Individual Carotenoid Content of SRM 1548 Total Diet and Influence of Storage Temperature, Lyophilization, and Irradiation on Dietary Carotenoids

Neal E. Craft* and Stephen A. Wise

Organic Analytical Research Division, Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899

Joseph H. Soares, Jr.

Nutritional Sciences Department, University of Maryland, College Park, Maryland 20742

A modified version of the AOAC procedure for the extraction of carotenoids from mixed feeds was coupled with an isocratic reversed-phase liquid chromatography (LC) method to measure individual carotenoids in SRM 1548 total diet and in a high-carotenoid mixed diet (HCMD). The major carotenoids identified in SRM 1548 were lycopene, β-carotene, lutein, α-carotene, and zeaxanthin in descending order of concentration. The concentration of all carotenoids in SRM 1548 decreased as storage temperature increased. Significant differences in carotenoid concentrations occurred between -80 and 4 °C storage temperatures. Lyophilization of the HCMD significantly decreased β-carotene and lycopene concentrations and produced an apparent increase in xanthophyll concentrations. Exposure to γ-irradiation significantly decreased α-carotene and β-carotene concentrations and led to an apparent increase in β-cryptoxanthin. SRM 1548 was found to be unsuitable for use as a reference material for carotenoid measurements, while HCMD has greater potential as a reference material.

As the association of carotenoids and retinoids with cancer (Peto et al., 1981; Ziegler et al., 1986; Ziegler, 1991), coronary artery disease (Gaziano et al., 1990), and morbidity and mortality (Rahmathullah et al., 1990) continues to be elucidated, awareness of dietary carotenoid consumption has heightened. The National Cancer Institute has recommended that people increase their intake of high-carotenoid foods (Butrum et al., 1988). However, food composition tables are severely lacking with regard to carotenoid composition of foods. Recently several groups (Khachik et al., 1986, 1989; Sweeney and Marsh, 1971; Heinonen et al., 1989; Bushway, 1986; Bureau and Bushway, 1986) have made strides to improve the quality and specificity of information available about the carotenoid content of foods. In most cases, the work involves the determination of individual carotenoids in high-carotenoid-containing fruits and vegetables. Little progress has been made in determining the individual carotenoid content of representative total diets from specific geographical areas. Such information would expedite epidemiologic comparisons between carotenoid consumption and the incidence of disease.

The United States Total Diet (USTD), which was collected as part of the U.S. Food and Drug Administration's Total Diet Study, consists of a mixed homogenate of representative proportions of 201 foods typically consumed by 25-30-year-old males in the United States (Pennington, 1983). The preparation and further treatment of the USTD is described by Wolf et al. (1990). Standard Reference Material (SRM) 1548 is a portion of USTD that was lyophilized, cryogenically homogenized, γ-irradiated for sterilization, and bottled in approximately 6.5-g quantities.

Recently Iyengar et al. (1987) demonstrated that elemental analysis of SRM 1548 was comparable to the sum of individual analyses of the requisite foods composing the mixed-diet material. Although the composition of individual foods cannot be determined by measuring composites, the analysis of composites is obviously less tedious, less time-consuming, and less difficult than for some individual foods.

The usefulness of total diet organic nutrient measurements in epidemiological studies is contingent on the final composite analyte concentrations being representative of the initial food materials. Unlike inorganic nutrients, many organic constituents, including carotenoids, are altered during handling and processing. Carotenoids are labile to oxygen, heat, acids, alkali, light, and oxidative metals (De Ritter and Purcell, 1981). The effects of storage temperature, lyophilization, and irradiation on the initial carotenoid concentrations are uncertain. Carotenoid losses in response to freeze-drying and irradiation are variable depending on the matrix and the processes used (Kläui and Bauernfeind, 1981; Boeh-Ocansey, 1984; Park, 1987; Bhushan and Thomas, 1990). These factors must be evaluated to determine the most suitable means of obtaining, preparing, and storing diet homogenates for organic analyses.

