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Fragmentation of β-Carotene in Autoxidizing Dehydrated Sweet Potato Flakes

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Autoxidation of carotenoids in dehydrated sweet potato flakes was studied using ^{14}C-β-carotene. The flakes were oxidized in the dark in sealed containers and analyzed periodically. The major portion of β-carotene was not attacked, indicating either unavailability for oxidation or formation of autoxidation retarding substances. β-Carotene which was attacked was rapidly oxidized mainly to lower molecular weight oxidation products, although some polymerization occurred. Oxidation products were separated into gaseous, steam volatile, water-extractable, acetone soluble, and insoluble fractions. Water-extractable, nonsteam volatile and acetone soluble fractions contained most of the radioactivity. Smaller amounts of radioactivity were found in insoluble, steam volatile, and gaseous fractions.

Oxidative deterioration with simultaneous color changes and off-flavor development in dehydrated sweet potato flakes (DSF) is of primary concern to sweet potato processors. The addition of antioxidants (Deobald *et al.*, 1964; Hoover, 1963) has been of limited value. Particularly vexing is the observation that different batches of DSF treated in the same manner may vary considerably in resistance to oxidative deterioration. There is a paucity of information concerning the manner in which autoxidation occurs in dehydrated food products such as DSF or dehydrated carrot flakes.

Studies of autoxidized dehydrated carrot flakes (Swain *et al.*, 1964) led to the conclusion that β-carotene (also the major pigment in DSF) destruction was related to off-flavor development. In contrast, other workers (Deobald *et al.*, 1964) found no direct relationship between carotene loss and off-flavor development in DSF. Such different findings concerning the role of β-carotene in off-flavor development in autoxidized dehydrated high-carotenoid vegetables were not explainable with present knowledge. An understanding of the pathway by which β-carotene is destroyed during oxidative deterioration of DSF is necessary if further progress is to be made in preventing such deterioration.

Initial attempts were made to study autoxidation of β-carotene in DSF by incorporating small amounts of highly radioactive ^{14}C-β-carotene into the puree before drum drying (Purcell and Walter, 1968b). Any fragments resulting from oxidation of β-carotene would be radioactive and thus distinguishable from other autoxidation products. This study showed that the added β-carotene behaved in the same manner as the native and that fragmentation was quite extensive. Later studies (Walter and Purcell, 1970) indicated the presence of two fractions of β-carotene in DSF. Addition of ^{14}C-β-carotene prior to drum drying of purees resulted in uneven distribution of radioactivity between these two fractions. With two fractions of β-carotene present, it is possible that two paths of oxidation exist, increasing the difficulty of interpreting results.

It is the purpose of this paper to describe the autoxidation of ^{14}C-β-carotene in DSF. The analysis of oxidized DSF was designed to give clearly definable fractions and provide a picture of the distribution of ^{14}C at different stages of oxidation.

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MATERIALS

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

Dehydrated Sweet Potato Flakes. The radioactive DSF were prepared by adding ^{14}C-β-carotene to a slurry of cooked Centennial sweet potatoes. The puree was then dried to flakes on an 8 × 10 in. double drum dryer heated with steam at 60 psig. The flakes were broken up to pass a 1-mm sieve and screened on a 0.175-mm screen to remove fine material which was discarded (Purcell and Walter, 1968b).

METHODS

Determination of Radioactivity. Radioactivity in various fractions was determined by the use of a Packard Model 3002 TriCarb liquid scintillation spectrometer. Quench correction was accomplished with an automatic external standard. Carotenes were decolorized before counting with the PPO-POPOP fluor system (Walter and Purcell, 1966). Aqueous samples were counted with a system containing Triton X-100 as a gelling agent and PPO-POPOP as the fluor (Lucier and Menzer, 1968). Insoluble solids were counted in thixotropic gel using the PPO-POPOP fluor system (Rapkin, 1963).

