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SELECTION OF CARROTS FOR CAROTENE CONTENT

by

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INTRODUCTION

For breeding purposes and variety trials a simple method of carotene estimation is indispensable for a satisfactory comparison of the carotene content of carrots. But it should be reliable. In this respect it is not only the chemical method that must be considered, but also the behaviour of the carrot. This study is concerned with this last point.

ESTIMATION OF TOTAL CAROTENOIDS

For the estimation of the carotene content the simplified procedure of BOOTH (2) was used, in which the content of total carotenoids is determined. According to BOOTH (2) and BOOTH and DARK (3) carotene comprises about 90 % of the total carotenoids of carrots. Only in very young, weakly coloured roots, this percentage is considerably lower.

A mixed sample of 20 roots was normally used for each determination, this being carried out as follows:

The roots were washed, dried and grated. After mixing thoroughly 2–3 grams was weighed out accurately and ground with pure sand in a mortar. Next the extraction was carried out (excluding day light as much as possible) with about 15 ml. of a mixture of 3 parts of petroleum ether (boiling-point 40–60 °C) and 2 parts of acetone, adding a small quantity of exsiccated sodium sulphate to promote a clear separation of the extractant. This liquid was then poured off and the extraction repeated until the liquid ceased to absorb pigment. The extracts obtained were diluted to a certain volume, and the colour intensity measured on a photo-electric colorimeter set at 480 mμ. With the aid of a standard curve, obtained by measuring solutions with standard concentrations of β carotene, the content of total carotenoids, expressed as β carotene, can be calculated.

General trend of total carotenoids in carrots

In the literature most authors hold the same view as to the general trend of total carotenoids in carrots. There is a tendency for the t.c. content to increase as the root grows until a maximum is reached, after which increases in root growth bring about no further increases in t.c. content (1, 3, 4, 6, 7).

It can be seen with the naked eye that in their earliest infancy roots are pale. With a variety like *Amsterdam Forcing* this pale juvenile period is short; with a *Nantes* it is longer, and with a winter carrot it is longer still. Smaller differences are found between varieties that are related more closely, and also between the various strains within a variety.

The maximum which can be reached also depends on variety. Varieties such as *Amsterdam Forcing*, *Vertou* and *Chantenay Red Core* have high maxima; *Slender Nantes* [1]) and *Berlikumer* have maxima that are slightly lower, while most of the strains of *Flakkeese* have maxima that are lower still. Strains of one and the same variety may also differ in this respect. Early colouring is frequently associated with a high maximum (e.g. with *Amsterdam Forcing* and *Vertou*), late colouring with a low maximum (as in the case of bad strains of winter carrots), but not necessarily so. For example *Nantes* colours earlier than *Chantenay*, but the latter reaches a higher maximum (Werner 7, Lamprecht & Svensson 6). It is therefore quite possible that maxima are higher in late strains than in early ones, provided the former have sufficient time to obtain their maxima.

Consequently, to be able to assess the carotene producing capacity of varieties and strains, it may seem desirable to compare the roots both when they have started to colour and when they have reached their maxima. It is difficult, however, to obtain representative figures for such a comparison. In addition to the hereditary tendency of the carrots, environment also exerts an influence.

Moisture content of the carrot

Carotene is produced in chromoplasts, which are contained in the cytoplasm. In large, watery, comparetively cytoplasm-deficient cells with large vacuoles, there is relatively less room for carotene than in small cells with small vacuoles. The consequence appears to be that in a succulent type of root the carotene content per unit of fresh tissue can never be increased to the same level as is possible in dry roots.

Indications are that carrots with a high content of total carotenoids can have a rather high content of dry matter, which is exemplified in Table 1. *Kieler Rote* has a high t.c. content but is also high in dry matter. *Meaux* is also higher than *Berlikumer B*, both in dry matter and total carotenoids.

Table 1. Total carotenoids in three varieties

Variety	No.	Total carotenoids in mg. per 100 grams of fresh weight	Dry matter as % of fresh weight	Total carotenoids in mg. per 10 grams of dry matter
Kieler Rote	53021	15.6	14.3	10.9
Meaux	53001	9.7	13.2	7.4
Berlikumer B	53013	8.3	10.6	7.8

[1]) This name is used to indicate the group of *Touchon* and related slender types.

After eliminating the moisture factor, *Kieler Rote* has still a higher t.c. content, but *Meaux* and *Berlikumer B* appear to be practically equal to each other in this respect. That *Meaux* has a higher t.c. content per unit of fresh weight is therefore only due to its lower moisture.

