hotrienol, rose oxides, nerol oxide, and linalool (Velišek et al., 1981; Toulemonde and Richard, 1983; Pfannhauser, 1986; Farkas et al., 1995), while the fresh green odor appears to be associated with hexanol, (*Z*)-3-hexenol, and hexanal (Poll and Lewis, 1986; Farkas et al., 1995). Fruity notes are related to esters of lower carboxylic acids and lower alcohols (Velišek et al., 1981; Askar and Treptow, 1985; Pfannhauser, 1986; Farkas et al., 1995). Relatively long chain alkanes (C_{14} – C_{31}) and fatty acid esters (C_{16} – C_{18}) may contribute to a waxy or fatty odor (Richter and Willühn, 1974; Toulemonde and Richard, 1983). The content of lower alcohols that give rise to fruity notes may be too low in elder flower to have any influence on the odor. The characteristic elder flower odor is associated with rose oxides, nerol oxide, hot-

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rienol, and linalool (Velíšek et al., 1981; Davídek et al., 1982; Toulemonde and Richard, 1983; Eberhardt and Pfannhauser, 1985a,b; Poll and Lewis, 1986; Farkas et al., 1995).

The development of more efficient dynamic headspace sampling techniques in recent years makes it possible to obtain more precise sampling of volatiles from juices and extracts. Combined with more sensitive and efficient equipment for identification and quantification of volatiles, the possibilities for optimization of raw materials for industrial processing of elder flower drinks are increased.

The aim of this project was to separate, identify, and quantify the contents of volatiles in elder flower drinks to be able to describe the quantitative and qualitative differences between the drinks processed from five elder cultivars, and to evaluate the significant contributors to the aroma profile of the drinks by the GC-sniffing technique.

MATERIALS AND METHODS

Plant Material. Florets of five elder cultivars, Allesoe, Donau, Sampo, Sambu, and Samyl, in full bloom were sampled from bushes on dry and sunny days in June 1998 at the experimental field at the Danish Institute of Agricultural Sciences, Research Centre Aarslev. Florets were picked from several different trees for every cultivar, at the bottom, middle, and top. The collected florets were then mixed within each cultivar to get randomized samples. The florets were cut where the major stalk splits.

Elder Flower Drink. Within a few hours after picking, 2 L of elder flower syrup was made from 300 g of florets, 255 g of peeled lemon slices, 1.5 kg of sucrose, 60 g of tartaric acid, and 2 mL of sodium benzoate solution (0.25 mg/mL). Florets, sugar, and lemon were placed in a 5 L stainless steel container and poured over with 1 L of boiling water, and then tartaric acid and preserving agent were added. The pH of the drink was 2.1. The mixture was stirred, and stored for extraction at 18 °C for 5 days with stirring twice a day. On the fifth day, the mixture was filtered through cheesecloth and 150 mL samples were tapped into 250 mL glass bottles, frozen, and stored at -22 °C until analysis. By use of all the ingredients except the elder flowers, a control mixture was made and stored by the same procedure as for the elder flower syrup. The drink was obtained by dilution of the elder flower syrup four times with tap water, but the analyses were carried out on the nondiluted syrups.

Dynamic Headspace Sampling (Purge and Trap). For collection of headspace volatiles, 150 mL of frozen elder flower drink was thawed for 2 h at 25 °C in a thermostated cabinet (Termaks series, Lytzen Lab, Denmark). All glass equipment was washed in an automatic cleaning machine (G 7783 CD Mielabor, Miele, Gütersloh, Germany) at 85 °C followed by rinsing twice with tap water followed by distilled water, thermostated at 25 °C, and purged with nitrogen for 1 h before sampling. The nitrogen was prefiltered through activated charcoal to remove contaminants and supplied through Teflon tubings (3.2 mm o.d., Microlab, Aarhus, Denmark), fitted with a one-way variable connector (1–11 mm tubing, 1.5 mm bore, Omnifit, Cambridge, England). Samples of 150 mL of thawed elder flower syrup were transferred to 300 mL conical bottles. The bottles were equipped with a magnetic bar, Teflon sleeves, and a gas washing bottle glass insert (NS 29/32, Kebo Lab, Denmark), allowing the gas to enter the liquid. The inserts were kept in place by metal clasps. The samples were placed in the thermostated cupboard at 25 °C on a magnetic stirrer and connected to the adsorbent traps. The traps were filled with 200 mg Porapak Q 50–80 mesh (Waters Inc., Milford, MA) inserted between two desiloxanized glass wool plugs in glass tubes (4 × 150 mm). No breakthrough of volatiles was detected by the used trap size. After the traps were mounted to the glass insert by a two-way variable connector (4–11 mm

tubing, Omnifit, Cambridge, England), the samples were purged for 1 h with nitrogen (150 mL/min) under constant stirring, for the sampling of headspace volatiles. For quantitative estimations, 10 μ L of (-)-*trans*-pinocarveol (Fluka Chemie AG, Switzerland, 1469 ng/ μ L) in double-distilled methylene chloride (HPLC grade) was added prior to the headspace collection of the volatiles.