Additionally, the validity and intercomparability of carotenoid measurements are dependent on the availability of a common "measuring stick". Currently there are no certified reference materials available for carotenoids in food matrices for verification of methods and for value assignment of in-house control materials.

The objectives of this investigation were to use liquid chromatography (LC) (1) to identify and quantify the major carotenoids in SRM 1548, (2) to determine the effects of sample handling and processing on carotenoid concentrations of a mixed-diet material (HCMD), and (3) to evaluate the use of SRM 1548 as a mixed-diet carotenoid reference material.

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* Author to whom correspondence should addressed.

Table I. Composition of High-Carotenoid Mixed Diet

ingredient	amount,[a] g	protein, g	carbohydrate, g	fat, g
apricots/tapioca	150	0	25	1
carrots	150	0	7	0
creamed corn	150	0	21	1
creamed peas	150	2	11	3
green beans	150	1	8	1
peaches	150	0	22	1
squash	150	1	10	0
sweet potatoes	150	1	23	1
tomato paste	200	3	22	3
vegetable oil	32	0	0	32
bovine albumin	42	42	0	0

[a] Proximate analysis supplied by manufacturer.

Table II. Comparison of Proximate Composition of USTD and HCMD

composition	USTD[a]	HCMD[b]
volatiles, %	83.8	82.5
carbohydrate, %	9.2	10.1
protein, %	3.4	3.4
fat, %	2.9	2.9

[a] Composition reported by Iyengar et al. (1987). [b] Composition determined by nutritional labeling of individual foods and addition of known amounts of protein (bovine albumin) and lipid (vegetable oil). Volatiles were determined by drying duplicate samples of HCMD overnight at 110 °C.

MATERIALS AND METHODS

Diet Samples. Samples of the SRM 1548 were obtained shortly after its preparation and were stored at 4, -20, and -80 °C for 1 year prior to analysis. Frozen samples of the USTD homogenate prior to lyophilization and irradiation were not available for analysis.

High-Carotene Mixed Diet. A surrogate high-carotene mixed diet (HCMD) homogenate was prepared, by mixing commercially available baby foods and tomato paste as listed in Table I, to simulate the composition of the USTD to evaluate the effects of lyophilization and γ-irradiation on carotenoid concentrations in a mixed-diet matrix. The mixture of commercially prepared baby foods and tomato paste was supplemented with protein and lipid to achieve the levels present in the USTD (Iyengar et al., 1987). The proximate composition of USTD as reported by Iyengar et al. (1987) and the composition of HCMD based on manufacturer labeling and supplementation of lipid and protein are listed in Table II.

Lyophilization and Irradiation. A portion of the frozen HCMD was lyophilized. Additional frozen samples of the HCMD in a container of dry ice were irradiated with 0, 10, 20, 35, and 50 kGy of γ-irrariation from [60]Co. The dose rate was approximately 111 Gy/min. The dry ice was replenished at each exposure interval to keep the sample frozen and to provide a reduced oxygen environment. All samples of HCMD were stored at -20 °C for less than 3 months prior to analysis.

Reagents. Ethanolic solutions of 0.05-1.0 μg/mL of lutein (Kemin Industries, Des Moines, IA), zeaxanthin, β-cryptoxanthin (Atomergic Chemetals, Farmingdale, NY), lycopene (extracted from tomato paste), α-carotene, and β-carotene (Sigma Chemical Co., St. Louis, MO) were prepared containing echinenone (Hoffmann-La Roche Inc., Nutley, NJ) as a volume correction standard. The solvents used were absolute ethanol, acetone, hexane, methanol, pentane, tetrahydrofuran (THF) stabilized with butylated hydroxytoluene (BHT, 0.01%), toluene, and water. All solvents were of HPLC grade or equivalent and were used without further treatment. Reagent grade pyrogallic acid, potassium hydroxide, and anhydrous sodium sulfate were used in the sample extraction.

