

Carotenoid Content of Thermally Processed Tomato-Based Food Products

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Tomato-based food products such as tomato paste, tomato sauce, and tomato-based soups are rich in carotenoid compounds and are frequently consumed in the United States. Foods such as these, which are high in carotenoid content, are of interest because of the demonstrated association between consumption of fruits and vegetables and reduced risk of lung and other epithelial cancers in humans. Limited analytical data on the carotenoid content of tomato-based products are available in food tables and data bases; however, they are usually reported only in terms of vitamin A activity. In this study name-brand and store-brand tomato-based food products purchased in three major U.S. cities were extracted and carotenoids were individually identified and quantified by reversed-phase HPLC according to methodology developed in our laboratory. The carotenoids that were detected and quantified included lycopene, lycopene-5,6-diol, lutein, α -, β -, γ -, and ζ -carotenes, neurosporene, phytoene, and phytofluene. As expected, lycopene was the most abundant carotenoid, ranging in concentration from 0.3 mg/100 g in vegetable beef soup to 55 mg/100 g in tomato paste. The concentration of β -carotene ranged from 0.23 mg/100 g in tomato soup to 1.51 mg/100 g in vegetable beef soup. Lutein was found at very low concentrations (less than 0.2 mg/100 g) in all products analyzed except tomato paste, which contained 0.34 g/100 g.

Keywords: *Lycopene; tomatoes; carotenoids; cancer; HPLC; antioxidant*

INTRODUCTION

Epidemiological studies have shown that an increased consumption of fruits (including tomatoes) and vegetables is associated with a reduced risk of lung and other epithelial cancers (Shekelle et al., 1981; Kvale et al., 1983; Le Marchand et al., 1989; Micozzi, 1989; Micozzi et al., 1990). Many fruits and vegetables have high concentrations of carotenoid compounds. Carotenoids are known to have antioxidant activity by quenching free radicals and singlet oxygen (Sies et al., 1992). Antioxidant functions are associated with lowering DNA damage, malignant transformations, and other parameters of cell damage in vitro (Sies et al., 1992). The ability of carotenoids to function as antioxidants may contribute to a reduction in disease risk (Colditz et al., 1985; Ziegler et al., 1986; Ziegler, 1989; Graham et al., 1990; Sies et al., 1992). Carrots, tomatoes, and green vegetables (i.e. spinach, kale, broccoli, and green beans) are examples of excellent sources of carotenoid compounds and are considered to be important contributors of carotenoids to the human diet.

Recently, interest in consumption of carotenoid-rich foods and in reduced cancer risk has led to further research to determine carotenoid content of foods frequently consumed by people in the United States. A data base was developed by the U.S. Department of Agriculture (USDA) and the National Cancer Institute (NCI) (Mangels et al., 1993) which contains carotenoid values obtained from the scientific literature for 120 individual foods. The data base contains median values

for five carotenoids (α - and β -carotenes, lycopene, lutein, and β -cryptoxanthin) in foods. Data were evaluated according to an artificial intelligence system known as CAREX and were given a confidence code indicating reliability of each value. Carotenoid values for the 120 individual foods were then used to estimate the carotenoid content of over 2400 foods (Chug-Ahuja et al., 1993) which are listed in the USDA Survey Nutrient Data Base Recipe File. This file provides the basis for the food composition data base used to calculate dietary intake of nutrients for both the USDA Surveys of food consumption and the National Health and Nutrition Examination Survey (NHANES) of diet and health. The foods listed in the recipe file include fruits, vegetables, and multicomponent foods containing fruits and vegetables.

During the development of the CAREX data base commercial products such as tomato-based soups containing vegetables and other tomato-based products (tomato paste, sauce, catsup, etc.) were identified as important candidates for carotenoid analysis. These analyses were necessary because of the lack of available carotenoid data, the lack of formulations or recipes, and the high frequency of consumption of these foods in the United States.

The objectives of this study were to (1) identify and quantify carotenoid compounds in frequently consumed commercially processed tomato-based food products from three major population centers in the United States (New York, Chicago, and San Francisco) and (2) examine results for relationships between carotenoid content and brand of product (name brand vs store brand) and geographic location.

