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# The Aroma of Black Currants

## II. Lower Boiling Compounds

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Black currants (*Ribes nigrum* L. *var.* Brödtorp) were analyzed for low boiling volatile compounds by means of gas chromatography, infrared spectrophotometry, and mass spectrometry. The following twenty-three compounds, not previously found in black currants, were identified: methanol, ethanol, propanol, 2-methylpropanol, butanol, 3-methylbutanol, pentanol, hexanol, 2-butanol, 2-pentanol, 2-methyl-3-buten-2-ol, 3-methyl-2-buten-1-ol, 1-penten-3-ol, acetaldehyde, hexanal, acetone, 2-butanone, methyl acetate, ethyl acetate, butyl acetate, ethyl butyrate, 1,8-cineol, and styrene. 2-Methylbutanol, pentanal, and 2,3-butandione were tentatively identified.

Twenty-five higher boiling compounds contributing to the aroma of black currants were identified previously.[1] An attempt has been made to identify some low boiling compounds in black currants with the aid of gas chromatographic retention data.[2] Although it is felt that the characteristic black currant aroma originates from the higher boiling part of the volatile complex, the more volatile compounds can doubtless contribute in a decisive way. This fraction has therefore now been subjected to analysis following the same general pattern of identification as that used for the higher boiling fraction. Owing to the difference in volatility, however, the concentration procedure used now is based on flash stripping of juice pressed from the berries, followed by vacuum distillation of the strip distillate and solvent extraction of the second distillate.

## EXPERIMENTAL

*Materials.* Fully ripe black currants *var.* Brödtorp harvested in August 1962 were stored at −40°C until used (spring 1964).

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

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*Concentrations of volatiles.* 5 kg of the berries was minced, 2.5 g of sodium fluoride was added to suppress fermentation, and the mash was treated with a depectinizing enzyme for 24 h. The juice was extracted in a hydraulic press exerting a pressure of 450 kg/cm². 146 kg of the berries yielded 120 kg (82 %) of juice.

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.[3] 7 kg of the juice was stripped in each pass at a feed rate of 4.5 kg per h. 22—25 % of the water was evaporated at 68—72 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. The first trap was cooled by solid carbon dioxide; the second by liquid air. After every second distillation the contents of the traps were added back to the main distillate. The distillates were pooled and the pH was adjusted to 8.5 with sodium carbonate.

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 highly volatile compounds were condensed in two cold-traps in series chilled by solid carbon dioxide and liquid air. 50 ml of distillate was collected for every 1.5 liter batch of strip distillate. The cold-traps were rinsed twice with distilled ethyl chloride at −40°C and +3°C, respectively. The rinsing liquid was added to the ethyl chloride used for subsequent extraction of the main distillate, which was saturated with sodium chloride at 0°C.

The extraction was carried out in a liquid-liquid extractor provided with a cooling mantle in order to enable extraction at room temperature [4] with very low boiling solvents. The distillate was maintained at +5°C by means of circulating methanol-water from a refrigerating unit. The extraction solvent was heated on a water-bath of 23°C, and the vapours were condensed in a Dewar-type condenser cooled with solid carbon dioxide. The extraction time was 6 h, and the extract was allowed to stand at −65°C for 2 days in order to freeze out residual water. The solvent was removed by distillation at a reflux ratio of 20:1 through a small mantled Vigreux column chilled by tap water of 12—14°C. 114 kg of the juice yielded 2.5 g aroma concentrate, which was stored in a stoppered tube under nitrogen at −25° C until analyzed.

Volatile compounds in 10 kg finely powdered press residue were concentrated in a manner similar to that previously described.[1] For 2 kg press residue three 3 liter portions of redistilled pentane were used, and the total extraction time was 46 h. After removal of the bulk of the solvent by careful distillation through a Vigreux column the concentrated extract was steam-distilled and the distillate extracted with diethyl ether. The extract was concentrated by distillation through a 150 mm Vigreux column at a reflux ratio of 10:1. 1.8 g of aroma concentrate containing large amounts of residual solvent was obtained. The concentrate was stored in a sealed ampoule under nitrogen at −25°C until used.

