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The Aroma of Cranberries

III. Juice of *Vaccinium vitis-idaea* L.

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Juice of lingonberries (*Vaccinium vitis-idaea* L.) was analysed for volatile compounds by means of gas chromatography and mass spectrometry. 44 compounds comprising 95 % of a concentrate of the volatiles have been conclusively identified. 15 of these are aliphatic alcohols, 8 aliphatic aldehydes and ketones, 5 terpene derivatives, 7 aromatic compounds, and 9 other compounds. 2-Methylbutyric acid amounting to 48 % of the concentrate and the aromatic compounds are likely to be the most important ones for lingonberry aroma.

The analyses of volatiles of edible fruits of the *Vaccinium* genus have been described in a series of papers.[1–3] The general procedure used was that the material was divided into press juice and press residue which were analysed for volatiles.

Eighty-two compounds comprising about 90 % of the volatiles contained in the press residue of *Vaccinium vitis-idaea* L. (lingonberries) were identified by gas chromatography and mass spectrometry.[1] Although most of these compounds can be expected to be present in the press juice, the volatiles of the latter may include some more volatile compounds. Nevertheless the volatiles are certainly present in other proportions. For this reason the juice was subjected to analysis. The procedure used was flash stripping followed by vacuum distillation of the strip distillate and subsequent solvent extraction of the last distillate.

Analysis of the volatiles of the gas phase in equilibrium with the juice and the berries will be the subject of a future paper.

EXPERIMENTAL

Materials. Ripe lingonberries (*Vaccinium vitis-idaea* L.) from Swedish forests were gathered in 1964 and stored at −30°C until used (juice pressed in winter of 1965 and stored at −30°C till spring 1967). The material was the same as that used in earlier experiments.[1]

Concentration of the volatiles. The berries were minced and the mash was treated with a pectinolytic enzyme preparation, Panzyme-Rapid Super (C. H. Boehringer Sohn), for 24 h. The juice was extracted in a hydraulic press. 190 kg of the berries yielded 170 kg of juice and 15 kg of press residue.

Volatiles in the juice were separated from non-volatile material and the bulk of water by rapid distillation in a flash stripper similar to that described by Weurman [4] and modified by Andersson and von Sydow.[5] 122 kg of the juice was stripped (8—15 kg in each pass) at a feed rate of about 4.2 kg per hour. 23 kg (19 %) of the water was evaporated at 70 mm Hg, giving an average temperature of 48°C at the top of the evaporator. The major part of the distillate was collected in a receptacle cooled by ice-water. The rest was condensed in two traps in series cooled by solid carbon dioxide and liquid air. The contents of the traps were added back to the main distillate.

The volatiles were concentrated further by batch distillation through a fractionating column (800 mm × 25 mm i.d.) packed with glass helices. The still was operated at 150 mm Hg with a reflux ratio of 20:1. The main distillate was collected in an ice-cooled receiver and the rest in a trap chilled by liquid air. All together 860 g distillate was collected from 21.5 l of strip distillate (11 batches of 2 l). The contents of the trap were added back to the main distillate.

The distillate was saturated with sodium chloride at 0°C and extracted with distilled ethyl chloride in a liquid-liquid extractor [6] provided with a cooling jacket in order to enable extraction at room temperature with very low boiling solvents. The distillate was maintained at 2°C by circulating methanol-water from a refrigerating unit. The extracting solvent was heated on a water-bath at 25°C and the vapours were condensed in a Dewar-type condenser cooled with solid carbon dioxide. The extraction time was 9 h, and the extract was allowed to stand at —65°C for 2 days to freeze out residual water. The solvent was removed by distillation through a small water chilled Vigreux column to a final volume of 0.9 ml. The content of residual solvent was determined by gas chromatography.

Gas chromatography. The equipment consisted of an Aerograph model 202/204 (hot-wire detector and flame ionization detector) and a Perkin-Elmer model 800 (flame ionization detector). A preparative column was used in the Aerograph 202/204: 25 % LAC 446 polyester on Chromosorb W AW 60—80 mesh, 3/8″ × 3 m Al tubing (denotion LAC 446 column).

