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Title RELATION OF COLOR IN COOKED CARROTS TO CAROTENE CONTENT AS DETERMINED BY CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS.

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Carrots were cooked to the just tender stage in a saucepan and in a pressure saucepan for appropriate lengths of time to make them approximately equal in tenderness as determined by a panel of judges and the Kramer Shear Press. A third lot of carrots was cooked in a pressure saucepan for approximately twice as long to represent overcooked carrots. Judges and the Hunter Color Meter indicated that the carrots cooked in the saucepan were more typically red-orange and bright and the carrots overcooked in the pressure saucepan were more yellow and dull.

Pigments extracted from the carrots from the three cooking treatments were chromatographed on a magnesia column and the principle fractions, α-carotene and β-carotene, eluted. The β-carotene was rechromatographed on an alumina column to separate

it into all-trans-β-carotene and neo-β-carotene B. In absolute amounts, carrots cooked in the saucepan had the highest concentration of all-trans-β-carotene and the highest total of all-trans-β-carotene, neo-β-carotene B and α-carotene, followed by those carrots cooked in the pressure saucepan for 50 seconds, with those cooked in the pressure saucepan for two minutes being lowest in both all-trans-β-carotene and total carotenes. However, when the α-carotene, the neo-β-carotene B and the all-trans-β-carotene were considered as percentages of the total, the percentage of a-carotene remained constant in the three treatments. Carrots cooked in the pressure saucepan for two minutes had a lower percentage of all-trans-β-carotene and a higher percentage of neo-β-carotene B than did carrots from the other two treatments. Thus, longer cooking in the pressure saucepan caused greater conversion of the more vivid all-trans-β-carotene to the paler cis-isomer, neo-β-carotene B. This isomerization plus loss of total pigment accounts for the differences in color of the cooked carrots from the three treatments.

RELATION OF COLOR IN COOKED CARROTS TO CAROTENE CONTENT AS DETERMINED BY CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS

by

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INTRODUCTION

Much of the aesthetic enjoyment of our foods is that conveyed through our sense of sight. The colors of vegetables and fruits in particular add variety and interest to our diet. During household and commercial preparation of food the pigments responsible for these colors should be treated in such a way as to enhance their beauty.

Carrots, one of our more common vegetables, provide vivid color to meals and constitute a high potential source of vitamin A in the diet. This is especially true because they have by far the highest carotene content of any food commonly eaten by man (49, p. 25) and at the same time are consumed in large quantities in the United States (50, p. 8-10).

Although the oxidative destruction of carotene has long been recognized, any possible effects of cooking on the carotene content of carrots has been considered slight. Callison and Orient-Keilles (10, p. 165) state that cooking caused no differences in either the carotene content of carrots analyzed chemically or the ability of the body to use the carotene. Since the carotenoids are insoluble in

water, loss of these pigments into cooking or canning water is slight during the processing of fruits and vegetables, according to Meyer (36, p. 234). She further says that the loss of carotenes, if it does occur, is small, in the order of five to ten percent. More recently, Griswold states that ordinary cooking conditions have little effect on the color or nutritive value of carotenoids and that the color is little affected by acid, alkali, the volume of water or cooking time (23, p. 174).

Hence, there has been a rather general concensus that, among the plant pigments, the carotenes are quite stable to cooking. Despite this, carrots from the same lot cooked in foods classes by different students and by different methods have been observed to differ in color. This study was undertaken to see whether cooking carrots does, in fact, affect the color of the final product.

REVIEW OF LITERATURE

Pertinent Characteristics of Carrots

Botanical Structure

The carrot, daucus carota L., is a member of the parsley family, umbelliferae (25, p. 451). The carrot is typical of the kind of plant in which there is special adaptation for storage by fleshy bodies in parts of the root system. In the carrot the hypocotyl and the base of the taproot form one fleshy structure through a massive development of parenchyma (storage) cells (14, p. 507).

The central portion of the root, the core, is composed of the xylem, the principle water conducting tissue. The xylem is a complex tissue containing many different types of cells: water-conducting cells, supporting elements and parenchymatous cells. The peripheral portion of the carrot surrounding the xylem is mostly phloem, the principle food-conducting tissue of vascular plants. The phloem is made up of several different types of cells, including sieve cells, parenchyma cells and fibers. Thus both the xylem and the phloem, which are separated from each other by a narrow translucent band of cells, the cambium, possess an abundance of parenchymatous cells (14, p. 221 and 51, p. 37).

Texture of Carrot Tissue as Altered by Cooking

The parenchyma cells of the carrot are held together by cementing substances which are composed principally of pectic constituents (36, p. 220). The effect of cooking on these cementing substances, which form the middle lamella, as well as on the cells themselves, must be considered. Carrots, like all fruits and vegetables, undergo softening when cooked, regardless of the cooking method. The degree to which carrots have been cooked may possibly affect the carotene content as well as the texture and this needs to be taken into consideration when evaluating the effects of cooking on the color of carrots.

The steaming of carrots, according to Simpson and Halliday (42, p. 194), has an effect similar to that which occurs during the storage of fruit. In both instances, as softening occurs, there is a decrease in total pectic substances accompanied by a decrease in protopectin and an increase in pectin.

As to the effect of cooking on the cells themselves, Sterling (44, p. 474) found only separation of intact cells in cooked apple fruit, carrot root and potato tuber. In carrots, after 20 minutes of steaming, he found some separation of the radial cells in the cambial region but no broken cells. After 40 minutes, he noted

radial separation of cells in the xylem and phloem as well, but still no broken cells. After 60 minutes of steaming he found extensive areas of radial cell separation throughout. At the same time he found a tangential separation of cells taking place in the more delicately-walled cambial region. No breakage of cell walls was apparent (44, p. 475-476).

Organic Acids in Carrots

In contrast with animal fluids, plant juices are slightly acid in reaction. The pH of carrots varies from 4.9 to 5.2 (29, p. 32). Any changes which may occur in the carotene pigments of carrots during heating may be due in part to the plant acids which are present.

The organic acids in carrots with their concentrations are fumaric, 0.06 percent, isocitric, 0.39 percent, malic, 3.2 percent, succinic, 0.65 percent and a trace of citric. (37, p. 85-86).

Carotenoids in Carrots

The name carotenoid is applied to all pigments chemically related to the carotenes, the first of the group to be isolated. The name carotene is derived from the fact that these pigments were first isolated from the root of the daucus carota by Wachenroder more than 130 years ago (16, p. 64).

The carotenoids (orange pigments) constitute one group of the ether-soluble or plastid pigments with the green chlorophylls making up the second as distinct from the red or purple anthocyanins and the yellow anthoxanthins both of which are water-soluble or vacuolar pigments (37, p. 50).

These yellow, orange and red-orange pigments are widely distributed in nature. A variety of foods such as yellow vegetables, yams, tomatoes, apricots and egg yolk owe their color to the presence of carotenoids. These pigments occur in green leaves but their presence is obscured by the chlorophylls except in the spring when the amount of chlorophyll is small and in the fall when the chlorophylls disintegrate (47, p. 28). In addition, the carotenoids have been found in nearly every part of the mammalian organism where they probably have been derived from the vegetable feeding stuffs. There is no record of any mammal manufacturing a "specific" carotenoid (30, p. 90). As to the purpose of the carotenoids, they have the general ability to absorb visible light and they may be involved in transferring energy for photosynthesis and in oxygen transport (9, p. 36). Frank (17, p. 84) suggests that the carotenoids serve as a pool for the phytol part of chlorophyll.

Chemistry of Carotenoids

Karrer's definition of carotenoids, accepted by the "Union Internationale de Chime", states that the "carotenoids are yellow to red pigments of aliphatic or alicyclic structure, composed of isoprene units (usually eight) (see Fig. 1) linked so that the two methyl

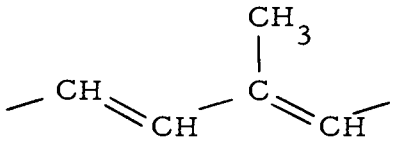

Figure 1. Isoprene unit

groups nearest the centre of the molecule are in positions 1:6 whilst all other lateral methyl groups are in positions 1:5; the series of conjugated double bonds constitute the chromophoric system of the carotenoids". (19, p. 1). This alternation of single and double bonds is responsible for the color of carotenoids, which varies not only from yellow through orange and red but even to violet, depending, in this order, on the increasing number of double bonds and on the presence of certain radicals (16, p. 64).

The biosynthesis of carotenoids is closely associated with the synthetic processes of non-saponifiable hydrocarbons such as terpenes and sterols. The isoprenoids arise from acetyl-Co-A by condensation and conjugation (18, p. 644).

