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Registry No. HyP, 51-35-4; HyL, 1190-94-9; L-Ala, 56-41-7; L-Tyr, 60-18-4; L-Met, 63-68-3.

Carotenoids and Provitamin A Activity of Carrot (*Daucus carota* L.) Cultivars

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The carotenoid and provitamin A content of 19 cultivars of orange carrot (*Daucus carota* L.) were analyzed by high-performance liquid chromatography. The amounts of the predominant carotenoids, α- and β-carotene, ranged from 2200 to 4900 and from 4600 to 10 300 μg/100 g of fresh weight, respectively. High α- and β-carotene contents were found especially in carrot cultivars Nantes Duke Notabene 370, Nantes Fancy Notabene 405, Narbonne F_1 BZ, Nelson F_1 BZ, Nantucket F_1 BZ, and Berlicum R. The tentatively identified 15-*cis*-β-carotene accounted for approximately 4.3% (range 1.3-12.9%) of the amount of *all-trans*-β-carotene. All carrot cultivars contained γ-carotene (range 630-2700 μg/100 g of fresh weight). Also, lutein (110-560 μg/100 g of fresh weight) was present. Expressed as retinol equivalents (RE, μg/100 g), the amount of provitamin A in carrot cultivars was between 1200 and 2300, an amount high enough to satisfy the human daily need of vitamin A.

Carrot is one of the most important vegetables grown and stored in Finland. Good orange color is essential for carrots sold on the fresh vegetable market and used for food processing. The main variation in the color of carrot is due to genotype (Bajaj et al., 1980; Gabelman, 1974), but the development stage of the plant (Banga et al., 1963; Bajaj et al., 1980; Evers, 1989; Hårdh et al., 1977) and temperatures during the growing season (Banga

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Table I. Carotenoids and Provitamin A Activity in Carrot (*D. carota* L.) Cultivars

cultivar	site[a]	n[b]	carotenoids, μg/100 g ± SD (fresh weight) α-carotene	β-carotene	γ-carotene	lutein	RE[c]
Bangor F_1 BZ[d]	B	5	2500	6600	850	160	1400
Bergen F_1 BZ	B	3	2500	5600	1200	130	1200
Berlicum N	C	3	2600	6000	800	400	1300
Berlicum R	C	3	4600	8500	2400	560	2000
Casey F_1 BZ	B	3	3500	6900	1200	220	1500
Chantenay R	C	5	2500	6100	630	450	1300
Flakkeer G	C	6	2200	5500	640	210	1200
Flakkeer R	C	3	2700	6300	950	340	1400
Flaxton F_1 BZ	D	5	2700	5600	1300	130	1300
Florence F_1 BZ	B	3	2700	4600	2500	120	1200
Fontana F_1 BZ	B	3	3000	6000	2700	110	1500
Nairobi F_1 BZ	B	2	3000	6000	930	360	1300
Nantes Duke Notabene 370	A	2	3900	8400	1700	200	1900
Nantes Fancy Notabene 405	B	3	4200	7900	1600	180	1800
Nantucket F_1 BZ	B	3	4200	7400	1600	260	1700
Napoli F_1 BZ	A	3	3600	4800	1200	190	1200
Narbonne F_1 BZ	B	3	4800	10300	1900	380	2300
Nelson F_1 BZ	B	3	4900	9000	1600	220	2000
Rondino F_1 BZ	B	3	3400	6600	1200	150	1500

[a] Growing site of carrot cultivar: A = Pikkiö, B = Hahkiala, C = Jokioinen, D = Säkylä. [b] Number of replicate analysis. [c] Retinol equivalents, μg/100 g = 0.167 (μg of β-carotene) + 0.083 (μg of α-carotene + μg of γ-carotene). [d] Hybride cultivar of Bejo Zaden.

et al., 1955, Hårdh, 1975; Hårdh et al., 1977) also cause color variation. Other variables such as fertilization have an effect.

Carrot (*Daucus carota* L.) contains a variety of carotenoids that form the typical color of the root. The main pigments of orange carrot are α- and β-carotene. According to Bajaj et al. (1980), the β-carotene content of carrots ranges from 850 to 8500 μg/100 g of fresh weight. Thus, carrot is an important dietary source of provitamin A.