Sample Preparation. Five 50 g samples of DSF each containing 2.0 × 10^7 disintegrations per min (dpm) of ^{14}C-β-carotene were weighed out. One sample, used for a 0 time study, was analyzed immediately. The remainder of the samples were placed in 3 l. wide-mouthed jars. Each jar was then fitted with a stopper equipped with gas inlet and outlet tubes. A 3 g sample of Ascarite in cheesecloth was suspended in the jar above the flakes. The incubation jars were flushed with oxygen gas and sealed with clamps. The jars were placed in the dark at 22° C and analyzed at intervals of 20, 34, 49, and 89 days.

ANALYSIS

Determination of Radioactive Gaseous Products. After the designated reaction period, the incubation jar was attached to an analysis train. The train consisted of the following parts: a gas scrubber containing 3*N* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine (2,4-DNP) to trap carbonyls; a second gas scrubber containing 4% aqueous sodium hydroxide to trap carbon dioxide and volatile acids; an oxidation furnace to oxidize hydrocarbons and ethers (neutrals); and a gas scrubber containing 4% sodium hydroxide to trap carbon dioxide from the oxidation furnace. The

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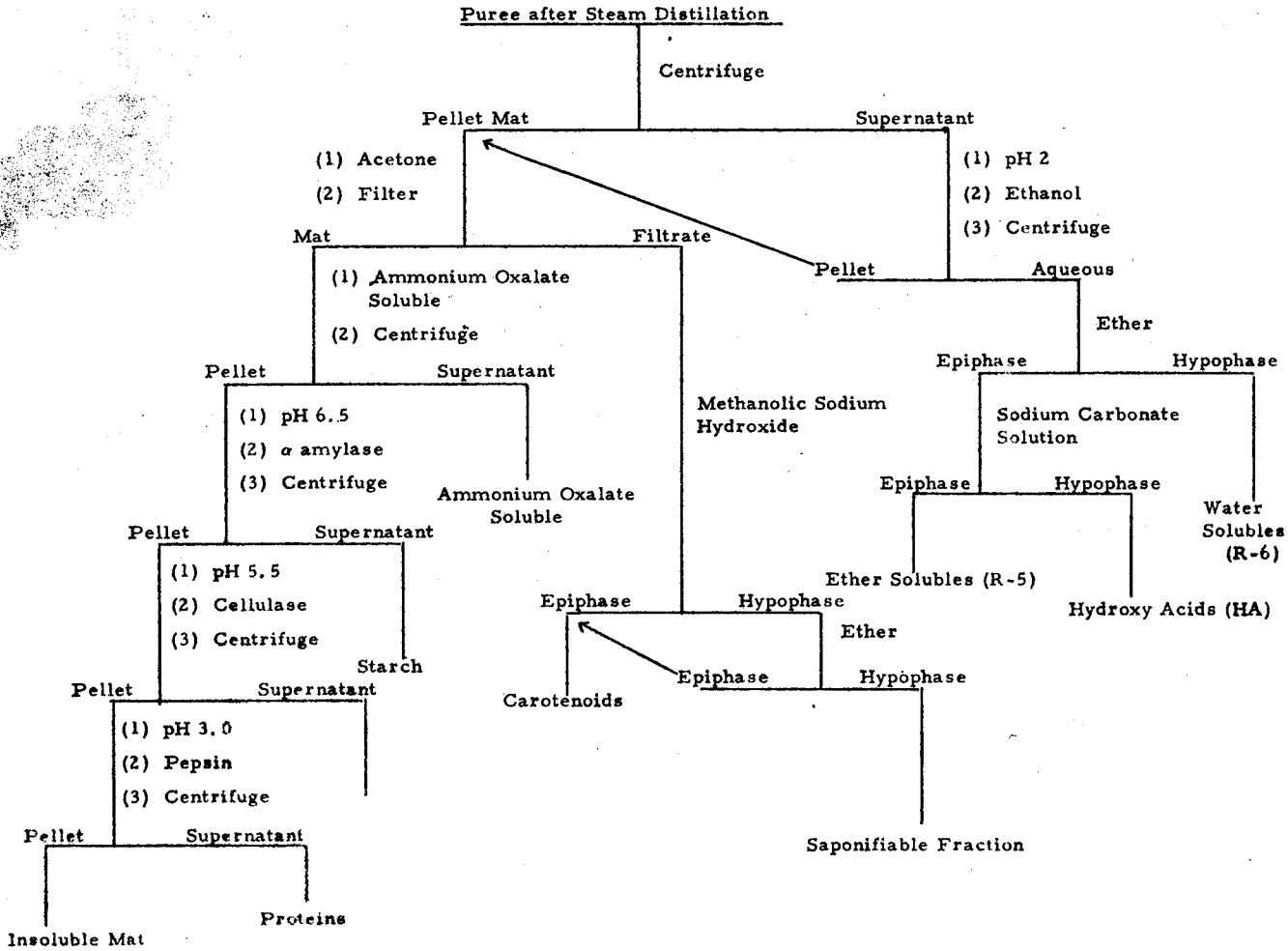