Most but not all carotenoids built up from eight isoprene units

possess 40 carbon atoms. The colored C_{40} isoprenoid hydrocarbons are termed "carotenes" and those that are not colored (phytoene and phytofluene) are designated as "colorless polyenes". The hydroxylated carotenes are termed "carotenols" or the more commonly termed "xanthophylls" (26, p. 459). Some 70 to 80 carotenoids have been found in nature up to the present time. All of these can be related to the parent substance, lycopene, (see Fig. 2) which is the

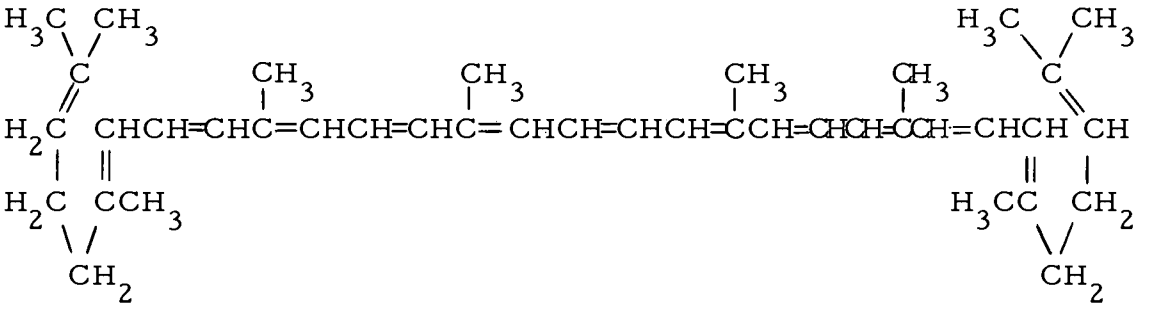

Fig. 2. Lycopene

principal pigment in ripe tomatoes, pink grapefruit, watermelon and rose hips (37, p. 56). By means of simple chemical changes such as cyclization, double bond migration, partial hydrogenation, introduction of hydroxyl-, keto-, or methoxyl groups or introduction of an oxygen bridge, etc., the whole range of pigments can be derived from this parent substance.

The most important isomers of carotene are α-carotene, β-carotene, γ-carotene and lycopene, all of which have the formula $C_{40}H_{56}$ (56, p. 268). These four carotenes are all composed of long

aliphatic chains containing methyl groups. Beta-carotene, α-carotene and γ-carotene can be formed from lycopene by ring closure at one or both ends of the molecule. See Figures 3, 4 and 5.

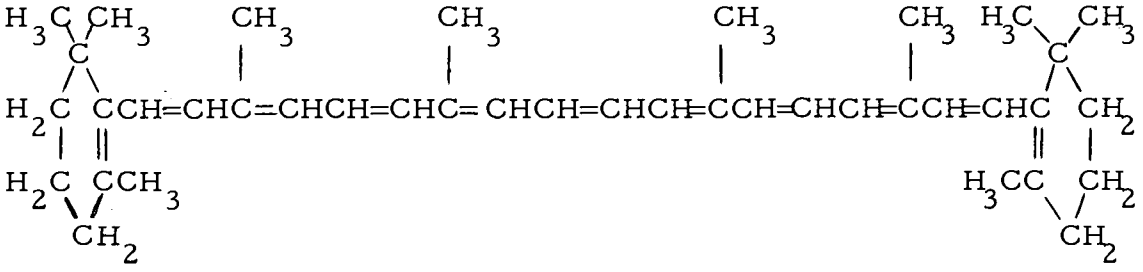

Fig. 3. β-Carotene

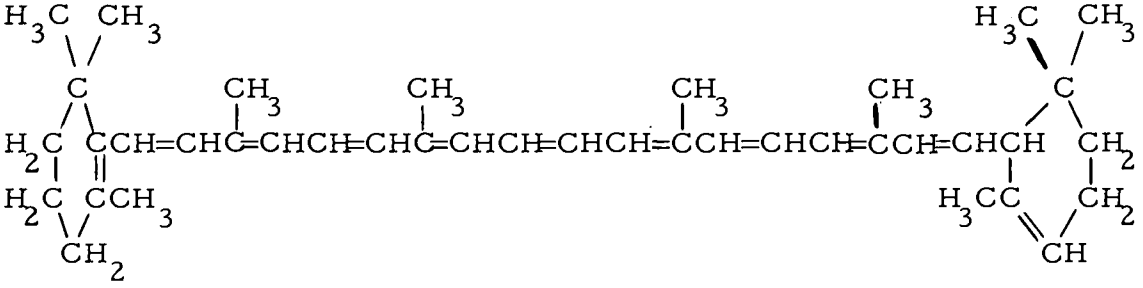

Fig. 4. α-Carotene

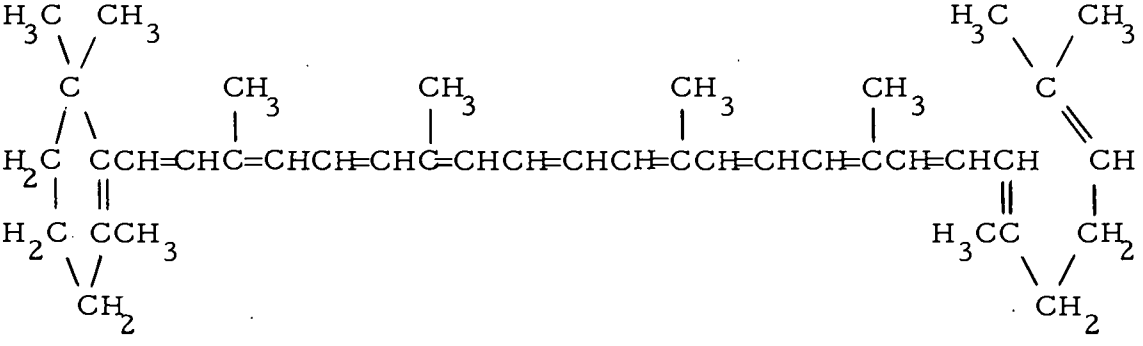

Fig. 5. γ-Carotene

Beta-carotene differs from lycopene only in the cyclization of the end carbons to form closed rings. Beta-carotene and lycopene are examples of symmetrical molecules. Asymmetrical carotenoids are those in which the terminal groups or rings are different, such as in α-carotene and γ-carotene.

Alpha-carotene differs from β-carotene only in the position of the double bond in the second ring, an α-ionone residue. The γ-carotene molecule is made up of one ending similar to lycopene and the other similar to β-carotene (37, p. 231). Alpha-carotene is optically active, because of the asymmetric C atom in position six of the α-ionone residue while β-carotene and γ-carotene are optically inactive (28, p. 375).

Beta-carotene is very widely distributed in nature, both throughout the vegetable and animal kingdoms. Alpha-carotene is almost as widely distributed in the vegetable kingdom as β-carotene, but never occurs in large concentrations. Mature green leaves usually contain approximately 90 percent β- and ten percent α-carotene (37, p. 56).

Alpha-, β-, and γ-carotene are called vitamin A precursors because they may be converted to this vitamin in the animal body (37, p. 56).

Another isomer, ζ - carotene, like lycopene, is open at

both ends of the chain. Zechmeister (57, p. 101) says the structure of ζ - carotene is 7, 8, 7', 8'-tetrahydrolycopene.

Kind and Distribution in Carrots

Most carotenoids of vegetables occur in the chromophores within the cytoplasm of the cells. These plastids are bodies of varied shapes, elongated, lobed, angled or spherical and are colored yellow or orange due to the presence of the carotenoids. Sometimes the pigment is diffused in the plastid, sometimes it occurs in the granules, sometimes it appears as isolated, well-formed crystals (53, p. 39). Certain carotenoids in the plastids are bound to or intimately associated with protein in the aqueous phase (8, p. 406). Non-crystalline carotenoids are present in colloidal suspension in the cell lipids or in admixture with solid or semi-solid fats (30, p. 5). The pigment bodies may also occur as optically anisotropic ribbons, plates, or spirals sometimes connected with starch grains (14, p. 21). In the latter case, the whole mass of starch grains (or various portions of the mass) is orange in color (51, p. 39).

Carotene is always found in higher concentration in the phloem than in the xylem of the carrot, the former containing approximately 80 percent of the total carotenoids. It has been well established that the carotene content decreases from top to tip. As in the case of

leaves, the concentration of the carotene in roots increases with growth and becomes greatest about 100 days after sowing (19, p. 56).

The optimum temperature for the production of carotene in roots such as carrots is between 60° and 70° F. At temperatures below this carrots are noticably less colored due to the absence of pigment from the peripheral cells (19, p. 75).