Extraction. The AOAC procedure (AOAC, 1980) for measuring carotenoids in mixed feeds was modified and used to extract the diet materials. Duplicate aliquots of approximately 1.5-2.0 g of SRM 1548, 0.5-0.6 g of HCMD, or 0.1 g of lyophilized HCMD were accurately weighed into 50-mL glass screw-top centrifuge tubes. To each aliquot were added 1.0 mL of water, 250 μL of echinenone in ethanol (as a volume correction standard), 250 μL

of 5% pyrogallol (w/v), and 15 mL of HEAT (10 hexane/6 ethanol/7 acetone/7 toluene). The mixture was vortex-mixed for 60 s. One milliliter of 40% KOH (w/v) in methanol was added to each sample and vortex-mixed for 60 s. The loosely capped tubes containing the mixtures were heated in an aluminum heating block at 56 °C for 30 min and then allowed to cool to room temperature. To each tube was added 15 mL of hexane and mixed; the solution was then diluted to 50 mL with 10% Na$_2$SO$_4$ solution (w/v) and vortex-mixed for 60 s. The samples were allowed to stand for 1 h at room temperature and then the upper organic phase was removed and washed with water to remove fatty acid soaps. The organic phase was dried by passing over anhydrous Na$_2$SO$_4$. Each extract was divided into two parts and concentrated using a centrifugal evaporator (Savant, Farmingdale, NY). Half of each extract was dissolved in 300 μL of THF/ethanol/methanol (1:1:1) for reversed-phase LC, and the other half was dissolved in 250 μL of hexane for normal-phase LC.

LC Analysis. All samples were analyzed by reversed-phase (RP) and normal-phase (NP) LC. The RPLC method of Craft et al. (1991) was used. Briefly, the LC system consisted of a dual-piston pump with a column oven regulated at 20 °C; a C$_{18}$, 5-μm particle size, 4.6 × 250 mm column (Vydac 201TP C$_{18}$, The Separations Group, Hesperia, CA); and 5% THF in methanol as the mobile phase at a flow rate of 1.0 mL/min. Carotenoids were monitored at 450 nm using a UV-vis detector (Model 206, Linear Instruments, Reno, NV) equipped with a tungsten lamp. All stainless steel frits were replaced with "biocompatible" materials (i.e., ceramic, titanium, or Hastalloy C).

NPLC was used as a second chromatographic technique to verify carotenoid peak identities. The NPLC system consisted of a single-piston, ternary solvent pump; a silica 5-μm particle size, 4.6 × 250 mm column (YMC Inc., Morris Plains, NJ); binary gradient elution; a flow rate of 1.5 mL/min; and detection at 450 nm. Solvent A was 100% pentane, and solvent B was 80% acetone/20% pentane. The following mobile phase gradient was used: 100% A for 3 min, then linear to 90% A/10% B in 10 min, linear to 45% A/55% B in 50 min, hold for 5 min, and then return to initial conditions by 60 min.

Semipreparative RPLC was performed on extracts of SRM 1548 using gradient elution, Vydac 201TP C$_{18}$, 10-μm semi-preparative LC column (22.5 × 250 mm), to collect individual carotenoid peaks. The gradient consisted of 100% methanol for 10 min and then linear to 30% THF in methanol over 50 min at a flow rate of 8.0 mL/min. The individual peaks were concentrated under nitrogen and reinjected onto the analytical reversed-phase system described above fitted with a UV-vis photodiode array detector (DAD, Model 990, Waters, Millipore, Milford, MA). Spectra were taken in 95% methanol/5% THF with the DAD from 305 to 550 nm during the LC analysis every 30 ms to assist in the identification of the carotenoid compounds.

Identification and Quantitation. Identification of dietary carotenoids was confirmed by coelution of carotenoid peaks with authentic standards using two modes of chromatography and UV-vis spectra of peaks eluted during semipreparative reversed-phase LC.

