

Characterization of a Stable Water-Dispersible Carotene Fraction from Dehydrated Sweet Potato Flakes

William M. Walter, Jr.* and Albert E. Purcell

A stable water-dispersible carotene fraction has been isolated from cooked sweet potato puree and reconstituted dehydrated sweet potato flakes. This fraction, formed when sweet potatoes are cooked and ground, is isolated from water soluble materials by precipitation with ethanol, and contains carbo-

hydrate and lipid-carotene components. The lipid-carotene portion exists in an aqueous medium as an emulsion with the carotene dissolved in the other lipid material. It is not known whether the carbohydrate component is associated with the lipid-carotene emulsion.

It was reported by Walter and Purcell (1970) that if ^{14}C - β -carotene is incorporated into dehydrated sweet potato flakes, the radioactivity is not equally distributed throughout these ^{14}C -labeled flakes. Upon reconstitution of this ^{14}C -labeled material, water dispersible and non-water-dispersible carotene containing fractions are isolated. It was found that the water-dispersible β -carotene fraction, although it contains a small amount of the total β -carotene, retains a disproportionately large amount of the added radioactivity. This finding, followed by a detailed study of cooked and uncooked sweet potatoes as well as dehydrated sweet potato flakes, indicated that β -carotene of cooked sweet potatoes and flakes is present in at least two fractions. This report concerns the isolation factors involved in formation, and the characterization of the water-dispersible β -carotene fraction.

MATERIALS AND METHODS

Dehydrated Sweet Potato Flakes. Centennial sweet potatoes were peeled, cut into cubes, and autoclaved at 121°C for 20 min in half their weight of water. The cooked cubes were ground into a smooth puree in a Waring Blendor. For those flakes which were to be made radioactive, an amount of ^{14}C - β -carotene calculated to be 0.1% of the carotene in the puree was dissolved in 5 to 10 ml of the specified solvent along with other designated compounds and added to a cooked puree with vigorous stirring. Experiments on the puree were performed at this stage. Where flakes were used, they were prepared by drying the purees on an 8×10 in. double drum dryer heated with steam at 60 psig (Purcell and Walter, 1968a).

^{14}C -Labeled- β -Carotene. This was obtained by growing mated cultures of *Blakeslea trispora* in the presence of 1,2- ^{14}C -sodium acetate (Purcell and Walter, 1968b). The specific radioactivity (sp. act.) was found to be 1.9×10^6 disintegrations per min per mg (dpm/mg).

Preparation of the Water-Dispersible Carotene Fraction. Purees of cooked Centennial sweet potatoes or reconstituted precooked dehydrated sweet potato flakes (flakes) were centrifuged for 35 min at $16,000 g$ (5°C). The supernatant was removed, the pellet suspended in a threefold (by weight) excess of boiling water, stirred for 10 min and the centrifugation repeated. Where additional aqueous extractions were used, the pellet resuspension and centrifugation steps were repeated. After the aqueous extractions were completed, the supernatants containing the water-dispersible

carotene fraction (DC) were combined, adjusted to pH 2 with hydrochloric acid, and mixed with sufficient 95% ethanol to give an ethanol-water ratio of 1 to 1. The resulting precipitate was collected by centrifugation as described above and designated as the precipitated water-dispersible carotene fraction (PDC).

Carotene remaining in suspension after ethanol precipitation was removed by mixing the ethanol-water supernatant with one-fourth volume of ether and adding two volumes of water. Suspended carotene was recovered in the resulting ether layer. That carotene left in the pellet after aqueous extraction was designated as non-water-dispersible (non-DC).

Analysis of Precipitated Water-Dispersible Carotene. Lipids including the carotenes were removed from PDC by exhaustive extraction with acetone followed by hexane (Figure 1). The amount of β -carotene was estimated by multiplying the absorbance at 450 nm by 4 to obtain the concentration in mg per l. Where flakes and puree containing ^{14}C - β -carotene were used, the β -carotene was quantitated and the sp. act. obtained as described earlier (Purcell and Walter, 1968a). After estimating the β -carotene content, the acetone-hexane extract was evaporated to dryness and held at 1 mm Hg for 2 hr. The residue was weighed and the ratio of lipids to carotene calculated.