Changes in carotene content during storage of carrots in the refrigerator at 4.4°C have been observed. Considerable deepening of carotene with storage has been reported (43, p. 145). Wide ranges in carotene content have been reported by Smith (43, p. 144). Values in micrograms per gram range from 100-170 for darkly pigmented carrots, 56-70 for medium orange colored carrots, 20-31 for rather pale carrots to 7-12 for very slightly colored carrots.

Lee (30, p. 1291) reported that garden varieties averaged 54 micrograms of carotene per gram of carrot. Carrots were found by Goodwin to contain generally between 60 to 120 micrograms of carotene per gram (wet weight) of carrot (19, p. 54). The total yellow pigment content of the cooked Imperator carrots studied by Callison was equivalent to 102 micrograms β-carotene per gram of cooked carrot with a standard deviation of 9.0 (10, p. 145). Maturity, sampling and length of storage all contribute to the wide variation in carotene content of carrots (43, p. 146).

The specific carotenoids present in daucus carota are α-, β-, and γ-carotene, xanthophyll and two hydrocarbons of unknown origin, according to Palmer as quoted in Karrer and Jucker (30, p. 67). In addition to α-carotene, β-carotene and xanthophyll, Harper and Zscheile (24, p. 90) reported the presence of ζ-carotene in considerable quantities and rather generally distributed and also the presence of lycopene in numerous varieties of carrots.

It is well known that the predominate carotenoids of carrot roots are α-carotene and β-carotene. Mackinney, Aronoff and Bornstein found α-carotene to constitute 5 to 15 percent of the total carotene fraction of carrots (35, p. 392). Callison et al. found that α-carotene made up 25.3 percent of the total pigments of the cooked Imperator carrots they studied (11, p. 145).

Quantities of β-carotene in carrots varied from 47 percent (30, p. 131) to the 63.2 percent of the total carotene content which Callison reported in cooked Imperator carrots (11, p. 145). The concentration of β-carotene present in Callison's sample of Imperator carrots was somewhat higher than the 51 micrograms per gram reported by Harper and Zscheile (24, p. 89) for the same variety (11, p. 146).

Gamma carotene is one of the rarest carotenoids. In carrots it occurs only to the extent of about 0.1 percent of the β-carotene

(30, p. 161).

The carotenol (xanthophyll) fraction of most commercial carrots is quite small, being only five to ten percent of the total carotenoids present (19, p. 55). Harper and Zscheile found the cis-isomers of α- and β-carotenes to be very low in fresh carrots (24, p. 88).

Changes in Carotenes Which May Affect Color of Carrots

Carotenoids crystallize readily and are subject to oxidation and isomerization (16, p. 64).

Oxidation. The high degree of unsaturation of the carotenoids makes them susceptible to oxidation with resulting loss of color after the food containing them has been dried. Carrots and apricots, for example, show loss of pigment on drying (36, p. 234). However, the carotenoids are very stable at low oxygen pressures, in darkness and at low temperatures, and in fact they have been found under such conditions in marine muds about 6000 years old (16, p. 64).

1. Enzymatic destruction. The well known fact that a preliminary blanching of harvested plant materials prevents subsequent oxidation of carotenoids indicates that the process is catalyzed by enzymes, probably lipoxidase. Enzymatic degradation occurs in plant tissues when the pigments and/or enzymes are liberated by destroying the cells by maceration. Oxidation is much more rapid when

cells are ruptured by freezing or when the plant wilts than in the intact plant.

2. Photochemical destruction. Photochemical destruction of carotene takes place in the presence of chlorophyll in acetone or petroleum ether. The chlorophyll itself takes part in the reaction and is not a catalyst (5, p. 10).

It is also possible that there are natural substances in the intact cell which protect carotenoids from oxidation. Weier found that if blanched carrots were leached with cold water prior to storage the stability of the carotenoids was considerably reduced, indicating the presence of a protective substance soluble in cold water after liberation from the cells by blanching (52, p. 537).

Isomerization. A long conjugated double-bond system, such as that of the carotenoid pigments, is subject to many spatial arrangements. Thus a large number of stereoisomers are found which differ from each other in biological potency and in certain physical properties such as adsorption affinity and absorption spectra. The all-trans configuration predominates in most natural as well as synthetic polyenes. The abundant occurrence of all-trans pigments is understandable because they possess the lowest energy content and the greatest stability of all possible spatial structures (56, p. 272). The all-trans configuration is maintained only when the carotenoids are

freshly extracted from natural sources or kept in the crystalline state (5, p. 5).

On the basis of theoretical considerations, Zechmeister, Pauling and others concluded that not all ethylenic groups of a carotenoid molecule are capable of taking part in cis-trans isomerization, but only those of the type $-C(CH_3)=CH-$, i. e., which carry a CH_3 side chain, and the double bond in the center of the molecule. In the case of β-carotene only the 3, 5, 6, 7 and 9 double bonds are capable of such isomerization. See Fig. 6. The remaining ethylenic groups

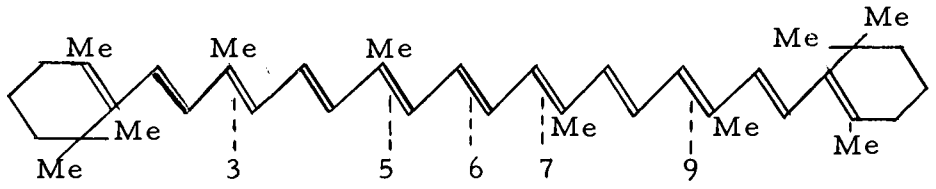

Fig. 6. All-trans-β-carotene

are believed always to assume a trans configuration due to steric hindrance (19, p. 10).

Although the all-trans configuration predominates in most fresh plant extracts, approximately one-third of the molecules may undergo spontaneous isomerization to the cis form. In solution some of the sterically unhindered double bonds slowly change configuration and a mixture of several cis-trans isomers is formed (5, p. 5). In addition, isomerization can be brought about or the rate of isomerization enhanced by any one of the following means: refluxing of a

solution of the carotenoid in an organic solvent, melting of the crystals, treatment with iodine, or exposure to acids and illumination (30, p. 39).

Upon such treatment the stereochemical uniformity of the molecule disappears and a complicated mixture of cis-trans isomers is found, in which ordinarily the unchanged portion of the initial all-trans molecules still predominate. Theoretically, each possible stereoisomer must be present in such an equilibrium mixture even if only in very small quantity (56, p. 270).

Transformation from the natural trans form brings about a number of changes. Those which are pertinent to this study include a decrease in the intensity of the color of the pigment in solution, a change in the adsorption affinity of the carotenoid on the chromatographic column, a decrease in the wave-lengths in the visible region of the spectrum at which the pigment absorbs, a lowering of the extinction coefficient and the appearance of a new maximum in the ultra-violet spectrum. Following Zechmeister and Polgár these new maxima are termed "cis-peaks" (30, p. 39).

If heat and acid cause isomerization, there is reason to suspect that heating the carotene in carrots in the presence of plants acids, as during cooking, might bring about the isomerization which has been demonstrated chemically.

Determining the Carotene Content of Carrots

Extraction. Carotene cannot normally be extracted adequately from carrots with nonpolar solvents because of a carotene-protein complex. This carotene-protein complex is salmon-pink in appearance. The carotene-protein complex must first be broken by denaturation of the protein with alcohol (34, p. 1291). Hence, to extract completely the carotenes from carrots the tissues are macerated, the protein denatured and the pigments extracted with suitable solvents (9, p. 37).

Separation of Pigments. The various terminal groups of the aliphatic chain of the different carotenoids not only determine the vitamin A activity but also the adsorbability and differential solubility properties by which they may be separated from one another (3, p. 45).

1. Phasic separation. The carotenoid pigments may be separated into two fractions, a petroleum ether or benzene soluble fraction and an alcohol soluble fraction. Pigments shaken in a mixture of these immiscible solvents separate into two layers. The upper or "epiphasic" layer contains the carotenes, including their epoxides, mono-, keto-, or monohydroxy-derivatives and/or any xanthophylls with their hydroxy group esterified or methylated. The lower or "hypophasic" layer contains xanthophylls containing two or more

free hydroxy or keto groups (5, p. 13).

Phasic separation is usually followed by identification of pigments by means of chromatographic separation followed by spectrophotometric analysis. The stereoisomers of a given carotenoid are very similar, but of their known physical properties, their adsorption affinities together with their absorption spectra, provide perhaps the best means of identifying them (56, p. 271).

