

## The Aroma of Cranberries

### I. *Vaccinium vitis-idaea* L.

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The volatiles of lingonberries (*Vaccinium vitis-idaea* L.) have been analysed using gas chromatography, mass spectrometry, and infrared spectrophotometry. Eighty compounds comprising 86 % of the concentrate and two acids have been conclusively identified. Of these are 13 aliphatic alcohols, 14 aliphatic aldehydes, 22 terpene derivatives, 24 aromatic compounds, and 9 other compounds, of which 2-methylbutyric acid is the most important single compound for the aroma.

Several edible fruits belong to the *Vaccinium* genus of the family *Ericaceae*. Examples are cranberries (large or American) (*V. macrocarpon* Ait.), small cranberries (*V. oxycoccus* L., in Swedish: tranbär) and mountain cranberries (red whortleberries, cowberries or lingonberries) (*V. vitis-idaea* L., in Swedish: lingon).

Perusal of the literature revealed no reports on volatiles of any of the cranberries. It is well known, however, that cranberries contain benzoic, quinic, citric, and malic acid,<sup>1</sup> from which aroma compounds may, perhaps, be derived. Virtanen and Alftan<sup>2</sup> reported the presence of a number of hydroxyketo and keto acids. As expected from our knowledge of plant material in general, Markley and Sando<sup>3</sup> found glycerides of linolenic, linoleic, and oleic acid in cranberries. These acids may also be precursors of aroma compounds in cranberries.

In this investigation lingonberries were treated with pectinase and the material was separated into press juice and press residue. The latter was extracted with pentane and a concentrate was prepared. This concentrate was separated by gas chromatography and the subfractions or sub-subfractions were analyzed by infrared spectrophotometry or in a combined gas chromatograph-mass spectrometer. The analysis of the press juice is in progress and the results will be reported later and compared with those presented here.

## EXPERIMENTAL

*Materials.* Ripe lingonberries (*Vaccinium vitis-idaea* L.) from Swedish forests were picked in 1964 and stored at  $-30^{\circ}\text{C}$  until used (winter 1965).

Reference compounds for infrared spectrophotometry and mass spectrometry were obtained from commercial sources and when necessary purified by gas chromatography.

*Concentration of volatiles.* The essential oil was concentrated mainly in the way used by Andersson and von Sydow for black currants.<sup>4</sup>

The berries were minced and the mash was treated with a pectinase: 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.

1.9 kg press residue was then extracted with redistilled pentane in a simple batch extractor consisting of a rotating 5 l separatory funnel. Three 2 l portions of pentane were used and the extraction times were 8, 14, and 24 h. The pentane solutions from eight batches were pooled and the pentane was evaporated on a water bath ( $45^{\circ}\text{C}$ ) through a short Vigreux column to a final volume of 250 ml.

The resulting concentrate was then subjected to steam distillation at atmospheric pressure and 6 l water-pentane distillate was collected. The distillate was saturated with ammonium sulphate and extracted with  $3 \times 800$  ml diethyl ether. This extract was washed with sodium carbonate solution to remove acidic compounds (mainly benzoic acid). The ether-pentane extract was dried over sodium sulphate, and the solvents were removed by evaporation through Vigreux columns of decreasing size until the solution volume was 1 ml. The concentrate was stored in a sealed ampoule at  $-25^{\circ}\text{C}$  until used. The content of remaining solvent was determined by gas chromatography.

For the examination of the acidic compounds 4 kg depectinized berry mash was extracted with pentane. This solution was washed with sodium carbonate solution which then was removed and acidified with dilute sulphuric acid. This acid solution was extracted with diethyl ether, dried and concentrated. The concentration of the volatile acids in relation to the non-acid volatiles was determined by a micro-method of vacuum steam distillation followed by extraction and gas chromatography.<sup>5</sup>

*Gas chromatography.* The equipment consisted of an Aerograph model 202 (hot-wire detector) and a Perkin-Elmer model 116 C (thermistor detector). The columns used were:

- (a) 20 % methyl silicon oil SF 96 on Chromosorb W AW 60–100 mesh,  $3/8'' \times 3$  m Al-tubing (denotation: SF 96 column)
- (b) 15 % LAC polyester on Embacel AW 60–80 mesh,  $1/4'' \times 4$  m Al-tubing (denotation: LAC 446 column)
- (c) 15 % FFAP modified carbowax 20 M on Chromosorb W AW DMCS 80–100 mesh,  $1/4'' \times 4$  m Al-tubing (denotation: FFAP column).

