THE MOST IMPORTANT QUALITY CRITERIA OF SOME HOME-GROWN BLACK-CURRANT VARIETIES

IV. Gas-chromatographic analysis of aroma

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In the study of the quality properties of black-currants, the main results which have been reported earlier in parts I, II and III of this series, the last part reported here is concerned with characterization of the aroma by gas-chromatographic methods.

Nowadays, gas-chromatography should be the method of choice for the investigation of volatile components. Application of this method makes it possible in principle to obtain a qualitative picture of the components which form the basis of the aroma; moreover, it should also be possible to evaluate the quantities of the different aroma components.

Here, it was necessary for reasons of methodology to divide the study in two parts, the first concerned with the lower-boiling part of the aroma, and the second the higher-boiling part. The former components were studied by means of a column which tolerated water, but not high temperatures; the higher-boiling compounds had to be studied with another column which tolerated higher temperatures, but not water, which was accordingly removed. As a result separate reports are given on these two groups of aroma compounds.

The material for this investigation was the same as in the rest of the series, although the analysis had to be limited so that lower-boiling compounds were analysed from the material obtained in 1962 and 1963, and higher-boiling compounds only from the material of 1962. Consequently the series obtained here are not so complete as in the other analyses reported. During the course of the investigation, it had been possible to improve some points in the methods, but as it was considered more important that the various series should be mutually comparable, changes in the methods were not adopted once a practicable procedure had been developed. In the comparison presented here the material was frozen. In addition, studies were also made with fresh berries; this led to some interesting results, but as in these studies the standing times of the samples to be analysed necessarily varied considerably in the different cases, the results obtained with the frozen berries seem to be more reliable.

Lower-boiling aroma compounds

Methods. The aim here was that of applying a method for the isolation of aroma compounds such that the secondary changes were as small as possible. Heating and the effect of air were consequently avoided, and the adsorbent employed in the concentration of aroma was the same mass as that later utilized in the gas-chromatographic column as filling material. It would probably be most advisable to use the so-called »head space» technique to avoid changes; here, this was not possible, as the amount of aroma compounds was not sufficient for the application of this method in the conditions available. As a result, concentration of the aroma before gas chromatography was inevitable; this was effected by means of gas stream and the cooling of filled adsorption tubes.

The sample consisted of 35 g of frozen berries, first thawed, after the addition of an equal amount of distilled water, in a water bath at 20°C. The thawed mixture was homogenized in a small closed plastic vessel, with a Bamix homogenizer, for 1—3 min. The aroma was collected from this sample by leading a slow nitrogen stream through it at room temperature. The stream was released into the sample through fritted glass to make the bubbles small. The adsorption tubes were cooled with running water, 10°C (column A), and salt-ice mixture at —18°C (column U); the diameter of the tubes was 4 mm, and the heights of the mass approximately 12 and 15 cm, respectively. The collecting time was 5 hours; the gas volume was then 2000 ml, and the flow rate 7 ml/min. If variations occurred, the gas volume was kept as constant as possible. The apparatus is illustrated in Fig. 1.

In the gas chromatography, a Perkin Elmer Fractometer 116 was used. For column filling, Mass W (polyethylene glycol/teflon) was chosen. To introduce the sample into the fractometer, the adsorption tube was heated with a water mantle

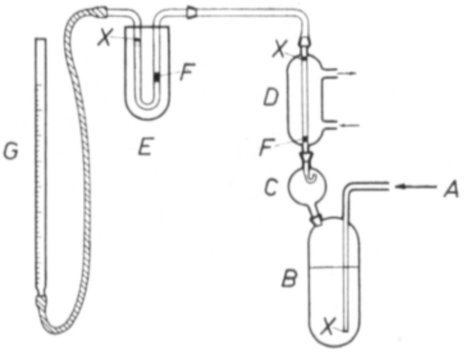

Fig. 1. Apparatus for collection of low-boiling aroma compounds from varieties of black-currant. A: gas inlet. B: sample container. C: connecting bulb. D: Column A, cooled with running water. E: Column U, cooled with NaCl-ice mixture. F: Glass wool. G: Flow-rate meter. X: Fritted glass.

to 100°C, and a helium gas stream led through for 2 minutes. The retention times were calculated from the starting point of the introduction. The following conditions were applied: temperature 80°C, and length of the column 2 m in both years; in 1962, P_{He_2} 0.8, P_{O_2} 0.8, P_{H_2} 0.5 and flow rate 4.4; in 1963, P_{He_2} 1.0, P_{O_2} 0.8, P_{H_2} 0.45 and flow rate 3.8. A slight difference exists in the column packing between the years; in 1962 the packing was done by the authors, whereas in 1963 a column packed by the producer was used. In the latter case, the packing was evidently tighter, which brought about some slight differences in the retention times. Notwithstanding this, the curves are well comparable, although exact superposition is not possible. Two different detectors were used simultaneously, viz. a thermistor and a flame ionization detector (FID). In general, the former has given only the macrocomponents (water, ethanol and methanol); the latter is more sensitive, and has thus given more components; it does not register water. With the macrocomponents as basis, it is possible to make an approximate evaluation of the boiling points of the aroma compounds present. As a rule, the time of analysis was at least 60 minutes; at this point, the water peak had already passed, and examination was concerned with whether something else would still come out.

Comparison of the results is founded upon the curves obtained from U-tubes. In 1962, the aroma in the A-tubes was also analysed to determine the extent to which the two tubes differed. It was concluded that the pictures are very similar, although the lowest-boiling compounds are better found in the U-tubes, whereas the bulk of the water is in the A-tubes. As it was impracticable to prevent the introduction of water into the fractometer, and the presence of water is a disturbing factor in analysis, it was considered advisable to employ the U-tubes for aroma analysis. Although the flame ionization detector does not register water, the more of it is present, the more the registration of other compounds in the same area is disturbed.