2. Chromatographic separation. The chromatographic technic, originally proposed by Tswett, a Russian botanist, in 1907, is a powerful and widely used tool for separating the various carotenes from one another as well as various isomers of a given carotene. Whereas paper chromatography has proven unsuccessful for the separation of individual carotenes, column chromatography has been so employed. Various pigments with even slight differences in structure appear in distinct adsorption zones when a petroleum ether solution flows through a column of properly activated adsorbent. These zones may be separated mechanically or eluted by the flowing chromatographic technique. The relative position on a chromatographic column gives a good indication of the identity of a particular pigment (5, p. 14).

An increase in the number of conjugated double bonds increases the adsorptive properties of a carotene. Also, acyclic compounds,

such as lycopene, are more strongly adsorbed than cyclic compounds, such as β-carotene. The shift of a double bond out of conjugation in a cyclic compound, such as α-carotene, further reduces the adsorption of the compound. Introduction of oxygen into the molecule increases the adsorption of the compound (26, p. 465).

Cis-trans isomers are named according to the positions they occupy on the adsorption column. Any cis-isomer is named by prefixing "neo-" to the name of the parent compound and then adding the letters T U V if adsorbed above the all-trans form, A B C if adsorbed below the all-trans form (56, p. 8). The three main stereoisomers of β-carotene, the predominate carotene found in plants, are neo-β-carotene U (the U for ultra as suggested by Polgár and Zechmeister), all-trans-β carotene (the main isomer), and neo-β-carotene B. The U isomer which has been observed in extracts of pumpkin, squash, carrots and others is included in the upper blurred part of the β-carotene zone. Zechmeister has confirmed the 9-mono-cis configuration of neo-β-carotene U. He also proposes that neo-β-carotene B is 9, 13'-dicis-β-carotene (57, p. 84). The carbon atom in the ring to which the two methyl groups are attached is numbered 1 or 1'. The two carbon atoms adjacent to the central double bond are numbered 15 and 15'.

Zeta-carotene forms a pale green band above neo-β-carotene

U and below γ-carotene should it be present on the column (48, p. 433).

Carotenes and the principal isomers of β-carotene can be resolved on columns of magnesia and subsequently on columns of alumina. The following schematic drawing illustrates the relative positions, on a suitably prepared adsorption column developed with petroleum ether, of the most important carotenes and the principal isomers of β-carotene if present in the same solution, see Fig. 7.

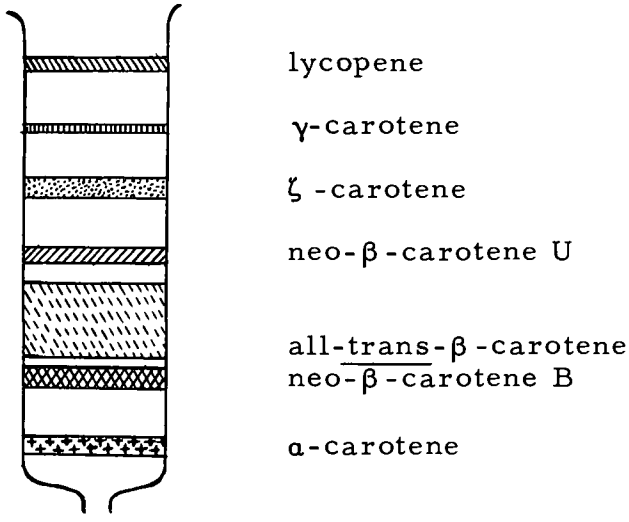

Fig. 7. Relative positions of carotenes and isomers of β-carotene on an idealized adsorption column

Broad bands on the column often need to be rechromatographed on the same column or a column containing a stronger adsorbent in order to secure spectroscopically pure compounds.

Spectroscopic Identification of Pigments. An important part of the identification of a carotenoid is the determination of its absorption

maxima. These pigments are sensitive to the blue radiations of the spectrum, while the chlorophyll system is sensitive to the yellow-orange radiations (27, p. 924). The visible light absorbancy of carotenoids comprises a range of about 300mμ (ca. 400-700mμ) (30, p. 4). A solution of a carotenoid, usually in petroleum ether or in carbon disulfide, can be examined in a spectrophotometer to determine absorption peaks. Usually two or three sharp absorption peaks can be determined to within 0.5mμ and these represent the wavelengths of the absorption maxima. These data are characteristic for each carotenoid and together with other physical constants are used for its identification (30, p. 8).

Thus, polyenes may be identified by their position on a chromatogram relative to other known polyenes and by the shape of the absorption curve. These are sufficient criteria, according to Goodwin, because (a) of considerable experience in his and other laboratories, i. e., Haxo, Trombley and Porter have shown that they always separate on a column in the order given, and (b) the absorption spectra maxima of adjacent adsorbed polyenes are quite distinct and characteristic (22, p. 347).

Quantitative Measurement of Pigments. Once the pigment is identified, the next step is to estimate the quantity. Spectrophotometric methods, when used for the determination of the concentration

of a substance in solution, are more sensitive, more accurate and more precise than visual or photometric methods (39, p. 559). To arrive at the concentration of a pigment in solution the optical density of the solution is first measured. Then the concentration of pigment present can be read from a standard curve. This calibration curve is prepared by plotting optical density against the concentration of a series of solutions of the pure compound.

An alternative method to determine the concentration of the pigment using its optical density is to calculate the concentration by use of the absorption coefficient for the specific pigment. The specific absorption coefficient is the optical density of a solution of the pigment at a concentration of one gram per liter (0.1 percent solution) read at the wavelength of maximum absorption in a cell one centimeter deep. Another notation frequently used is the expression $E^{1\%}_{1\,Cm}$ which is the optical density of a solution of the pigment at a concentration of one gram per liter (1.0 percent solution) read at the wavelength of maximum absorption in a cell one centimeter deep. The specific absorption coefficients for the isomers studied in this report are as follows:

α-carotene (in petroleum ether)	270 (21, p. 552)
neo-β-carotene B (in iso-octane)	192 (20, p. 296)
all-trans-β-carotene (in iso-octane)	251 (5, p. 20)

EXPERIMENTAL METHOD

In this study, Imperator carrots* were used. At the beginning of the experiment, a sufficient quantity was purchased at a local market for the entire experiment. In this attempt to assess the effects of cooking on the color of carrots, three cooking treatments were used. One lot of carrots was cooked to the just done stage in a saucepan, a second to the same stage but in a pressure saucepan, while a third lot was cooked for two minutes, a time midway between the minimum one minute and the maximum three minutes recommended for pressure saucepanning sliced young carrots (1, p. 23). Before attempting to evaluate the effects of cooking on the color of carrots it was deemed essential to control or at least to know the degree of doneness. Therefore tenderness of carrots from each of the three treatments was measured objectively and subjectively. Then the color of carrots from the three treatments was determined objectively and subjectively. In order to account for any changes observed in the color of the carrots the carotene content was analyzed chemically.

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*The author is indebted to Dr. James R. Bagget, assistant Professor of Horticulture, Oregon State University, for identification of the variety of carrots.

Preparing the Samples

Imperator carrots were pared and then diced on a Hobart institutional dicing attachment to a uniform size of half an inch. Pieces of carrots which were noticeably off color (green) were removed.

From the batch of diced carrots three 400-gram cooking lots were weighed. For the subsequent assay of pigments in the cooked carrots, three 5-gram portions of diced carrots, weighed before cooking, were tied loosely in cheesecloth bags and included in each cooking lot.

Cooking the Carrots

One lot was cooked in a small amount of water (150 milliliters) in a covered aluminum saucepan to the just done stage. The carrots were added when the water first boiled and cooking time was counted from return to the boiling point. When this quantity of carrots was cooked under these conditions it took 19 minutes to produce carrots which were just tender. A second lot of carrots was cooked on a rack in a two-quart aluminum pressure saucepan with 120 milliliters of water to the just done stage, also. After many trials, it was found that a cooking time of 50 seconds at 15 pounds pressure in the pressure saucepan made the carrots as tender as those cooked for 19

minutes in a saucepan. A third lot was cooked on a rack in the same pressure saucepan with 120 milliliters of water for two minutes. The cooking of the carrots by the three methods was repeated five times.

Subjective Ranking of Carrots for Color and for Tenderness

After the cooked carrots were drained, cooled and coded, they were ranked under a MacBeth lamp* with a daylight filter by the four judges. Judges ranked the three samples for hue (from red-orange to yellow) and for intensity (from bright to dull). Judges also ranked the three samples from most tender to least tender, indicating any samples considered either overdone or underdone. The evaluation of the cooked carrots for tenderness and for color by the four judges for each replication gave a total of 20 judgements.

Objective Assessment of Carrots for Color

The Hunter Color and Color-Difference Meter** is a tristimulus colorimeter which measures color on three scales to give uniform values for the visual perceptibility of differences. It evaluates

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*MacBeth Daylight Lamp, Type B BX-36, MacBeth Corporation, Newburgh, New York.

