PHYSICAL AND CHEMICAL CHANGES OCCURRING IN THE CARROT ROOT DURING GROWTH

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Parallel to the elongation and the thickening of the carrot (*Daucus carota* L.) root, there is an active synthesis of sugars, mainly sucrose, and carotenes, mainly β-carotene. The contents of both groups of substances reach their maximum at the end of the 3rd mo after seeding, then remain almost constant. Organic and amino acids, which are present in rather low concentration, increase slowly with age. In a growing carrot, pyruvic acid is the most plentiful acid, followed by isocitric and malic. Phenolic compounds, abundant in the very young root, decrease rapidly during the first 3 mo, then remain at a plateau level up to harvest date.

Parallèlement à l'élongation et à la tubérisation de la racine de carotte (*Daucus carota* L.), il se produit une synthèse active de sucres, principalement du saccharose, et de carotènes, surtout de la β-carotène. Les teneurs respectives de ces deux groupes de substances atteignent leurs maxima à la fin du troisième mois suivant l'ensemencement, puis demeurent quasi constantes. Les acides organiques et aminés, dont les concentrations sont toujours faibles, augmentent quelque peu avec l'âge. Dans la carotte en croissance, l'acide pyruvique est le plus abondant, suivi par l'acide isocitrique et l'acide malique. Les composés phénoliques, abondants dans la très jeune racine, diminuent rapidement pendant les 3 premiers mois, puis demeurent pratiquement inchangés jusqu'à la date de la récolte.

INTRODUCTION

Although the carrot (*Daucus carota* L.) root has great nutritional and economic value, there is no recent comprehensive review of the physiological and biochemical changes occurring during its growth. Apart from Plantenius' (1934) data and two more recently published articles by Goris (1969*a*, *b*) relating data obtained between 1946 and 1949, most of the papers found in the literature are concerned with particular or abnormal aspects of carrot physiology (Marsh and Goddard 1939*a*, *b*; Gautheret 1938; Adams 1970; Chubey and Nylund 1970). In this research, which was ultimately aimed at better conditions for carrot storage, we have tried to gather comprehensive information on the carrot root as a whole organ during its growth up to harvest.

MATERIALS AND METHODS

Carrots of the cultivar Imperator 11 were used in this study. They were grown at the University of Alberta Parkland Farm in Edmonton.

Methods of Sampling

To obtain the most detailed information possible, we analyzed the xylem and the phloem separately. This could be done only with roots that had reached the weight of 20 g, and on carotenes and sugars for which the estimation techniques were very sensitive. In these instances, three carrot roots were used for each sample. They were cut into 1-inch (2.54-cm) long segments and the xylem and phloem parts were separated by hand. All the fragments of the same tissue were collected and ground in a Waring blender without addition of any solvent. Three aliquots of 0.5 g were taken from the slurry for sugar and carotene extraction.

For organic and amino acids, which are present in low concentrations, larger samples were necessary. Also, three carrots were used for each sample. They were cut into 1–2-mm thick slices, which were picked out at random to make two samples of 50 g. For phenol estimation, three samples of 5 g were made in a similar way.

Methods of Extraction

EXTRACTION OF PIGMENTS AND SUGARS. The 0.5-g sample of carrot tissues was transferred

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into a mortar and ground finely with a pestle in the presence of sea sand and acetone. After filtering through a Büchner funnel, the filtrate was transferred to a separatory funnel and partitioned with petroleum ether. The aqueous acetone (lower) layer was concentrated and used for sugar determination. The petroleum ether layer, dehydrated, evaporated to dryness, and redissolved in petroleum ether, was used for carotene estimation.

EXTRACTION OF ORGANIC AND AMINO ACIDS. The 50-g sample of carrot slices was introduced into an Erlenmeyer flask containing 100 ml of boiling water and refluxed for 5 min. After cooling, the mixture was ground in a Waring blender and filtered on a large Büchner funnel. The cake was reextracted twice with water. All the filtrates were collected, concentrated, and made to a known volume. This solution served for organic and amino acid determination, and to the subsequent identification of these and of the sugars.

EXTRACTION OF TOTAL PHENOLS. The 5-g sample of carrot slices was homogenized and extracted by refluxing with 70% methanol for 1 h. The extract was then evaporated to remove the methanol. Because of the eventual development of a green color due to a reaction between amino acids and the phenols, the extract was passed through a cation exchange resin column (Rexyn 101, H^+ form, 16–50 mesh). The effluent was then concentrated and made up to a known volume, generally 100 ml, and a 1-ml aliquot was used to determine the phenolic content.