Xanthophyll stock standards were prepared in ethanol containing 30 μg/mL butylated hydroxytoluene (BHT) as an antioxidant, and carotene stock solutions were prepared in hexane containing 30 μg/mL BHT. Standard concentrations were assigned using the absorptivities in the appropriate solvents listed by Davies (1976) and were corrected for LC purity at the wavelength maximum for a given carotenoid (Craft et al., 1991). Stock solutions were combined to provide four calibration solutions with concentrations ranging from 0.05 to 1.0 μg/mL of each carotenoid. Quantitation was accomplished according to the internal standard method using echinenone as the internal standard (volume correction standard). The correlation coefficients of the calibration curves for each of the carotenoid standards was 0.99 or better.

Statistical Analysis. Duplicate aliquots of all samples were carried through the complete analysis. Statistical analysis of the data was performed using the SAS (Cary, NC) statistical package. Storage temperature data for SRM 1548 were analyzed using a one-way analysis of variance. When a significant effect was detected among the variances of the group means, Tukey's
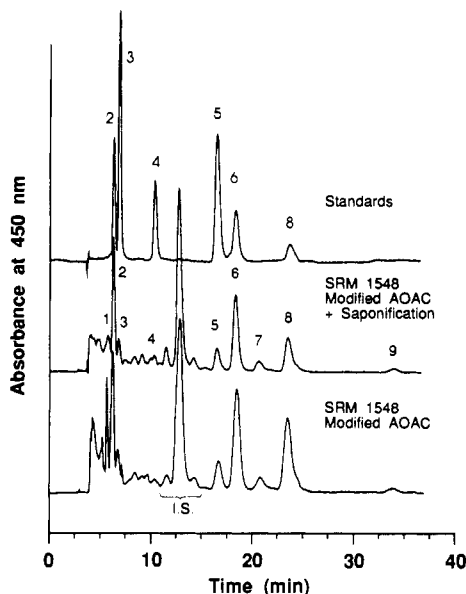
Figure 1. Isocratic reversed-phase LC separation of carotenoids in SRM 1548 total diet. Conditions: Vydac 201 TP C_{18} column, 95% methanol/5% THF, 1.0 mL/min, 20 °C, detection at 450 nm. Peak identifications: (1) unidentified; (2) lutein; (3) zeaxanthin; (4) β-cryptoxanthin; (5) α-carotene; (6) β-carotene; (7) 13-cis-β-carotene; (8) lycopene; (9) 15,15′-cis-lycopene; IS, internal standard.

comparison of means was used to determine which groups were significantly different from each other (Scheffé, 1959). Analysis of covariance was used to determine whether the amount of exposure to γ-irradiation altered carotenoid concentrations in HCMD. Student's t-test was used to determine if lyophilized samples of HCMD were different from frozen samples. All statistical tests were done at the 0.05 level of significance.

RESULTS

Carotenoid Identification. A representative chromatogram of the RPLC separation of carotenoids in SRM 1548 is illustrated in Figure 1. The major carotenoids identified in the SRM 1548 were as follows: peak 2, lutein (λ_{max} 445, 473 nm); peak 3, zeaxanthin (λ_{max} 449, 475 nm); peak 4, β-cryptoxanthin (λ_{max} 450, 478 nm); peak 5, α-carotene (λ_{max} 445, 473 nm); peak 6, β-carotene (λ_{max} 451, 478 nm); peak 7, 13-cis-β-carotene (λ_{max} 340, 447, 474 nm); peak 8, lycopene (λ_{max} 444, 470, 501 nm); peak 9, 15,15′-cis-lycopene (λ_{max} 360, 438, 464, 494 nm). Peak 1 was not identified due to sample limitations but exhibited maxima similar to those of lutein (λ_{max} 445, 471 nm). UV-visible spectra for nine peaks are illustrated in Figure 2. Authentic xanthophyll standards coeluted with the identified xanthophylls listed above using both NPLC and RPLC corroborating their identification. Authentic carotenoid standards for α-carotene, β-carotene, and lycopene coeluted with the carotenes listed above with RPLC corroborating their identification. Cis isomers of β-carotene and lycopene were prepared by thermal isomerization and isolated by separation on an LC column packed with calcium hydroxide. RPLC and NPLC separations of the HCMD carotenoids are illustrated in Figure 3.