**Hunter Color and Color-Difference Meter, No. 106, Gardner Laboratories, Bethesda, Maryland.

the color of a sample in the same way that a skilled observer would under optimum conditions. The instrument compares an unknown specimen with a standard of predetermined color characteristics. The "L" scale measures visual lightness or the "value" of the sample. The "a_L" scale measures redness when plus, gray when zero and greenness when minus. The "b_L" scale measures yellowness when plus, gray when zero and blueness when minus (29, p. 1898). The machine is calibrated so that one unit is equivalent to a just noticeable difference discernible to the human eye.

The instrument was standardized against a National Bureau of Standards white porcelain plaque having the following "L" scale values: "L", 85.6; "a", -0.4; and "b", 0.5. After the samples were put through the shear press they were placed in a polystyrene dish and placed on the illuminated area of the instrument. The dish was rotated over the area at approximately 30 rpm by use of a spinning attachment to insure more reliable and precise measurements (7, p. 49).

Objective Assessment of Carrots for Tenderness

The Kramer-Shear Press* measures the force required to

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*Kramer Shear Press, L. E. E. Incorporated, 625 New York Avenue, Washington 1,, D. C.

push the shear blades through a sample in the cell. This force is plotted as a function of time. Each product has its own characteristic curve pattern (12, p. 343). The area under the curve is measured, in square inches, using a polar planimeter. The amount of work needed to shear the sample is proportional to the area under the curve and the greater the area the tougher the product.

For each test, 150-gram portions (drained weight) of cooked carrots at room temperature were placed in the cell of the Shear Press. The instrument was set for a working pressure of 400 pounds.*

Determination of Carotenoid Pigments in Cooked Carrots

Extraction

Cold extraction of carotene is necessary because in extracts from fresh tissues, where the carotene is predominately in the all-trans form, heating causes formation of cis isomers with consequent decrease in intensity of color (5, p. 12).

Glass apparatus with non-lubricated joints was used throughout. The extraction and subsequent handling of the pigments were carried

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*The author is appreciative of the assistance of Dr. Robert F. Cain, Professor of Food Science and Technology, in the use of the Hunter Meter and the Kramer Shear Press.

out in the minimum amount of light.

Extraction of carotene from the carrots was accomplished by a slight modification of the method of Moore and Ely (34, p. 135). Pigments were extracted from two of the three five-gram samples in each cooking lot; the third five-gram sample served as a spare. Thus ten assays of carotenes for each cooking treatment were made. Each five-gram sample was extracted in a Waring blendor with a foaming mixture of two volumes of 95 percent ethanol to one volume of petroleum ether.* Both the blendor and the extractant were chilled to lessen overheating during the extraction. One hundred milliliters of foaming mixture and nine milliliters of methanolic potassium hydroxide (one pound of potassium hydroxide in 1400 milliliters methanol) to improve foaming were used for the first extraction (54,p. 135). The blendor was run at low speed until the carrot was mascerated and then run with the transformer** set at 60 volts for 10 minutes. Intermittently, the sides of the blendor were scraped with a jar scraper and washed down with ethanol. The contents of the blendor were then filtered through a glass wool plug in a funnel into a separatory funnel. The residue of pulp on the glass wool was washed

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*Skellysolve B, b. p. 60-80, Skelly Oil Company.

**Powerstat Variable Transformer, The Superior Electric Company, Bristol, Conneticut.

alternately with petroleum ether and ethanol. The pulp, freed from the glass wool by washing with ethanol, was returned to the blendor with an additional 35 milliliters of foaming mixture. The blendor was run at high speed with the transformer set at 110 volts for two minutes. The sides of the blendor were again scraped and rinsed with ethanol and the blendor run an additional two minutes. The residue was allowed to settle and the supernatant liquid transferred into the separatory funnel containing the original extraction. If necessary, a small amount of water was added to cause separation of the petroleum ether and ethanol phases. The hypophase was transferred to another separatory funnel and washed with 30 milliliters of petroleum ether. The latter petroleum ether was combined with the original epiphase. The ethanol phase was twice again treated first with 20 milliliters and then with ten milliliters of petroleum ether. The combined petroleum ether extracts were washed with tap water six times to remove the alcohol. The extract was concentrated under vacuum with the flask immersed in tepid water to hasten the evaporation. During the evaporation the flask was wrapped in foil to exclude light. The extract was made up to 50 milliliters with petroleum ether.

Chromatographic Separation

A 20 milliliter aliquot of the petroleum ether extract from

each of the three treatments was then chromatographed on a 1:1 (w/w) column of magnesium oxide* and diatomaceous earth**. The adsorbents were dried in an oven at 80°C overnight. They were then mixed well and stored in the same oven. The column was 175 millimeters by 19 millimeters with a sintered glass stopper in the bottom. Each column was filled with petroleum ether into which the adsorbent was introduced. Adsorbent was added and the solvent allowed to flow through the tube until the adsorbent was packed ten centimeters deep (2, p. 155). A filter disk was placed upon the adsorbent to help prevent eddies. A one centimeter layer of anhydrous sodium sulfate was placed above the disk to remove any traces of water which might be in the petroleum ether extract. Additional solvent was added to keep air from the contents of the column until the 20 milliliters of carotene extract were introduced into the column. The extract was allowed to flow through the column until the top level of the liquid had reached the sodium sulfate layer; immediately, then, a developer of four percent acetone in petroleum ether was added to the column. Approximately 150 milliliters of developer was used to effect the separation of the pigments into four distinct, visible bands.

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*Sea Sorb 43, Adsorptive Magnesia, Westvaco, Fisher Chemical Company.

**Celite 545, Johns-Manville Company.

The extracts from the three cooking lots each gave the same distinctive zones on the adsorption columns. See Table 1. The

Table 1

Carotenes from Imperator Carrots

Band	Color	Carotene	Absorption Maxima in Petroleum Ether in mμ		
1	Orange	γ-carotene	495	463	
2	Pale-green	ζ- carotene	423	400	378
3	Red-orange	β-carotene	483	452	426
4	Yellow	α-carotene	478	447.5	

bands as identified by their relative position on the column and their absorption peaks on the spectrophotometer were as follows: typically at the top of the column appeared a very fluorescent residue of debris or "impurities" and unsaponified material. Bickoff states that if colored oxidation products of β-carotene are present, they remain firmly adsorbed near the top of the column (5, p. 23). Slightly below this an orange band of γ-carotene appeared. Below the γ-carotene, a pale green band of ζ-carotene was evident. Below the ζ-carotene, a broad red-orange band of β-carotenes appeared. Toward the bottom of the column a narrower band of α-carotene appeared. Below the α-carotene a colorless band of

phytoene or phytofluene was apparent only under ultra-violet light.

After separation of the pigments on the magnesium oxide column, each band was then eluted by the same developer and collected as a series of eluents.

The α-carotene band, the first to be eluted, was made up to a volume of 50 milliliters with additional petroleum ether.

The principal zone, a wide, bright orange β-carotene band, was the second to be eluted. If spectrophotometric measurements are to be made in the cis-peak region, it is essential that any acetone present in the eluent be removed (45, p. 463). Also, acetone in the developer was found to carry the entire β-carotene fraction in its solvent front during subsequent attempts at chromatographic separation of the β-carotenes. Therefore, the β-carotene band after elution from the column was washed free of acetone with six washes of distilled water and then made up to a volume of 100 milliliters with petroleum ether before rechomatographing on an Al_2O_3 column.

The ζ-carotene and γ-carotene fractions were not collected as they constitute only a small fraction of the total carotene content of carrots and would thus have little influence on the color.

Twenty-five milliliters of the fraction containing the β-carotene

were rechromatographed on an aluminum oxide* column. The column was 300 millimeters by 10 millimeters with a sintered glass stopper in the bottom. To prepare the column it was filled with petroleum ether and the aluminum oxide poured in. This adsorbent settled and gave a uniformly packed column. Enough aluminum oxide was used to provide a column about ten centimeters deep. Over the adsorbent a one centimeter layer of sodium sulfate was placed and the aliquot of the fraction containing the β-carotenes then introduced. The column was developed with three percent para-methylanisole** in petroleum ether as suggested by Bickoff et al. (6, p. 766). This developer caused the separation of a pale yellow band, neo-β-carotene B, below the all-trans-β-carotene band on the column. There was no separation of neo-β-carotene U, at least in quantities that could be seen. The same developer was used to elute the neo-β-carotene B which was collected as it washed off the column. This fraction was made up to a volume of 25 milliliters with petroleum ether.

Next, the aluminum oxide column was flushed with two five

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*Neutral Alumina AG7, Activity Grade I according to Brockman, 100-200 mesh, Calbiochem, Los Angeles, California.

