Autoxidation of Carotenes in Dehydrated Sweet Potato Flakes Using ^{14}C-β-Carotene

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Autoxidation of carotenoids in food products is different from autoxidation in vitro. Radioactive carotenoids can be used to study autoxidation of carotenoids in food products when it can be shown that the added carotenoid behaves the same as native carotenoids. Xanthophylls are much more resistant to autoxidation in vitro than carotenes but only slightly more resistant in foods. Lutein added to dehydrated sweet potato autoxidized at about the same rate as carotene. When purified ^{14}C-β-carotene was added to dehydrated sweet potato flakes, the specific radioactivity of the total β-carotene fraction did not change during autoxidation of 45% of the total β-carotene. Label from the ^{14}C-β-carotene was distributed among saponifiable and nonsaponifiable lipids, methanol-water-soluble extract, and insoluble residues.

The oxidation of pure carotenoids in vitro has been extensively studied (Bodea, 1956; Bodea and Nicoară, 1956; Drozdova and Balakhovskis, 1955; Hunter and Krakenberger, 1947; Tsukida *et al.*, 1966; Wachs and Kockert, 1959). These studies show that autoxidation of β-carotene is initiated at the 3 or 3′ position, yielding carotene epoxides and 3- or 3′-hydroxycarotenes. The epoxides are further oxidized to carbonyls. The 3,3′-dehydroxycarotenes resist further oxidation, so that they accumulate as a result of the oxidation of β-carotene (Bodea, 1956; Bodea and Nicoară, 1956).

In studies of the change of carotenoids in precooked dehydrated sweet potato flakes (DSF), Purcell (1962) found no evidence that any group of carotenoids was preferentially oxidized or spared. Quackenbush (1963) found that the dehydroxy carotenoids of yellow corn were oxidized, although at slower rates than carotene. These data suggest that the mechanism of carotene autoxidation in food products is different from autoxidation in vitro.

The great difficulty of studying autoxidation of carotene in impure systems is that the origin of isolated compounds cannot be determined and in such complex systems as food products there is no way of knowing where to look for products of oxidation.

For example, Swain *et al.* (1964) isolated a number of compounds from autoxidized carrot flakes. It was possible to attribute only α- and β-ionone and their epoxides to carotene oxidation. It is believed that this problem can be eliminated by use of ^{14}C-labeled β-carotene added to the food products. If β-carotene were the only source of radioactivity, it must be assumed that any radioactive compound came from the carotene and that radioactivity in any noncarotene fraction indicates the presence of a product of carotene oxidation.

A study of the feasibility of using dehydrated sweet potato flakes containing ^{14}C-β-carotene for study of carotene autoxidation in food products is reported.

MATERIALS

Labeled β-Carotene. Carbon-14-labeled β-carotene was obtained by growing mated cultures of *Blakeslea trispora* (Anderson *et al.*, 1958) in the presence of sodium acetate-1,2-^{14}C (Purcell and Walter, 1968).

Lutein. Five kilograms of spinach were blended in 5 liters of water, mixed with 10 liters of methanol and 500 grams of Hyflo Super Cel, and filtered to dryness. The dry mat was extracted with acetone-hexane (1 to 1) until colorless. The acetone-hexane extracts were transferred to separatory funnels. Sufficient water was extracted from the mat to cause the acetone-hexane mixture to form two layers. The bottom layer, acetone-water, was drawn off and extracted with ether. The hexane upper layer was washed with water to remove the remaining acetone and shaken for 2 minutes with 1/4 volume of methanol saturated with potassium hydroxide. After about 20 minutes of standing distinct layers formed. The lower saponified layer was passed through ether. Both the hexane and ether extracts were washed free of alkali, combined and dried with sodium sulfate, evaporated to dryness, and taken up in hexane. The extract was chromatographed on magnesium oxide and developed with hexane-acetone-methanol, 89:10:1, until the xanthophylls were well separated. The major band, lutein, was carved out and eluted from the adsorbent with hexane-acetone-methanol, 50:40:10. The solvents were evaporated and lutein was crystallized twice from hot benzene. Melting point and visible spectra were similar to those previously

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reported (Karrer and Jucker, 1950). The ^{14}-C-β-carotene and lutein were recrystallized less than 24 hours before addition to the DSF.

METHODS

To establish the feasibility of studying autoxidation of carotenoids in food products by adding labeled carotene, it was necessary to establish that added carotene behaves the same as native carotene and that the label of oxidized carotene can be recovered in noncarotene fractions. Two methods were used to determine the similarity between added and native carotenoids: adding lutein to sweet potato flakes and following the carotene and lutein degradation during storage in an oxidizing atmosphere, and adding labeled β-carotene to sweet potato flakes and following the specific activity of β-carotene extracted from flakes stored in oxidizing atmospheres.

About 200 grams of DSF were prepared containing 7.534×10^5 disintegrations per minute (d.p.m.) of ^{14}C-β-carotene. After 3 weeks of storage under oxygen the DSF were extracted and the radioactivity in the various fractions was determined (Figure 1).