The Porapak traps were eluted with 2 mL of methylene chloride into 15 mL glass tubes with a scale (Silber Brand, Germany). Prior to vaporization of excess solvent 100 μ L of methyl-3-butenolate (Aldrich, Germany, 5.64 ng/ μ L) in methylene chloride was added to ensure that every sample was concentrated equally. By use of a gentle flow of nitrogen (50 mL/min) the samples were evaporated to a volume of 200 μ L, transferred into 300 μ L glass microvials, and preconcentrated at ambient temperature (approximately 20 °C) to a final volume of 50 μ L. After use the Porapak columns were regenerated with 5 mL of methylene chloride. The last 2 mL of the rinsing eluate was concentrated to 50 μ L and checked for impurities.

Analysis of Volatiles. A Hewlett-Packard 5890 Series II Plus gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector (FID) was used. Volatiles were separated on a Chrompack (Middletown, The Netherlands) WCOT fused silica capillary column (50 m × 0.25 mm i.d., DF = 0.2 μ m liquid phase, CP-WAX 52CB). Helium was applied as a carrier gas with a flow rate of 1.40 mL/min and 155 kPa column head pressure. One microliter of each sample was injected onto the column in splitless mode, with a splitless purge time of 60 s. The oven temperature was kept at 33 °C for 1 min, programmed to 130 °C at 2 °C/min and further to 220 °C at 10 °C/min, followed by constant temperature for 10 min. The injector temperature was 200 °C, and the FID detector temperature was 230 °C. Yields of individual aroma compounds in the Porapak eluates were estimated from the FID peak areas, and the internal standard (-)-*trans*-pinocarveol was used. The response factor was set to 1 for all compounds.

GC retention indices (relative retention index, RI) were determined externally with a series of *n*-alkanes (C₁₀–C₂₅) (van den Dool and Kratz, 1963). The GC conditions were the same as described above with the exception of the oven temperature, which was linearly programmed: 30 °C (1.3 min, isothermal) to 220 °C at 2 °C/min.

A Varian Saturn 2000 ion trap mass spectrometer operated at an ionization potential of 70 eV and interfaced to a Varian Star 3400 CX gas chromatograph (Varian chromatography systems, California) equipped with a CP-WAX 52CB column was used for the GC-MS analysis of volatiles. The GC conditions were the same as described above. Compounds were identified from the GC retention data and by comparing the mass spectra of the entrained compounds with those of authentic compounds (Table 1) unless otherwise noted. Authentic compounds were supplied by Aldrich (Germany), Fluka Chemie AG (Switzerland), and TCI Tokyo Organic Chemicals (Japan).

Olfactometry. The olfactory evaluation of single aroma compounds was performed by GC-sniffing on a Shimadzu 14A gas chromatograph (Shimadzu, Kyoto, Japan). The column was mounted directly onto the sniff insert to increase the concentration levels of volatiles guided to the sniff insert. An SGE OSS-2 splitter system with an air humidifier in the sniff insert was mounted onto the gas chromatograph to reduce extreme drying of the mucus membrane in the noses of the judges. Six judges noted descriptors induced by the compounds eluting from the GC column. The panel of judges was a mixed group of both sexes with an age ranging from approximately 20 to 60 years. Column and GC setup was as described above. The GC-sniffing sessions were performed once by each judge, and only odor descriptions which three or more judges agreed on were considered to be valid (Table 1).

Statistics. The results were analyzed by the use of one-way analysis of variance (ANOVA) including the test for homogeneity of variance, calculation of the coefficient of variation (CV), and least significant difference (LSD_{0.05}) at the

Table 1. Odor Description of Aroma Compounds Isolated from the Headspace of Elder Flower Drink