When carrots are only selected for colour, or carotenoid content calculated on the fresh-weight basis, such a selection may automatically lead to a dryer carrot. It is then possible that the carrot selected has either a higher dry matter and a higher carotenoid content or one of these properties. This demonstrates that in selecting for root colour two things have to be considered viz. colour and succulence, or if a chemical method is applied, carotenoid content and dry matter content.

The same holds good for the varous strains within a variety, as appears from the following example:

TABLE 2. TOTAL CAROTENOIDS IN TWO STRAINS OF BERLIKUMER (EXP. 52G)

Strain	No.	Total carotenoids in mg. per 100 grams of fresh weight	Dry matter as % of fresh weight	Total carotenoids in mg. per 10 grams of dry matter
Berlikumer	52006	8.09	11.5	7.04
Berlikumer B	52023	7.27	10.2	7.13

Obviously, the two strains have practically the same carotenoid producing capacity. That 52006 is higher in carotenoids than 52023 is only due to the lower moisture content of the former.

If it is intended to grow a carrot only for its carotene (chicken-food and the like), it does not matter if the carrot is non-succulent. Carrots for human consumption, however, should be succulent, according to their use.

Not only is the moisture content of a carrot determined by its genotype, but also by environment. The conditions which alter the moisture content of carrots also alter the carotenoid content calculated on the fresh-weight basis.

This is clearly shown from periodic investigations by BROWN (4) during which a heavy shower obviously made the carrots so much more watery that the carotenoid content per 100 grams of fresh weight temporarily decreased by nearly 15–20 % of the carotenoid content determined before the rain.

From this it may be concluded that the carotenoid content per 10 grams of dry matter, also with a view to the influence of the growing conditions, is a better indicator than the carotenoid content per 100 grams of fresh weight, for thus at least one source of error is avoided.

DEGREE OF DEVELOPMENT OF THE CARROT

What has been said above does not imply that the carotenoid content in mg. per 10 grams of dry matter obtained for a variety or strain is always fully representative. This appears for instance from figures published by BARNES (1), and from some experiments carried out by the authors themselves. BARNES (1) tried to indicate the influence of soil moisture by making use of pot-trials. Carrots in pots with relatively much water were lower in dry matter than carrots in pots with less water. However,

the former contained more carotenoid per 100 grams of fresh weight so that conversion into carotenoid content per 10 grams of dry matter dit not give identical results. Field trials carried out by the authors produced similar results.

TABLE 3. TOTAL CAROTENOIDS REPRESENTING THE MEAN OF FOUR STRAINS OF AMSTERDAM FORCING, GROWN IN THREE LOCALITIES

Locality	Soil	Trial	Total carotenoids in mg. per 100 grams of fresh weight	Dry matter as % of fresh weight	Total carotenoids in mg. per 10 grams of dry matter
Elst	clay	52 H	8.48	9.1	9.32
Wag. Berg	dry sand	52 E	7.78	9.7	8.02
De Goor	moist sand	52 B	5.60	8.6	6.51

The three localities differed widely in soil moisture, which was lowest at Wageningse Berg and highest at De Goor. These differences were also reflected in the dry matter content (the dryer the soil the dryer the carrot) and in the carotene values. Even after the figures were converted into terms of dry matter, great differences remained.

It was shown, however, that these differences disappeared if the differences in root development of the carrots grown in the three localities were also taken into account. In this respect the way in which the development of the root is expressed is important.

For one of the four strains shown in Table 3 the course of the carotenoid content of the carrots grown in the above localities has been plotted in different ways against a certain standard for the degree of development of the carrots (fig. 1–3):

a. the carotenoid content in mg. per 100 grams of fresh weight against the age of the roots in days from sowing until harvest (fig. 1);
b. the carotenoid in mg. per 100 grams of fresh weight against the mean fresh weight of the carrots (fig. 2);
c. the carotenoid content in mg. per 10 grams of dry matter against the mean root-weight expressed in grams of dry matter (fig. 3).

On comparing the three figures it will be seen that the age of the root is the less suitable criterion for the carotenoid content, since the curves for the course of this content are widest apart.

The fresh weight of the root is a slightly better criterion, for the curves run a little closer to each other. It is, however, far from being satisfactory.

Fig. 3 reveals that the best criterion is afforded by the weight of the root expressed in grams of dry matter, for the three curves practically coincide. Independently of the locality, the carotenoid content increases as the root, with respect to its dry matter, becomes heavier.

Similar curves drawn for the other three strains show the same phenomenon.