Figure 1. Flow sheet for fractionation of puree after steam distillation of dehydrated sweet potato flakes

oxidation furnace was constructed of a ¼ in. i.d. copper tube 12 in. in length packed with copper wire and wrapped in a heating tape. The gas stream from the second trap was mixed with an equal amount of oxygen and passed through the furnace which was heated to 300° C.

After the incubation jar was attached to the train, it was flushed with a total of 12 l. of nitrogen gas over a 2 hr period. When the indicated amount of nitrogen had passed through the incubation jar, the Ascarite was removed and counted. Each trap in the train was counted also.

Determination of β-Carotene. A 3-g sample was removed from the incubation jar and analyzed for carotene content, total radioactivity, and specific radioactivity of the remaining β-carotene (Purcell and Walter, 1968b). The data from this analysis was used to calculate the amount of carotene destruction and the total amount of radioactivity in the sample.

Determination of Steam-Volatile Radioactivity. The remaining 47 g were reconstituted with distilled water and steam distilled under vacuum (Cobb, 1969). The aqueous steam distillate, usually about 2 l., was removed and counted. A 500 ml aliquot was treated with 5 g of sodium chloride and 1 ml of concentrated hydrochloric acid. This solution was extracted three times with 100 ml portions of carbonyl-free benzene. All radioactivity was extracted into the benzene, indicating that no alkaline radioactive fragments were present. The benzene extract was extracted with 40 ml of 5% sodium bicarbonate to remove organic acids. Both phases were counted. Aliquots of the bicarbonate extracted benzene were used in the carbonyl and alcohol determination described below. One 25 ml aliquot was analyzed for carbonyl content (Henick *et al.*, 1954). Three 25 ml aliquots were withdrawn and treated as follows: no treatment, used as a blank; added to excess 2,4-DNP and trichloroacetic acid; and added to 59 mg of 3,5-dinitrobenzoyl chloride (3,5-DNB). These samples were sealed and allowed to stand overnight at room temperature. The benzene was then removed *in vacuo* and the samples vacuum distilled at 1 mm Hg for 2 hr at 65° C. The material remaining in each of the three samples was counted. Radioactivity in the 2,4-DNP treated sample was attributed to carbonyls, and counts in the 3,5-DNB treated samples were attributed to alcohols.

The net radioactivity due to carbonyls and alcohols was obtained by subtracting the counts found in the blank from the counts found in each of the treated samples. The difference between the radioactivity measured in the benzene and that found in carbonyls, alcohols, and acids was believed due to radioactive ethers and hydrocarbons (neutrals).

Radioactivity in the Puree after Steam Distillation. After steam distillation the puree was centrifuged at 14,600 g for 45 min at 4° C. The pellet material was washed by suspending it in water and recentrifuging. The combined supernatants were adjusted to pH 2 with hydrochloric acid, mixed with an equal volume of ethanol, and centrifuged as above. The small amount of pellet material was combined with the larger amount of pellet material obtained above (Figure 1).