RPLC Validation. Recovery of individual carotenoid standards from the LC column was 98 ± 6% (Craft et al., 1992). The reproducibilities (% RSD) of measured carotenoid concentrations in the HCMD for six replicates of the complete analysis were lutein 6%, zeaxanthin 9%, β-cryptoxanthin 5%, α-carotene 3%, β-carotene 3%, and lycopene 6%. The retention time of lycopene, the last eluting carotenoid peak, varied by less than 1%.
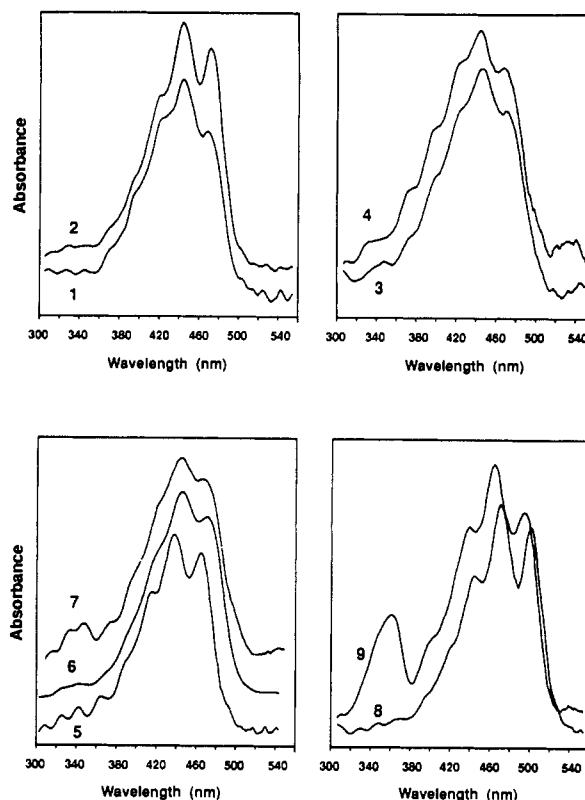

Figure 2. UV–visible spectra in 95% methanol/5% THF of selected carotenoids in SRM 1548 total diet. Spectra numbers correspond to peak identification in Figure 1.

Effect of Storage Temperature. The influence of storage temperature on carotenoid concentrations in SRM 1548 is presented in Table III. A significant decrease in the concentrations of all carotenoids, except lutein, occurred when diet samples were stored for 1 year at 4 °C compared to –80 °C. The concentrations of all measured carotenoids stored at –20 °C were less than those of carotenoids stored at –80 °C; however, the differences were not significant.

Effects of γ-Irradiation and Lyophilization. The influence of γ-irradiation and that of lyophilization on the carotenoid concentrations of the HCMD are demonstrated in Table IV. Lyophilization resulted in a significant increase in measured β-cryptoxanthin concentration and significant decreases in measured β-carotene and lycopene concentrations. The apparent concentration of all xanthophylls increased as a result of lyophilization.

The effect of irradiation on mean carotenoid concentrations is presented in Table IV. As the dose of γ-irradiation increased, a significant upward trend occurred for the peak eluting with the retention time of β-cryptoxanthin, while α-carotene and β-carotene experienced significant downward trends.