The quantity of berries taken here for aroma analysis was constant, and the fraction collected in the U-tubes has been taken for the run as a whole. Thus quantitative comparisons between the different samples are possible in these series, although standardization of the method was probably not quite sufficient for exact quantitative determinations.

Results. As a general conclusion it has been found that in the different analyses a total of 13 peaks was present, although they did not occur everywhere. The first 10 of these were comparatively sharp, but the last 3, coming after the water

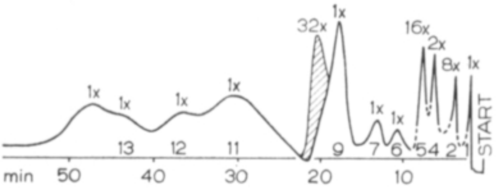

Fig. 2. Aromagram of Brödtorp black-currants from Piikkiö, low-boiling compounds. (80° C).

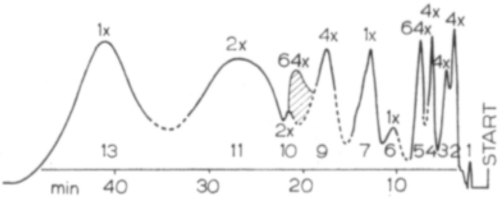

Fig. 3. Aromagram of Black of Lepaa black-currants from Piikkiö, low-boiling compounds. (80° C).

peak, were flattened. It may be assumed that these last peaks represent considerable amounts of aroma compounds, although these are not well separated under the conditions applied.

The results obtained are exemplified by the aromagrams of Brödtorp (Fig. 2) and Black of Lepaa (Fig. 3) from Piikkiö, 1962, arrived at with the FID; in addition, the water peak recorded with the thermistor detector is indicated as a striped peak. At the base, the numbers of the peaks are given, the sensitivity used being given at the top of peaks; e.g. 8 x means that the real height is obtained by multiplication by this factor. The dotted lines represent areas in which the sensitivity was changed.

In this study it was not possible exactly to identify the compounds, which are designated only by numbers in the order of issue from the fractometer. In some cases it may be possible to guess which is the main component of a peak, although it might be that one peak represents several components not separated under the prevailing conditions. Thus, peak 5 probably corresponds to ethanol, and peak 4 to methanol, the principal components of the low-boiling aroma. In part, the peaks are well separated, and in part they are discernible only as small elevations on the side of a higher peak. In some cases the retention times exhibit variations, e.g. peak 1, ordinarily rather low, may vary somewhat in its location; accordingly it has been designated as 1a and 1b. No more than a few of the peaks are comparable in height with the macrocomponents; thus at the level of the 1962 methanol peak these are only Nos. 2 and 9; in 1963, such heights appear even more seldom, and then these come into question Nos. 1, 6 and 11.

After these general observations, it is important to consider which factors may influence the aroma composition, and in which way. Factors which may influence the aroma include the variety, weather conditions, northernness of the growing locality, the degree of ripeness and the condition of the berries. The aroma composition again may be characterized by different features: 1) presence or lack of some peaks in the aromagrams, 2) relative amounts of the component parts, evaluated from the height or area of the peaks, and 3) differences in the general level of the aromagrams. Of these criteria, the first two are mainly qualitative, and the third quantitative in nature.

As regards the effect of variety, it is concluded that there are to be found no differences based on the variety factor, evident as the characteristic presence or lack of any component; in the same variety, the presence of the peaks may vary in different years and in different localities, and thus the aroma composition of the varieties in the low-boiling range is not sufficiently constant to be characteristic of the variety. Neither is the amount of aroma, as evaluated on the basis of the sum of heights of all peaks, typical of the variety, but varies between wide limits in the different years and in the various localities. It thus seems that the effect of variety on the aroma composition cannot be demonstrated by virtue of the strong influence of the other factors although the existence of such effect is well known; cf. LARSSON (12) concerning varieties in northern Sweden. However, it must be remembered that the aroma composition considered here represents only the low-boiling part of the aroma.

In contrast, the effect of the weather conditions seems to be very clear-cut. In 1962, the summer was cool, whereas in 1963 it was warm and sunny. This difference is discernible in the aroma picture, first in the fact that the frequency of some peaks is changed. Thus, in 1963, peaks No. 6, 7 and 9 are less frequent than in 1962, whereas peak No. 8 shows contrary behaviour. This is illustrated by the following consideration, in which the total number of samples studied for each case was 17:

Peak	6	7	9	8
Frequency in 1962	14	11	17	4
Frequency in 1963	3	4	9	14

Secondly, a great difference between the years is discernible also in the total aroma, calculated from the sum of the peak heights, as is shown below:

	Average of the total aroma	Limits of variation
1962	14098	1840—38229
1963	1886	354— 8093

This comparison evidences that the level of the total aroma was much higher in 1962 than in 1963. This result appears unexpected in that it is well known, that the berry aroma develops more strong and more typical in a warm and sunny summer than in a cool and rainy one. Nevertheless, it should be borne in mind that the aroma compounds studied here are easily volatile, and consequently secondary losses may occur more easily in a warm than in a cool summer.

As regards the effect of the groving locality, it might be anticipated that it would correspond to that of the weather conditions, i.e. a southern location would correspond to a warm summer, which would here mean a diminution in the total aroma, and changes in the frequencies of some peaks. When the results are examined with these guiding lines in mind, some similarity is found in that the peaks No. 6, 7 and 8, which were more frequent in a cool summer, are commoner towards the north than towards the south whereas peak No. 8 again exhibits contrary behaviour. However, this result is only of preliminary nature, and exceptions exist. Also, the total aroma, measured as the sum of all peak heights, increases towards the north, as is evident in the following:

Locality	Piikkiö	Pälkäne	Laukaa	Ylistaro	Maaninka	Rovaniemi
1962, average	7002			17822	21553	18684
1963, »	974	815	1429		5391	1718

The increase is irregular, and the years under study differ from each other; the causes of such irregularities might be not only climatic conditions, but also the effect of the degree of ripeness and the transport conditions, which may have raised the values at Maaninka. Although these samples were not definitely fermented, the longer transport had rendered them riper than samples for which transport period was shorter.