Estimation Techniques

SUGAR ESTIMATION. An aliquot of the sugar solution was hydrolyzed with 6 N formic acid for the estimation of total sugars. Another aliquot was used for the determination of free reducing sugars. Estimations were made by Somogyi's (1945) technique. The difference between the total and reducing sugar values corresponds to nonreducing sugars.

ORGANIC AND AMINO ACID ESTIMATION. An aliquot of the water extract was used for a combined titration of free carboxylic acids and of amino acids. Another aliquot was allowed to pass through a cation exchanger column and the effluent was used for total acidity determination. Organic acids were titrated with barium hydroxide in the presence of phenol phthalein. To the neutralized sample resulting for free acidity determination, an adequate volume of formaldehyde was added and amino acids were titrated according to Sørensen (1907).

CAROTENE ESTIMATION. Carotene was estimated spectrophotometrically by measuring the light absorption at 446 mμ. The corresponding amount of carotene was given by comparison with a standard curve. The solvent used was petroleum ether.

TOTAL PHENOL ESTIMATION. The total phenols were estimated by the method of Swain and Hillis (1959) except that Folin-Ciocalteau reagent was used in place of Folin-Denis reagent (Sarkar 1972). Chlorogenic acid was used to prepare the standard curve.

Qualitative Analysis

Sugars and amino acids were identified by paper chromatography, using the n-butanol–acetic acid–water (4:1:5, v:v:v) solvent system. The sugars were revealed by spraying with a phosphoric solution of para-anisidine and the amino acids with ninhydrin. Organic acids were studied first by paper chromatography (solvent system: n-butanol–formic acid–water (4:1:5, v:v:v) spray: 0.04% bromocresol green in ethanol), and later by gas chromatography, as methyl esters on a column of Reoplex 400, with temperature programmed from 50 C (isothermal for 5 min then) 4 degrees C per min, up to 170 C. The pigments were separated on a column of magnesium oxide; elution was made with hexane mixed with various proportions of acetone.

RESULTS

Physical Changes During Growth

The anatomical changes during the development of the so-called "carrot root" has been described by Esau (1940). Our biometric data are in agreement with her description. The first organs to grow very actively were the leaves. These reached 13–18 cm long within 2 wk. During the same time, the stem remained almost unchanged whereas the lateral roots became more and more numerous. It was not before 1 mo later that the main root started to grow longer, but it remained thin for another month. The root growth was then faster than that of the leaves. Then there is a second active extension of the leaves followed by a noticeable elongation of the root. Finally both leaves and roots, which have reached their maximal length (about 26–39 cm) stop growing. The root began to enlarge laterally when it had reached about half of its maximal length. The thickening was slow at the start but became faster and faster. The rate of growth

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reached a maximum at the time of "biochemical maturity (see below) but growth did not cease and the root continued to thicken slowly after that date. This is the economic reason for late harvesting.

Chemical Changes During Growth

CAROTENE. The roots of the seedlings were colorless. Only about 1 mo after seeding will the yellow color appear. The carotene content increased steadily up to a maximum of about 3 mo after seeding, then decreased slightly. This maximum was reached at 100–150 g, then remained practically constant (Table 1). The carotene content of the phloem was almost double that of the xylem.

On the magnesium oxide column six bands were separated. Two of them were eluted with hexane, and had the spectra of α- and β-carotenes. The third component, eluted with hexane mixed with 10% acetone, was identified as ε-carotene. The three other bands, eluted successively with hexane mixed with 20, 50, and 80% acetone, have not yet been identified with certainty. β-carotene and α-carotene and the "80% acetone-component" are the prominent pigments of the carrots (75, 63, and 13%, respectively, of total carotene extract).

SUGARS. In a preliminary experiment, it has been found that the sugar content of carrot roots increased steadily to reach a plateau about 3 mo after seeding. In a later experiment, we attempted to estimate separately the sugars from the phloem and from the xylem. The contents of reducing and nonreducing sugars are higher in the phloem than in the xylem (Table 2). The ratio of nonreducing sugars to reducing sugars in the xylem increases slowly to become similar to that of the phloem at the later stages of development. In both tissues the reducing sugar content remained rather constant, whereas the ratio of nonreducing sugars to reducing sugars increased exponentially. During its growth the carrot root seems to actively synthesize or accumulate, or both, the nonreducing sugars, mainly sucrose. The only reducing sugars found were fructose and glucose.

ORGANIC ACIDS. Carrot roots have a low content of organic acids. These represent about 0.2% of the fresh weight. The variations of the acidity during growth are given in Table 3. There seems to be a maximum, somewhat later than that of the carotene, then a decrease. The third column of Table 3 shows that nontitratable acidity is high in carrot. Eleven organic acids have been separated by gas chromatography of which 10 have been identified. The compound that emerges out of the column just before malic acid has not been identified with certainty; it is tentatively given the name of oxaloacetic acid. Table 4 gives the variations of each acid (as a percentage of total acidity) at various stages of growth. The most abundant acids are, in decreasing order, pyruvic, oxaloacetic, isocitric, and malic acids.