peak no.	compound ^a	suggested origin of compound ^c	odor description ^d (detected)	odor description ^e (not detected)	retention time (min)	RI _{CP-WAX 52 CB}
1	unknown		spicy, sour		8.17	< 1000
2	pentanal	FA	fruity, vanilla		8.55	< 1000
3	unknown (<i>m/z</i> 97, 55, 43, 67, 85)				8.77	1009
4	unknown (<i>m/z</i> 71, 43, 59, 53)		candy, fruity		9.52	1030
5	unknown (<i>m/z</i> 41, 72, 99, 59, 115)				10.07	1045
6	hexanal	FA	green grass		11.88	1090
7	unknown (<i>m/z</i> 54, 55, 41, 84)				12.19	1101
8	1-penten-3-one	FA		pungent, mustard	12.41	1105
9	linalool oxide (pyran) ^b	T		flowery	13.08	1115
10	unknown terpene (<i>m/z</i> 67, 137, 43, 93, 82)	T			13.42	1123
11	unknown terpene (<i>m/z</i> 91, 77, 119, 65, 105, 134)	T			13.84	1132
12	4-methyl-3-penten-2-one	FA	sweet, flowery, sickening		14.24	1139
13	butanol	FA	sweet, fusel oil		14.88	1152
14	α-phellandrene	T		sweet, flowery, citrus	15.75	1168
15	α-terpinene	T		lemon	16.07	1173
16	heptanal	FA	citrus, fruity		17.19	1194
17	limonene	T		citrus, orange, lemon	17.77	1204
18	1,8-cineole	T	peppermint, menthol		18.14	1211
19	unknown terpene (<i>m/z</i> 67, 96, 81, 109, 137, 55, 123, 152)	T			18.46	1214
20	3-methylbutanol	A		wine, whisky	18.60	1216
21	unknown terpene (<i>m/z</i> 95, 137, 79, 43, 77, 67, 152, 109)	T			19.11	1225
22	2-pentylfuran	FA		green bean, vegetable	19.99	1240
23	(<i>Z</i>)-β-ocimene	T	sweet, flowery		20.29	1245
24	γ-terpinene	T		lemon, lime	20.62	1250
25	(<i>E</i>)-β-ocimene	T		sweet, tropical	21.29	1261
26	<i>p</i> -cymene	T		fresh, citrus	22.13	1274
27	unknown terpene (<i>m/z</i> 91, 79, 77, 119, 105, 65, 51, 134)	T			22.63	1280
28	terpinolene	T		citrus, pine	22.90	1283
29	3-hydroxy-2-butanone	FA		buttery, creamy, fatty	23.02	1287
30	octanal	FA		citrus, fatty	23.56	1298
31	1-octen-3-one	FA	mushroom		24.38	1317
32	(<i>Z</i>)-3-hexenyl acetate	FA		green, fruity	25.47	1327
33	unknown (<i>m/z</i> 83, 55, 41, 69, 57, 97, 50)				25.71	1331
34	6-methyl-5-hepten-2-one	T		sweet, fruity, green	26.69	1347
35	<i>cis</i> -rose oxide	T	elder flower syrup		27.47	1359
36	hexanol	FA	fresh grass, fruity		27.77	1364
37	<i>trans</i> -rose oxide	T		flowery, elder flower	28.41	1373
38	(<i>E</i>)-3-hexenol	FA	green, fatty, spicy		28.92	1388
39	(<i>Z</i>)-3-hexenol	FA	fresh green grass		29.70	1393
40	nonanal	FA	faint elder flower		30.30	1403
41	(<i>E</i>)-2-hexenol	FA	green pepper		31.23	1417
42	(<i>E</i>)-2-octenal	FA		green grass	32.58	1438
43	<i>cis</i> -linalool oxide (furan)	T		elder flower, earthy, floral	33.24	1448
44	1-octen-3-ol	FA		mushroom, earthy	34.14	1463
45	heptanal	FA		green	34.41	1467
46	unknown (<i>m/z</i> 67, 110, 95, 81, 43)				35.03	1477
47	nerol oxide ^b	T	elder flower syrup, floral		35.16	1479
48	6-methyl-5-hepten-2-ol	T			35.52	1484
49	(<i>E,E</i>)-2,4-heptadienal	FA		fatty, green	36.67	1502
50	camphor	T		medicine	37.20	1510
51	unknown (<i>m/z</i> 179, 69, 95, 109, 123, 137, 152, 161, 194)				37.94	1523
52	benzaldehyde	S	candy, sweet		38.41	1531
53	unknown (<i>m/z</i> 55, 93, 67, 43, 81, 110, 125, 153)		green		39.34	1546
54	linalool	T	flowery, freesia, sweet		40.34	1561
55	octanol	FA	sharp, fatty, spicy		40.74	1567
56	dimethyl sulfoxide	A		mild garlic	41.69	1576
57	β-caryophyllene	T	woody, spicy, sweet		42.38	1600

Table 1 (Continued)

peak no.	compound ^a	suggested origin of compound ^c	odor description ^d (detected)	odor description ^e (not detected)	retention time (min)	RI _{CP-WAX 52 CB}
58	unknown (<i>m/z</i> 109, 79, 81, 43, 53)				42.60	1602
59	hydroxylinalool ^b	T			42.87	1604
60	terpinen-4-ol	T		woody, earthy, sweet	43.40	1609
61	hotrienol	T	faint flowery, elder flower		44.18	1623
62	safranal	T		herbal sweet	45.75	1648
63	unknown (<i>m/z</i> 55, 67, 83, 41, 98)				47.49	1677
64	<i>p</i> -methoxystyrene	S	sweet		48.04	1686
65	α -terpineol	T	faint, sweet, flowery		49.19	1707
66	1,1,6-trimethyl-1,2-dihydronaphthalene ^b	T	licorice		50.80	1737
67	unknown terpene (<i>m/z</i> 67, 94, 59, 79, 43, 109, 137, 155)	T			51.19	1748
68	methyl salicylate	S		minty, sweet	51.81	1764
69	citronellol	T		sweet, rose-like	52.41	1780
70	nerol	T		sweet, floral, citrus	53.48	1812
71	β -damascenone	T	elderberry, fruity		53.95	1827
72	geraniol	T		sweet, floral	54.81	1864
73	benzyl alcohol	S		floral, rose	55.48	1893
74	2-phenylethanol	S		floral, rose	56.13	1921
75	β -ionone	T		woody, balsamic, fruity	56.77	1949
76	eugenol	S		spicy, clove	60.82	>2100

^a Mass spectra and GC retention times were consistent with those of reference compounds unless otherwise noted. Mass spectra of unknown compounds are listed in parentheses with descending intensities of fragment ions. No mass spectrum could be obtained for peak no. 1 (unknown) as this compound coeluted with the solvent on the MS. ^b Tentatively identified. The mass spectra were consistent with published data (NIST, 1998). ^c A, amino acid derivative; FA, fatty acid breakdown product or derivative of these; S, shikimic acid derivative; T, terpene derivative or breakdown product of these. ^d Odor detected in GC-sniff analysis. ^e Odor information obtained from the literature, but not detected in the GC-sniff analysis (Poll and Lewis, 1986; Bauer et al., 1990; Curtis and Williams, 1994; Aldrich, 1996).