In order to obtain a representative figure for a variety or strain it seems therefore indispensable to take also the mean root-weight in grams of dry matter into account. Also in determining the carotenoid content e.g. in manuring trials, this content is in itself of no value if it cannot be compared with the weight of the root, preferably expressed in grams of dry matter.

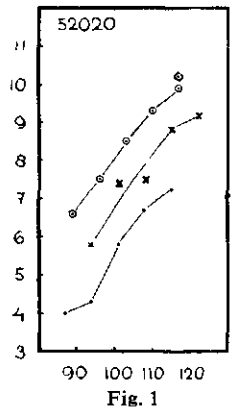

Fig. 1

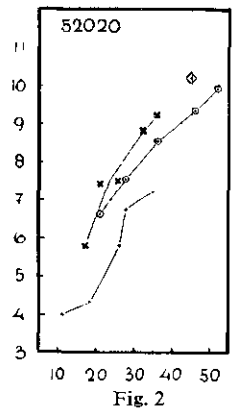

Fig. 2

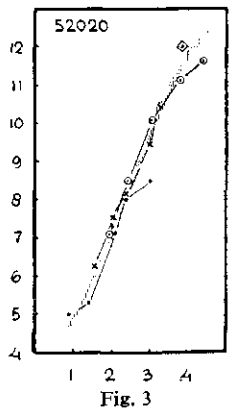

Fig. 3

FIG. 1. COURSE OF THE TOTAL CAROTENOID CONTENT OF A STRAIN OF AMSTERDAM FORCING IN THREE LOCALITIES AND ONE ESTIMATION IN A FOURTH LOCALITY. T.C. IN MG. PER 100 GRAMS OF FRESH WEIGHT (ORDINATE) PLOTTED AGAINST DAYS FROM SOWING TO HARVEST (ABSCIS)

◇ Bergum × Wageningse Berg

⊙ Elst (O.B.) . De Goor

FIG. 2. SAME AS IN FIG. 1, BUT NOW T.C. IN MG. PER 100 GRAMS OF FRESH WEIGHT (ORDINATE) PLOTTED AGAINST AVERAGE FRESH WEIGHT OF THE HARVESTED CARROTS (ABSCIS)

FIG. 3. SAME AS IN FIG. 1 AND 2, BUT NOW T.C. IN MG. PER 10 GRAMS OF DRY WEIGHT (ORDINATE) PLOTTED AGAINST AVERAGE WEIGHT OF HARVESTED CARROTS EXPRESSED IN GRAMS OF DRY MATTER (ABSCIS)

DIFFICULTIES OF THE MAXIMUM

If the maximum carotenoid content that is reached during the growing season had a constant value, this might be an important help in assessing the carotenoid content. It is therefore to be regretted that this maximum, even within the same variety or strain, can differ so widely from year to year.

BOOTH and DARK (3) expressed the maximum carotenoid values (per 100 grams of fresh weight), which they had found over a period of seven years, in index-figures. Representing the mean maximum by 1, the index in successive years from 1942–48 was as follows:

Year	1942	1943	1944	1945	1946	1947	1948
Index	1.12	1.04	0.92	1.18	0.79	1.09	0.86

Evidently, in different years the maximum values can fluctuate between about 80 % and 120 % of the mean value for a certain number of years. With carrots harvested towards the winter, this maximum mostly falls in October, but may also occur in August or as late as the end of November (BOOTH and DARK, 3; see also earlier references cited by these authors). Consequently the time when the maximum is reached is variable too.

From data of BARNES (1) it can be calculated that the phenomenon of the maximum remains if the carotenoid content, in mg. per 10 grams of dry matter, is plotted against the mean root-weight, expressed in grams of dry matter.

The maximum can best be determined if the carrots are sown early in the season, especially the late varieties. If sowing is done too late, the time required for attaining

the maximum will be insufficient. (See figures published by BOOTH and DARK (3), and by LAMPRECHT and SVENSSON (6)).

For carrots sown at the same time and in the same year the maximum may also differ with locality. Fig. 3 shows that the curve for the locality "De Goor" turns downwards as soon as the dry root-weight averages 2½–3 grams. The curve for Elst dips down when this weight is 4 grams.

The precise cause of the variation in the maximum is at present unknown. The maximum may be correlated with a stage of root development in which maturity is completed; and this stage may be caused to occur earlier or later not only by genetical but also by environmental factors.

Although the occurrence of a maximum is an interesting fact, it causes some difficulties in the search for a representative figure. In fact, the maximum fluctuates too easily to afford a reliable guidance. In addition it may disturb the relationship between the carotenoid content and the dry rootweight, for as long as the maximum has not been attained the carotenoid content appears to increase with the dry weight of the root. Once the maximum has been reached, this no longer holds good.