The concentrate from the press residue was separated into 19 main fractions on the SF 96 column. The temperature was programmed  $50-200^{\circ}\text{C}$  at  $3^{\circ}\text{C}/\text{min}$ , the helium carrier gas flow rate was 200 ml/min and the sample size 200  $\mu\text{l}$ . The fractions were collected in glass U-tubes which were chilled by liquid air and stored in solid carbon dioxide when not in use. After four successive collections, each fraction was dissolved in 100  $\mu\text{l}$  ether.

These main fractions were separated into subfractions on the LAC 446 column (fraction 1–11) or on the FFAP column (fraction 12–19). The LAC 446 column was used in the Perkin-Elmer 116 C. The helium flow rate was 65 ml/min and the temperatures were  $60^{\circ}$ ,  $80^{\circ}$ ,  $100^{\circ}$ ,  $120^{\circ}$ , and  $140^{\circ}\text{C}$  (fraction 1–2, 3–4, 5 and 7, 6 and 8–9, resp., 10–11). The FFAP column was used in the Aerograph 202. The helium flow rate was 65 ml/min and the temperatures were  $200^{\circ}$  and  $220^{\circ}\text{C}$  (fraction 12 and 14, resp., 15 and 18–19). The subfractions were collected as described but dissolved in 25  $\mu\text{l}$  carbon tetrachloride (spectro grade). All fractions were stored in sealed ampoules at  $-25^{\circ}\text{C}$ .

The acidic fraction was deprived of benzoic acid on the FFAP column. One fraction was collected ahead of the benzoic acid peak.

The relative amounts of the various fractions were estimated from the areas of the chromatographic peaks. For the main fractions and some of the subfractions the peaks were cut out and weighed and for the remaining subfractions the product of the peak heights and the retention times was used.

*Infrared spectrophotometry.* Infrared spectra were recorded in a Perkin-Elmer model 221 spectrophotometer. The subfractions and reference compounds were analyzed in carbon tetrachloride solution in a microcell (path length 0.2 mm) with the use of solvent compensation.

*Mass spectrometry.* The subfractions and/or the main fractions were analyzed in a combined gas chromatograph-mass spectrometer, LKB 9000. The gas chromatographic separation was done on a modified carbowax column (5 % FFAP on Chromosorb W AW DMCS 80–100 mesh, 1/8" × 2.65 m stainless steel tubing). The temperatures were selected between 10° and 225°C depending on the fraction examined. Temperature programming was generally used. Mass spectra were recorded at 70 eV. The separator temperature was usually 200°C and the ion source temperature 250°C. The fractions were identified by comparison with our own reference spectra or spectra given in the literature.

## RESULTS

The results of the separation of the volatiles on the SF 96 column is shown in Fig. 1. Fractions were collected on a preparatory scale as denoted in Fig. 1. As an example Fig. 2 demonstrates the separation of fraction No. 8 on the LAC 446 column. Identification was carried out by mass spectrometry for almost all compounds successfully identified. When the concentrations were high enough, infrared spectrophotometry was used for confirming the results. The data obtained were invariably checked by the retention times observed on the various columns. The data obtained are presented in Tables 1–5, in which the numerals refer to the main fractions obtained on the SF 96 column and the letters to the subfractions, in alphabetical order, obtained on the LAC 446 column for fractions 1–11 and on the FFAP column for fractions 12–19. The concentrations given refer to the total neutralized ether free concentrate. As several fractionations are involved and since the separations were often not complete the concentrations given are only approximate. Two acids were identified in the lingonberries from a pentane extract which was treated by