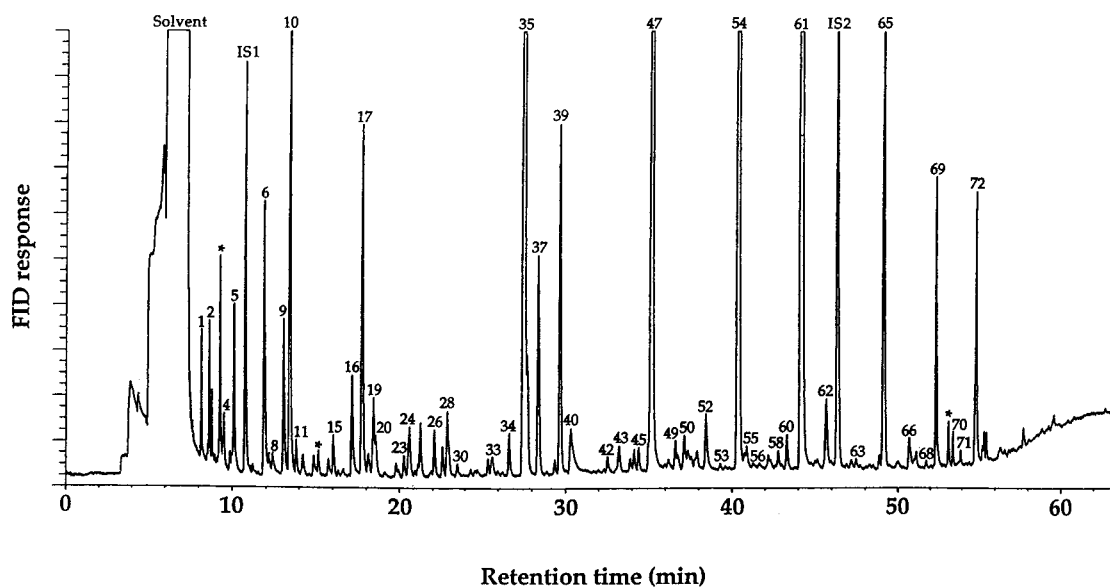


Figure 1. Gas chromatogram of volatiles collected from elder flower drink processed from the cultivar Sampo. Peak numbers refer to those in Tables 1 and 2. IS1 represents the internal standard methyl-3-butenolate. IS2 represents the internal standard (–)-*trans*-pinocarveol. Peaks labeled with an asterisk refer to impurities.

95% significance level in a two-way test according to Zar (1996). All headspace sampling experiments were carried out in quadruplicate.

RESULTS AND DISCUSSION

Identification of Aroma Compounds. A total of 76 compounds were detected repeatedly in the headspace of elder flower extracts (Figure 1). Fifty-nine of these were identified by comparison of their mass spectral data with those from authentic compounds and/

or mass spectra suggested by the NIST database (NIST, 1998) and GC retention indices (Table 1).

The majority of the constituents were of terpenoid origin and included monoterpenes, irregular terpenes, and one sesquiterpene, β -caryophyllene (Table 1). Most of the monoterpenes detected are common constituents of the essential oil and headspace of flowers (Knudsen et al., 1993; Lawrence and Shu, 1993), although α -phellandrene, α -terpinene, γ -terpinene, terpinolene, and safranal have not previously been reported among elder

flower volatiles. Terpenoid alcohols and oxides, such as linalool oxide (pyran), *cis*- and *trans*-rose oxide, *cis*-linalool oxide (furan), nerol oxide, linalool, hydroxylinalool, terpinen-4-ol, hotrienol, and α -terpineol, have all previously been detected in both elderberry fruits and flowers (Velíšek et al., 1981; Davídek et al., 1982; Toulemonde and Richard, 1983; Eberhardt and Pfannhauser, 1985a,b; Pfannhauser, 1986; Poll and Lewis, 1986). Some of these compounds may, however, be artifacts produced during processing of the elder flower drink by acid hydrolysis of monoterpene diols and/or triols, derived from epoxides or glycosides (Williams et al., 1980; Eberhardt and Pfannhauser, 1985b; Wintoch et al., 1993; Vasserot et al., 1995). The irregular terpenes are constituted of β -damascenone, β -ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene (1,1,6-TDN), 6-methyl-5-hepten-2-one, and 6-methyl-5-hepten-2-ol. The former has previously been identified as a contributor to elderberry odor (Poll and Lewis, 1986), whereas this is the first report of 6-methyl-5-hepten-2-ol, 6-methyl-5-hepten-2-one, and 1,1,6-TDN in elder flower products. β -Damascenone and 1,1,6-TDN are also known from wine and are mainly produced during aging (Rapp, 1992). In wines 1,1,6-TDN is regarded as an off flavor contributing to bottle-aged character. The irregular terpenes from elder flower are likely derivatives of carotenoids (Buttery, 1981; Rapp, 1992).