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MATERIALS AND METHODS

Food Product Sampling. Name-brand and store-brand tomato-based thermally processed foods (soups, whole tomatoes, catsup, spaghetti sauce, tomato paste, tomato sauce, tomato puree, tomato juice, and vegetable juice) were chosen for carotenoid analysis on the basis of frequency of consumption of these foods in the United States as estimated from the USDA Continuing Survey of Food Intake for Individuals, 1986, and sales volume data (Nielsen Scantrack Data, A. C. Nielsen Co., Northbrook, IL, 1990). Products were purchased from three U.S. cities (New York, Chicago, and San Francisco), which were chosen to represent three distinct regions of the United States (northeast, north-central, and west). These are major population centers within the United States with New York and Chicago ranking two and three in the nation, respectively (Market Scope, MacClean Hunter Media, Inc., Stamford, CT, 1993). Although Los Angeles is ranked as the no. 1 population center in the nation and San Francisco is ranked as no. 29, San Francisco was chosen to represent the western region of the United States. This was justified because product brand names and relative sales figures were similar for Los Angeles and San Francisco. Designated name-brand and store-brand products were purchased from the two grocery chains having the largest sales volume in each of the three cities. Analyses were performed on at least one name-brand and one store-brand product from each of the three cities. For example, at least two units (cans) of tomato soup (*name brand* having the same lot number) and at least two units (cans) of tomato soup (*store brand* having the same lot number) were obtained from each of the three cities (New York, Chicago, and San Francisco). Name-brand or store-brand products having the same lot numbers were combined, from which aliquots were taken for extraction. The six samples (name brand from each of the three cities and store brand from each of the three cities) were extracted and analyzed for carotenoids. This sampling scheme was repeated for each of the tomato products. Tomato puree and vegetable juice were the two exceptions. Sales volume data for all brands of tomato puree were similar, and the total sales for this product were low compared to other products such as tomato paste; therefore, a single composite of five brands for all of the cities was analyzed for carotenoid content. The sales volume data for one name brand of vegetable juice was much greater compared to all other name brands and store brands; therefore, only one brand of vegetable juice from each of the three cities was analyzed.

Reagents. HPLC grade solvents [tetrahydrofuran (THF), methylene chloride, acetonitrile, methanol, and hexane; Burdick & Jackson, Baxter Healthcare Corp., Muskegon, MI] were used without further purification. The THF was stabilized with 0.01% butylated hydroxytoluene (BHT; Fluka Chemical Corp., Ronkonkoma, NY). The methylene chloride contained 0.1% *N,N'*-diisopropylethylamine (Aldrich Chemical Co., Milwaukee, WI).

The internal standard, β -apo-8'-carotenal (Fluka Chemical) was dissolved in methylene chloride, filtered through Whatman No. 42 filter paper, and stored at -20°C until needed. The stock solution was regularly replaced with a fresh solution, as needed, and the purity of the internal standard was verified by HPLC and UV-vis spectroscopy. β -Apo-8'-carotenal was also injected as an external standard after every 10 sample injections to verify operation of the chromatographic instrumentation.

Extraction and Chromatography. The extraction procedure was similar, with a few minor modifications, to a published procedure for extraction of carotenoids from tomatoes and tomato paste (Khachik et al., 1992b). The extraction was carried out at 0°C and under gold fluorescent lights. An appropriate amount of internal standard was added to each food sample before extraction (Table 1) to indicate the extent of losses as a result of extraction and chromatography. Magnesium carbonate and Celite used as a filter aid (each at 10% of the weight of the sample) were added, and the sample was blended for 20 min in an Omni Mixer with THF. The mixture was filtered through Whatman No. 1 filter paper on

Table 1. Quantity of Tomato-Based Product Extracted and Internal Standard Added before Extraction^a

tomato-based product	quantity extracted (g)	β -apo-8'-carotenal (mg)
soups	300	0.91
whole tomatoes	100	0.91
catsup	100	0.93
spaghetti sauce	100	0.88
tomato paste	50	1.15
tomato puree	100	0.85
tomato juice	200	0.93
vegetable juice	200	0.93
tomato sauce	50	0.89

^a Final volume of extract = 50 mL in methylene chloride.