DISCUSSION

Carotenoid Identification. The major carotenoids identified in SRM 1548 were lycopene, β-carotene, lutein, α-carotene, and zeaxanthin, in decreasing order of concentration (Table III). These carotenoids and their relative order of prevalence coincide well with the proportions that occur in human serum of healthy 20–45-year-old males (Brown et al., 1989) and middle-aged men (Stacewicz-Sapuntzakis et al., 1987). Minor carotenoids (<2% of the total peak area at 450 nm) were not identified. We are unaware of any study in which a direct comparison has been made of individual carotenoids in a representative
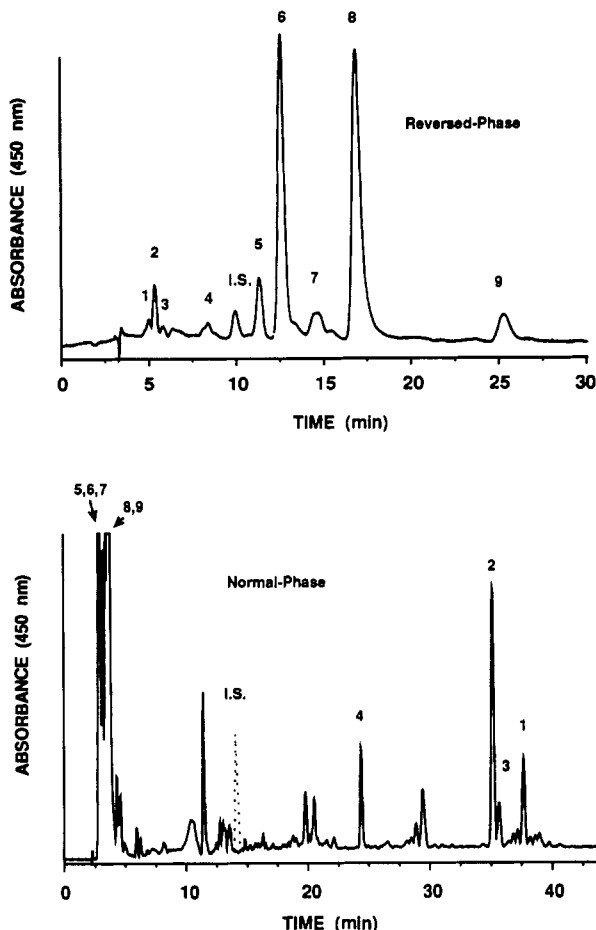
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Figure 3. Normal-phase and reversed-phase LC separation of carotenoids in the high carotenoid mixed diet. Peak identifications are as listed in Figure 1.

total diet with serum carotenoid levels in the population consuming the diet. This type of comparison would be of great interest to determine whether mean serum carotenoid concentrations of a population correspond to individual carotenoids consumed in the diet.

Methodological Considerations. The greatest difficulty associated with measuring individual carotenoids in a mixed-diet matrix is the removal of interfering lipid materials. We investigated the effects of extraction solvent, saponification conditions, and alternative extraction methods (e.g., size exclusion chromatography and supercritical fluid extraction) on the recovery of carotenoid compounds. Although alternative techniques look promising, quantitative recoveries were not achieved and no single combination of solvent and saponification conditions yielded maximum carotenoid recovery and lipid hydrolysis. Therefore, the described modification of the AOAC method (AOAC, 1980) for measuring xanthophylls in animal feeds was employed since (1) it appeared to be effective, (2) it is well established, and (3) it is not prone to difficult emulsions. Pyrogallol was added to minimize oxidative losses, the saponification time was increased to enhance the lipid hydrolysis, and an internal standard was incorporated to adjust for volume differentials throughout the extraction and injection of samples.

In spite of using alkaline hydrolysis during the extraction procedure, the presence of large amounts of lipid material persisted. Due to the presence of lipid, injection volumes during RPLC analysis were limited to 20 μL and, as a result of the solvent volume necessary to dissolve the lipid, the concentrations of carotenoids in the injection solution were less than optimal. The presence of lipid was less