Another major group of the constituents arise from autoxidation of fatty acids and are constituted primarily of aldehydes, ketones, and alcohols, of which 1-penten-3-one, butanol, 1-octen-3-one, 3-hydroxy-2-butanone, (*E*)-2-octenal, (*E*)-3-hexenol, (*E*)-2-hexenol, 1-octen-3-ol and (*E,E*)-2,4-heptadienal have not previously been reported from elder flowers, although some of these compounds have been detected in elderberry (Askar and Treptow, 1985; Poll and Lewis, 1986). Among esters derived from fatty acids, only (*Z*)-3-hexenyl acetate was identified (Table 1).

Only a few benzenoids and phenylpropanoids derived from shikimate were found in quantifiable amounts and included benzaldehyde, *p*-methoxystyrene, and methyl salicylate. The major part of benzaldehyde probably arises by decomposition of cyanogenic glycosides. Eugenol, benzyl alcohol, and 2-phenylethanol were also detected but only found in traces (Table 2). Benzyl alcohol and 2-phenylethanol have earlier been described as contributors to the odor of elderberry (Miková et al., 1984; Askar and Treptov, 1985; Poll and Lewis, 1986).

One sulfur-containing compound, dimethyl sulfoxide (DMSO), was detected, and either is formed during extraction by degradation of amino acids, or is an impurity that originates from the added ingredients (Table 2). It has not previously been reported in elder flowers, but is a well-known character impact compound in cabbage and onions (Freemann and Whenham, 1975).

Aroma Composition of Cultivars. A total of 72 compounds were quantified (Table 2). Significant differences among cultivars were observed for all compounds, except for limonene, 1,8-cineole, γ -terpinene, (*E*)-2-octenal, DMSO, β -caryophyllene, and four unidentified compounds. The coefficient of variation (CV) varied from 12.7% to 19.3% for 13 compounds, from 20.0% to 29.7% for 42 compounds, from 30.2% to 39.8% for 13 compounds, and finally above 40% for four compounds (Table 2). For some of the minor aroma compounds it was not possible to make a precise quantification as they occurred in very low amounts and

were not fully resolved in the gas chromatogram (Table 2, Figure 1). This could explain the differences between the investigated cultivars with regard to the content of some of the minor volatiles. From measurement of the volatile content in the control samples that were processed without application of elder flowers, it appears from Table 2 that the lemon slices contribute to considerable quantities of hexanal, 2-pentylfuran, *p*-cymene, (*E*)-2-octenol, and safranal. Almost the whole content of limonene arose from the lemon slices.

Terpenoid alcohols and oxides constituted the major part of the volatiles emitted from elder flower drink. Hotrienol was the largest contributor comprising on average 18.4% of the trapped volatile mass, followed by linalool (15.0%), *cis*-rose oxide (11.4%), nerol oxide (5.1%), *cis*-linalool oxide (furan) (4.5%), *trans*-rose oxide (2.5%), citronellol (1.8%), and α -terpineol (1.7%). The largest concentration differences among cultivars were observed for *cis*-linalool oxide (furan) ranging from 6.3 ng/mL drink in Sampo to 210.8 ng/mL in Donau and for *cis*-rose oxide ranging from 84.8 ng/mL drink in Donau to 439.9 ng/mL in Allesoe (Table 2). (*E*)- β -Ocimene was one of the major compounds among the nonoxidized terpenes comprising on average 0.6% of the trapped volatile mass (Table 2).

In the group of compounds arising from autoxidation of fatty acids, (*Z*)-3-hexenol was the largest contributor comprising on average 8.9% of the trapped volatiles, followed by hexanal (2.5%), hexanol (2.2%), and heptanal (1.8%) (Table 2). These compounds are frequent constituents of the essential oil and headspace of flowers and leaves (Knudsen et al., 1993; Lawrence and Shu, 1993). (*Z*)-3-Hexenol is, for example, emitted from most green plant tissues and is known as "leaf alcohol". The largest concentration differences among cultivars were observed for (*Z*)-3-hexenol and hexanol ranging from 61.3 and 20.5 ng/mL, respectively, in Sampo to 408.8 and 130.1 ng/mL, respectively, in Donau (Table 2).

Benzaldehyde was the most abundant aromatic compound in the headspace comprising on average 1.6% of the headspace volatiles. The remaining aromatic compounds were only present in minute amounts (Table 2).

Classification of Aroma Compounds. The identified aroma compounds of the elder flower drink were classified into 10 of 16 odor classes defined by Curtis and Williams (1994) and furthermore classified with respect to the measured concentrations in the drinks (Table 3). The terpenoid oxides *cis*-linalool oxide (furan), nerol oxide, and *cis*- and *trans*-rose oxide, hotrienol, and nonanal were placed in the elder flower aroma group, whereas terpene alcohols such as linalool, citronellol, α -terpineol, nerol, and geraniol were placed in the group with flowery notes together with other monoterpenes, 4-methyl-3-penten-2-one, benzyl alcohol, and 2-phenylethanol, in accordance with literature odor descriptions (Velíšek et al., 1981; Toulemonde and Richard, 1983; Eberhardt and Pfannhauser, 1985a,b; Pfannhauser, 1986; Poll and Lewis, 1986; Bauer et al., 1990; Mosandl, 1992; Curtis and Williams, 1994; Aldrich, 1996). β -Damascenone with the typical aroma of elderberries was placed in the group with fruity odor together with pentanal, heptanal, octanal, (*Z*)-3-hexenyl acetate, 6-methyl-5-hepten-2-one, and six nonoxidized monoterpenes (Tables 1 and 3). The grassy group is composed of well-known aliphatic aldehydes and alcohols with typical odors of green grass. 1-Octen-3-ol, 3-hydroxy-2-butanone, 1-octen-3-one, 2-pentylfuran, and DMSO