The effect of the degree of maturity and condition seems to have been evident, although precise evaluation of these factors is difficult. It is clear that ripening occurs more slowly in a cool summer and in a more northern locality, which in principle induce delay in the ripening. The clearly unripe berries were eliminated from the berry samples studied. In such unripe berries the aroma is less developed, as is well known; this was also confirmed here by some gas-chromatographic analyses of unripe berries. However, in all the samples, slow after-ripening without doubt occurred during frozen storage before the aroma was studied, which may have to some degree levelled out the differences. When the notes concerning the condition of the berries are compared with the aroma picture obtained, it is discernible that often the number of aroma components is less in samples characterized as unripe; this result is not without exceptions, possibly because the definitely unripe berries were eliminated. In contrast, in samples characterized as crushed or over-ripe, the number of components exceeds the average, in any case as regards 1962, when the summer was cool. No. 3 was often absent from the peaks when the sample was designated as unripe; it is possible that this component develops only in ripe berries. Again, peak No. 10 might be characteristic of over-ripe berries, although the correlation is not completely regular. The degree of ripeness exercises a clear effect on the sum of peak heights, although sometimes after-ripening changes the situation. Thus, in samples designated as unripe, the peak heights are lower than usual, and in over-ripe samples are again higher than the average. The over-ripeness is particularly clearly evident in peak No. 5, i.e. ethanol, of which the amount is increased appreciably if fermentation takes place, but also increases as a result of over-ripeness as such, although no definite fermentation has occurred.

An interesting question in consideration of the aroma picture of black-currant, is the proportion of ethanol and methanol in total aroma, and possible variation of this relation in the different cases. The proportion of ethanol is of particular interest. Alternatives might exist here: 1) when the amount of ethanol increases, the amount of other aroma components does not increase at the same rate, and thus ethanol reaches a dominant role, or 2) when the amount of ethanol increases, this simultaneously induces a rise also in the other aroma components, or perhaps in their »loosening» from the berries, and thus the aroma content is increased in its entirety. To decide between these alternatives, a comparison was made of the ethanol content and the total aroma content, evaluated from the peak height in the aromagrams. This comparison is illustrated in Fig. 4. It can be seen that a relatively good positive correlation exists between the ethanol content and the amount of total aroma. This result would lead to the conclusion that under the experimental conditions applied the rise in the ethanol content in general enhanced the typical black-currant aroma, the ethanol not having taken on any dominant postition. In these samples, no definite fermentation had taken place; the result may of course be divergent if this happens. Moreover, in the diagram the two years under study clearly differ from each other: in 1962 both ethanol content and total aroma were high, whereas in 1963 both values remained low.

Another interesting point in connection with the aroma of black-currant is the possible correlation between the aroma number and the height of the ethanol

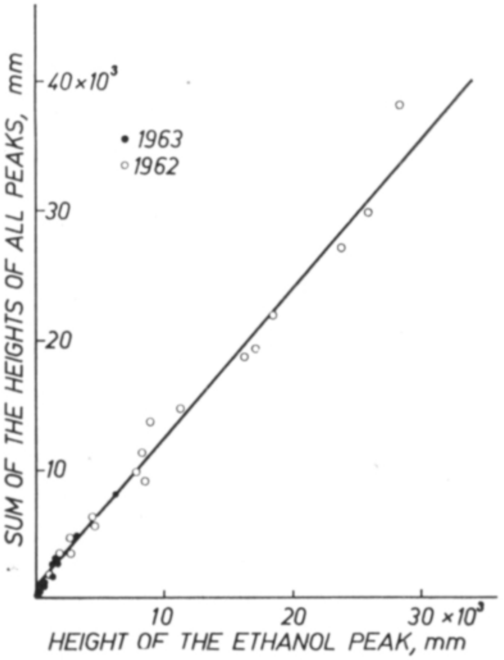

Fig. 4. Relation between total aroma and ethanol content, measured as peak heights, in the black-currant aroma of the different varieties.

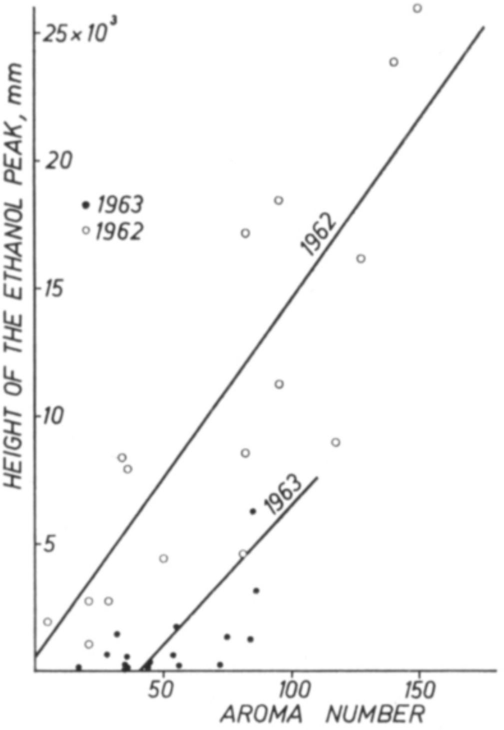

Fig. 5. Relation between the ethanol content, measured as peak height of aromagrams, and aroma number of different varieties of black-currant.

peak. A comparison of these two factors is shown in Fig. 5. As the aroma number reflects the volatile reducing substances, the most prominent of these in black-currant being ethanol, a positive correlation is to be expected. Such a correlation does seem to exist, although in this case, where the aroma numbers vary relatively little, the values of the two years investigated differ somewhat, and give two separate lines; these are nearly parallel. The correlation found is positive, as was expected: the higher the ethanol peak, the higher the aroma number. Nevertheless, considerable variation exists in the values.