In Perkin-Elmer 800 the following column was used: 25 % Carbowax 20M on Chromosorb W AW DMCS 80—100 mesh, 1/8″ × 2.6 m S.S. tubing (denotion CW 20M column).

The concentrate from the juice was separated into 19 fractions on the LAC 446 column. The temperature was programmed 50—160°C at 2°C/min; the carrier gas (helium) rate was 200 ml/min and the sample size 80—175 µl. The fractions were collected in stainless steel U-tubes (1/8″ × 25 cm) filled with Chromosorb W. The traps were chilled by liquid air and were stored in solid carbon dioxide when not in use. After four successive collections the U-tubes were straightened out while still cold, and the volatile material was transferred to small glass traps, as described by Shearer *et al.*[7] The glass traps were rinsed with 40—50 µl diethyl ether and the fractions were stored in sealed ampoules at —25°C.

To get a better chromatogram for quantitative estimation the preparative LAC 446 column was used with a flame ionizing detector. The relative amounts of the various fractions were estimated from the areas of the chromatographic peaks by cutting them out and weighing them.

The fractions were quantitatively investigated with the use of the CW 20M column in Perkin-Elmer 800. The nitrogen carrier gas flow rate was 25 ml/min and various temperature programs in the interval 50—200°C were used depending on the fraction examined.

Mass spectrometry. The fractions were qualitatively analyzed in a combined gas chromatograph-mass spectrometer LKB 9000. The gas chromatographic separation was done on a CW 20 M column giving chromatograms almost identical to those obtained in the Perkin-Elmer 800. Mass spectra were recorded at 70 eV. The separator temperature was 200°C and the ion source temperature 270°C. The fractions were identified by comparison with our own reference spectra or spectra given in the literature.

RESULTS

The volatile complex was separated in 19 fractions on the LAC 446 column. Each of these fractions was analysed in the combined gas chromatograph-mass spectrometer using the CW 20M column. The data obtained were invariably checked by the retention times observed on the different columns. The compounds identified are given in Tables 1—5.

For the quantitative estimation of the components the 19 fractions were gas chromatographed separately on a CW 20M column using a flame ionization detector. The concentrations given refer to a solvent-free concentrate. Since the analysis involved repeated fractionations and since the separations were often incomplete, the figures given are only approximate. For comparison the amounts of the compounds found in the press residue of lingonberries [1] are given in Tables 1—5.

All together 44 compounds were identified. The known artefacts with regard to impurities in the solvent were removed and the quantitative data were corrected accordingly. The 44 compounds constituted 95 % of the solvent-free concentrate. The four largest unidentified compounds were at most 0.1 % each. There were several below 0.05 % noticeable by gas chromatography which have not been identified. The total amount of volatiles in the juice was calculated as 6 ppm.

Table 1. Aliphatic alcohols.

Compound	Main fraction	%	% (acid free)	% in ess. oil (acid free) from press residue
Ethanol	2	1.3	3	—
1-Propanol	3	0.09	0.2	—
1-Butanol	6	0.9	2	0.3
1-Pentanol	8	1.1	2	1.7
1-Hexanol	10	0.8	2	2.3
2-Methyl-1-propanol	5	1.1	2	—
2-Methyl-1-butanol	7	1.1	2	0.2
3-Methyl-1-butanol	7	1.1	2	0.8
2-Pentanol	6	0.05	0.1	0.8
3-Hexanol	7	0.04	0.1	—
3-Methyl-2-buten-1-ol	9	0.6	1	—
2-Methyl-3-buten-2-ol	4	3.6	7	3.6
1-Penten-3-ol	6	0.4	1	—
trans-2-Hexen-1-ol	11	0.1	0.2	—
cis-3-Hexen-1-ol	11	2.1	4	1.0
		14.4		

DISCUSSION

A comparison with the data obtained with the press residue reveals that there are large differences with regard to absolute and relative quantities. In order to allow comparison of the data, the percentages obtained for the juice were recalculated with omission of the large amounts of volatile acids which were not included in the data from the analysis of the press residue. In comparison of the data it should be remembered that the amount of volatiles in the juice, excluding acids, is approximately three times that in the press residue.