*Gas chromatography.* The equipment consisted of an Aerograph model 202/204 (hot-wire detector and flame ionization detector) and a Perkin-Elmer model 116 C (thermistor detector). Three columns were used in the Aerograph 202/204:

(a) 25 % LAC 446 polyester on Chromosorb W AW 60—80 mesh, 3/8″ × 3 m aluminium tubing (denotion: LAC 446 25 % column).
(b) 20 % silicone oil SF 96 on Chromosorb W AW 69—80 mesh, 3/8″ × 3 m aluminium tubing (denotion: SF 96 column).
(c) 18 % polyethylene glycol 1000 + 0.5 % Tween 80 on Chromosorb W AW DMCS 80—100 mesh, 1/8″ × 2.7 m stainless steel tubing (denotion: PEG 18 % column).

In Perkin-Elmer 116 C the following columns (aluminium tubing, 1/4″ × 3.9 m) were employed:

(d) 25 % polyethylene glycol 1000 on Embacel acid washed 60—100 mesh (denotion: PEG 25 % column).
(e) 15 % LAC 446 polyester on Embacel acid washed 60—100 mesh (denotion: LAC 446 15 % column).
(f) 10 % SAIB (sucrose diacetate hexaisobutyrate + 5 % Quadrol (N,N,N′,N′-tetrakis (2-hydroxypropyl)ethylene diamine) on Embacel acid washed 60—80 mesh (denotion: S/Q column).

Helium was used as carrier gas for all work with the thermal conductivity detectors. For the PEG 18 % column used in connection with the flame ionization detector the carrier gas was moist nitrogen at a flow rate of 30 ml/min.

The concentrate from the juice was separated into 24 fractions on the LAC 446 25 % column. The temperature was programmed 53—150°C at 2°C/min, the helium rate was 200 ml/min. and the sample size 0.15 ml. The fractions were collected in stainless steel U-tubes (1/8″ × 250 mm) partly filled with Chromosorb W. The traps were chilled by liquid air and were stored in solid carbon dioxide when not in use. After eleven successive collections the U-tubes were straightened out while still cold, and the volatile material was transferred to a small glass trap as described by Shearer *et al.*[5] The glass trap was rinsed with 40—50 µl diethyl ether and a minor part of each fraction was reserved for combined gas chromatography-mass spectrometry. The major part was separated into subfractions on the PEG 25 % column for infrared spectrophotometry at suitable column temperatures between 60°C and 100°C. The helium rate was 65 ml/min. The subfractions were collected and transferred to the glass trap as described and were dissolved in 40 µl carbon tetrachloride (spectro grade). All fractions were stored in sealed ampoules at —25°C. The entire fractionating procedure was tested with 0.2 µl of acetone and a recovery of 85 % was found. The S/Q column was used for check-analyses of some of the fractions.

Volatiles in the press residue were separated on the SF 96 column. Temperature was programmed 50—200°C at 3°C/min, the helium rate was 200 ml/min and the sample size 0.2 ml. Ten low boiling fractions preceding the monoterpene hydrocarbons were collected in seven successive chromatographic runs. The fractions were separated into subfractions on the LAC 446 15 % column for infrared spectrophotometry at suitable column temperatures between 50°C and 80°C. The subfractions were collected and stored as described.

The relative amounts of the fractions were determined by cutting out and weighing the peaks in separate chromatograms run at a high paper velocity. No corrections were made for varying detector response for different compounds.

The headspace vapours of berries, juice, press residue, and the various condensates were analyzed on the PEG 18 % column, operated at 65°C. A 10 ml sample in a 20 ml injection bottle closed with a rubber stopper was equilibrated in a water-bath of 50°C for 25 min. 2.5 ml of headspace vapour was withdrawn with a heated syringe and injected into the gas chromatograph. Some of the major volatile components in the juice were quantitatively determined by the technique described by Kepner *et al.*[6] Calibration curves were obtained by adding increasing amounts of the compound under investigation to samples of stripped juice, which had been diluted with distilled water to the original volume. 2 ppm of 3-heptanone was used as internal standard.