Thin-layer chromatography of the PDC lipid-carotene mixture was performed on silica gel G with three solvent systems selected to separate neutral lipids and polar lipids. They were as follows: (1) naphtha:ethyl ether:acetic acid (90:10:1); (2) same as 1 except (70:30:2); (3) chloroform:methanol:water (75:22:2.5) (Stahl, 1965). After development, the plates were visualized with sulfuric acid-dichromate or molybdenum blue reagents.

The acetone-hexane insoluble residue was a water-soluble, white amorphous material. A 2% aqueous solution was tested with Benedict's reagent before and after hydrolysis with 0.1N hydrochloric acid. A portion of the acid hydrolyzate was studied for sugar content by paper chromatography (Block *et al.*, 1958). Another part of the aqueous solution was treated with potassium iodide-iodine solution (Bailey and Whelan, 1961) and the visible spectrum recorded with a Cary Model 15 spectrophotometer. Kjeldahl analysis for nitrogen was performed on weighed samples of the original residue.

Molecular Size. A portion of DC was filtered through 1.2 and 0.32μ filters. Additional information regarding the molecular size of the components of DC was obtained by gel filtration chromatography. One milliliter samples of aqueous 2% PDC were chromatographed on a column containing Biogel P-100 (Calbiochem) using distilled water or a 1%

*Department of Food Science, North Carolina State University at Raleigh, and Southern Utilization Research and Development Division, U.S. Department of Agriculture, Raleigh, N.C. 27607

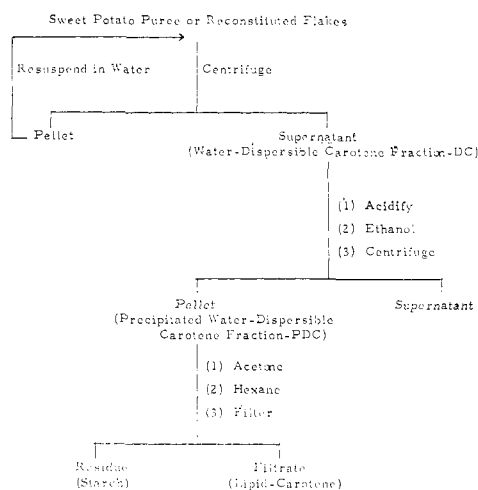


Figure 1. Flow sheet for isolation of the water-dispersible carotene fraction

sodium chloride eluant. The effluent was monitored for starch content with potassium iodide-iodine reagent (Bailey and Whelan, 1961), for carbohydrates with phenolsulfuric acid (Dubois *et al.*, 1956), and for carotene by extraction with ether-ethanol followed by spectrophotometry. An identical procedure was also used for chromatography on Sepharose 2B (Pharmacia Fine Chemicals).

Spectral Properties. The spectrum of the DC from 600 nm to 400 nm was obtained. The spectral absorbance maxima (λ max) were compared to the λ max of an aqueous emulsion of the acetone-hexane soluble portion of PDC and to those reported for colloidal and crystalline β -carotene, and cooked and raw sweet potato slices. The aqueous emulsion obtained from the PDC fraction was prepared by adding 1.0 ml of a solution of the lipid-carotene extracted from PDC in ethanol-ether (1 to 1) dropwise into 20 ml of boiling water. The volume was then reduced to 10 ml and the yellow solution filtered and diluted. The final concentration of β -carotene in the aqueous lipid-carotene emulsion was 32 μ g per ml.

In order to test for interaction between the lipid and starch components, a series of emulsions containing varying amounts of the PDC starch was prepared by diluting the emulsion described above and adding PDC starch. This series contained from 0 to 468 mg starch per mg carotene. The spectrum from 600 to 400 nm for each emulsion was obtained.

Fractional Precipitation of the Water-Dispersible Carotene Fraction with Ethanol. An aqueous emulsion containing DC was prepared from 10 g of flakes. A 50-ml aliquot was removed, treated with 5 ml of 95% ethanol, and centrifuged at 17,000 *g* for 10 min at 5° C. The pellet was removed, and the carotene was extracted and measured. The extracted residue was dried and weighed. This procedure was repeated

with increasing increments of 95% ethanol being added to the supernatant obtained from the centrifugation of the preceding fractionation.