**Eastman Organic Chemicals, Rochester 3, New York.

milliliter portions of petroleum ether to remove the para-methylanisole. Four percent acetone in petroleum ether was put on the column to elute the all-trans-β-carotene. This all-trans-β-carotene was collected and made up to a volume of 25 milliliters with petroleum ether.

Quantitative Estimation

A quantitative estimation of pigments was done on the original petroleum ether extracts, and on the following fractions after chromatographic separation and removal from the columns: α-carotene, total β-carotenes, neo-β-carotene B and all-trans-B-carotene. Since the original extracts contained α-, β-, ζ- and γ-carotene as well as any cis-isomers which might have been formed, the optical densities of these original extracts were read at a wavelength of 436 mμ on a Beckman DU Spectrophotometer to determine the absorbency. This is the best wavelength for mixtures of α- and β-carotenes and for the isomers of β-carotene (3, p. 61-62 and 4, P. 25). The quantity of pigment, reported as carotene, per gram of raw carrot was calculated according to the AOAC formula (2, p. 655):

$$C = \frac{\text{absorbancy x vol}}{196 \times L \times W}$$

where C equals the concentration of carotene in milligrams per gram, vol equals the total final volume of the eluate at times of reading, the figure 196 equals the specific absorption coefficient at 436 mμ, L equals the length of the cell in centimeters and W equals the weight

of sample in grams.

After removal from the column, fractions of the original extract were read at appropriate wavelengths to determine their absorbency. The α-carotene solution, in four percent or less acetone in petroleum ether, was read at 443mμ, the wavelength of maximum absorbency (21, p. 270). Four percent acetone in petroleum ether was found neither to affect readings at the wavelengths used nor to destroy carotene at this concentration. The total β-carotene fraction was read at 436mμ and the neo-β-carotene B fraction was read at 443mμ (20, p. 294). The all-trans-β-carotene fraction was read at its absorption maximum of 452 mμ (30, p. 135). In each case, the concentration of the particular pigment in grams per liter was calculated by dividing the absorbency of the test solution by the specific absorption coefficient. The specific coefficients used are found on page 23 of this paper.

In addition to calculating the concentration of β-carotene in this way, it was also read from a calibration curve of β-carotene, as a check on the calculated values. To prepare the curve, ten milligrams of 100 percent β-carotene* was made up to 100 milliliters in petroleum ether. Dilutions of 0.2, 0.5, 1.0, 1.5 and 2.0

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*Eastman Organic Chemicals, Rochester 3, New York.

micrograms per milliliter were made from the stock solution. These dilutions were read on the spectrophotometer at a wavelength of 436mμ, which is the best wavelength for the isomers of β-carotene and also at 452mμ which is the wavelength of maximum absorption for all-trans-β-carotene. Optical density readings were plotted against the concentrations of β-carotene in petroleum ether to obtain the standard curve. See Fig. 8.

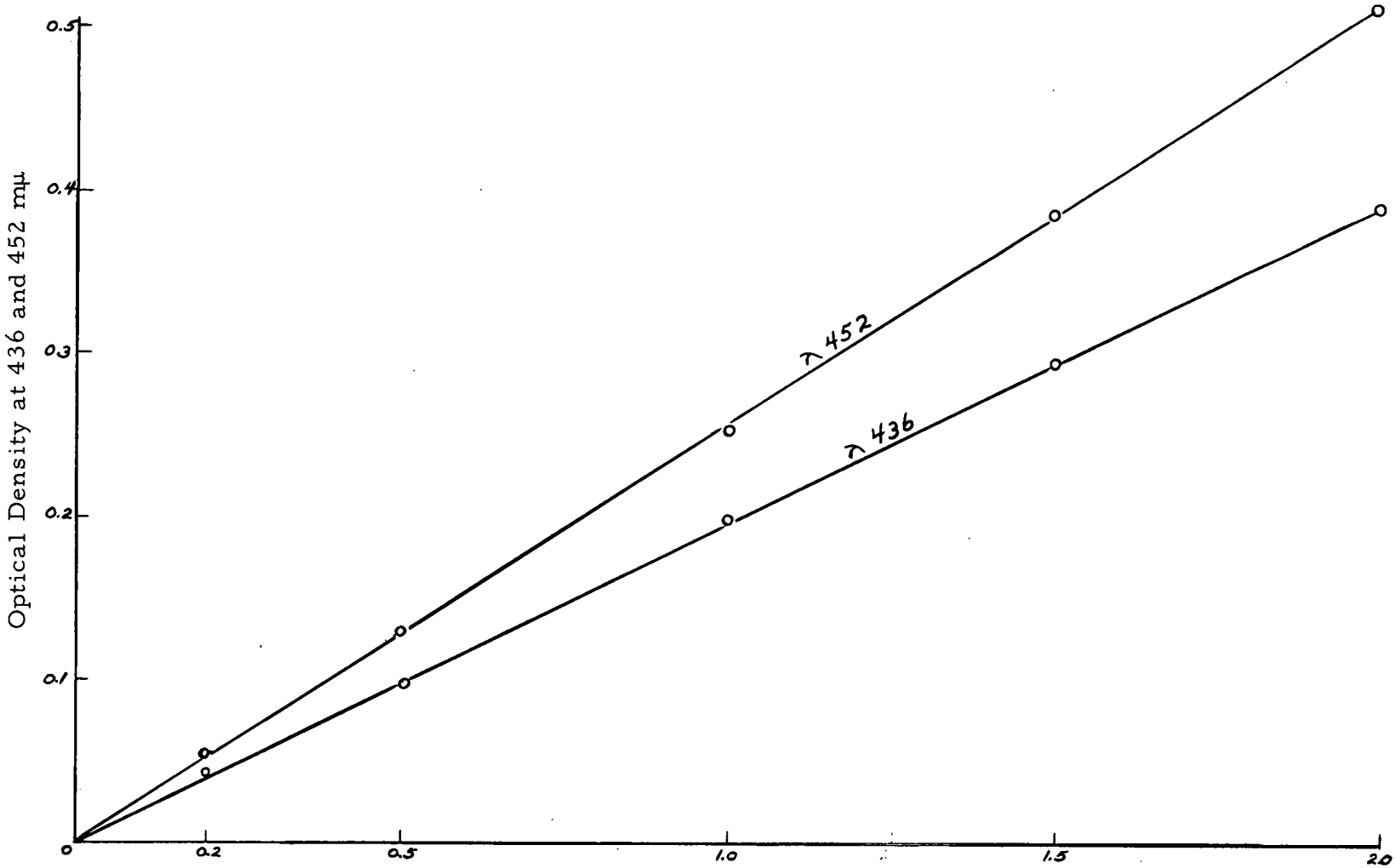

Figure 8. Standard Curve for β-Carotene

RESULTS AND DISCUSSION

Doneness of Cooked Carrots

Subjective Evaluation

The carrots cooked for two minutes in a pressure saucepan were judged most tender 19 times out of 20 and considered overdone 14 times. Carrots cooked in a covered saucepan for 19 minutes were judged least tender nine times out of 20. The same four judges ranked the carrots cooked in a pressure saucepan for 50 seconds as least tender 11 out of 20 times. In other words the latter two cooking procedures, in the opinion of the judges, produced carrots cooked to approximately the same tenderness.

Objective Measurement

Measurements made on the Kramer Shear Press are given in Table 2. For carrots cooked in a pressure saucepan for two minutes this area averaged 0.7 square inch. This small area under the curve indicates that relatively less work was required to shear these carrots; hence they were most tender. For carrots cooked in the saucepan for 19 minutes the area under the curve averaged 1.96 square inches. Carrots cooked in a pressure saucepan for 50

seconds averaged 1.93 square inches. Thus, this instrument showed that the carrots cooked in a saucepan and in a pressure saucepan for 50 seconds were about equally tender while those cooked in a pressure saucepan for two minutes were much more tender. Essentially, this was in agreement with the way the judges ranked the samples.

Table 2

Tenderness of Cooked Carrots as Measured by Shear Press*
(square inches)

Treatment	Replications					Mean
	I	II	III	IV	V	
Saucepan, 19 minutes	2.04	2.81	--	1.57	1.42	1.96
Pressure Saucepan, 50 seconds	2.52	1.92	1.35	2.12	1.78	1.93
Pressure Saucepan, 2 minutes	.95	.59	.46	.87	.69	.71

* Area under the curve is proportional to work required to shear the sample.

Typical work curves (Replication V) for carrots from the three cooking treatments are given in Fig. 9.