*Infrared spectrophotometry.* Infrared spectra were recorded in a Perkin-Elmer model M 221 spectrophotometer. Collected fractions 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 concentrate of volatile compounds in the juice and fractions from the LAC 446 25 % column were analyzed in a combined gas chromatograph-mass spectrometer placed at our disposal by Dr. R. Ryhage, Stockholm. An Atlas CH 4 mass spectrometer was used. The apparatus and analytical technique have been described previously.[7] The gas chromatographic separation was done on a polyethylene glycol column (17.5 % Carbowax 20M on Chromosorb W AW DMCS 60-80 mesh, 3 m × 3 mm i.d. glass tubing). The separation of the concentrate was carried out by programmed temperature operation (42—110°C, 2°C/min), while the fractions were analyzed at isothermal temperatures ranging from 42°C to 120°C. Mass spectra were recorded at 20 eV, and in most cases fractions were identified by comparison with spectra given in the literature.

## RESULTS

The gas chromatographic separation of the volatiles in the juice on the preparative LAC 446 column is shown in Fig. 1 and that of the concentrate from the press residue on the SF 96 column in Fig. 2. Fractions were collected

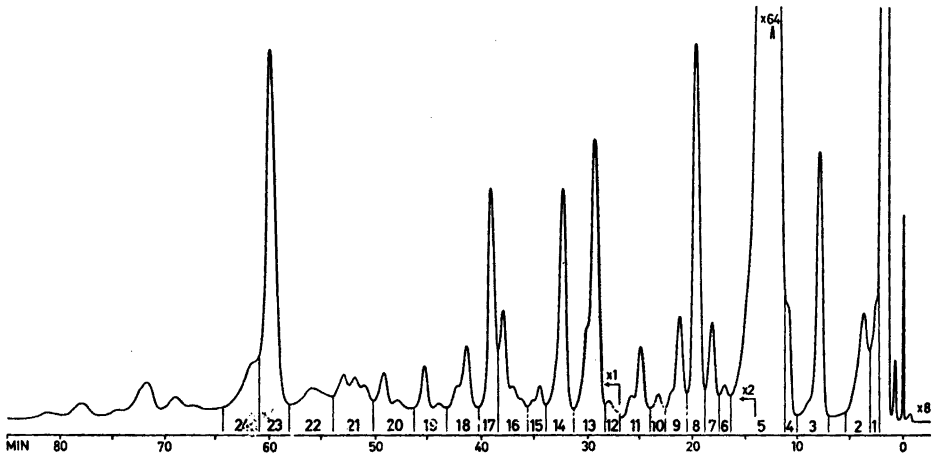


*Fig. 1.* Volatiles in cold-pressed black currant juice separated by preparative gas chromatography (LAC 446 column).

as indicated in the figures. The data obtained in analysis on the juice are given in Table 1, and supplementary comments are given below.

*Fraction No. 1.* As it was suspected that this fraction contained acetaldehyde, a derivative was prepared with 2,4-dinitrophenylhydrazine. The hydrazone was identified by thin layer chromatography.

*Fraction No. 3.* This fraction was shown by IR and MS to consist of a mixture of acetone and methyl acetate. The ratio between the amounts of the two components was estimated by comparison of the IR spectrum of the fraction with spectra of known mixtures of these compounds.

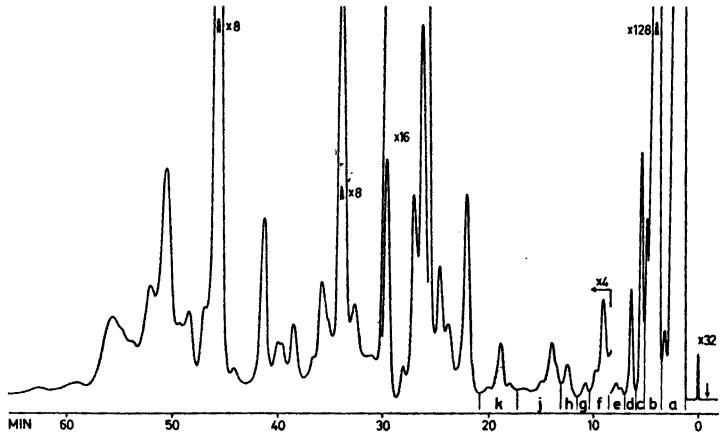


*Fig. 2.* Volatiles in press residue separated by gas chromatography (SF 96 column).