RESULTS AND DISCUSSION

The centrifuged supernatant from cooked sweet potato puree or reconstituted flakes contained water soluble materials (*e.g.*, sugars, flavonoids, etc.) as well as the water dispersible carotene fraction (DC). Treatment of this supernatant with sufficient 95% ethanol to give a concentration of 50% ethanol caused the precipitation of PDC (Figure 1). Ether extraction of the supernatant showed over 98% of the water-dispersible carotene was removed by ethanol precipitation.

The PDC consisted of acetone-hexane insoluble (99% by weight) and lipid-carotene (1% by weight) components. PDC is obtained only from cooked, ground sweet potatoes or from reconstituted flakes. Attempts to obtain it from raw sweet potato puree prepared by grinding raw potatoes in the presence of an amylase inhibitor (0.1% mercuric chloride solution) were unsuccessful, indicating that cooking is essential for formation.

Formation and Isolation of the Water-Dispersible Carotene Fraction. The amount of PDC obtained from sweet potatoes varied according to the temperature of the cooked cubes when the puree was prepared. When puree was prepared from potatoes at 98–100° C, 32% of the total dry matter was found in the PDC. However, for puree prepared at 25° C, 7% of the dry matter was isolated in PDC. There was very little change in the amount of lipid-carotene component, regardless of the puree preparation temperature. The major change was in the acetone-hexane insoluble residue. Possibly grinding the cooked potatoes at the higher temperature fragments the swollen starch granules, causing large amounts of starch to be colloiddally dispersed in the aqueous medium and isolated in PDC when ethanol is added. When cooked sweet potatoes are allowed to cool before grinding, the starch molecules aggregate and thus are not dispersed by the grinding process.

Preparation of flakes from puree considerably increased the total amount of carotene isolated in PDC (Table I), although little weight increase was noted in the fraction. This is because the amount of acetone-hexane insoluble component (99% by weight) is not affected by the preparation of flakes. After six aqueous extractions, approximately 4% of the total carotene of puree was located in the PDC compared to 22% in the PDC of flakes. A plausible explanation for this observed increase of carotene in PDC from flakes is that heat decreases the viscosity of the lipid-carotene solution, and thus upon drum drying it is excluded from the larger starch aggregates as particles small enough to be suspended upon reconstitution of the flakes. An analogous situation was

Table I. Distribution of Carotene, Radioactivity, and Specific Radioactivity Ratio in ¹⁴C- β -Carotene Labeled Puree and Flakes^a

	Number of Aqueous Extractions	% Carotene in Fraction		% Radioactivity in Fraction		Specific Activity Ratio PDC/Non-DC
		Non-DC	PDC	Non-DC	PDC	
Puree	2	97.43	2.57	29.15	70.85	91.6
	6	95.58	4.42	20.39	79.61	83.9
Flakes	2	92.34	7.76	72.01	27.99	4.4
	6	77.92	22.08	46.66	53.34	3.8

^a Isolated from non-water-dispersible (non-DC) and precipitated water-dispersible (PDC) fractions.

found by another worker (Mitchell, 1969) who noted that a spray dried emulsion of acid dextrin and cottonseed oil when reconstituted with water contained particles of a much smaller particle size distribution than before drying.

The DC appears to be a discrete fraction. Four aqueous extractions of cooked puree or reconstituted flakes remove 75–80% of the total DC, and six extractions remove essentially all that can be removed. This suggests that the DC and non-DC are distinct and separable fractions. Addition of small amounts of ^{14}C - β -carotene to cooked purees showed that the added carotene was unequally distributed between the DC and non-DC, again suggesting discrete fractions (Walter and Purcell, 1970). Distribution and differences in the resulting sp. act. of the two fractions are shown in Table I. In the puree, about 80% of the added radioactivity remains in the DC (six extractions) causing the sp. act. of the carotene in this fraction to be 83 times as great as the sp. act. of the non-DC carotene. With flakes, some 53% of the radioactivity remains in the DC (six extractions) where the sp. act. of the carotene is 3.8 times that of the non-DC carotene. These data also suggest that preparation of flakes greatly modifies the environment of the lipid-carotene material of the puree.