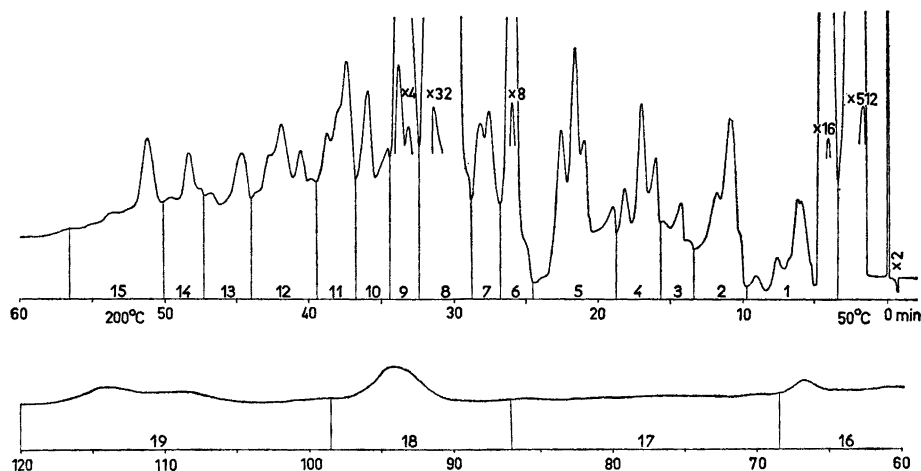


Fig. 1. The aroma concentrate of lingonberries separated on a preparatory SF 96 column.

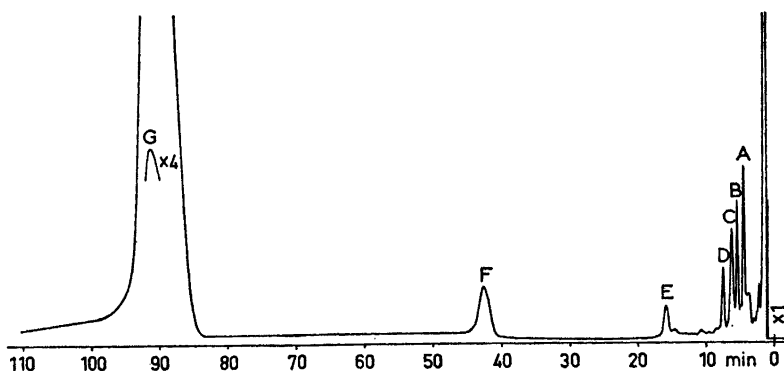


Fig. 2. Main fraction No. 8 separated on the LAC 446 column.

sodium carbonate solution, from which the acids were obtained for mass spectrometric analysis. The acids were benzoic acid and 2-methylbutyric acid. The concentration of the latter one was roughly 75 % of the concentration of benzyl alcohol which is the major volatile compound.

Altogether 94 compounds have been identified. Five of these are only tentatively determined and seven more likely originate from the solvent which

Table 1. Aliphatic alcohols.

Compound	%	Fraction	Identification by	
			IR	MS
1-Butanol	0.3	2J	(+)	+
1-Pentanol	1.7	4F	(+)	+
1-Hexanol	2.3	5E	+	+
1-Octanol	0.7	9B	(+)	+
1-Nonanol	0.3	11E	(+)	+
2-Methyl-1-butanol	0.2	3E		+
3-Methyl-1-butanol	0.8	3E	+	(+)
2-Pentanol	0.8	2I	+	+
3-Pentanol	0.3	2H	(+)	+
(2-Methyl-2-butanol)	0.7	1E		(+)
2-Methyl-3-buten-2-ol	3.6	1H	+	+
3-Methyl-3-buten-2-ol	0.4	2L	+	+
<i>cis</i> -3-Hexen-1-ol	1.0	5F	+	+
1-Octen-3-ol	0.5	7E	(+)	+

Total 13.6

+ means definite identification  
 (+) means tentative identification

also may be the case with *p*-cymene and styrene. Of major fractions not identified there is one of 0.6 % namely 15 S. This compound has the molecular weight 180 and the formula  $C_{11}H_{16}O_2$ . Preliminarily, it is assumed to be a  $\gamma$ -lactone fused to a sixmembered ring. There are six compounds of 0.5–0.3 % not identified and below that concentration, but still observable by gas chromatography, about three hundred compounds more not identified. Quite a number of these are sesquiterpene derivates.