*Table 1.* Analytical data and identified compounds of the low boiling fraction of black currant aroma

| Fraction No. | Subfraction No. | %[a] | Infrared data | Mass spectrometry data | Identified compound | %[a] |
|---|---|---|---|---|---|---|
| 1 | | trace | | | acetaldehyde[b] | trace |
| 2 | I | 1.2 | diethyl ether | diethyl ether | diethyl ether[c] | 1.2 |
| | II | 1.3 | ethyl bromide | ethyl bromide | ethyl bromide[c] | 1.3 |
| 3 | I | 31 | acetone (70 %) and methyl acetate (30 %) | acetone and methyl acetate | acetone<br>methyl acetate | 22<br>9 |
| 4 | I | 10 | ethyl acetate | ethyl acetate | ethyl acetate | 11[d] |
| | II | 1.5 | | ethanol | | |
| 5 | I | 0.9 | ethyl acetate | ethyl acetate | | |
| | II | 0.4 | 2-butanone | 2-butanone | 2-butanone | 0.4 |
| | III | | ethanol | | ethanol | |
| 6 | I | 0.2 | C=O (1718 $cm^{-1}$) | (2,3-butandione) and other compound(s) | | |
| 7 | I | 0.3 | OH | 2-butanol | 2-butanol | 0.3 |
| | II | 4.4 | propanol | propanol | propanol | 4.4 |
| 8 | I | 11 | 2-methyl-3-buten-2-ol | *m/e* = 86, 85, **71**, 59, 58, 53, 43, 41, 31, and | 2-methyl-3-buten-2-ol | 10.5 |
| | | | C=O (1735 $cm^{-1}$ | ethyl butyrate | ethyl butyrate | 0.5 |
| 9 | I | 0.7 | C=O (1735 $cm^{-1}$) | unknowns | | |
| | II | 4.0 | 2-methyl-propanol | 2-methylpropanol | 2-methyl-propanol | 4.0 |
| 10 | I | 0.3 | hexanal and other compound(s) | hexanal and other compound(s) | hexanal | 0.2 |
| | II | 0.1 | | 2-pentanol | 2-pentanol | 0.1 |
| 11 | I | 2.0 | butanol | butanol | butanol | 2.0 |
| | II | 0.7 | OH,-CH=$CH_2$ | *m/e*=86, 85, 71, 68, 67, 59, 58, **57**, 44, 43, 41, 31, 29 | 1-penten-3-ol | 0.7 |
| 12 | I | 0.2 | | unknown(s) | | |
| 13 | I | 0.3 | | unknowns | | |
| | II | 3.0 | 3-methylbutanol and other compound(s) | 3-methylbutanol and other compound(s) | 3-methylbutanol | 2.5 |
| 14 | I | 0.3 | | pentanol | pentanol | 0.3 |
| | II | 0.5 | | unknown(s) | | |
| | III | 1.2 | | 1,8-cineol | 1,8-cineol | 1.2 |
| | IV | 1.0 | | styrene | styrene | 1.0 |
| 16 | I | 1.0 | 3-methyl-2-buten-1-ol | *m/e*=86, 85, **71**, 68, 67, 53, 43, 41, 31, 29, 27 | 3-methyl-2-buten-1-ol | 1.0 |
| 17 | | 2.5 | | hexanol | hexanol | 2.3 |
| 18 | | 1.4 | | *cis*-3-hexen-1-ol | *cis*-3-hexen-1-ol | 0.8 |
| 19 | | 0.7 | | 1-octen-3-ol | 1-octen-3-ol | 0.5 |
| 23 | | 8.3 | | terpinen-4-ol | terpinen-4-ol | 8.0 |
| | | 90.4 | | | | 85.2 |

[a] % in the concentrate less ethanol and solvent.
[b] see text.
[c] impurities in ethyl chloride.
[d] including fraction No. 5:I.