Various means were tried to incorporate the added ^{14}C - β -carotene into flakes so that the labeled carotene was uniformly distributed throughout the flakes. However, as Table II shows, the sp. act. of the water-dispersible carotene is invariably greater than that of the main body. The only case in which the sp. act. ratio is less than 3.8 is with sweet potatoes which were ground raw and the amylosic enzymes allowed to hydrolyze the starch before flaking (Deobald *et al.*, 1968; Hoover, 1967).

Characterization of the Water-Dispersible Carotene Fraction. ACETONE-HEXANE INSOLUBLE COMPONENT. A 2% aqueous emulsion of this material gave a blue coloration with iodine-potassium iodide solution with a λ max at 550 nm. Treatment with Benedict's reagent gave a negative test for reducing substances. Hydrolysis of the 2% emulsion with 0.1 *N* hydrochloric acid followed by treatment with Benedict's reagent gave a strong positive test. Paper chromatography of the hydrolyzate indicated glucose to be the only sugar present. The protein content (Kjeldahl analysis) was less than 3%. A thick aqueous slurry of this acetone-hexane insoluble substance was hydrolyzed when treated with α -amylase (fungal, Calbiochem). From the above information it is concluded that the major portion of PDC is made up of starch or high molecular weight dextrans.

ACETONE-HEXANE SOLUBLE COMPONENT. Thin-layer chromatography indicated that in addition to β -carotene, neutral lipids and polar lipids were present. The lipid-carotene ratio was found to be 60 to 1 compared to 34 to 1 in the non-DC portion.

Physical Characteristics of the Water-Dispersible Carotene Fraction. An aqueous dispersion of the water-dispersible carotene fraction was found to be very stable with no flocculation noted even after standing several days at 5° C. This stability was noted for DC or a portion of PDC which had been dehydrated by freeze-drying and reconstituted with warm water. Three successive extractions of the aqueous dispersion with equal volumes of ethyl ether removed 25% of the carotene. Particle size distribution was obtained by passing the aqueous dispersion through 1.2 and 0.32 μ filters. The larger filter retained 17% of the carotene, while the smaller filter retained 97%.

When a 2% aqueous emulsion of PDC was chromatographed on a column of Biogel P-100, all components were

Table II. The Effect of Methods of Addition of ^{14}C - β -Carotene^a Upon Incorporation into Dehydrated Sweet Potato Flakes

Treatment ^{b,c}	Specific Radioactivity Ratio (DC β -Carotene/Non-DC β -Carotene)
^{14}C - β -Carotene in Ether-Ethanol (1:1)	4.02
^{14}C - β -Carotene in Ether	13.07
^{14}C - β -Carotene in Methylene Chloride	15.20
^{14}C - β -Carotene + 20 mg Methyl Stearate in Ether-Ethanol (1:1)	12.04
^{14}C - β -Carotene in Ether-Ethanol (1:1) added to raw grind puree ^d	2.34

^a ^{14}C - β -carotene added was approximately 0.1% of total carotene in the sample. ^b ^{14}C - β -carotene solution was added with vigorous stirring to the cooked puree before flaking. ^c The pellet was resuspended in water and recentrifuged once after the initial centrifugation. ^d Sweet potatoes were ground raw and held at 80° C for 5 min before cooking. After cooling, the label was added and flakes were made.