Water-Extractable Materials. The ethanol-water super-

natant was extracted with ether to remove nonpolar organic materials such as carotenes and nonionized acidic components. The ether was extracted with 1% sodium carbonate solution to remove the acidic components which were designated as hydroxy acids (HA). The ether extracted aqueous layer was designated as R-6 and the ethereal sodium carbonate extracted layer as R-5. Radioactivity in each fraction was determined. The ether fraction was evaporated *in vacuo*, taken up in 20 ml hexane, and β-carotene present was estimated from the absorbance at 450 mμ. Radioactivity in R-5 was corrected by subtraction of counts due to residual β-carotene.

Pellet Mat. The pellet mat obtained by centrifugation was extracted with acetone in a Buchner funnel until the extracts were colorless. The mat was then treated with 250 ml of 0.25*M* ammonium oxalate. The resulting suspension was centrifuged at 30,000 g for 10 min at 0° C. All subsequent centrifugations were performed in the same manner. The supernatant was removed and the pellet resuspended in ammonium oxalate solution and recentrifuged. The combined supernatants were designated as ammonium oxalate soluble material and counted.

The ammonium oxalate insoluble material was successively digested with: 2.0 g of α-amylase (bacterial, Calbiochem) in 500 ml 0.02*M* disodium phosphate and 0.006*M* sodium chloride, pH 6.5; 1.0 g cellulase (Calbiochem) in 500 ml 0.02*M* sodium acetate, pH 5.5; and 1.0 g pepsin (porcine stomach mucosa, Calbiochem) pH 3.0. Each digestion was continued overnight at room temperature. The digested mixture was centrifuged and the pellet washed once with the buffer. The supernatants were designated starch, cellulose, and protein, respectively. The undigested mat weight after drying was in the range of 5 to 6 g or roughly 12% of the starting weight. Radioactivity in each fraction was determined.

Pellet Filtrate. The acetone filtrate was evaporated to dryness, taken up to 500 ml ether, and saponified by vigorous shaking with 100 ml of saturated methanolic sodium hydroxide. The lower saponifiable phase was removed and extracted with ether. This ether extract contained any carotenoids and other materials which partitioned into the saponifiable phase. These extracts were combined with the nonsaponifiable upper phase, evaporated to dryness, and subjected to counter current distribution on a 50 tube Post CDCD apparatus, using hexane-95% methanol as the solvent (Purcell and Walter, 1968c). When the distribution was complete, tubes corresponding to separate fractions were pooled. It was found that combining every five tubes was a satisfactory method of resolving the fractions. Each fraction was evaporated to 20 ml, the concentration determined as β-carotene (Purcell and Walter, 1968b), and an aliquot counted.

Tubes 41–50 contained β-carotene, epoxy carotenes, and nonpolar lipids. These tubes were combined and chromatographed on magnesium oxide. The band due to β-carotene was carved out and purified by recrystallization. The specific radioactivity was determined by measuring the amount of radioactivity as dpm in a known weight of ^{14}C-β-carotene (Purcell and Walter, 1968b). Radioactivity in the remainder of the magnesium oxide column was quantitated by eluting all radioactive materials with acetone-hexane (1 to 1) and counting the eluate. This radioactivity was added to that found in tubes 1–40 to give the radioactivity of the nonsaponifiable fraction not due to ^{14}C-β-carotene. Tubes 1–40 contained hydroxy carotenoids and other polar lipids. The ether extracted lower saponifiable phase was counted also.

RESULTS AND DISCUSSION

Dehydrated food products containing small amounts of fats and a relatively high concentration of carotenoids deteriorate when stored in the presence of oxygen. Carotenoid destruction with its resulting loss of color has been used as a measure of the progress of autoxidation in DSF (Deobald *et al.*, 1964) and chili peppers (Chen and Gutmanis, 1968). Although other important changes in chemical composition are undoubtedly occurring along with color loss, very little is known about these changes and even less about the fate of the destroyed carotene.