Again, as concerns the methanol, it might be anticipated that it would show a correlation with the degree of esterification of the pectin, as a lowering of the degree of esterification of pectin during its breakdown means that methanol is liberated from the methoxyl groups. The amount of methanol would thus be higher the more breakdown had taken place in the pectin, and thus the lower was the degree of its esterification; of course this assumes that there had been no loss of methanol through evaporation. Fig. 6 illustrates the correlation between the height of the methanol peak and the degree of esterification of pectin. Is it observable that there is the expected negative correlation, although the variability is great, and the two years investigated again differ from each other; however, the lines obtained are nearly parallel. Since methanol is even more easily volatile than ethanol, it is no matter for surprise that the correlation is not better. Here again it may be noted that the height of the methanol peak was in general higher in 1962 than in 1963.

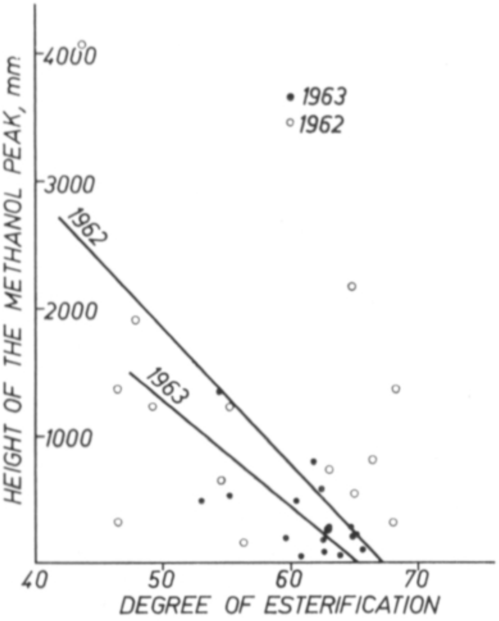

Fig. 6. Relation between the methanol content, measured as peak height of aromagrams, and degree of esterification of pectin in different varieties of black-currant.

Higher-boiling aroma compounds

Methods. It might be assumed from those aroma compounds, which have a boiling-point higher than that of water, that they are to a great part of terpene nature. In the buds of black-currants leaves, some terpenes and sesquiterpenes, such as nopinene, l-sabinene, d-caryophyllene and d-cadinene were found 1937 by Glichitch and Igolen (8); it seems evident that similar aroma compounds are found in both black-currant berries and in the leaves, although other compounds are probably also present in berries. Such terpene type and other higher-boiling compounds might be separated from the lower-boiling compounds by fractional distillation, generally employed in recovery of the aroma. However, it was noted in preliminary experiments that when an attempt at aroma recovery was made with steam distillation, marked changes took place in the aroma. The same conclusion was drawn by Tinner (13). It was accordingly considered advisable to avoid a high temperature, and the distillation was performed in vacuum. Further, it was borne in mind that the waxy materials found in the berry skin should be removed already at this phase; these waxes probably have little to do with the aroma, and in addition their presence is harmful to the chromatographic column. However, the distillate obtained cannot be studied as such by gas-chromatographic methods, as the columns do not tolerate water. The aroma compounds were therefore extracted from the distillate with an organic solvent. Such a procedure does not fully guarantee that the aroma obtained corresponds in all respects to the original aroma. It was thus necessary to check, by organoleptic methods, whether the aroma obtained had changed in its properties.

The material for this part of the study consisted of frozen berries of the year 1962. For the analysis, 250 g of berries were taken, an equal amount of distilled water added, and the berries were thawed on a water bath at 20°C. The mixture was homogenized in 3 lots with Bamix in a closed plastic vessel; the homogenization time was 1 minute. Isolation of the aroma from the homogenate was effected by vacuum distillation, with the temperature of the water mantle 50°C. The receiving vessel was chilled with ice-salt mixture at —18°C. The vacuum varied between 50 and 80 mm Hg, and the boiling point was correspondingly 38—46°C. The desired amount of distillate was 100 ml, but this varied in the different cases between 80 and 160 ml; the time required varied between 45 and 120 minutes. At the end of the distillation, part of the distillate in the receiving vessel was usually in the frozen state. The distillates were ordinarily kept at +4°C in vessels closed with fritted-glass cork until the following day. The extraction was performed in a liquid extraction apparatus holding 100 ml of distillate. The extraction time was 5 hours, and 100 ml of ethyl ether (p.a.) was used as extractant. The extract was dried with anhydrous Na_2SO_4; the bulk of the ether was evaporated in a Rotavap evaporator, following which approximately 6 ml of concentrated extract remained. Immediately before gas-chromatographic analysis, the rest of the ether was removed, so that only one drop of the aroma sample remained. — An endeavour was made to use ethyl cloride in the extraction (cf. 6); this solvent has the advantage of a boiling point as low as +12°C, and thus the aroma losses which occur in the evaporation of the solvent are minimal. However, in comparison of the two gas-chromatographic analyses it was discovered that ethyl chloride extracts evidenced no more components than the corresponding ether extracts. Ether was consequently chosen as the extractant, as it is simpler in use. — In gas-chromatography, a Perkin Elmer Fraktometer 116 was used with a 2 m column, filled with Celite (60—100 mesh), and as the stationary phase 15 per cent butandiol succinate polyester (BDS). The following conditions were chosen: P_{He} 0.8, P_{O_2} 0.8, P_{H_2} 0.5, flow rate 3,1, the temperature in the the main series being 120°C. Before gas-chromatography, the sample was concentrated to as small a volume as possible; it was drawn in an injection needle, which then contained partly liquid and partly gaseous phase, with a total volume of 25 μl. On the average, the time of the run was 2 hrs. — Apart from the main series, a checking run was effected at 150°C, to speed up the separation of the highest-boiling components. For this series, a new sample, 5 μl in size, was taken for the gas chromatography, by adding some drops of ether, from the sample tubes kept in the refrigerator. The conditions were as follows: P_{He} 0.8, P_{O_2} 0.8, P_{H_2} 0.5, flow rate 2.6 and temperature 150°C. The column was the same as above.