Unlike the extraction technique used on the press residue [1] the concentration technique used here does not favour the determination of the less polar compounds, but it gives more correct data on the more volatile compounds (Tables 1 and 2). On the other hand, the least volatile compounds are not included as is examplified by the presence and absence, respectively, of several aromatic hydroxy compounds (*cf.* Table 4 in this paper and Ref. 1).

Generally speaking, differences must be expected between the juice and press residue with regard to both quality and quantity of volatile compounds. From a practical point of view the data obtained from the juice are of interest in themselves, besides they give a much better picture of the situation within the berries since they contain 10 times as much juice as solid materials (press residue) and since the concentration of total volatiles in the juice is six times higher than that in the press residue.

Of the alcohols (Table 1), 2-methyl-3-buten-2-ol is the major component as in the press residue. This compound and the other unsaturated ones are probably important for the total aroma. As is common with plant materials, the concentration of ethanol was found to be quite variable depending on the treatment of the specimen and on slight differences in the concentration procedure. This is, however, irrelevant to the present investigation.

Table 2. Aliphatic aldehydes and ketones.

Compound	Main fraction	%	% (acid free)	% in ess. oil (acid free) from press residue
2,4-Heptadienal	13	0.2	0.4	0.8
Acetone	1	0.7	1	—
2-Butanone	2	0.1	0.2	—
2-Heptanone	8	0.02		—
3-Penten-2-one	7	0.1	0.2	—
6-Methyl-5-hepten-2-one	11	0.3	0.6	0.4
Diacetyl	3	0.7	1	0.3
Acetoin	9	0.2	0.4	—
		2.3		

Table 3. Terpenes.

Compound	Main fraction	%	% (acid free)	% in ess. oil (acid free) from press residue
Linalool	13	1.3	3	0.3
4-Terpinenol	15	1.5	3	0.9
α-Terpineol	17	1.4	3	0.7
Linalool oxide	13	0.5	1	—
1,8-Cineole	8	0.6	1	0.2
		5.3		

Of the many aliphatic aldehydes found in the press residue only one — 2,4-heptadienal — was identified in the juice. This was also the major alkadienal in the press residue.

As expected, the terpene hydrocarbons found in small amounts in the press residue, were not identified in the juice, owing to their low water solubility (Table 3). The three major, relatively important monoterpene alcohols found in the press residue were also obtained in fairly large amounts in the juice.

As in the press residue the aromatic compounds were found to be abundant in the juice (Table 4) and they certainly contribute in a decisive way to the aroma.

Of the remaining compounds (Table 5) 2-methylbutyric acid is by far the most interesting because it occurs in large amounts and because of its relatively strong odour. The total amount of this acid is 3 ppm of the juice and remaining compounds total 3 ppm.

Supplementary studies are in progress to analyse the gas phase over juice and over berries.

Table 4. Aromatic compounds.

Compound	Main fraction	%	% (acid free)	% in ess. oil (acid free) from press residue
Benzaldehyde	14	2.1	4	3.3
Acetophenone	17	2.6	5	1.1
Benzyl alcohol	19	9.4	19	40.2
Methyl benzoate	16	7.4	15	1.5
Benzyl formate	18	1.5	3	0.1
Benzyl acetate	18	0.3	0.6	0.4
Methyl salicylate	18	0.1	0.2	0.3
		23.4		

Table 5. Other compounds.

Compound	Main fraction	%	% (acid free)	% in ess. oil (acid free) from press residue
Ethyl acetate	2	0.3	0.6	4.1
Ethyl 2-methylbutyrate	5	0.07	0.1	—
2-Methoxyethyl acetate	9	0.1	0.2	—
2-Ethoxyethyl acetate	10	0.1	0.2	—
Furfural	12	0.3	0.6	0.5
2,2,4-Trimethyl-1,3-dioxolane	3	0.1	0.2	—
1,1-Diethoxyhexane	8	0.1	0.2	—
Isobutyric acid	12	0.3	—	—
2-Methylbutyric acid	14	47.8	—	—
		49.2		

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