Table 2. Quantitative Determinations of Aroma Compounds Isolated from the Headspace of Elder Flower Drink Processed from Five Cultivars^a

peak no.	compound	concentration, ng/mL					significance ^b	CV (%)	LSD ^c (ng/mL)	control ^d concn (ng/mL)
		Allesoe	Donau	Sambu	Sampo	Samyl				
1	unknown	16.7	11.9	15.9	11.3	17.8	*	19.0	6.2	2.6
2	pentanal	32.3	22.7	102.3	9.0	12.0	***	26.7	7.5	0.1
3	unknown	5.5	10.0	7.0	6.8	4.7	ns	42.0	10.8	1.9
4	unknown	11.8	20.1	11.7	10.3	12.7	***	17.1	5.4	1.5
5	unknown	17.3	22.7	20.7	24.0	17.4	ns	16.9	6.9	8.1
6	hexanal	84.9	46.1	65.4	40.0	81.1	**	24.7	34.0	16.2 ^e
7	unknown	4.2	4.4	3.5	4.7	4.6	ns	18.8	1.6	1.1
8	1-penten-3-one	5.2	3.9	14.4	5.1	4.4	***	29.5	3.8	nd ^f
9	linalool oxide (pyran)	3.7	9.2	9.1	18.1	6.6	***	33.4	6.5	0.3
10	unknown terpene (pyran)	10.3	24.5	16.4	59.9	9.6	***	35.0	19.1	0.2
11	unknown terpene	1.4	2.6	1.7	5.2	2.1	***	26.8	1.4	0.7
12	4-methyl-3-penten-2-one	4.4	3.2	4.4	4.8	3.0	*	23.6	1.7	0.3
13	butanol	8.4	21.0	5.3	3.9	4.9	***	17.0	4.5	0.8
14	α -phellandrene	8.1	7.4	6.1	4.0	9.7	***	17.4	2.7	0.5
15	α -terpinene	5.9	4.3	8.8	7.6	5.0	***	19.3	2.5	0.4
16	heptanal	80.7	28.3	46.1	15.5	58.6	***	26.6	27.3	1.1
17	limonene	30.8	35.4	31.7	45.0	33.2	ns	32.3	23.3	33.2 ^e
18	1,8-cineole	4.0	2.9	4.1	4.8	4.2	ns	22.8	1.9	0.7
19	unknown terpene	16.5	138.7	24.1	14.6	20.1	***	13.0	39.9	3.1
20	3-methylbutanol	6.4	nd	7.0	6.7	2.3	**	68.1	4.8	0.4
21	unknown terpene	9.6	8.5	11.7	1.2	12.1	***	22.6	4.3	0.5
22	2-pentylfuran	1.6	0.7	1.2	1.2	1.2	**	25.0	0.6	0.5 ^e
23	(<i>Z</i>)- β -ocimene	2.5	2.3	4.9	3.5	3.6	**	23.6	1.6	0.2
24	γ -terpinene	4.4	7.3	5.3	8.8	5.8	ns	30.2	4.4	2.2
25	(<i>E</i>)- β -ocimene	8.0	8.0	28.3	8.4	25.1	***	31.9	12.9	0.2
26	<i>p</i> -cymene	3.6	3.2	3.7	7.3	4.3	**	29.5	2.5	15.6 ^{e,g}
27	unknown terpene	2.6	3.2	3.2	5.8	2.1	***	17.2	1.3	0.2
28	terpinolene	1.9	4.5	5.4	7.1	3.3	*	39.8	3.8	0.5
29	3-hydroxy-2-butanone	1.1	6.4	2.1	5.4	2.4	***	30.2	2.5	0.5
30	octanal	6.0	2.4	9.4	2.5	8.1	**	28.7	4.4	1.5
31	1-octen-3-one	2.0	0.9	1.7	1.1	1.8	*	29.7	1.1	0.1
32	(<i>Z</i>)-3-hexenyl acetate	4.6	4.0	11.6	2.2	33.6	***	25.5	11.3	0.1
33	unknown	6.9	7.9	19.7	5.3	8.3	***	18.7	3.9	1.4
34	6-methyl-5-hepten-2-one	9.3	6.6	17.6	6.7	22.9	***	21.2	6.9	0.6
35	<i>cis</i> -rose oxide	439.9	84.8	355.4	290.9	263.6	***	26.9	145.5	0.7
36	hexanol	61.4	130.1	45.0	20.5	32.8	***	18.0	33.2	0.5