As in these experiments the amount of distillate taken for the ether extraction was in all cases 100 ml irrespective of the total volume of the distillate, and since in addition the transfer of the aroma concentrate in the gas chromatograph was not quantitative, the results obtained can be compared with each other only qualitatively; quantitative comparisons cannot be performed. If such comparisons had been required, the amount of starting material should have been greater, and aliquots taken for further treatment at the various phases; for this, the amount of berries available was inadequate. If, again, the ethereal extract were to have been used quantitatively for gas chromatographic analysis, a precise injection technique would have become necessary; no reliable method had been developed for this at the time of the study.

Results. When the curves obtained at 120°C are compared, 18 clear-cut and well separated peaks are discernible; in the last part, the peaks become flattened, and their comparability is poor. In all, approximately 25 peaks are present. Some of these are much higher than others; prominent peaks of this type are Nos. 10, 15, 16, 18, and 19. In the earliest part of the curves some very high peaks are noticeable, but their separation is not good. It was found from some model runs that easily volatile substances such as ethanol, propanol and butanol would in these conditions come out before peak No. 3; this area shows a rather high level. There exist slight differences between the different samples in respect to the occurrence and relative heights of the peaks; the reasons for such differences are considered below.

As regards the curves obtained at 150°C, the purpose was that of improving the picture in the last section; this, of course, led to poorer separation of the peaks at the start than was the case at 120°C. The identification and comparison of the peaks at 120 and 150°C is rendered somewhat difficult by the fact that some of the peaks may be missing at 150°C. However, the general course of the curves is so similar, that it may be possible to conclude from the highest peaks which of them correspond to each other in the two instances. Thus, at both temperatures, the highest peaks were Nos. 10, 15, and 19. The peaks were thus the same at both temperatures, except as regards the last high peak at 150°C, which would be missing at 120°C. In part, some other peaks may also be missing at 150°C, although they occur at 120°C; this is not very astonishing, as the samples were left standing for a rather long period before being analysed at 150°C. Peaks Nos. 16, 17 and 18 in particular

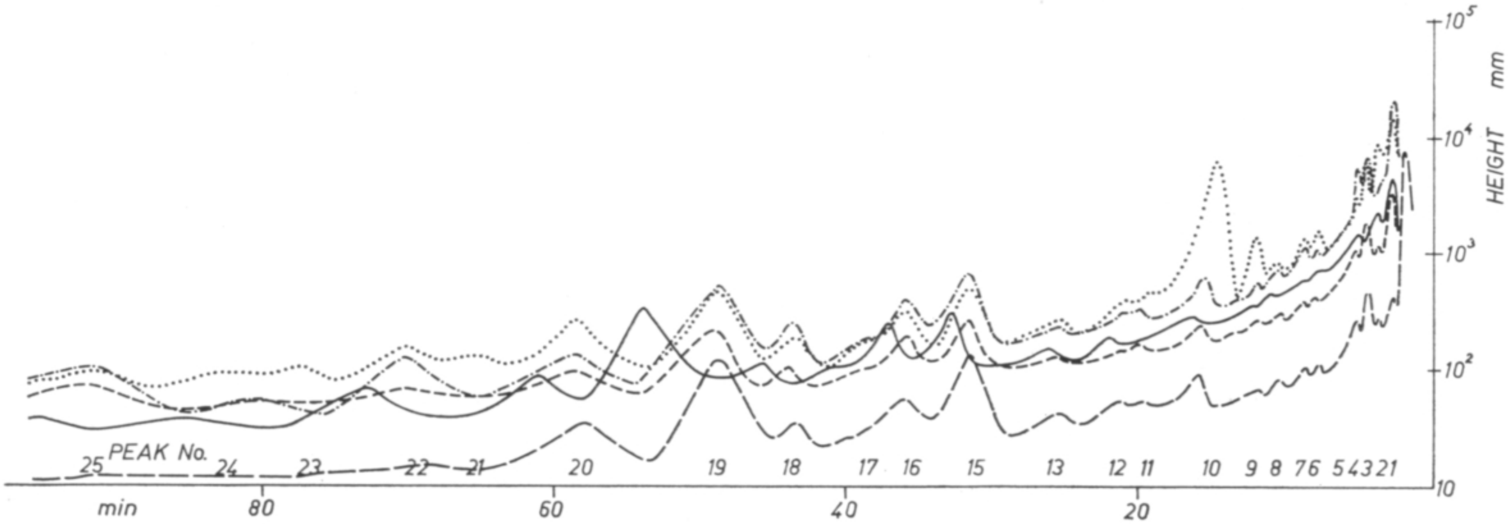

Fig. 7. Comparison of aromagrams of Brödtorp samples obtained from 5 different growing places in 1962. Higher-boiling compounds run at 120° C.