DISCUSSION

From the foregoing it can be concluded that the best representative values are obtained when:

a. a carrot is analyzed for total carotenoids when sufficiently coloured but before the maximum carotenoid content has been reached;
b. the carotenoid content is calculated on the dry-weight basis;
c. the weight of the carrot, in grams of dry matter, is taken into account.

Now the problem is how to know the moment that the carrots have sufficiently coloured but the maximum is far enough away. When comparing varieties or strains, from which many roots are available for investigation, periodical carotenoid estimations can be made. They will then show the course of the carotenoid content and the best moment for comparison can easily be selected afterwards.

But, when individual carrots must be compared, each root can be sampled only once. In this case it is thought that standard curves might be useful.

Fig. 4 shows the carotenoid curves for a number of early, midseason, and late varieties. They have been composed from the data from four replicated experiments in four different localities in 1953, in the same way as shown in fig. 3. Again, the total carotenoids in mg. per 10 grams of dry matter have been plotted against the root weight in grams of dry matter.

The varieties are *Amsterdam Forcing*, *Vertou*, *Slender Nantes*, *Chantenay Red Cored*, *Imperator*, *Berlikumer*, *Improved Flakkee* and *Leviathan*.

Amsterdam Forcing is represented by three strains, the numbers 52020, 52004 and 52010. The curves of the numbers 52020 and 52004 fall together. The carotenoid content should be estimated when the dry root weight is about 2–2½ grams.

Vertou is represented by one strain, no 52001. It has a high carotenoid content, but not so high as *Amsterdam Forcing*. The best moment for carotenoid estimation seems to be when the dry weight of the roots is about 2–3 grams.

Slender Nantes is represented by two strains, the numbers 52022 and 52009. The carotenoid content is lower than that of *Vertou*. A dry weight of the roots of about 3–5 grams seems to be adequate.

Chantenay Red Cored (52013) has a high carotenoid content. It should be assessed before the dry root weight is about 16 or 17 grams.

The same may be good for *Imperator*. Its maximum is lower than that of *Chantenay Red Cored*.

Berlikumer is represented by two strains, the numbers 52023 and 52006. Being somewhat later than the last two varieties, its maximum may fall later too. But it seems safest to estimate the carotenoid content before the dry root weight is about 18 grams.

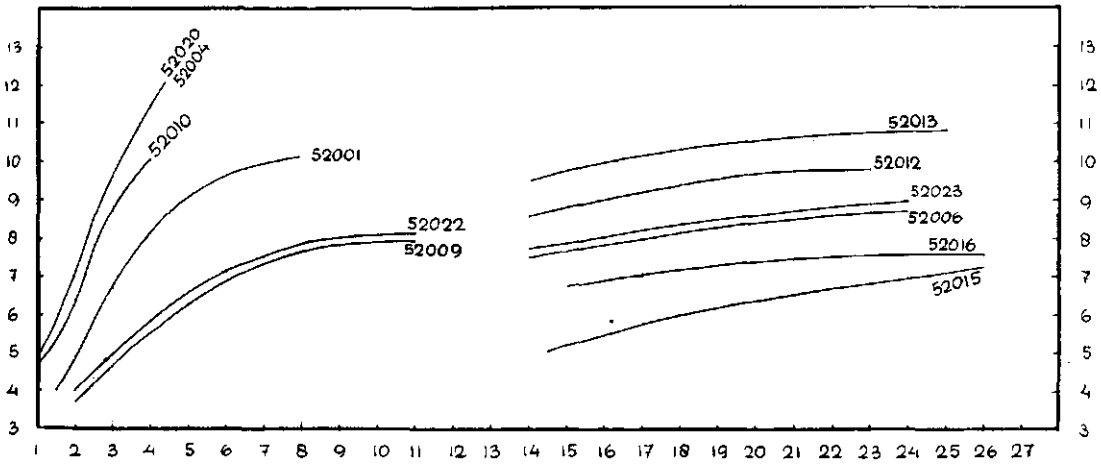

FIG. 4. COURSE OF THE TOTAL CAROTENOID CONTENT OF A NUMBER OF EARLY, MIDSEASON, AND LATE VARIETIES OF CARROT. T.C. IN MG. PER 10 GRAMS OF DRY WEIGHT (ORDINATE) PLOTTED AGAINST AVERAGE WEIGHT OF HARVESTED CARROTS EXPRESSED IN GRAMS OF DRY MATTER (ABSCIS). CURVES COMPOSED FROM THE DATA FROM FOUR EXPERIMENTS IN THE SAME WAY AS SHOWN IN FIG. 3.