Fig. 9. Typical Work Curves

Color of Cooked Carrots

Subjective Evaluation

In judging hue, the panel ranked the carrots cooked in the saucepan most red-orange 19 out of 20 times. In every case, they ranked the carrots cooked in the pressure saucepan for two minutes the most yellow. In regard to intensity, the judges selected the carrots cooked in the saucepan as brightest in every instance. The carrots cooked for two minutes in a pressure saucepan were judged dullest 17 times and the carrots cooked in a pressure saucepan for 50 seconds as intermediate 17 times. Thus, the judges found the carrots cooked in a saucepan to be brightest and the most red-orange and those cooked in a pressure saucepan for two minutes to be dullest and most yellow. Judges were not asked to score the carrots as to

value, since the red-orange hue is inherently of lower value than yellow in the natural value order of the hues.

Objective Measurement

Colors of the cooked carrots as measured by the Hunter Color and Color Difference Meter are recorded in Table 3. Readings made on the "L"or value scale which show the amount of light reflected off the surface of the sample averaged 46. 4 for the carrots cooked for two minutes in the pressure saucepan and 46. 0 for those carrots cooked for just 50 seconds in the pressure saucepan. Those carrots cooked in a saucepan averaged 44. 8. The carrots cooked in the pressure saucepan were lighter (by two just noticeable differences) than those cooked in the saucepan and the cooking time in the pressure saucepan made no difference in lightness.

Readings made on the "a_L" scale, which measures red when positive, averaged 34. 0 for carrots cooked for two minutes in the pressure saucepan, 36. 5 for carrots cooked for 50 seconds in the pressure saucepan, and 38. 2 for carrots cooked in a saucepan, indicating that carrots cooked in a saucepan were definitely redder than those cooked under pressure. The carrots cooked in the saucepan evidenced nearly two units more redness than the carrots cooked in the pressure saucepan for 50 seconds and four units more than those

Table 3

Color of Cooked Carrots
(Hunter Color and Color-Difference Meter)

Hunter Scale	Treatment	Replications					Mean
		I	II	III	IV	V	
L	Saucepan	44.9	44.5	44.9	45.4	44.5	44.8
	Pressure Saucepan, 50 seconds	46.5	45.6	46.0	46.4	45.7	46.0
	Pressure Saucepan, 2 minutes	45.8	46.2	46.7	46.7	46.4	46.4
"a_L"	Saucepan	37.5	37.6	41.0	36.4	38.5	38.2
	Pressure Saucepan, 50 seconds	35.6	36.2	36.0	36.7	37.8	36.5
	Pressure Saucepan, 2 minutes	32.7	33.3	35.6	33.8	34.8	34.0
"b_L"	Saucepan	28.5	27.0	28.3	28.9	28.1	28.2
	Pressure Saucepan, 50 seconds	30.0	28.8	29.9	29.9	28.7	29.5
	Pressure Saucepan, 2 minutes	29.7	29.2	30.1	30.3	29.9	29.8

cooked for two minutes. This is in agreement with the judges who found the carrots cooked in the saucepan most red-orange 19 times out of 20.

Readings made on the "b_L" scale, which measures yellow when positive, averaged 29.8 for carrots cooked for two minutes in a pressure saucepan, 29.5 for carrots cooked for 50 seconds in a pressure saucepan and 28.2 for carrots cooked in a saucepan. The similarity of the "b_L" values for the carrots cooked in the pressure saucepan for both 50 seconds and two minutes would indicate that the time in the pressure saucepan was a less significant factor in the shift from typical red-orange color to yellow than is the method of cooking the carrots.

Thus, readings made on the "a_L" and "b_L" scales indicate that carrots cooked in a pressure saucepan appeared more yellow at the same time as they became less red. Although the judges appeared to find more differences in yellowness of the carrots than did the Hunter Meter, the judges and this instrument substantially agreed on the color of the carrots from the three treatments.

Carotenoids in Cooked Carrots

Total Carotenoids

The concentration of the total pigments in the cooked carrots

are shown in Table 4. The mean concentrations, in micrograms per gram of raw carrot, were 118, 107.7, and 102 for carrots cooked in a saucepan, in a pressure saucepan for 50 seconds and in a pressure saucepan for two minutes, respectively. The total carotenoids were lowest in the carrots cooked for two minutes in a pressure saucepan and 16 micrograms per gram of raw carrot higher in carrots cooked in a saucepan. The individual values varied from 84.7 to 156.1 micrograms per gram of raw carrot. This wide range was due in part to the inherent differences in carotene content of the carrots making up the five gram portions. These five gram sub-samples were made up of phloem and/or xylem pieces taken from various parts of the carrot. This no doubt accounts for much of the variation from replication to replication for any one treatment and between sub-samples within a replication. Aside from this variation, these figures represent a crude measurement, only, of the carotenoids in the carrots cooked by the three treatments.

α-Carotene

The concentrations of α-carotene in the cooked carrots are shown in Table 5. The mean concentrations of α-carotene in micrograms per gram of raw carrot were 12.6, 11.4 and 10.5 for carrots cooked in a saucepan, in a pressure saucepan for 50 seconds and in

Table 4

Total Carotenoids in Cooked Carrots
(mcg/gm)

Replication		Treatment		
		Saucepan 19 minutes	Pressure saucepan 50 seconds	Pressure saucepan 2 minutes
I	a	140.3	119.9	116.8
	b	103.0	90.8	93.8
II	a	86.2	85.7	84.7
	b	125.5	104.1	103.6
III	a	128.1	77.6	94.3
	b	156.1	119.2	103.3
IV	a	102.1	106.9	113.7
	b	88.8	123.9	85.2
V	a	138.3	122.2	120.4
	b	111.5	127.6	104.3
Mean		118.4	107.7	102.0

Table 5

α -Carotene in Cooked Carrots
(mcg/gm)

Replication	Treatment		
	Saucepan 19 minutes	Pressure saucepan 50 seconds	Pressure saucepan 2 minutes
I			
a	18.3	12.0	12.9
b	9.0	9.2	9.4
II			
a	8.3	8.3	10.7
b	14.9	10.9	11.4
III			
a	14.3	8.8	9.0
b	17.5	12.9	9.4
IV			
a	10.2	10.2	11.3
b	9.0	13.0	7.3
V			
a	14.2	14.5	12.5
b	10.8	14.1	10.9
Mean	12.6	11.4	10.5

a pressure saucepan for two minutes, respectively. While the average for the carrots cooked in a saucepan was highest and that for the carrots cooked in a pressure saucepan lowest, the averages were quite similar and there was considerable variation from replication to replication.

Neo-β - Carotene B

The concentrations of the neo-β-carotene B in the cooked carrots are shown in Table 6. The mean concentration of this cis-isomer in carrots cooked in the saucepan was 10. 7 micrograms per gram of raw carrot, with a standard deviation of 7. 0. The mean quantity of this cis-isomer in carrots cooked in a pressure saucepan for 50 seconds was 9. 4 micrograms per gram of raw carrot, with a standard deviation of 4. 1. The mean quantity of this same isomer in carrots cooked in a pressure saucepan for two minutes was 13. 5 micrograms per gram of raw carrot, with a standard deviation of 4. 5. The differences in values for this isomer are not great for the three treatments and the standard deviations are high, particularly in the carrots cooked in the saucepan.

All-trans-β-Carotene

The concentrations of all-trans-β-carotene in the cooked carrots are shown in Table 7. The mean concentration of all-trans-β-

Table 6

Neo-β -Carotene B in Cooked Carrots
(mcg/gm)

Replication	Treatment		
	Saucepan 19 minutes	Pressure saucepan 50 seconds	Pressure saucepan 2 minutes
I			
a	.8	4.2	15.1
b	5.2	14.3	7.8
II			
a	13.5	8.6	11.7
b	11.7	14.8	19.5
III			
a	25.0	9.9	16.1
b	17.4	15.1	21.3
IV			
a	8.1	5.5	15.6
b	10.2	9.1	10.9
V			
a	13.0	3.6	9.4
b	1.5	7.8	7.8
Mean	10.7	9.4	13.5

Table 7

All-trans-β-Carotene in Cooked Carrots
(mcg/gm)

Replication	Treatment		
	Saucepan 19 minutes	Pressure saucepan 50 seconds	Pressure saucepan 2 minutes
I			
a	62.4	58.5	47.0
b	57.0	40.8	43.0
II			
a	42.0	43.8	29.2
b	48.4	43.2	35.0
III			
a	53.4	27.4	33.0
b	63.8	44.6	35.0
IV			
a	53.0	54.2	45.0
b	35.6	56.4	32.2
V			
a	64.0	55.4	54.4
b	60.2	60.0	50.4
Mean	54.0	48.4	40.4

carotene in carrots cooked in a saucepan was 54. 0 micrograms per gram of raw carrot, with a standard deviation of 7. 0. For carrots cooked for 50 seconds in a pressure saucepan the mean was 48. 4 micrograms per gram of raw carrot, with a standard deviation of 9. 7. For carrots cooked for two minutes in a pressure saucepan the mean was 40. 4 micrograms per gram of raw carrot, with a standard deviation of 8. 2. Thus more of the original brightly-colored all-trans- β -carotene was found in the carrots cooked in a saucepan. The least amount was found in the carrots cooked in a pressure saucepan for two minutes and an intermediate amount in carrots cooked for 50 seconds in a pressure saucepan. However, the standard deviations are high and, therefore, the evidence is not clear-cut.