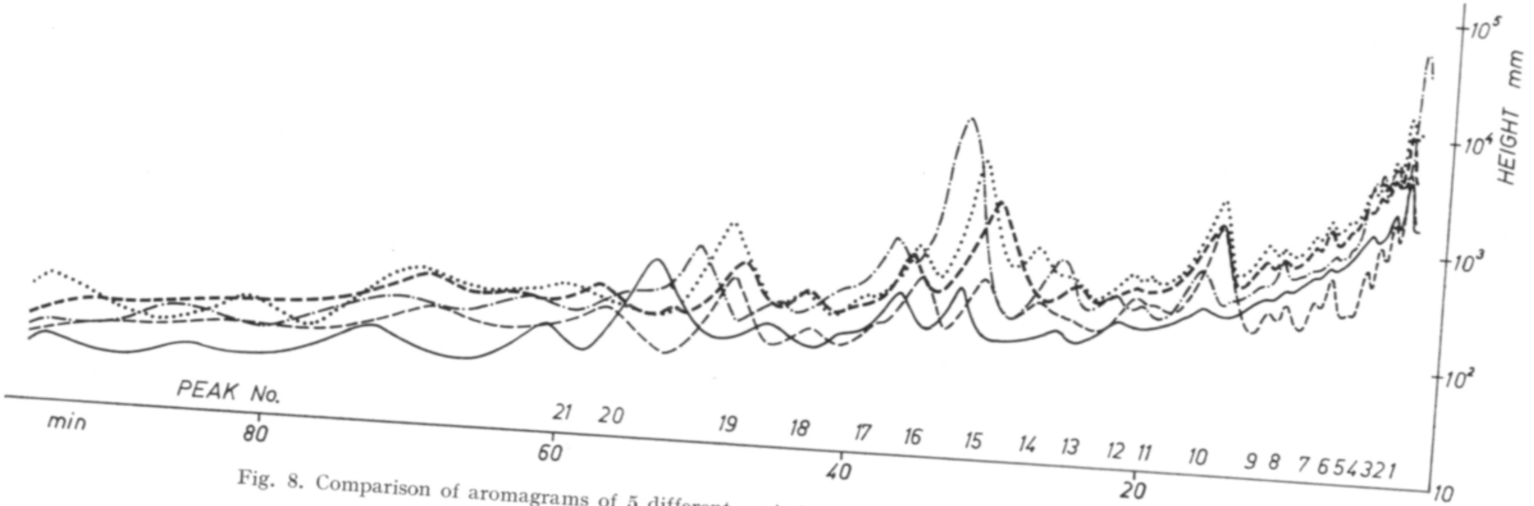

Fig. 8. Comparison of aromagrams of 5 different varieties from Piikkiö in 1962. Higher-boiling compounds run at 120° C.

— — — Wellington XXX
- - - - Wellington X
-·-·-·- Janslunda
· · · · · Goliath
——— Brödtorp

are poorly presented at 150°C. In contrast, the peaks following No. 19 are more clearly seen at 150°C than at 120°C.

To illustrate the results arrived at, comparison of the 5 samples of Brödtorp is presented at both 120 and at 150°C (Fig. 7, Fig. 9). For the comparison, the curves of these samples, obtained from different localities, have been drawn on a semilogarithmic paper, so that even the highest peaks are clearly visible, and the various curves are easily mutually comparable. In the same way, a comparison of 5 different varieties obtained from Piikkiö is presented at the two temperatures applied (Fig. 8, Fig. 10).

On examination of these illustrations, it must be borne in mind that the retention times are not always exactly the same, by reason of small variations in temperature regulation and other conditions during the run. This is seen in the aromagrams of the different varieties at 120°C, where peak No. 10 has still almost the same location for the varieties, although the peaks of Janslunda and Brödtorp are slightly more advanced than the others; this difference becomes more conspicuous towards the end. Nevertheless, the curves are well comparable, as in all cases the general features are relatively similar, and the differences between the varieties are easily detectable.

The results obtained here rendered it possible, in the same way as with the low-boiling compounds, to consider the effect exerted on the aroma picture by the variety, locality of growing, degree of ripeness and condition. In contrast, the effect of weather conditions is more difficult to assess, as the material is from the year

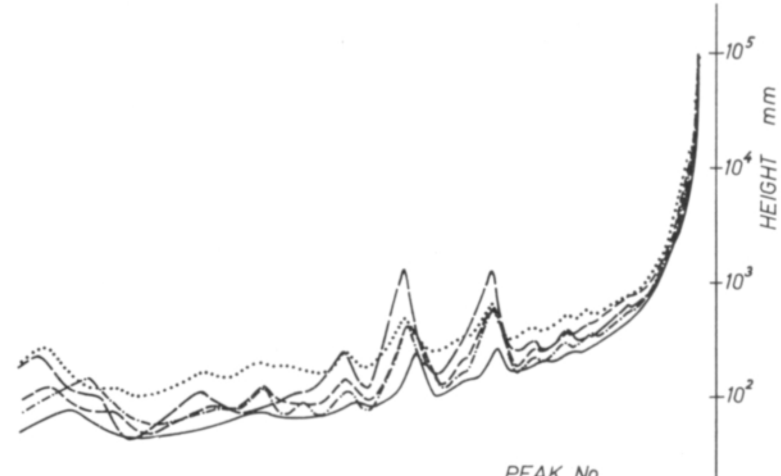

Fig. 9. Comparison of aromagrams of Brödtorp samples obtained from 5 different growing places in 1962. Higher-boiling compounds run at 150° C.