Table III. Spectral Absorption Maxima of Tissue Slices and Water-Dispersible Carotene from Centennial Sweet Potatoes, Aqueous Emulsion of PDC Lipids, Crystalline, and Colloidal β -Carotene

Source	Maxima, nm
Sweet Potato	
Raw Slice ^a	455, 480, 517
Cooked Slice ^a	455, 485
Water-Dispersible from Flakes	438, 457, 490
Water-Dispersible from Cooked Puree	437, 459, 492
Aqueous Emulsion from PDC Lipids	436, 460, 487
Crystalline β -Carotene ^b	435, 462, 492, 538
Colloidal β -Carotene Suspension ^b	428, 452, 478, 519

^a Purcell *et al.* (1969). ^b Tachibana and Nakamura (1965).

eluted in the void volume. The exclusion limit for this gel is 1×10^5 molecular weight. In contrast, Sepharose 2B gel gave two starch fractions. One fraction eluted in the void volume and the other appeared after three column volumes. The lipid-carotene component was also eluted in the void volume along with high molecular weight starch. This lipid-carotene material is thus of such size as to be excluded from the pores of Sepharose 2B which has an exclusion limit of 2×10^7 molecular weight (determined on random polysaccharide polymers). The stability of an aqueous emulsion of this water-dispersible carotene fraction and the small particle size involved indicate that β -carotene in this fraction exists in the colloidal state.

Spectral absorption maxima of the DC fraction, an aqueous emulsion of the lipid-carotene components of PDC, colloidal and crystalline β -carotene, and cooked and raw sweet potato slices are given in Table III. All components of Table III contain β -carotene as the major pigment. Differences in the absorption maxima can only be due to differences in the physical state of β -carotene. The data show that the maxima of the aqueous emulsion are very similar to those of the water-dispersible carotene from both flakes and cooked puree. Both crystalline β -carotene and colloidal β -carotene suspensions have a maximum at 519–538 nm which is totally absent in the other members of Table III. This maximum is due to the crystalline structure of β -carotene (Tachibana and Nakamura, 1965). The general shape of the absorption spectra is informative also. The spectra of water-dispersible carotene and the lipid-carotene emulsion have the appearance of a typical carotene-oil solution. That is, the central peak at 458 to 460 nm is the most intense, with lesser peaks at 435 and 490 nm. The crystalline and colloidal β -carotene spectra

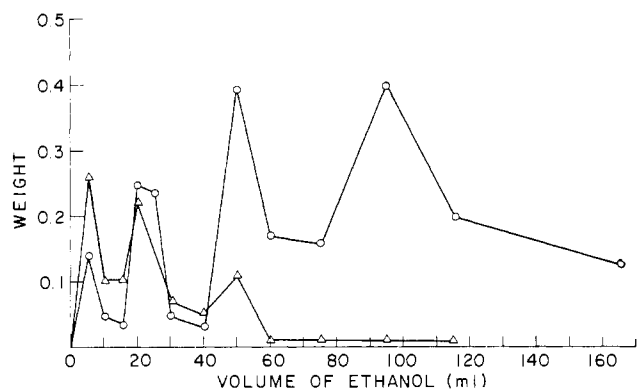


Figure 2. Fractional precipitation of the water-dispersible carotene fraction from precooked dehydrated sweet potato flakes with ethanol. \circ Carbohydrate weight in g; Δ β -carotene weight in mg

have the greatest absorbance at 428 to 435 nm, with less absorbance for the other peaks.

The absence of the crystalline absorbance maxima coupled with the similarity between the spectra of the lipid-carotene aqueous emulsion and the spectra of the water-dispersible carotene indicates that β -carotene of the water-dispersible carotene fraction is dissolved in the other lipid material. Purcell *et al.* (1969) postulated a dissolution of β -carotene in cellular lipids as being responsible for the change in color which high carotenoid vegetables undergo during cooking. They also noted a disappearance of a 518 nm peak when sweet potato slices were cooked.

Considering the stability and small particle size of an aqueous dispersion of the water-dispersible carotene fraction as well as evidence that the carotene is dissolved in lipid material leads to the conclusion that this lipid-carotene solution is colloiddally dispersed in water. In an aqueous medium the water-dispersible carotene fraction is made up of a lipid-carotene emulsion and starch components or aggregates of high molecular weight.

Evidence that the two components of the water-dispersible carotene fraction are associated in an aqueous medium is not definitive. Serial addition of the starch component to an aqueous emulsion of the lipid-carotene component followed by spectral examination of each emulsion indicated that there was no interaction between the chromophore of the lipid-carotene emulsion and the starch component. No shift in the λ max was noted even in the dispersion containing 469 mg starch component per mg of carotene.