Food products containing large amounts of unsaturated fats have been studied quite extensively (Lundberg, 1962). In these foods, oxidative attack leads to high levels of peroxides whose concentration is used as an index of oxidative deterioration. On the other hand, in DSF with a fat content of 0.6% (Watt and Merrill, 1963) and carotene content of 0.07%, peroxides were either absent or at undetectable levels during oxidation. This investigation into oxidative deterioration of DSF uses ^{14}C to follow the pathway of carotene destruction and color loss as an index of the progress of autoxidation.

Probable Mechanism of β-Carotene Destruction. Autoxidation of ^{14}C-β-carotene labeled DSF leads to a wide distribution of label among the various fractions (Table I). Drum drying of the puree alone alters about 6% of the carotene, causing fragments from this processing to be found in most fractions.

An unusual characteristic of autoxidation of β-carotene in DSF is that a large portion of β-carotene is not available for oxidative attack (Table II). This finding is in contrast to a similar study of autoxidation of freeze dried carrots (Swain *et al.*, 1964) in which it was found that β-carotene was rapidly and completely destroyed. We found that during the first 20 days, 20.2% of the β-carotene was destroyed, while 6.8% was lost during the next 69 days. This trend of β-carotene destruction in DSF suggests oxygen depletion, formation of autoxidation retarding substances, or possibly protection of a large part of the carotene by materials native to the sweet potato. Oxygen depletion is unlikely since after 89 days the molar ratio of oxygen to carotene double bonds was 41. It is not possible at present to distinguish between the other possibilities.

Throughout the storage period, water-extractable and pellet filtrate fractions contained the largest amount of radioactivity, indicating that polar, water-extractable, and less polar longer-chain fragments were the primary autoxidation products. Polar, short-chain fragments were isolated in the water-extractable fraction. These nonsteam volatile compounds remained in the aqueous phase after centrifugation, followed by treatment of the supernatant with an equal volume of ethanol and recentrifugation (Figure 1). The major portion of the radioactivity in this fraction was ether extractable, indicating differences in polarity within the fraction.

The pellet filtrate, separated into nonsaponifiable and saponifiable components, contained less polar, longer-chain, colorless fragements from β-carotene oxidation. The nature of these fragments is deduced from the fact that although radioactivity in the polar portion of the nonsaponifiable fraction (tubes 1–40 of CDCD separation) rapidly increased during oxidation, the amount of carotenoid material (excluding unreacted β-carotene) exhibited no such increase. If there was any conversion of β-carotene into hydroxy carotenoids (xanthophylls) the radioactivity in this portion should increase. Table III shows that there is an increase in radio

Table I. Distribution of Radioactivity in DPM by Fractions in Autoxidizing Dehydrated Sweet Potato Flakes

Fraction	Time, Days 0	20	34	49	89
Gaseous Products					
Carbon Dioxide	...	2,912	5,655	6,257	10,684
Neutrals	...	717	524	629	436
Total	...	3,629	6,179	6,886	11,120
Steam Volatiles					
Carbonyls	962	16,316	16,913	14,737	12,439
Alcohols	983	4,935	4,486	3,298	3,125
Acids	...	...	1,098	300	318
Others	...	...	85	865	1,310
Total	1,061	21,251	22,582	19,200	17,192
Residue from Steam Distillation					
Water Extractable					
R-5	44,514	88,010	121,192	147,726	103,928
R-6	5,422	51,353	43,306	45,453	43,204
Hydroxy Acids	2,724	21,910	28,212	30,034	39,736
Total	52,660	161,273	192,710	223,213	186,868
Pellet Mat					
Starch	907	4,768	8,018	7,464	7,967
Cellulose	318	1,926	2,700	816	2,790
Protein	612	2,352	1,702	3,781	5,508
Mat	1,607	5,512	6,961	8,983	6,360
Ammonium Oxalate Soluble	11,457	19,103	26,865	32,692	32,352
Total	14,901	33,571	46,246	53,736	54,977
Pellet Filtrate					
Carotenoids[a]	52,085	124,649	151,351	110,251	107,539
Saponifiable Fraction	14,973	52,717	67,031	77,217	75,533
Total	67,058	177,366	218,382	187,468	182,892

[a] Excluding unreacted β-carotene radioactivity.