a Büchner funnel. The solid material was extracted two or three more times until it was devoid of red/orange color after filtering and the filtrate was colorless. The THF extracts were combined, and the volume was reduced by about two-thirds under vacuum at 35°C on a rotary evaporator. Components of the combined extract were partitioned into methylene chloride (250 mL) and salt water (150 mL) in a separatory funnel. The organic layer was removed and washed with water (3×150 mL) containing sodium chloride. If color remained in the water layer, it was washed with methylene chloride until carotenoids were completely removed. The methylene chloride layer containing carotenoids was dried over anhydrous sodium sulfate (powder) and filtered through Whatman No. 42 filter paper on a Büchner funnel. The volume of the filtrate was reduced under vacuum to approximately 10 mL. Preliminary studies demonstrated that lycopene was lost if the solution was permitted to go to complete dryness (L.H.T., unpublished observations). The 10 mL concentrated solution was quantitatively filtered through a $0.45\ \mu\text{m}$ filter and the volume brought up to 50 mL in methylene chloride in a volumetric flask. Appropriate dilutions were made by transferring aliquots of this solution into HPLC injection solvent [acetonitrile (40%), methanol (20%), methylene chloride (20%), hexane (20%)]. Twenty microliters of the final dilution was injected onto the HPLC column.

Conditions for the HPLC separations were the same as those reported by Khachik et al. (1992b) with a few exceptions. A stainless steel Microsorb-MV C_{18} column (25 cm length, 4.6 mm i.d., $5\ \mu\text{m}$ spherical particles; Rainin Instrument Co., Inc., Woburn, MA) was used. In addition, a column inlet filter ($0.5\ \mu\text{m}$, 3 mm i.d.) was placed in front of the Brownlee C_{18} guard column. A Waters Model 990 photodiode array detector and Model 5200 printer/plotter were used as the HPLC detection system.

Calibration curves based on peak area were established for the internal standard and for each carotenoid and were used to determine concentration. The wavelength used for calibration of standards and integration of peaks from sample extracts depended on the wavelength of optimum absorption for each carotenoid. Wavelengths other than the monitoring wavelength (450 nm) were accessible because of the diode array detection system. All carotenoids were quantified at 450 nm with the exception of the following: lycopene at 470 nm, β -cryptoxanthin at 445 nm, ζ -carotene at 400 nm, phytofluene at 350 nm, and phytoene at 290 nm.

Establishment of a Control Sample. Aliquots from a single large pool of vegetable juice were stored at -60°C until needed. These were extracted and analyzed at the beginning, after every 10 sample extractions, and at the end of the study to give an indication of the reproducibility of the laboratory technique over time. Vegetable juice was considered to be a suitable product for a control sample because it contained all of the carotenoids of interest in concentrations that could be easily measured.

Statistical Analysis of Data. A mean value of carotenoid content (milligrams per 100 g) for each of the three cities (New York, Chicago, and San Francisco) was calculated for each carotenoid and for each product (name brand and store brand). A table containing individual values can be obtained by writing to L.H.T. Analysis of variance (ANOVA) and the General