37	<i>trans</i> -rose oxide	112.5	23.3	82.3	30.4	66.1	***	24.0	30.1	0.2
38	(<i>E</i>)-3-hexenol	4.0	7.8	3.4	0.7	2.8	***	28.4	2.7	nd
39	(<i>Z</i>)-3-hexenol	213.0	408.8	207.5	61.3	257.4	***	19.3	118.7	0.4
40	nonanal	25.3	17.1	33.2	13.1	31.7	**	30.7	14.7	4.2
41	(<i>E</i>)-2-hexenol	4.5	12.7	9.4	0.7	2.3	***	27.1	4.0	0.2
42	(<i>E</i>)-2-octenol	5.1	3.2	5.0	3.3	5.2	ns	25.0	2.4	2.3 ^e
43	<i>cis</i> -linalool oxide (furan)	124.5	210.8	89.0	6.3	130.7	***	21.9	6.8	1.5
44	1-octen-3-ol	9.6	11.6	7.2	3.7	14.6	***	20.3	4.3	0.6
45	heptanol	8.6	8.0	5.4	4.0	8.5	**	22.1	3.3	0.5
46	unknown terpene (pyran)	3.3	8.1	5.8	3.4	4.2	***	22.0	2.5	0.2
47	nerol oxide	63.3	124.7	108.4	288.7	58.8	***	25.3	72.3	1.6
48	6-methyl-5-hepten-2-ol	6.6	4.1	3.3	nq	3.7	***	34.2	2.7	0.1
49	(<i>E,E</i>)-2,4-heptadienal	3.6	17.5	5.1	5.6	3.1	**	26.3	9.0	2.0
50	camphor	4.1	5.9	5.4	7.0	3.2	*	30.2	3.0	1.3
51	unknown	9.3	11.2	3.9	3.7	13.5	***	27.1	5.2	nd
52	benzaldehyde	31.5	93.3	12.0	13.5	49.4	***	21.3	24.5	1.1
53	unknown terpene	1.4	1.6	1.5	1.6	1.8	ns	25.7	1.0	0.8
54	linalool	305.5	272.0	624.5	383.8	343.0	***	21.0	160.2	3.3
55	octanol	2.6	2.4	2.6	3.1	2.3	*	12.7	0.6	1.5
56	dimethyl sulfoxide	nd	0.9	0.7	1.5	0.9	ns	78.8	1.4	0.2
57	β -caryophyllene	0.7	2.1	1.0	3.0	1.5	ns	30.7	1.3	1.1
58	unknown	4.6	2.3	0.7	0.7	0.6	***	33.0	1.4	nd
59	hydroxylinalool	2.3	2.6	2.6	3.3	1.5	***	21.7	1.1	0.4
60	terpinen-4-ol	2.0	3.4	2.7	6.6	2.9	**	20.0	1.6	2.5
61	hotrienol	358.9	547.1	452.0	747.9	260.9	***	21.3	215.7	1.2
62	safranal	9.1	12.2	7.1	14.9	8.7	***	21.3	4.6	7.8 ^e
63	unknown	4.6	0.8	3.8	1.7	3.7	***	36.6	1.7	nd
64	<i>p</i> -methoxystyrene	5.1	0.4	7.9	0.5	11.4	***	27.1	4.9	nd
65	α -terpineol	17.8	38.6	48.3	87.6	29.9	***	22.1	20.5	6.9
66	1,1,6-trimethyl-1,2-dihydronaphthalene	15.0	1.5	4.2	7.2	9.6	**	47.0	8.2	1.1
67	unknown terpene (pyran)	99.7	197.5	61.8	2.7	86.6	***	22.5	64.3	0.6
68	methyl salicylate	3.3	1.7	1.2	1.1	2.1	***	20.8	1.7	0.5
69	citronellol	62.2	6.7	59.7	35.2	56.3	***	29.1	27.0	0.8
70	nerol	5.2	3.1	13.5	3.9	7.3	***	25.6	3.5	0.4
71	β -damascenone	3.2	6.6	0.9	2.0	3.9	***	24.2	1.9	0.4
72	geraniol	9.8	4.6	20.3	6.9	8.4	***	23.6	5.9	0.3
73	benzyl alcohol	nq ^h	nq	nd	nq	nq				nd
74	2-phenylethanol	nq	nd	nd	nq	nq				nd
75	β -ionone	nq	nq	nq	nq	nq				nd
76	eugenol	nq	nd	nq	nd	nd				nd
total concentration (ng/mL) of volatiles		2465	2779	2834	2441	2241				142