One means of checking on this variation was to calculate the percentages of the carotene of the β band recovered as all-trans- β -carotene plus the neo- β -carotene B from the aluminum oxide column. Values for the total β-carotene band as separated on the first magnesium oxide column averaged in micrograms per gram of raw carrot 66. 6 for those carrots cooked in a saucepan, 59. 7 for those cooked for 50 seconds in a pressure saucepan and 56. 1 for those cooked two minutes in a pressure saucepan. The sum of the two isomers, neo- β -carotene B and all-trans- β -carotene, recovered off the second aluminum oxide column was between 96 and

97 percent of the total quantity of the pigment in the β-carotene band from the carrots for each cooking method. The percentage recovery was 90 and 94 percent, respectively, for replications Ia and Vb where the values for neo-β-carotene B were extremely low.

Interpretation of the Data

Perhaps a clearer picture of the chemical changes which occurred as a result of the cooking treatment is given by considering the totals of the concentrations for the three fractions, all-trans-β-carotene, neo-β-carotene B and α-carotene, for each. See Table 8.

Table 8

All-trans-β-Carotene, Neo-β-Carotene B and α-Carotene in Cooked Carrots*
(mcg/gm)

Treatment	All-trans β-Carotene	Neo-β-Carotene B	α-Carotene	Total
Saucepan	54.0	10.7	12.6	77.3
Pressure saucepan 50 seconds	48.4	9.4	11.4	69.2
Pressure saucepan 2 minutes	40.4	13.5	10.5	64.4

*Average values of five replications.

The total concentration of all three fractions was highest in carrots cooked in a saucepan, intermediate in carrots cooked in a pressure

saucepan for 50 seconds and approximately 13 micrograms per gram of raw carrot lower in carrots cooked in a pressure saucepan for two minutes. Presumably, these progressively lower values can be attributed to oxidative losses. Any such oxidized carotene so formed would remain at the top of the first column. Thus part of the loss of color in carrots overcooked in the pressure saucepan, as found by the judges and the Hunter Meter, can be accounted for in this manner.

One contributing factor to this variation is undoubtedly due to variation in the initial carotene content of the pieces of carrot which constituted the raw sample. This inhomogeneity could account, in part at least, for variations between sub-samples for any one replication and treatment. Furthermore, it is not known whether the ratio of α-carotene to β-carotene is uniform throughout the various portions of the carrot. Therefore, it seemed advisable to calculate the percentages of the all-trans- β-carotene, the neo- β-carotene B and the α-carotene for each replication of each treatment, based on the total of the three. The averages of these percentages, for each of the ten analyses, are given in Table 9.

Table 9

Percentages of All-trans-β-Carotene, Neo-β-Carotene B and α-Carotene in Cooked Carrots*

Treatment	All-trans-β-Carotene	Neo-β-Carotene B	α-Carotene
Saucepan	69.8	13.8	16.3
Pressure saucepan 50 seconds	69.9	13.6	16.5
Pressure saucepan 2 minutes	62.5	21.2	16.3

*Based on the sums of the three for each of ten analyses

Figures in this table show that, on this basis, the α-carotene represented a constant fraction of the total pigments in the carrots from each cooking treatment. In regard to all-trans-β-carotene those carrots cooked in the saucepan or in the pressure saucepan for 50 seconds had the higher percentage and those cooked in a pressure saucepan for two minutes the lower percentage (69.8 and 69.9 percent vs. 62.5 percent). In regard to neo-β-carotene B, those cooked in the saucepan and in the pressure saucepan for 50 seconds had the lower percentage and those cooked in the pressure saucepan for two minutes the higher percentage (13.8 percent and 13.6 percent vs. 21.2 percent). Comparing carrots cooked in a saucepan with those cooked in a pressure saucepan for two minutes, the higher percentage of

all-trans- β -carotene, 7.9 percent for the former, was approximately the same as the higher percentage of neo- β -carotene B in the latter (7.4 percent). In other words, the differences in percentages of all-trans- β -carotene (7.9 percent) was approximately balanced by differences in percentages of neo- β -carotene B (7.4 percent), since the α -carotene fraction was the same in all three.

The data suggest that the differences in color as a result of the cooking treatment may be due in part to differences in destruction of pigments, especially the all-trans- β -carotene. Also contributing to the differences in the color of carrots from the three treatments, however, is the ratio of the neo- β -carotene B to the all-trans form. In this regard, the carrots cooked in a pressure saucepan for two minutes have a higher ratio of neo- β -carotene B to the all-trans form, while the ratio in the other two treatments is essentially the same. This conversion to the cis-isomer may be attributed to the effects of prolonged temperature in the presence of plant acids with a consequent loss in the bright, red-orange color of the carrots.

While the data show that there was an observable and measurable difference in the color of the carrots from the three cooking treatments, the quantity of carotene in the raw carrot is so high that changes in the color due to oxidation and/or isomerization do not materially impair the acceptability of the cooked product.

SUMMARY

1. Imperator carrots were cooked to the just done stage in a saucepan and in a pressure saucepan. A third lot was also cooked in a pressure saucepan for a time midway between the maximum and the minimum recommended. As defined in this experiment these carrots were considered overcooked. Doneness was determined by a panel of four judges and by the Kramer Shear Press.

2. For color the four judges ranked the carrots cooked in the saucepan as most red-orange 19 times out of 20 and brightest every time. The judges ranked the carrots which were overcooked in the pressure saucepan as most yellow every time and dullest 17 times out of 20.

3. The Hunter Color-Difference Meter showed that carrots which were cooked in a saucepan were two units redder than those cooked to the same doneness in a pressure saucepan and four units redder than those overcooked in a pressure saucepan. Thus cooking carrots in a pressure saucepan produced carrots which were yellower than those cooked in a saucepan. In addition, carrots which were overcooked in a pressure saucepan were two units lighter in value than the carrots cooked by either method to the just done stage.

4. Five-gram samples (raw weight) were included in each of the

cooking lots. Carotenes in these samples were extracted into petroleum ether for the subsequent chemical analysis. These extracts were chromatographed on magnesia columns to form several distinct fractions, of which the principal bands, α-carotene and β-carotene, were collected separately. The β-carotene band was rechromatographed on an aluminum oxide column to effect separation of the neo-β-carotene B and all-trans- β-carotene.

5. To determine the concentrations of each pigment, the optical densities of the original extractions, the α-carotene, the all-trans-β-carotene and neo-β-carotene B were read on a spectrophotometer at the wavelengths of their maximum absorbencies. The concentrations were calculated from the optical density readings, using the specific absorption coefficients.

6. The α-carotene averaged in micrograms per gram of raw carrot 12.6, 11.4 and 10.5 for carrots cooked in the saucepan, in a pressure saucepan for 50 seconds and in a pressure saucepan for two minutes, respectively. The neo- β-carotene B averaged in micrograms per gram of raw carrot 10.7, 9.4 and 13.5 for carrots cooked in the saucepan, in a pressure saucepan for 50 seconds and in a pressure saucepan for two minutes, respectively. The all-trans- β-carotene averaged in micrograms per gram of raw carrot 54.0, 48.4 and 40.4 for carrots cooked in the saucepan, in a pressure saucepan for

two minutes, respectively.

7. When the absolute values were totaled for each cooking treatment the lower totals for carrots cooked in a pressure saucepan could be accounted for by loss of all-trans- β-carotene.

8. When the α-carotene, the neo- β-carotene B and the all-trans- β-carotene were considered as percentages of the total for each treatment, it was apparent that the percentage of α-carotene remained constant. Carrots cooked in a pressure saucepan for two minutes had a lower percentage of all-trans- β-carotene and a higher percentage of neo- β-carotene B than did carrots cooked by the other two methods. The longer cooking in the pressure saucepan caused considerable conversion from the more vivid all-trans- β-carotene to the paler neo- β-carotene B. Apparently, the high temperature in the pressure saucepan plus the presence of plant acids was responsible for this conversion.

9. While cooking carrots brings about some loss of pigment and some isomerization with an accompanying decrease in the bright red-orange color of the carrots still, because of the high initial concentration of carotene, cooking does not materially impair the acceptability of the product.

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