*Fraction No. 6.* This fraction was not pure but had a strong odour reminiscent of 2,3-butandione. IR showed a carbonyl absorption in agreement with this compound (1718 $cm^{-1}$) and the MS contained as prominnet peaks the most important peaks of the spectrum of 2,3-butandione ($m/e$ = 86 and 43). It is therefore likely that this fraction contains 2,3-butandione.

*Fraction No. 8.* 2-Methyl-3-buten-2-ol was identified by IR. In addition to the absorptions of this compound the spectrum contained an ester carbonyl absorption at 1735 $cm^{-1}$. Besides a number of peaks, which could easily be attributed to the alcohol, the MS contained all peaks belonging to the spectrum of ethyl butyrate. The quantitative relationship was very roughly estimated from IR.

*Fraction No. 11.* IR of subfraction II showed a secondary or tertiary aliphatic alcohol. The spectrum also revealed the presence of a vinyl group and the absence of a gem-dimethyl grouping. The MS gave the molecular formula $C_5H_{10}O$ with the base peak $m/e$ = 57, all other peaks having relative abundances of less than 15 %. The only compound compatible with these analytical data is 1-penten-3-ol.

*Fraction No. 13.* The appearance of the IR spectrum of subfraction II indicated the presence of one or more minor components besides the major one, 3-methylbutanol. Gas chromatographic analysis of this subfraction on the S/Q column revealed a component with slightly shorter retention time than 3-methylbutanol. On the basis of retention data obtained with all three columns used, this component is tentatively identified as 2-methylbutanol.

Headspace analysis was employed in order to check the influence of the technical procedures on the most volatile fraction and to determine the amounts of some of the major components in this fraction in the juice. Quantitative data were obtained for the concentrations of ethanol (200 ppm), acetaldehyde (0.9 ppm), acetone (2.0 ppm), methyl acetate (0.9 ppm), and ethyl acetate (1.4 ppm). Headspace analysis also revealed the presence of large amounts of methanol in the juice (800 ppm). As methanol is not extracted by ethyl chloride from a dilute aqueous solution, it is not found in the concentrate. As expected, the analysis revealed that substantial losses of some compounds occurred during the concentration procedure, but that no new compounds were formed. The mean loss could be roughly estimated as 50 % of the total volatile fraction. As the concentrate (less ethanol and solvent) was 3 ppm of the berries, the total amount of low boiling volatiles can be estimated as 6 ppm, methanol and ethanol not included.

Despite careful purification of the pentane used, a number of compounds identified in the analysis of the highly volatile fraction of the press residue proved to be aliphatic and aromatic hydrocarbons originating from the solvent. The following components in the press residue were identified by IR: ethyl acetate (fraction *d*), pentanal (tentatively, fraction *g*), ethyl butyrate, butyl acetate, hexanal (fraction *j*), and styrene (fraction *k*). Pentanal and butyl acetate were not identified in the analysis of the juice, but the latter compound is probably identical with the carbonyl compound in fraction 9:I, the identity being supported by retention data.

## DISCUSSION

The low boiling fraction of the volatile complex of black currants studied here was roughly 6 ppm of the fresh weight, compared with 9 ppm of essential oil in the same berries.[1] The major part of the low boiling components was found in the juice expressed from the berries with only traces of low boiling volatiles remaining in the press residue.

The concentrate of low boiling volatiles from the juice had a very strong odour without any resemblance to that of fresh black currants. A powerful "green" odour note, possibly due to the presence of *cis*-3-hexen-1-ol, was observed. Some of the low boiling compounds identified are known to possess strong odours, *e.g.* hexanal, but the majority of the components in the concentrate have comparatively low odour intensities. As very little is known about odour thresholds of the individual compounds, and particularly of mixtures, it is at present impossible to draw any conclusions about the extent to which the aroma of black currants is dependent upon the compounds with a low boiling point.

Judging from the results of gas chromatography, the composition of the concentrate from the press residue, most of which consisted of higher boiling material, was nearly identical with that of the essential oil previously described.[1] The odours of the essential oil and the concentrate studied here were also almost identical and suggestive of black currants, although some odour notes were apparently missing. It may thus be concluded that the characteristic odour of black currants is dependent mainly on those compounds of the volatile complex with a higher boiling point and that the contribution by the components with a low boiling point is small but probably necessary for the true aroma of the berries.

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