## DISCUSSION

The concentration technique used was chosen in order to obtain a fairly large sample in a short period of time. In using such a procedure several low boiling compounds are missed and the composition, both qualitatively and quantitatively, is not necessarily representative of the whole berries or the juice from the berries. A comparison with data obtained for the juice will be the subject of a later publication but preliminary experiments indicate that the differences between the whole berries and the press residue are not important. The lingonberries studied here contained 0.8 ppm volatiles of the fresh weight. The 80 neutral compounds identified amount to totally 86 % of the neutral concentrate. As pointed out above there are a few hundred more volatile compounds observable by gas chromatography.

Table 2. Aliphatic aldehydes.

Compound	%	Fraction	Identification by	
			IR	MS
Pentanal	1.3	2E	+	+
Hexanal	1.6	4D	(+)	+
Heptanal	0.3	5B	(+)	+
Octanal	0.4	7C	(+)	+
Nonanal	2.4	9A	(+)	+
Decanal	0.4	11D	(+)	+
<i>trans</i> -2-Hexenal	0.06	5C	(+)	(+)
<i>trans</i> -2-Heptenal	0.5	6B	+	+
<i>trans</i> -2-Octenal	0.3	8E	+	+
<i>trans</i> -2-Nonenal	0.3	10C	+	+
<i>trans</i> -2-Decenal	0.4	12G	+	+
<i>trans,cis</i> -2,4-Heptadienal	} 0.7	7F	+	+
<i>cis,trans</i> -2,4-Heptadienal		7	+	+
<i>trans,trans</i> -2,4-Heptadienal	0.1	9C		(+)
<i>trans,cis</i> -2,4-Decadienal	0.08	12K		(+)
<i>trans,trans</i> -2,4-Decadienal	0.5	12L	+	(+)
2,6-Nonadienal	0.08	10E	(+)	+

Total 9.4

It is clear from Tables 1—5 that the aromatic compounds (Table 4) dominate the complex of volatiles. They amount to 52.3 % of the neutral fraction plus the content of benzoic acid. This large amount of aromatic volatile compounds is not common in fruits and berries. As benzoic acid and benzyl alcohol are important constituents it is quite natural that several esters of these compounds are present. Benzyl alcohol, although most abundant, is not likely to play a dominating role in the aroma of lingonberries as it is known to have a fairly low specific odour intensity.<sup>6</sup> Several of the aromatic compounds, such as benzaldehyde, anisaldehyde, eugenol, and others are known to have high specific odour intensities and also characteristic odours. Although they are present in much smaller amounts, they are thus still probably more important for the total aroma of lingonberries than benzyl alcohol.

Of the 13 conclusively identified aliphatic alcohols (Table 1) the four unsaturated ones are rather abundant. As they have a high specific odour intensity they may play an important role in the aroma of lingonberries, while the saturated ones are relatively unimportant.

Table 3. Terpenes.

Compound	%	Fraction	Identification by	
			IR	MS
Myrcene	0.02	7		+
Limonene	0.3	8B	+	+
( $\gamma$ -Terpinene)	0.1	8C	(+)	(+)
$\alpha$ -Pinene	0.9	6A	+	+
$\beta$ -Pinene	0.2	7A	(+)	+
3-Carene	0.3	8A	+	+
Camphene	0.1	6		+
Verbenone	0.1	11L		+
1- <i>p</i> -Menthene-9-al	0.3	11G	(+)	+
Camphor	0.3	10D	+	+
Linalool	0.3	9C		+
4-Terpinenol	0.9	11F	+	+
$\alpha$ -Terpineol	0.7	11I	+	+
Perilla alcohol	0.1	12R		+
Borneol	0.06	11H		+
1,8-Cineole	0.2	8C	+	+
Longifolene	0.06	14F		+
$\alpha$ -Muurolene	0.06	15G		+
$\delta$ -Cadinene	0.03	15H		+
Cuparene	0.05	15J	(+)	+
Isopimaradiene	0.2	19J		+
Pimaradiene	0.1	19H		+
Manoyloxide	0.8	19L		+

Total 6.2

Table 4. Aromatic compounds.