Table 1. Carotene contents of growing carrots

Fresh wt (g)	Carotene (μg/g fresh wt)			
	Whole carrot	Xylem	Phloem	P/X
0–2	88.37			
2–5	103.07			
5–10	147.54			
10–15	205.39			
15–20	248.31			
20–25	281.56	160.26	336.47	2.3
25–30	290.72	166.60	380.56	2.3
40–60	387.29	235.90	497.90	2.1
60–100	375.70	241.32	488.63	2.0
100–150	591.34	333.14	648.61	1.9
150–300	534.04	260.10	598.33	2.3

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Table 2. Sugar contents of growing carrots

Fresh wt (g)	Reducing sugars (mg/g fresh wt)				Nonreducing sugars (mg/g fresh wt)				Nonred./Red.		
	Whole carrot	Xylem	Phloem	P/X	Whole carrot	Xylem	Phloem	P/X	Whole carrot	Xylem	Phloem
0–2	16.83				6.53				0.39		
2–5	20.72				3.50				0.17		
5–10	17.00				6.61				0.39		
10–15	21.14				7.52				0.36		
15–20	16.67				7.78				0.47		
20–25	17.77	11.96	23.53	1.9	16.09	3.09	8.14	2.6	0.91	0.26	0.35
25–30	19.01	15.41	24.41	1.6	13.09	4.48	15.02	3.3	0.69	0.29	0.62
30–40	17.96	15.59	19.44	1.2	15.31	10.96	21.63	1.9	0.85	0.70	1.11
40–60	14.00	13.30	15.72	1.2	17.03	16.46	21.09	1.3	1.22	1.24	1.34
60–100	19.48	15.14	18.56	1.2	20.60	16.94	24.30	1.4	1.06	1.12	1.31
100–150	13.90	10.25	14.02	1.4	15.52	17.23	21.78	1.3	1.12	1.68	1.55
150–300	22.92	15.39	24.69	1.6	49.22	31.51	53.37	1.7	2.15	2.09	2.16

Table 3. Organic acid, amino acid, and phenol contents of growing carrots

Fresh wt (f.w.) (g)	Titratable acidity (meq/100 g f.w.)	Total acidity (meq/100 g f.w.)	Nontitr. / titr.	Amino acids† (meq/100 g f.w.)	Phenols (meq/100 g f.w.)
15–20	1.136	8.720	6.7	1.03	527.26
30–40	1.194	8.005	5.7	1.09	426.69
60–100	1.072	9.467	7.3	1.26	421.62
100–150	1.528	9.222	5.0	2.17	452.87
150–300	1.338	8.364	5.3	2.17	296.68

‡Sørensen technique gives only the amount of carboxyl groups.

Table 4. Changes in organic acid composition of carrots during growth (% of total acids)

Acid	Weight (g)				
	2–5	25–30	40–100	150	150–300
Pyruvic	22.6	18.3	16.4	45.3	41.6
Glyoxylic	0.8	0.2	0.2	0.1	1.3
Fumaric	9.0	4.2	10.0	1.7	5.5
Succinic	14.4	10.6	13.3	7.1	12.8
Oxaloacetic (?)	13.6	13.7	22.3	7.1	14.0
Malic	22.1	31.3	7.1	13.1	15.1
Citric	7.9	8.7	3.4	1.5	7.9
Isocitric	9.3	11.6	10.0	29.2	8.4

AMINO ACIDS. Amino acids also are present in rather low concentration in carrot roots. They tend to increase with growth (Table 3). Their composition is similar to the amino acid fraction of most plant tissues. There are about 20 components, among which the most important are, in decreasing order, alanine, glutamic acid, and aspartic acid.

PHENOLIC COMPOUNDS. Their content decreased rapidly then remained almost constant as the carrot matured (Table 3). The main phenol found in growing carrots was chlorogenic acid.

DISCUSSION

It appeared from the results obtained with sugars and carotene, which are the most significant compounds in the carrot roots, that these organs reached their maturity about 3 mo after seeding. These results are in accordance with those of Platenius (1934) and Goris (1969*a*, *b*). The latter, who reported first a sudden reversal of the proportions of the reducing sugars and of sucrose, found later that such a change did not occur with all the cultivars of carrots studied. Goris also noticed, as we did in this work, that the sugar content increased rapidly towards a certain value, and then remained on that plateau up to the harvest date.