Addition of Carotenoids. Centennial sweet potatoes were peeled, cut into about 1-cm. cubes, and autoclaved at 125° C. for 15 minutes in half their weight of water. The cooked cubes were slurried in a Waring Blendor and the desired carotenoid, dissolved in ether-ethanol (1 to 1), was stirred into the slurry with rapid stirring. The puree was then dried to flakes on an 8 × 10 inch double drum dryer heated with steam at 60 p.s.i.g. Food material remained in contact with the drum for 45 seconds. The DSF were broken up to pass a 1-mm. sieve and then screened on a 0.175-mm. screen to remove the fine material, which was discarded. Samples were weighed into bottles, providing at least 10 ml. of head space for every gram of flakes. Air in the bottles was replaced with oxygen by evacuating the bottles in a desiccator and relieving the vaccum with oxygen. The bottles were stored in the dark at about 20° C.

Extraction of Carotenoids. Carotenoids were extracted from samples as previously described (Purcell, 1962), except that each water-soluble extract—methanol-water, acetone-water, and methanol-sodium hydroxide—was extracted with ether to assure quantitative recovery of the xanthophylls.

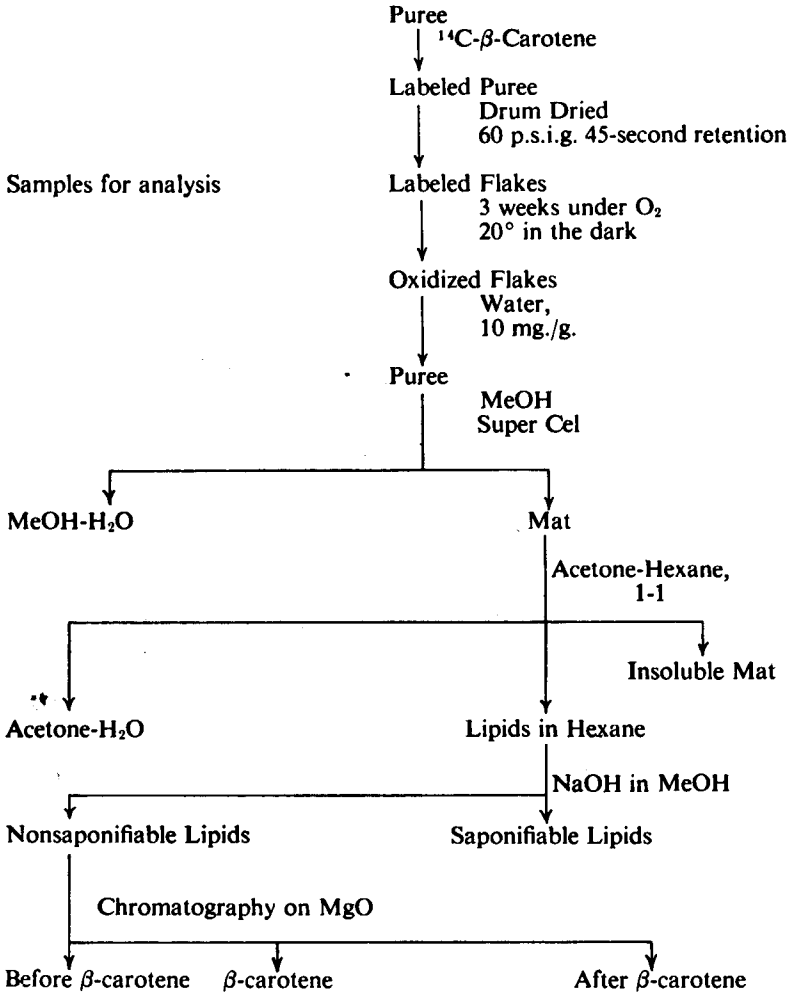

Figure 1. Flow diagram for withdrawing samples and analysis of fractions during study of autoxidation of ^{14}C carotene in sweet potato flakes

Estimating Carotene and Lutein. Hexane solutions of the nonsaponifiable extracts were partitioned with 95% methanol as described by Petracek and Zechmeister (1956). The amounts of carotene and lutein were calculated from absorbance at 450 mμ. This is believed to give more reproducible estimates than can be obtained by chromatography.

Determination of Carotene Oxidation and Specific Radioactivity. Carotenes were extracted as described previously. Absorbance of the nonsaponifiable extract at 450 mμ was used to calculate the amount of β-carotene, using an absorption coefficient of 0.250 per mg. per liter. The extracts were then chromatographed on magnesium oxide and the β-carotene bands carved out. The β-carotene was crystallized once from methanol and twice from hexane. Specific radioactivity expressed as disintegration per minute per milligram was determinined by measuring the amount of carotene spectrophotometrically and determining the amount of radioactivity.