^a Concentrations of aroma compounds determined relative to the internal standard (*-trans*-pinocarveol). ^b ns, nonsignificant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. ^c LSD, least significant difference at $P < 0.05$. ^d Extract without elder flower, but with slices of lemon, sugar, and preserving agent. ^e Arose from lemon. ^f nd, not detected. ^g Impurity. ^h nq, not quantified, present in trace amounts only.

Table 3. Elder Flower Aroma Compounds Grouped in 10 Odor Classes According to Odor and Concentration^a

odor classes	concentration (ng/mL)	compounds
flowery		
elder flower	> 100	<i>cis</i> -linalool oxide (furan), <i>cis</i> - and <i>trans</i> -rose oxide, nerol oxide, hotrienol
	30–99	nonanal
flowers only	> 100	linalool
	30–99	α -terpineol, citronellol
	10–29	linalool oxide (pyran), nerol, geraniol
	5–9	α -phellandrene, 4-methyl-3-penten-2-one
	0–4	(<i>Z</i>)- β -ocimene
	trace	benzyl alcohol, 2-phenylethanol
fruity	30–99	limonene, (<i>Z</i>)-3-hexenyl acetate, heptanal
	10–29	pentanal, (<i>E</i>)- β -ocimene, 6-methyl-5-hepten-2-one
	5–9	α -terpinene, γ -terpinene, <i>p</i> -cymene, terpinolene, octanal, β -damascenone
grassy	> 100	hexanol, (<i>Z</i>)-3-hexenol
	30–90	hexanal
	10–29	(<i>E</i>)-2-hexenol, (<i>E,E</i>)-2,4-heptadienal
	5–9	(<i>E</i>)-3-hexenol, (<i>E</i>)-2-octenal, heptanol
	0–4	octanol
agrestic	10–29	1-octen-3-ol
	5–9	3-hydroxy-2-butanone
	0–4	2-pentylfuran, 1-octen-3-one, dimethyl sulfoxide
minty	5–9	1,8-cineole, methyl salicylate
medicated	5–9	camphor
spicy	trace	eugenol
herbaceous	10–29	safranal
woody	5–9	terpinen-4-ol
	0–4	β -caryophyllene
	trace	β -ionone
miscellaneous	30–90	benzaldehyde
	10–29	butanol, <i>p</i> -methoxystyrene, 1,1,6-trimethyl-1,2-dihydronaphthalene
	5–9	1-penten-3-one, 3-methylbutanol, 6-methyl-5-hepten-2-ol
	0–4	hydroxylinalool

^a The flowery group is split into two subgroups.

occurred in the agrestic group, whereas the woody group included terpinen-4-ol, β -caryophyllene, and β -ionone. The medicated, minty, spicy, and herbaceous groups contained only one or two compounds each, whereas eight compounds of different origins were placed in the miscellaneous group (Table 3).

GC-Sniff Evaluation of Aroma Compounds. The GC-sniff method was used to be able to determine which compounds and odor classes contributed significantly to the aroma of the elder flower drink, which generally has a typical odor of fresh and sweet elder flower as previously described (Eberhardt and Pfannhauser, 1985a,b; Jacksic and Daffertshofer, 1985; Treptow, 1985; Prendergast and Dennis, 1997; Viberg and Sjöholm, 1997).

According to the GC-sniff test, the characteristic elder flower odor was related to those of *cis*-rose oxide, nonanal, nerol oxide, and hotrienol of the elder flower subgroup with clear “elder flowery” tones, whereas *cis*-linalool oxide (furan) and *trans*-rose oxide from this subgroup did not contribute significantly to the elder flower aroma as perceived by GC-sniff (Table 1). According to earlier findings other flower odors in elder flower products are correlated with most of the compounds listed in the flowery subgroup with flowery notes only (Velišek et al., 1981; Toulemonde and Richard, 1983; Pfannhauser, 1986); however, the GC-sniff test indicated that only linalool, α -terpinenol, 4-methyl-3-penten-2-one, and (*Z*)- β -ocimene of this group contribute significantly with “flowery” notes. Other consistent GC-sniff descriptors of the flowery group were “sweet” notes for 4-methyl-3-penten-2-one, linalool, α -terpinenol, and (*Z*)- β -ocimene. The sweet notes were also found for butanol, *p*-methoxystyrene, and benzaldehyde all belonging to the miscellaneous group.

Many of the compounds identified in the headspace of elder flower drink belong to the fruity group, but only pentanal, heptanal, and β -damascenone were found to contribute significantly with “fruity” notes as shown by GC-sniff. The latter compound also had notes of “elderberry” and seems to be important for the aroma of elder flower drink, which is in accordance with previous investigations (Poll and Lewis, 1986). The fresh odor of elder flower drink appeared to be related to high concentrations of hexanal, hexanol, and (*Z*)-3-hexenol with strong notes of “green grass”, although other members of the grassy group such as (*E*)-2-hexenol and (*E*)-3-hexenol also contributed to the very attractive aroma of the drink (Table 1).

Other consistent GC-sniff descriptors were “mushroom” for 1-octen-3-one of the agrestic group, which is known to be an important character impact compound in mushrooms together with 1-octen-3-ol (Pyysalo and Suihko, 1976). Furthermore, the sniff test revealed notes such as “peppermint and menthol” for 1,8-cineole (minty group) and “licorice” for 1,1,6-TDN (miscellaneous group), and a few notes from unknown compounds (Table 1).

GC-sniff is an excellent method to find important aroma compounds. The drawback of this method is, however, the loss of synergistic effects among aroma compounds. It is therefore difficult to determine the importance of the less attractive compounds in the agrestic, minty, medicated, spicy, and herbaceous groups. The absence of notes in the sniff test of aroma compounds that normally have an odor can be explained by a relatively low content and/or high threshold values, and therefore these compounds are not detectable by the human nose in the present investigation. Some of these compounds may still be important due to synergistic effects.

The flavor differences among elder flower drinks processed from the five cultivars investigated are despite significant differences in their content of aroma compounds difficult to describe without a comprehensive sensory evaluation. However, on the basis of the olfactory evaluation of single aroma compounds it appears that the most important volatiles in elder flower drinks occur among compounds in the flowery subgroup with the characteristic elder flower odor. Contribution to the fruity and sweet odor of elder flower drinks is found primarily among compounds belonging to the fruity and miscellaneous groups, and the flowery subgroup with flowery notes. Compounds from the grassy group clearly contribute to the freshness of the drink.

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