The carotene content of carrot increases with the length of the growing season. Therefore, the color of young and small carrots is pale, and they contain little α- or β-carotene (Evers, 1989; Gabelman, 1974). The carotene content increases when the root is maturing (Banga et al., 1963; Fritz and Habben, 1977), and at the same time the proportion between α- and β-carotene changes. β-Carotene accounts for about 60% of the total carotenoid content in ripe orange carrot and α-carotene for about 20%, the rest being due to lycopene, γ-carotene, ζ-carotene, and/or β-zeacarotene (Banga and De Bruyn, 1964; Gabelman, 1974; Simon and Wolff, 1987).

The aim of the present study was to determine the carotenoid and provitamin A content of 19 cultivars of the orange carrot (*D. carota* L.).

EXPERIMENTAL SECTION

Sampling. The samples of 18 cultivars of orange carrot were obtained from three different locations in Southern Finland; one variety was grown in Säkylä, Western Finland (Table I). The sampling time was October 1988, after an average growing period of 4 months outdoors. Approximately 10 carrots were randomly selected from each carrot variety and stored in the dark at +4 °C for no more than 3 days before the carotenoid analysis.

Extraction. Fresh and cleaned carrot halves were homogenized in a blender. The sample homogenates (3 g) were extracted with acetone (100 mL) in a Waring Blendor using Na_2SO_4 (20 g) as desiccant, vacuum-filtered, and concentrated prior to saponification. The samples were extracted two to three times to remove all color. The extraction and saponification procedures have been described earlier by Heinonen et al. (1989).

High-Performance Liquid Chromatography. The carrot samples were analyzed with use of the nonaqueous reversed-phase (NARP) system introduced by Nelis and De Leenheer (1983). A Varian Vista 5500 liquid chromatograph (Varian) was equipped with a Varian UV-200 detector and a Varian 4270 integrator. The column was a Zorbax ODS (5–6 μm, 25 × 0.46 cm (i.d.)) (DuPont) preceded by a guard column (5 × 0.46 cm (i.d.)) packed with Bondapak AX/Corasil (37–50 μm) (Waters). The elution mixture was acetonitrile–dichloromethane–methanol (70:20:10); the flow rate was 1 mL/min, and the running temperature was 30 °C. The UV detection was set at 450 nm. Both standards and samples were injected via full loop, approximately 55 μL.

Identification and Quantitation. The carotenoids were identified by comparing their retention times with those of authentic standards. Quantitation was based on an external standard method where the calibration curves ranged from 70 to 2100 ng/mL. *all-trans-* and 15-*cis*-β-carotene were obtained from F. Hoffmann La Roche, α-carotene was from Sigma Chemical Co., and lutein was from Roth. γ-Carotene was a gift from Dr. F. Khachik from USDA, Beltsville. The concentrations of stock standard solutions were determined spectrophotometrically (Davies, 1976) with appropriate extinction coefficients ($E^{1\%}_{1cm}$) (Heinonen et al., 1989).

Some carrot extracts were also run through a chromatographic system equipped with diode array detection (Hewlett-Packard 1090 liquid chromatograph, UV/vis diode array detector, and 85B data system) for purity checking and identification of the chromatographic peaks. The tentative identification of 15-*cis*-β-carotene was based especially on the characteristic subsidiary peak in the near-ultraviolet region.

The mean, standard deviation, and coefficient of variation were calculated from the data of usually triplicate analysis. A Q-test was used to reject the error results (Fritz and Schenk, 1979). The individual carotenoid values were converted into retinol equivalents, RE (μg/100 g) (Food and Nutrition Board, 1980).

RESULTS AND DISCUSSION

The HPLC chromatogram of carotenoids in carrot is shown in Figure 1. The NARP chromatographic system used gave a good resolution of lutein and α-, β, and γ-carotene; also the tentatively identified 15-*cis*-β-carotene could usually be separated from *all-trans*-β-carotene.