Fractional precipitation from the DC fraction with increasing concentration of ethanol (Christensen and Smith, 1957) gave three fractions of starch or long chain dextrans associated with the lipid-carotene component and one very large carbohydrate fraction with virtually no associated carotene (Figure 2). These data indicate that the lipid-carotene emulsion is associated with three discrete starch or dextrin fractions, but it is possible that each precipitating fraction merely coprecipitates a part of the lipid-carotene component.

Observation of the presence of a lipid-carotene solution is of considerable value in the study of autoxidation of carotene in dehydrated sweet potato flakes since it allows the possi-

bility that part of the carotene is destroyed by a fatty acid-coupled oxidation mechanism. The starch component of the PDC fraction also may exert a considerable influence on carotene autoxidation in flakes when one recognizes the ability of starch to serve as a protective matrix toward lipids during autoxidation (Maywald and Schoch, 1957).

CONCLUSIONS

The stable water-dispersible carotene fraction isolated from cooked sweet potato puree or reconstituted dehydrated sweet potato flakes consists of starch and lipid-carotene components which are precipitated from solution when the concentration of ethanol is adjusted to 50%.

During cooking and grinding of sweet potatoes, some of the carotene is dissolved in cellular lipids and part of this solution is emulsified with water. Drying a puree of cooked sweet potatoes into flakes causes a larger portion of the lipid-carotene solution to be emulsified when the flakes are reconstituted. Some 15–25% of the carotene is water extractable as an aqueous lipid-carotene emulsion from flakes. The emulsion so formed has a particle size between 1.2 and 0.32 μ and is fairly stable. The lipid-carotene weight ratio of this emulsion is 60 to 1, while that of the non-water-dispersible carotene is 34 to 1, indicating that there is a difference in the environments of the water-dispersible and non-water-dispersible.

The major portion of the water-dispersible carotene fraction is made up of starch or dextrans of fairly high molecular weight, or at least aggregates of high molecular weight. It is not known whether this carbohydrate material acts merely as a protective colloid toward the lipid-carotene emulsion, or if a stable adduct is formed during the processing.

LITERATURE CITED

- Bailey, J. M., Whelan, W. J., *J. Biol. Chem.* **236**(4), 969 (1961).
 Block, R. J., Durrum, E. L., Zweig, G., "Paper Chromatography and Paper Electrophoresis," pp. 170–196, Academic Press, New York, 1958.
 Christensen, G. M., Smith, F., *J. Amer. Chem. Soc.* **79**, 4492 (1957).
 Deobald, H. J., McLemore, T. A., Hasling, V. C., Catalano, E. A., *Food Technol.* **22**, 627 (1968).
 Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F., *Anal. Chem.* **28**, 350 (1956).
 Hoover, M. W., *Food Technol.* **21**, 322 (1967).
 Maywald, E. C., Schoch, T. J., *J. Agr. Food Chem.* **5**, 528 (1957).
 Mitchell, W. A., "Symposium on Foods: Carbohydrates and Their Roles," H. W. Shultz, Ed., Section VI, pp. 428–429, Avi Publishing Co., Westport, Conn., 1969.
 Purcell, A. E., Walter, W. M., Jr., *J. Agr. Food Chem.* **16**, 650 (1968a).
 Purcell, A. E., Walter, W. M., Jr., *J. Label. Compounds*, **IV**, 94 (1968b).
 Purcell, A. E., Walter, W. M., Jr., Thompkins, W. T., *J. Agr. Food Chem.* **17**, 41 (1969).
 Stahl, E., "Thin Layer Chromatography," pp. 137–183, Academic Press, New York, 1965.
 Tachibana, T., Nakamura, A., *Kolloid-Z. Z. Polym.* **203**(2), 130 (1965).
 Walter, W. M., Jr., Purcell, A. E., *J. Agr. Food Chem.* **18**, 550 (1970).

Received for review August 14, 1970. Accepted October 26, 1970. Paper number 3164 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, N.C. 27607. Use of trade names of specific materials does not constitute a recommendation by the U.S. Department of Agriculture to the exclusion of others which may also be available.