——————— Piikkiö
— — — — — Ylistaro
- - - - - - - - - Maaninka I
-·-·-·-·-·-·- Maaninka II
· · · · · · · · · · · · Rovaniemi

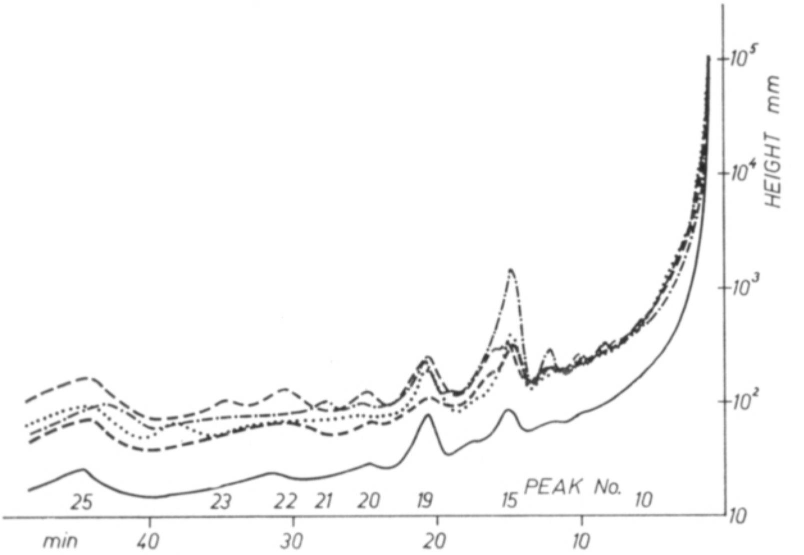

Fig. 10. Comparison of aromagrams of 5 different varieties from Piikkiö in 1962. Higher-boiling compounds run at 150° C. The curve of Brödtorp has been drawn at a lower level to facilitate comparison.

— — — — — Wellington XXX
_ _ _ _ _ _ _ _ _ Wellington X
_._._._._._._ Janslunda
. . . . Goliath
——————— Brödtorp

1962 only; the analyses of the 1963 material are lacking. Neither is quantitative comparison of the aroma practicable, as the amounts analysed do not correspond exactly to each other. Consequently, the qualitative aroma picture is employed for comparative purposes.

As concerns the effect of variety on the aroma picture, conclusions may be drawn from the comparison shown in Figs. 7, 8, 9, and 10. When a study is made of the aroma of the same variety at different localities — as an example of this, Brödtorp has been chosen, since the material was greatest here — it is noticeable that the aroma picture is very much alike in the different cases. For instance, peaks Nos. 14 and 24 are missing in all of them. Small differences discernible may be owing to the effect of locality, degree of ripeness and condition. By contrast, a comparison between various varieties from the same locality shows more differences. In particular, the relative amounts of components in the area of peaks Nos. 14—16 differ, although the peaks in the last part, best visible at 150°C, also evidence dissimilarities.

Conclusions on the effect of locality are rather difficult to draw, as it is not easy to be certain which is in fact the effect of locality, and which that of degree of ripeness and condition. However, it might be assumed that in more northern localities, more numerous components would be present; an indication in this direction is given by the comparison between the variety Brödtorp at Piikkiö and Rovaniemi, of which the former is southern, the latter northern, and where the berries were to some extent unripe in both cases. A similar general trend is observable in the comparison of the samples from Piikkiö with corresponding samples from Maaninka in the north, although here the possible effect of the prolonged transport in the latter case cannot be ignored.

Very few conclusions may be drawn in respect of the effect of degree of ripeness and condition. If an additional sample of Wellington XXX, originating in Great Britain and having by virtue of the long transport period definitely been fermented, is employed for comparative purposes, it is seen that in this case peaks Nos. 3, 9 and 10 were particularly high. However no similar features were noted in the other samples studied where long transport might have caused corresponding changes; thus in the Brödtorp samples, peaks Nos. 9 and 10 were higher than usual in the sample of Rovaniemi, which was more or less unripe. It thus appears that in the higher-boiling compounds the effect of degree of ripeness and the condition do not exercise such a marked effect upon the aroma picture as they evidenced in the lower-boiling compounds.

Organoleptic checking experiments. When the aroma is isolated from the starting material at room temperature by means of a nitrogen stream, it is probable that the aroma so obtained corresponds to the aroma composition, as it influences the sense of smell. This would accordingly also be the case here in respect of the lower-boiling compounds. By contrast, when isolation of the aroma is effected by vacuum distillation and subsequent ether extraction, it is less clear that the aroma obtained corresponds to that originally present in the berries. Some organoleptic tests were accordingly made to check the extent to which isolation of the aroma had been successful in retaining the original properties.

In smell-testing, it was found that there was still present a strong and rather typical smell of black-currant in the residue of the vacuum distillation; in the opinion of the judges it would have been difficult to make a mistake in this respect. Evidently, separation of the aroma by vacuum distillation had not been quantitative. However, it may be mentioned that isolation of the lower boiling compounds by the aid of a nitrogen stream had not been superior in this respect. In the distillate, the smell was not so characteristic as that of the residue: some of the judges referred to the smell as only »ester-like», others mentioned the smell of some other berries, and no more than a small proportion of the judges evaluated the smell as typical of black-currant. Thus the aroma of the distillate seems to correspond only to one part of the original aroma both qualitatively and quantitatively. The aroma of a distillate sample, which had been left standing, was evaluated as weakened. The residue of the ether or ethyl chloride extract was found even more divergent; here the descriptions of the judges hinted to the presence of terpenes: »bitter», »sticking smell», »orange peel», »terpene-like», »something pine-like». Again, the water phase of the distillate after ether extraction was characterized as »berry-like», although one of the judges even recognized the black-currant aroma in it.

It thus seems that the method applied in isolation of the higher-boiling compounds separated into the organic solvent mainly compounds of the terpene type, whereas at least a proportion of those substances which form the basis of the general berry aroma remained in the aqueous phases. These conclusions are, of course, of a preliminary nature, as identification of the compounds has not been effected.

Discussion

Although a vast quantity of information is available on gas-chromatographic aroma studies in general, and also a considerable amount of work has been done on fruit and berry aroma, very few studies have been concerned with the aroma of black-currant. In fact, the only significant contribution in this field is constituted by the investigations made in Sweden by von Sydow and his group (cf. 7; 1; 2; 3, 4, and 5), which may be useful for comparison. However, in this case as well the methods applied differ so greatly that comparison seems to be rather difficult. In the Swedish studies, the main aim was that of making precise identification of the aroma components, and measurement of their relative amounts, and a long list of various compounds in the higher-boiling range, mostly terpenes, has been reported. Nevertheless, not so much attention was attached to such factors as variety, locality, weather conditions and so on, as in the present study. Notwithstanding the considerable differences in methods, in both cases similar results have been found to the extent that definite varietal differences in the higher-boiling compounds have been noted (7; 3 and 5).