significant in NPLC used to verify peak identity. The solubility of the lipid is greater in hexane than in ethanol/ methanol/THF, thereby requiring less dilution of the extracts, and the polarities of the lipid and the injection solvent are similar, causing less aberration in the chromatography. No attempt was made to identify the class- (es) of nonsaponifiable lipids that remained in the samples after the hydrolysis step, although the presence of sterols has been reported (Noga and Lenz, 1983). Davies (1976) suggested that sterols could be removed by precipitating from light petroleum at -10 °C, by precipitating as sterol digitonides, or by chromatography on alumina. Attempts to remove the nonsaponifiable lipid component by precipitating at -10 °C and using alumina chromatography were unsuccessful. The formation of digitonides was not attempted since one would expect less than quantitative recovery of carotenoids exposed to boiling ethanol. It is also possible that the saponification conditions were not sufficiently rigorous to hydrolyze all of the lipid esters present in the food matrix. To test this possibility, additional saponification was performed on the lipid extract of SRM 1548 obtained from the modified AOAC procedure. The resulting residue was less oily and had less volume. Upon addition of mobile phase, a white precipitate (possibly sterols) formed, which required filtration prior to injection onto the LC. Possibly the precipitation of sterols did not occur during initial attempts because of their solubility in residual nonpolar lipid. An improvement is evident in the chromatography between the center and lower chromatograms in Figure 1; however, substantial losses of carotenoids (up to 50% for lycopene) also occurred. The apparent loss of peaks at 5.0 and 5.4 min (Figure 1) in response to additional saponification was due to the elimination of refractive index effects resulting from disappearance of a large peak absorbing in the UV region. The peak remaining at 5.4 min after additional saponification (peak 1, Figure 1) could not be identified; however, it exhibited the same λ_{max} as lutein with slightly less spectral fine structure and did not test positive for 5,6-epoxides.

Effects of Storage and Processing. The measured concentrations of the carotenoids in SRM 1548 are only approximations of the actual carotenoid content of the USTD prior to lyophilization, γ-irradiation, and storage. Although portions of SRM 1548 were maintained under optimum storage conditions (-80 °C, protected from oxygen and light), changes that occurred during processing prior to receipt of the material cannot be determined. In spite of losses incurred during processing and storage, carotenoid concentrations in SRM 1548 were measurable. Unfortunately, for the reasons cited above, the carotenoid concentrations determined in SRM 1548 should not be used for verification of analytical methods.

Due to the labile nature of carotenoid compounds, one would anticipate better stability as storage temperature decreases (Table III). After 1 year of storage, the concentrations of all carotenoids (with the exception of lutein) were significantly lower in the diet sample stored at 4 °C compared to -80 °C. Even at -20 °C, the measured concentrations of all carotenoids were lower than the diet at -80 °C. Similarly, Crandall et al. (1983) found that the β-carotene content of orange oil decreased as storage temperatures increased above -18 °C. Craft et al. (1988) reported that carotenoids in serum samples stored at -70 °C were stable for at least 2 years; however, significant losses occurred in samples stored at -20 °C between 6 and 15 months.

The effects of lyophilization and irradiation on caro-

Table III. Effect of Storage Temperature on Carotenoid Concentrations in SRM 1548

storage temp, °C	concentration,[a] ng/g of dry wt					
	lutein	zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	lycopene
-80	134[a] (22)[b]	25[a] (4)	6[a] (1)	85[a] (14)	270[a] (44)	326[a] (53)
-20	112[a] (18)	20[ab] (3)	5[ab] (1)	66[ab] (11)	214[ab] (35)	278[ab] (45)
4	114[a] (18)	15[b] (2)	3[b] (<1)	56[b] (9)	201[b] (33)	176[b] (29)
pooled SEM[c]	16	3	0.5	6	23	43

[a] Represents the mean carotenoid concentration of two sample extracts. Values in the same column with different letter superscripts are significantly different ($p \leq 0.05$) using analysis of variance and Tukey's multiple comparison test. [b] Values in parentheses are expressed as ng/g of wet wt. [c] Pooled standard error of the mean across treatments.