Determination of Radioactivity. Radioactivity in various fractions was determined by use of a Packard Model 3002 TriCarb liquid scintillation spectrometer. Carotenes were decolorized before counting (Walter and Purcell, 1966). Aqueous extracts were counted by adding 1 ml. of extract, 9 ml. of absolute ethanol, and 10 ml. of a phosphor solution to each vial. Insoluble solids were counted in thixotropic gel (Rapkin, 1963).

RESULTS AND DISCUSSION

The validity of the study of autoxidation using radioactive material rests upon the assumption that native and added material have the same fate during autoxidation. Studies testing this assumption were conducted on DSF containing added lutein and ^{14}-C-β-carotene.

Studies of the autoxidation of DSF containing lutein added at two different levels (Table I) show that lutein is neither preferentially oxidized nor spared. This is in agreement with previous reports that native lutein and other xanthophylls are not preferentially destroyed or spared during oxidation (Purcell, 1962). Plotting linear regression lines for the loss of carotene and lutein shows that the loss of carotene is 0.313% per day, while the loss of lutein is 0.246% per day.

Samples of DSF containing small amounts of ^{14}C-β-carotene were extracted at intervals. The concentration and specific activity of β-carotene were determined (Table II).

Table I. Carotene and Lutein Concentration during Storage of Dehydrated Sweet Potato Flakes

Time, Days	Carotene, Mg./G.			Lutein, Mg./G.		
	Control[a]	Low[b]	High[c]	Control[a]	Low[b]	High[c]
0	0.458	0.464	0.461	0.014	0.052	0.164
7	0.449	0.460	0.462	0.017	0.056	0.160
20	0.421	0.425	0.436	0.019	0–054	0.155
34	0.406	0.404	0.410	0.015	0.050	0.149
48	0.381	0.369	0.381	0.013	0.048	0.140

[a] Control, no added lutein.
[b] Lutein added at 10% of carotene estimated in sweet potato.
[c] Lutein added at 40% of carotene estimated in sweet potato.

The specific radioactivity remained fairly constant during significant destruction of carotene, offering strong evidence that native and added carotene behaved similarly. With nearly 40% destruction of carotene in 35 days a change in specific activity of more than 4.8% would be expected if there were a significant difference in the behavior of added and native β-carotene. In a subsequent experiment 3.78 mg. of ^{14}C-β-carotene (13×10^6 d.p.m.) were added to about 1 kg. of sweet potato and incubated for 3 weeks. During this time 45% of the carotene was oxidized. The initial and final specific radioactivity were 9.73×10^4 and 9.64×10^4 d.p.m. per mg., respectively.

Microscopic comparisons were made of untreated sweet potato puree, sweet potato puree stirred with ether-ethanol, and sweet potato puree with large amounts of added β-carotene, 50% of native. No difference was found. No cells or identifiable plastids were found, indicating that the native carotene was released from the chromoplasts and distributed throughout the slurry. In all cases most of the carotenoids were observed in small droplets, and not associated with any type of structure. In all samples occasional small birefringent colored areas were found which appeared to be microcrystals of carotenoids. These observations indicate no reason to suspect that native and added carotenes would be distinguishable from each other.

Appreciable amounts of radioactivity were found in all fractions (Table III). Routine monitoring showed some volatile radioactivity upon opening the incubation jar. The 91% recovery of added label indicates that volatile components may represent a significant part of carotene oxidation products.

Table II. Specific Activity of ^{14}C-β-Carotene in Sweet Potato Flakes and Loss of β-Carotene during Storage at 18° to 23° C.

Time, Days	Total Carotene, Mg./G.	Specific Activity, D.P.M./Mg.	% Loss
0	0.71	1092	...
14	0.46	1204	35.2
35	0.43	1144	39.4

Table III. Distribution of ^{14}C from ^{14}C-β-Carotene Oxidized in Sweet Potato Flakes after 41% Loss of β-Carotene

Fraction	Total D.P.M. × 10^4	% of Added Radioactivity
MeOH-H_2O	4.38	5.81
Acetone-H_2O	4.366	5.79
Nonsaponifiable	48.958	64.98
Saponifiable	6.6198	8.79
Mat	4.1216	5.47
Total recovered	68.4454[a]	90.85
MgO chromatographic fraction		
Before β-carotene	1.48	1.96
β-Carotene	45.6	60.52
After β-carotene	1.756	2.33
Pure β-carotene	47.1685[b]	62.61

[a] Estimate 75.34 × 10^4 d.p.m. in total sample.
[b] Calculated from specific activity and amount measured spectrophotometrically in hexane extract.

CONCLUSIONS

Labeled β-carotene can be used to study autoxidation of carotene in food products. The wide distribution of label throughout all fractions suggests that the mechanism of carotene autoxidation in a food product is more complex than oxidation of pure carotene in glass. The use of labeled compounds offers the possibility of studies of autoxidation reactions of various compounds in any system in which the biostructure is destroyed. Further studies are under way to determine the identity of labeled fractions arising from β-carotene in autoxidized dehydrated sweet potato flakes.

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