The carotenoid composition and the provitamin A activity of 19 cultivars of carrot are shown in Table I. As expected, cv. Nantes Duke Notabene 370 and cv. Nantes Fancy Notabene 405, usually grown for a longer time than the F_1 cultivars, were rich in both α- and β-carotenes. Nantes Duke Notabene 370 is the principal carrot cultivar used by the Finnish food industry. According to results from the growing season of 1986 in Finland (Evers, 1989),

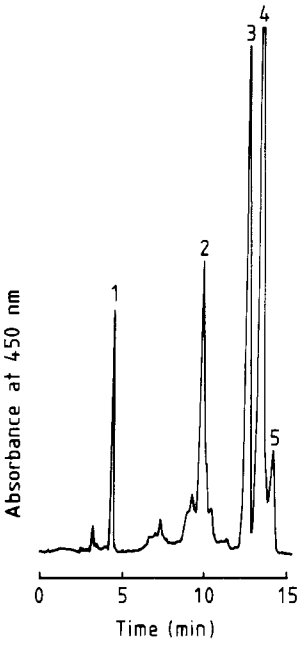

Figure 1. Liquid chromatogram of carrot carotenoids separated on a Zorbax ODS column with use of acetonitrile–dichloromethane–methanol (70:20:10) as the mobil phase. Peak identification: lutein (1), γ-carotene (2), α-carotene (3), *all-trans*-β-carotene (4), 15-*cis*-β-carotene (5).

this particular cultivar contained below 7000 μg/100 g of fresh weight of α- and β-carotene. Due to the exceptionally warm summer of 1988, the carotenoid content in the present study was high in all carrot cultivars. For example, cv. Nantes Duke Notabene 370 contained α- and β-carotenes at 3900 and 8400 μg/100 g of fresh weight, respectively. The accumulation of carotenoids favors warmth, and that is why in the south of the country there are regularly more carotenes in vegetables than in the north (Hårdh, 1975, Hårdh et al., 1977). Hårdh (1975) found that when the carotene (α- and β-carotene) content of cv. Nantes Fancy Notabene 405 was 5300 μg/100 g of fresh weight in southern Finland (60°11′ N), it was only 2400 μg/100 g of fresh weight in the north of the country (69°05′ N). The ranges of α- and β-carotene in cv. Nantes Fancy Notabene 405 were 1900–7300 and 1500–12000 μg/100 g of fresh weight, respectively (Hårdh et al. 1977). α-Carotene accounted for 43–126% of the amount of β-carotene. However, Gabelman (1974) states that the primary cause of variation in carrot color is genetic, not environmental. In the present study, cv. Nantes Fancy Notabene 405 contained 4200 μg/100 g of fresh weight of α-carotene and 7900 μg/100 g of fresh weight of β-carotene.

High carotene content was also found in one feed type carrot cultivar, Berlicum R, and in the F_1 cultivars Narbonne, Nelson, and Nantucket. The amount of β-carotene in carrots ranged from 4600 to 10 300 and that of α-carotene from 2200 to 4900 mg/100 g of fresh weight. α-Carotene accounted for about 50% (range 38–75%) of the amount of β-carotene. The amount of the tentatively identified 15-*cis*-β-carotene was approximately 4.3% of that of *all-trans*-β-carotene (range 1.3–12.9%). All carrot cultivars contained γ-carotene ranging from 630 to 2700 μg/100 g of fresh weight. Also lutein was present, 110–560 μg/100 g of fresh weight. According to Simon and Wolff (1987), β-carotene accounts for 44–79% of all carotenes quantitated in typical and dark orange inbred carrot cultivars, and 94–97% is due to α-, β-, and ζ-carotene.

Apart from Nantes Duke Notabene 370, the other two carrot cultivars analyzed that are recommended for cultivation in Finland are Nantes Fancy Notabene 405 and Nantucket F_1. Altogether there are seven recommended carrot cultivars (Pessala, 1988). The concentration of carotenoids in commercial cultivars of carrots purchased at different seasons of the year have been studied earlier (Heinonen et al., 1989). It was found that the β-carotene content was at its lowest (2600–5500 μg/100 g of fresh weight) in summer (June to August) and highest (4600–7700 μg/100 g of fresh weight) in the fall and during the first months of storage (October to March). α-Carotene ranged from 500 to 2900 μg/100 g of fresh carrot. According to Booth and Dark (1949) the maximum carotenoid content is not the same in all seasons, but the fluctuation can be up to 20% above or below the average.

In selecting carrots for carotene content, both size and degree of maturity have to be taken into account, since the carotene content increases with the development of the root until a (varying) maximum is reached (Banga et al., 1955). The carrot cultivars contained 1200–2300 RE (μg/100 g) of provitamin A. Thus, independent of the carrot cultivar, 100 g of fresh carrot will satisfy the recommended dietary need of vitamin A (women, 800 RE/day; men, 1000 RE/day) (Food and Nutrition Board, 1980). In Finland, the average daily consumption of carrot is 18.3 g (Agricultural Economics Research Institute, 1989); carrot accounts for approximately 10% of the dietary intake of vitamin A.