Compound	%	Fraction	Identification by	
			IR	MS
(Benzene) <sup>a</sup>	0.9	1C		+
(Toluene) <sup>a</sup>	0.1	3C		+
(Xylene) <sup>a</sup>	0.09	5A		+
(Trimethylbenzene) <sup>a</sup>	0.2	7C		+
<i>p</i> -Cymene	0.2	8D	(+)	+
Styrene	1.0	5D	+	+
(Naphthalene) <sup>a</sup>	0.07	11L		+
Benzaldehyde	3.3	6C	+	+
Anisaldehyde	0.2	12T		+
Acetophenone	1.1	8F	+	+
Benzyl alcohol	40.2	8G	+	+
2-Phenyl ethanol	0.7	10K	+	+
<i>p</i> -Cumyl alcohol	0.3	12U		+
Carvacrol	0.1	12X		+
Thymol	0.08	12V		+
<i>p</i> -Cymene-8-ol	0.03	9		+
Phenol	0.2	6C		+
Ethylphenol	0.3	10		+
Eugenol	1.0	13Q	+	+
<i>o</i> -Hydroxydiphenyl	0.07	15AA	+	+
Benzoic acid	see text	15Y	+	+
Methyl benzoate	1.5	9D	+	+
Ethyl benzoate	0.2	10H	+	+
Hexyl benzoate	0.04	15Q		+
<i>cis</i> -3-Hexenyl benzoate	0.08	15R	+	+
Benzyl benzoate	0.2	16Z		+
Benzyl formate	0.1	8G		+
Benzyl acetate	0.4	10I	+	+
Methyl salicylate	0.3	11O	+	+
(Diethyl phthalate) <sup>a</sup>	0.02	15W		+
(Dibutyl phthalate) <sup>a</sup>	0.8	18G	+	+

Total 54.5

<sup>a</sup> may originate from pentane solvent.

Most of the 14 aliphatic aldehydes (Table 2) contribute to some extent to the aroma. They may originate from the oxidation of unsaturated fatty acids, which has been well established concerning linoleic and linolenic acid, which have been found to be present in lingonberries.<sup>3</sup>

There is also the possibility that some of the aldehydes have been formed through  $\alpha$ -oxidation of fatty acids and subsequent oxidative decarboxylation.<sup>7</sup>

Eriksson<sup>8</sup> has reported that the enzyme alcohol: NAD<sup>+</sup> oxidoreductase of the pea fruit has a low substrate specificity. If this is a general observa-

Table 5. Other compounds.

Compound	%	Fraction	Identification by	
			IR	MS
Ethyl acetate	4.1	1B	+	+
Methyl palmitate	0.4	18D	+	+
Methyl stearate	0.02	17L		+
2-Pentylfuran	0.1	7B		+
Furfural	0.5	5G	+	+
Diacetyl	0.3	2F	(+)	+
6-Methyl-5-hepten-2-one	0.4	7D	(+)	+
2-Octadecanone	0.01	17N		+
2-Methylbutyric acid	see text	—	+	+

Total 5.8

tion for plant material this may be a link between some of the alcohols and aldehydes found in lingonberries.

Terpenes are very common in fruits and berries and have also been found in lingonberries (Table 3). They amount to 6.1 % compared with 70–80 % of the volatiles of black currants.<sup>4</sup> The hydrocarbons are probably not very important for the total aroma but the terpene alcohols and carbonyls may be of some importance.

Of the remaining compounds, 2-methylbutyric acid is no doubt the most important one. It is almost as abundant as benzyl alcohol and it has as related aliphatic acids also a high specific odour intensity. Its contribution to the aroma of lingonberries is obvious from the fact that the typical lingon aroma disappears from the pentane extract on neutralization.

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