Amsterdam Forcing	52020, 52004, 52010	Imperator	52012
Vertou	52001	Berlikumer	52023, 52006
Slender Nantes	52022, 52009	Improved Flakkeese	52016
Chantenay Red Cored	52013	Leviathan	52015

The same may be true of *Improved Flakkee* (52016) and *Leviathan* (52015). *Leviathan* may grow out to a very large root. Evidently its maximum is later than that of any of the other varieties considered.

Reliable standard curves may be obtained by periodical carotenoid estimations of samples of the population from which individual roots are selected. In order to be able to compare the total carotenoids of individual roots with the standard curves the fresh weight of the whole root and representative values of its dry matter and total carotenoids must be estimated. From these data the root weight in grams of dry matter and the t.c. in mg per 10 grams of dry matter can be calculated. To get representative values for the dry matter and t.c. the cylinder method of BOOTH (2) seems to be the best usable.

There are indications that the relation t.c. content – dry root weight may be influenced by an abnormally early or late ripening of the root. This problem will be considered in a later article, after some more experiments will have been made.

SUMMARY

1. For breeding purposes and variety trials a study has been made of how to get representative values of the carotene content of carrots. For the estimation of the carotene content the simplified procedure of V. H. BOOTH was used, in which the content of total carotenoids is determined.
2. Calculation of the carotenoid content on a dry-weight basis gives more representative values than that on a fresh-weight basis. Differences in carotenoid content, when calculated on a fresh-weight basis, may partly or wholly be the result of the selection of a dryer type of carrot, or they may be influenced by those growth conditions which modify the moisture content of the root.
3. There is general agreement that the carotenoid content of normally growing carrots increases with their development till a maximum is reached. After this the carrot may grow larger without a further increase in carotenoid content. However, since the maximum carotenoid content may vary with season and locality, it is in itself not a representative character. The degree of development of the root must also be taken into account.
4. From the evidence produced it is concluded that the best representative values of carotenoid content are obtained, when:
   a. the carotenoid content is determined in sufficiently coloured roots before the maximum is reached;
   b. this content is calculated on a dry-weight basis; and
   c. it is compared with the root weight, expressed in grams of dry matter.
5. For a number of carrot varieties graphs are given in which the relation between carotenoid content and root weight (in grams of dry matter) is shown.

SAMENVATTING

Selectie op caroteen-gehalte bij wortels

1. Voor veredelingsdoeleinden en rassenonderzoek werden de mogelijkheden ter verkrijging van representatieve waarden voor het caroteengehalte van wortels bestudeerd. Dit gehalte werd bepaald volgens de vereenvoudigde methode van V. H. BOOTH, waarbij het gehalte aan totale carotenoïden bepaald wordt.
2. De waarden verkregen door berekening van het carotenoïden-gehalte op basis van het drooggewicht zijn representatiever dan die welke berekend worden op basis van het versgewicht. Verschillen in het carotenoïden-gehalte, indien berekend op het versgewicht, kunnen geheel of gedeeltelijk het gevolg zijn van de selectie van een droger worteltype, of teweeggebracht zijn door die groei-omstandigheden welke het vochtgehalte van de wortel veranderen.
3. Er is algemene overeenstemming dat het carotenoïden-gehalte van normaal groeiende wortels tegelijk met de groei van de wortel stijgt tot een maximum is bereikt, waarna wel de wortel kan doorgroeien maar geen verdere stijging van het

carotenoïden-gehalte plaats heeft. Daar verschillen in groeiplaats en groeiseizoen ook in het maximum tot uitdrukking kunnen komen, is het maximum op zichzelf geen vast richtsnoer. De ontwikkelingsgraad van de wortel dient eveneens in aanmerking genomen te worden.

4. Het bovenstaande leidt tot de conclusie dat de beste representatieve cijfers voor het carotenoïden-gehalte verkregen worden, indien:
   a. het carotenoïden-gehalte bepaald wordt bij voldoend gekleurde wortels, alvorens het maximum bereikt is;
   b. dit gehalte berekend wordt op basis van het drooggewicht; en
   c. het vergeleken wordt met het wortelgewicht, uitgedrukt in grammen droge stof.
5. Voor een aantal wortelrassen worden diagrammen gegeven, waarin de verhouding tussen carotenoïden-gehalte en wortelgewicht (in grammen droge stof) tot uitdrukking komt.

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