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Registry No. Vitamin A, 68-26-8; α-carotene, 7488-99-5; β-carotene, 7235-40-7; 15-*cis*-β-carolene, 19361-58-1; γ-carotene, 472-93-5; lutein, 127-40-2.

Hydrogen Ion Equilibria of α-Globulin from *Sesamum indicum* L.

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The hydrogen ion equilibrium of the high molecular weight protein α-globulin of sesame seed has been investigated in the pH range 2.0–12.0. Analysis of the data gave pK_{int} values of 4.37, 7.47, and 9.60 for carboxyl, imidazole, and ε-amino groups, respectively. The number of residues containing these groups agreed well with the values obtained from the amino acid analysis. Tyrosyl phenolic groups had a pK_{int} of 9.41 as determined from spectrophotometric titration. The value of the electrostatic interaction parameter, w, varied with pH, and this is discussed in relation to the hydrodynamic properties of the protein.

The major protein of *Sesamum indicum* L. seed is α-globulin, which constitutes nearly 65% of the total protein (Nath et al., 1957; Prakash and Nandi, 1978). The protein has been isolated to homogeneity and characterized for its physicochemical properties. Its behavior in solutions of electrolytes, detergents, urea, guanidine hydrochloride, polyhydric alcohols, acid, or alkali has also been studied in detail (Prakash and Narasinga Rao, 1986; Prakash, 1985; Lakshmi et al., 1985a,b). The protein undergoes association–dissociation and denaturation in the presence of acid or alkali. Thus the binding of hydrogen ion by the protein has an effect on its stability and quaternary structure.

In this study we have investigated the hydrogen ion equilibrium of the protein by electrometric titration and have attempted to interpret the data in terms of the changes that the protein undergoes at acid/alkaline pH.

MATERIALS AND METHODS

(a) Isolation of α-Globulin. Sesame seeds were obtained from the National Seeds Corp. Ltd., Bangalore, India, and α-globulin was isolated by the method of Prakash and Nandi (1978). The protein was found to be homogeneous by analytical ultracentrifugation, polyacrylamide gel electrophoresis, and ion-exchange chromatography (Prakash and Nandi, 1978). It was dialyzed repeatedly against 0.1 M KCl solution.

(b) Protein Concentration. The concentration of α-globulin was determined by macro-Kjeldahl nitrogen estimation and using a factor of 6.25 to convert nitrogen to protein value (Prakash and Nandi, 1978). A calibration curve relating the milligrams of protein present in the solution with the ultraviolet absorbance of the protein at 280 nm was obtained; proper correction for scattering between 380 and 310 nm was applied. The absorption coefficient of $E_{280}^{1\%,1cm} = 10.0$ obtained thus was used for the routine determination of protein concentration. This was also confirmed by a gravimetric method.

(c) Amino Acid Analysis. The amino acid analysis of the protein was performed in an LKB amino acid analyzer by following the standard procedure of hydrolyzing the protein in 6 N HCl (Spackman et al., 1958).

(d) Hydrogen Ion Titration. The pH meter was standardized with standard buffers of pH 4.01, 6.50, and 9.05. All solutions were prepared with distilled and deionized water. The hydrogen ion titration of the protein was conducted at 28 ± 0.1 °C in 0.1 M KCl solution using extensively dialyzed protein solution. To 25 mL of 1% protein solution in 0.1 M KCl was added either standard HCl (0.1 M) or standard NaOH (0.1 M) in increments of 10 μL via an Agla micrometer syringe. After each addition, sufficient time was allowed for stabilization of pH and the pH value was noted. Nitrogen gas was flushed over the solution for measurements above pH 6.0. A blank titration was carried out with 25 mL of 0.1 M KCl in a fashion similar to that described above for the protein solution. From the difference in pH values, the number of hydrogen ions bound/dissociated per 250 000 g of protein was calculated (Prakash and Nandi, 1978; Prakash, 1985; Tanford, 1962).

(e) Spectrophotometric Titration. This was carried out by adjusting the pH of the protein solution in 0.1 M KCl with 1 M NaOH to different pH values and measuring the absorbance of the solution at 295 nm in a Perkin-Elmer 124 double-beam spectrophotometer (Tanford, 1962; Wetlaufer, 1962). Nitrogen was used to flush the solutions. The pH of the solution was measured both before and after absorbance measure-

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