Table II. Destruction of β-Carotene in Dehydrated Sweet Potato Flakes

Time, Days	Mg β-Carotene in 3 g Sample	% Loss of β-Carotene
0	1.78	...
20	1.42	20.2
34	1.36	23.6
49	1.35	24.2
89	1.30	27.0

Table III. Relationship between Radioactivity and Carotene Content in the Polar Carotenoid Fraction[a]

Time, Days	Total Dpm × 10^4	Total Carotene[b] (Mg)	Specific Activity Dpm/Mg × 10^6
0	1.448	0.570	0.254
20	5.204	0.405	1.283
34	4.963	0.370	1.334
49	4.440	0.367	1.224
89	5.856	0.408	1.435

[a] Determined as β-Carotene. [b] Tubes 1–40 of CDCD separation of the nonsaponifiable fraction from the pellet filtrate.

activity; however, the amount of carotene decreased slightly during the oxidation. Additionally, from a study of Table I, it is evident that radioactivity in the saponifiable component also increased during storage. The fragments isolated in this fraction are colorless compounds which are solubilized by the strong alcoholic base.

Polymer formation was indicated by radioactivity in the pellet mat. These polymers could have resulted from polymerization of the β-carotene itself or from copolymerization between β-carotene oxidation fragments and polymeric material native to the sweet potato, such as pectins, starch, cellulose, and proteins. The amount of radioactivity found in this fraction suggests smaller amounts of β-carotene are involved in polymerization than in the fragmentations discussed above.

Still smaller amounts of radioactivity were found in the steam volatile fraction. A large part of this radioactivity was found in the carbonyl portion. Smaller amounts of activity appeared in the alcohols with very little in the acids and hydrocarbons. Total steam volatile carbonyls as measured by the Henick-Benca method paralleled radioactivity in the carbonyls throughout the reaction. Saturated carbonyl content was greater than unsaturated carbonyls at every analysis period.

The spectral and thin-layer chromatographic properties of the 2,4-DNP derivative of authentic β-ionone were compared to those of the 2,4-DNP derivative of the most radioactive carbonyl isolated from the steam volatile fraction. It was impossible to distinguish any difference between the two by these methods, indicating that β-ionone is an important component of the steam volatile carbonyls. A compound tentatively identified by thin-layer chromatography as acrolein also contained appreciable radioactivity. This indicates that at least a small portion of β-carotene was oxidized to short-chain carbonyls and alcohols. After the oxidation of β-carotene slowed, the production of these materials essentially ceased.

The least amount of radioactivity was found in gaseous

products. Radioactivity in this fraction was due to carbon dioxide and/or volatile organic acids. The steady increase of radioactive fragments in the gaseous products even after β-carotene destruction has slowed could indicate that some fragments from β-carotene destruction are themselves oxidized into gaseous products.

Although radioactivity in gases and steam volatiles was small, it should be noted that these materials are very important to aroma acceptance. It remains to be seen whether these radioactive components and therefore indirectly β-carotene was responsible for the off-flavor associated with autoxidized DSF.

With two fractions of β-carotene present, the possibility of different paths and rates of oxidation exists. Consequently, the results of this study could be more representative of the more highly labeled fraction than of the total carotene. A model system was prepared from lipid-free insoluble solids of sweet potato, sucrose, and ^{14}C-β-carotene in peanut oil. In this system it was known that the entire β-carotene component had the same specific activity. The model system was oxidized and studied by the methods described. The pattern of distribution of radioactivity in the model system was found to be similar to that in DSF, indicating that both fractions of β-carotene oxidize in essentially the same manner.

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