Table IV. Effect of γ-Irradiation and Lyophilization on the Carotenoid Concentration of a High-Carotenoid Mixed Diet

treatment	concentration,[a] μg/g of wet wt					
	lutein	zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	lycopene
control	1.75	0.333	0.218	2.68	15.4	34.3
lyophilized	2.15	0.389	0.378[c]	2.61	14.3[c]	14.2[c]
10 kGy	1.74	0.319	0.251	2.71	15.5	34.0
20 kGy	1.80	0.334	0.280	2.68	15.5	35.8
35 kGy	1.60	0.296	0.273	2.63	15.2	28.6
50 kGy	1.65	0.329	0.360	2.49	14.9	29.0
pooled SEM[b]	0.02	0.006	0.006	0.01	0.05	0.52
			trend[d]	trend[e]	trend[e]	

[a] Represents the mean carotenoid concentration of two sample extracts. [b] Pooled standard error of the mean across treatments. [c] Lyophilized values with superscripts are significantly different ($p \leq 0.05$) from control values using Student's t-test. [d] Using analysis of covariance, a significant ($p \leq 0.05$) upward trend exists as γ-irradiation increases. [e] Using analysis of covariance, a significant ($p \leq 0.05$) downward trend exists as γ-irradiation increases.

tenoid concentration were more variable and appear to depend on the matrix and subsequent storage (Bauernfeind, 1981; Simon and Wolff, 1987; Bhushan and Thomas, 1990; Park, 1987). The degradative effects of lyophilization and irradiation on the hydrocarbon carotenoids are shown in Table IV. Interestingly, the measured concentrations of the xanthophylls increased in response to these treatments. At first this phenomenon appears to be contradictory; however, lyophilization probably leads to more efficient hydrolysis of xanthophyll esters. Intact xanthophyll esters elute intermingled with lycopene in this method. Upon complete hydrolysis an apparent decrease in lycopene concentration may represent hydrolysis of esters and a concomitant increase in the concentration of free xanthophylls. This occurrance does not negate the possibility of true oxidation of hydrocarbon carotenoids during these processes which may also contribute to an apparent increase in xanthophyll concentrations. Oxidative products of carotene degradation may occur as xanthophylls, epoxides, and/or carotenals which may coelute with the xanthophylls (Marty and Berset, 1988; Kanasawud and Crouzet, 1990).

Considerations in the Development of a Total Diet Reference Material. Although it is not the intent of this paper to thoroughly explain the physical processes responsible for altered carotenoids, an awareness of the effects of processing is important to the use of total diet composites for the assessment of nutrient intake and for the development of reference materials. If total diet composites are to be used for the correlation of nutritional intake and the incidence of disease, analysis of dietary constituents must be representative of the concentrations as consumed. SRM 1548 would be inappropriate for such use; however, a similar material could be used as a SRM for carotenoid method development and validation. The concentrations certified for analytes in a SRM are assigned on the basis of measurements performed on the matrix after all processing is completed. The primary considerations in the development of a SRM include (1) a matrix representative of the actual material being analyzed, (2) analyte concentrations in the matrix at levels comparable to analyte concentrations in the unknown material, (3)

analyte (matrix) stability for a practical time period (>1 year), and (4) SRM homogeneity. SRM 1548 meets all of these criteria for inorganic constituents but falls short for the carotenoids. Due to the carotenoid losses that occurred during processing, the concentrations are no longer at a level comparable to fresh diet homogenates and the storage conditions (4 °C) were not adequate to maintain the stability of the carotenoids. Therefore, the values reported in Table III for carotenoid concentrations in SRM 1548 should not be used as reference values for this material.

Alternatively, a material similar to HCMD would appear to be a suitable candidate material for a mixed-diet matrix carotenoid and vitamin SRM. The individual food constituents used to prepare HCMD are well-homogenized and in the form of stabilized suspensions prior to blending. The components can be custom blended to provide appropriate concentrations of all analytes of interest. When vacuum packed by the manufacturer, the products have approximately a 2-year shelf life; however, carotenoid and vitamin stability has not been determined in such a material. An evaluation of the stability and usefulness of a similar material is currently underway.

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