Summary

A gas chromatographic study has been made of the volatile aroma components of black-currant. The material consisted of 12 home-grown varieties from 6 localities taken over a period of 2 years, although the series are not complete. Separate investigation was made of the low-boiling and high-boiling parts of the aroma; in the former case, material of two years was studied, and in the latter case, that of one year only.

The lower-boiling components were isolated in chilled adsorption tubes from a nitrogen stream led through a berry homogenate at room temperature. Analysis was effected at 80°C with a polyetylene glycol/teflon column (the same mass served as filling for the adsorption tubes). In all, 13 components were registered, although their occurrence, and the amounts in the different samples, were somewhat variable. As regards the different factors, the variety exercised no more than a slight effect on the aroma picture, whereas weather conditions were important, influencing the frequency of the peaks and the general aroma level; a cool summer increased the level. The northernness of the growing locality had a similar effect, although this result its of preliminary nature. The degree of ripeness and the condition had a considerable effect; the level of aromagrams was higher with more ripe samples, and in particular the ethanol content, which forms the bulk of the low-boiling aroma, was increased. A positive correlation was detected between the height of the ethanol peak and the total aroma or aroma number. By contrast, the height of the methanol peak exhibited a slight negative correlation with the degree of esterification of the pectin.

The higher-boiling compounds were isolated by vacuum distillation and subsequent extraction with ether. In the gas chromatography, use was made of a butandiol succinate polyester/celite column, and the temperatures were 120 and 150°C.

In all, 25 components were observed. Of the various factors, the variety showed a clear-cut influence on the aroma picture, as differences in the occurrence of the peaks and the relative heights were noted between the varieties; in this series, quantitative comparisons between the samples were impossible. The effect of locality and degree of ripeness is less well defined.

Organoleptic checking showed that the changes were small in isolation of the lower-boiling components. In contrast, isolation of the higher-boiling compounds induced more changes, so that an aroma fraction obtained in the organic solvent was more terpene-like than the original aroma.

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SELOSTUS:

MUSTAHERUKAN TÄRKEIMMISTÄ LAATUOMINAISUUKSISTA ERÄILLÄ KOTIMAASSA VILJELLYILLÄ LAJIKKEILLA

IV. Aromin kaasukromatografinen analyysi

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Mustaherukan haihtuvia aromiaineita on tutkittu kaasukromatografian avulla. Tutkimusaineistona oli 12 kotimaassa viljeltyä lajiketta, joista saatiin näytteitä 6 eri paikkakunnalta 2 vuoden aikana, vaikkakaan sarjat eivät ole aukottomat. Alhaalla kiehuvat ja korkealla kiehuvat aromiaineet tutkittiin erikseen; edellisistä saatiin kahden vuoden sarja, jälkimmäisistä yhden vuoden.

Alhaalla kiehuvat aromiaineet eristettiin jäähdytettyihin adsorptioputkiin johtamalla huoneenlämmössä typpivista marjahomogenaatin läpi. Kaasukromatografia-ajossa käytettiin polyetylenglykoli/teflon pylvästä; ajolämpötila oli 80°C (adsorptioputkien täytteenä käytettiin samaa massaa). Kaikkiaan 13 komponenttia saatiin esille, vaikkakin niiden esiintyminen ja määrät eri näytteissä vaihtelivat jonkin verran. Eri tekijöistä lajikkeella oli vain vähäinen vaikutus alhaalla kiehuvien aromiaineiden suhteen, kun taas sääolosuhteilla oli voimakas vaikutus sekä huippujen esiintymisfrekvenssiin että yleiseen aromimäärään; viileänä kesänä aromimäärä oli suurempi. Kasvatuspaikan pohjoisuudella oli samansuuntainen vaikutus, mutta tätä tulosta on pidettävä alustavanluontoisena. Kypsyysasteella ja kunnolla oli melkoinen vaikutus; aromagrammien taso oli korkeampi kypsemmillä näytteillä, erityisesti etanoli, joka muodosti pääkomponentin alhaalla kiehuvassa fraktiossa, oli määrältään suurentunut. Positiivinen korrelaatio todettiin etanolihuipun korkeuden ja kokonaisaromimäärän sekä aromiluvun välillä. Päinvastoin taas metanolihuipun korkeus osoitti lievää negatiivista korrelaatiota pektiinin esteröitymisasteen kanssa.

Korkealla kiehuvat aromiaineet eristettiin vakuumitislauksella ja senjälkeen suoritetulla eetteriuutolla. Kaasukromatografiassa käytettiin butandiolsukkinaattipolyesteri/celiitti pylvästä, ajolämpötilat olivat 120 ja 150°C. Kaikkiaan todettiin 25 aromikomponenttia. Lajikkeella oli selvä vaikutus aromikuvaan, mikä näkyi huippujen esiintymisessä ja niiden suhteellisissa korkeuksissa; tässä koesarjassa ei ole mahdollista suorittaa varsinaisia kvantitatiivisia vertailuja eri näytteiden aromin kesken. Kasvatuspaikkakunnan ja kypsyysasteen vaikutus on vähäisempi.

Organoleptiset tarkistuskokeet osoittivat, että huoneenlämmössä suoritetussa aromieristyksessä aromin muutokset jäivät vähäisiksi. Sensijaan korkealla kiehuvien aromiaineiden eristys aiheutti enemmän muutoksia siten, että orgaaniseen liuottimeen saatu aromifraktio oli enemmän terpeenityyppinen kuin alkuperäinen aromi.