However, this "biochemical maturity" is reached while the carrot root is still growing, especially in diameter, and can therefore not be taken as a criterion for determining the harvest date. Subsequent experiments did not show any significant effects of late harvest dates on the storage physiology of the carrots.

There was a high proportion of nontitratable organic acid. Chemically this means that the cellular content of the carrot root is remarkably buffered. This has a bearing on the organoleptic characteristics of the carrot. In comparing the acid composition of the growing carrots with that of the stored ones, a large amount in the growing carrots of the compound was tentatively identified as oxaloacetic acid and pyruvic acid. Malic acid is relatively less abundant in the growing than in the stored carrots. Also noticeable is the rather high proportion of isocitric

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acid. It is difficult to make relevant comments on these results without specific biochemical experiments, but the accumulation of oxaloacetic acid, as well as of the other abundant acids that all belong to the Krebs cycle, seems to indicate a certain degree of inhibition of this respiratory pathway. This can be reckoned for by the fact that carrot roots are underground organs; they normally grow in an environment where oxygen is not as freely available as in air. It is known that there is a close relationship between oxaloacetic acid and pyruvic acid. The latter may be produced by the catabolism of sugars, which are very abundant in the carrot root, and converted to oxaloacetic acid. There is also a close relationship between malic and isocitric acids through the glyoxylate pathway. Accumulation of isocitric acid would indicate that its conversion to malic acid via isocitrate lyase is not so active. Although the neutral lipid content of the carrot root is rather low, the lipids must play an important role because of the prominent occurrence of carotene, which, being fat-soluble, must be associated with lipids, and which has common precursors with lipids.

Our results on the phenolic content of growing carrots contradict those of Chubey and Nylund (1970), who found that the phenolics increased with age up to the harvest. We have no explanation for this discrepancy. However, our qualitative analysis is in agreement with Chubey and Nylund's (1970) data, i.e., that the major component of carrot phenols is chlorogenic acid.

CONCLUSION

In trying to understand the changes that occur in the carrot root during its growth, we should bear in mind that the harvesting of the carrots is different from the "harvesting" of a fruit. The carrot plant has a 2-yr cycle. Thus, the carrot that we dig out for our consumption is a root, which is in full metabolic activity, of which the life cycle is far from terminating, and which, therefore, would normally undergo a long series of transformations: lignification, winter rest, spring reactivation, if left in the soil. Its structure as a storage organ makes it possible for the carrot root to preserve most of its biochemical regulatory systems long after harvest. Comprehension of this point will help us understand the physiology of stored carrots.

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LITERATURE CITED

ADAMS, P. B. 1970. Effect of adenine nucleotides on the respiration of carrot root slices. Plant Physiol. **45**: 495–499.

CHUBEY, B. B. and NYLUND, R. E. 1970. The effect of maturity and environment on phenolic compounds and oxidative browning in carrots. J. Amer. Soc. Hort. Sci. **95**: 393–394.

ESAU, K. 1940. Developmental anatomy of the fleshy storage organ of *Daucus carota*. Hilgardia **13**: 175–224.

GAUTHERET, R. J. 1938. Recherches sur la culture de tubercules de carotte. C. R. Acad. Sci., Paris **206**: 457–459.

GORIS, A. 1969*a*. Les sucres de la racine de carotte cultivée. Qual. Plant. Mat. Veg. **18**: 283–306.

GORIS, A. 1969*b*. Métabolisme glucidique de la racine de carotte cultivée (Variété Nantaise demi-longue) au cours du cycle végétatif de la plante. Qual. Plant. Mat. Veg. **18**: 307–330.

MARSH, P. B. and GODDARD, D. R. 1939*a*. Respiration and fermentation in the carrot, *Daucus carota*. I. Respiration. Amer. J. Bot. **26**: 724–728.

MARSH, P. B. and GODDARD, D. R. 1939*b*. Respiration and fermentation in the carrot, *Daucus carota*. II. Fermentation and the Pasteur effect. Amer. J. Bot. **26**: 767–772.

PLANTENIUS, H. 1934. Chemical changes in carrots during growth. Plant Physiol. **9**: 671–680.

SARKAR, S. K. 1972. Ethylene and phenolic metabolism of stored carrots. Ph.D. Dissert. Univ. of Alberta, Edmonton, Alta.

SOMOGYI, M. 1945. A new reagent for the determination of sugars. J. Biol. Chem. **160**: 61–68.

SØRENSEN, S. P. L. 1907. *In* C. A. Streuli and P. R. Averell, eds., The analytical chemistry of nitrogen. Wiley-Interscience, New York, N.Y., 1970, Part 2, pp. 635.

SWAIN, T. and HILLIS, W. E. 1959. Phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agr. **10**: 63–68.