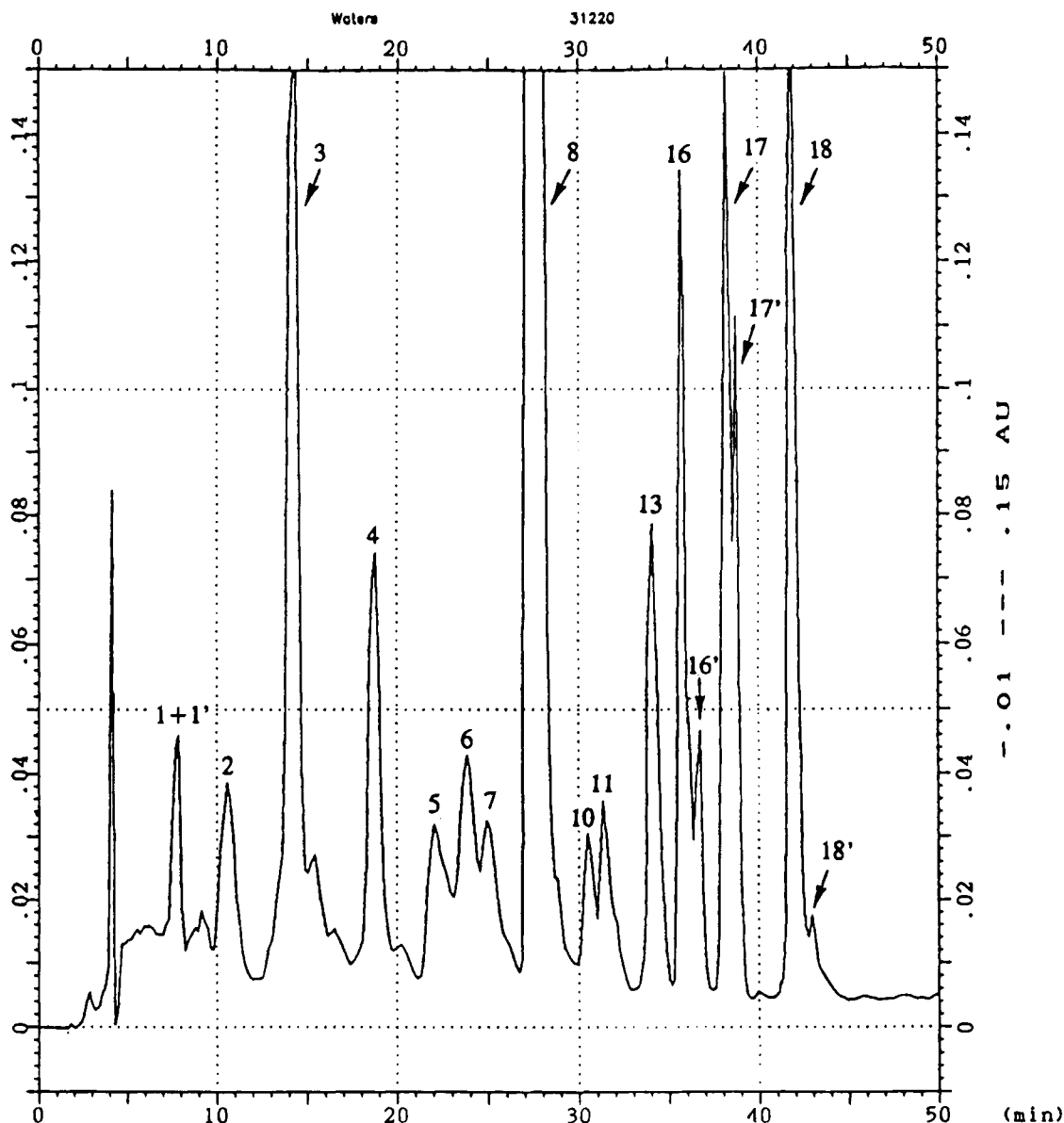


Figure 1. HPLC profile of carotenoids in an extract of tomato paste. For peak identification, see Table 4. HPLC conditions are described in the text.

Linear Models (GLM) were used to test the effect of geographic region (city) and brand on β -carotene and lycopene mean values. Personal Computer Statistical Analysis Systems (SAS) version 6.04 was employed.

RESULTS AND DISCUSSION

Chromatographic Profiles, Recovery, and Statistics. The chromatographic profile for tomato paste (Figure 1) and profiles of other tomato products (tomato soup, tomato juice, whole tomatoes, catsup, spaghetti sauce, tomato puree, and tomato sauce), which were devoid of green vegetable ingredients, were similar to the tomato paste profile reported by Khachik et al. (1992b). In the present study, concentrations of all of the detected carotenoids (β -, γ -, and ζ -carotene, lutein, lycopene, neurosporene, phytoene, phytofluene, and lycopene-5,6-diol) were determined with the exception of the epoxides (lycopene 1,2-epoxide and lycopene 5,6-epoxide), which were present in the extracts but were not quantified because their concentrations were low (peaks 4 and 5, respectively, in Figure 1). Furthermore, the epoxides were not considered to be important to this study because they are not found in human plasma

(Khachik et al., 1992a). Therefore, it is likely that the epoxides are not directly absorbed from the diet or, if absorbed, they do not remain intact. It was suggested by Khachik et al. (1992b) that lycopene-5,6-diol (5,6-dihydroxy-5,6-dihydrolycopene, peak 2 in Figure 1) could be the result of acid-catalyzed ring opening of lycopene 5,6-epoxide in acidic tomatoes; however, its true origin remains uncertain. Lycopene-5,6-diol has also been identified in extracts of human plasma (Khachik et al., 1992a), and in this case may be the result of ring opening of lycopene 5,6-epoxide in the acidic stomach during digestion.

The identification of 1,2-dimethoxypropyllycopene (peak 6 in Figure 1) is only tentative at the present time. Previously, the methoxylated lycopenes had been identified by Khachik et al. (1992b). A more detailed structural elucidation of the methoxylated lycopenes will be published elsewhere.

A typical chromatographic profile for tomato-based food products that contained vegetables (vegetable beef soup, minestrone soup, vegetarian vegetable soup, and vegetable juice) is shown in Figure 2. These chromatograms contained peaks that interfered with the quan-

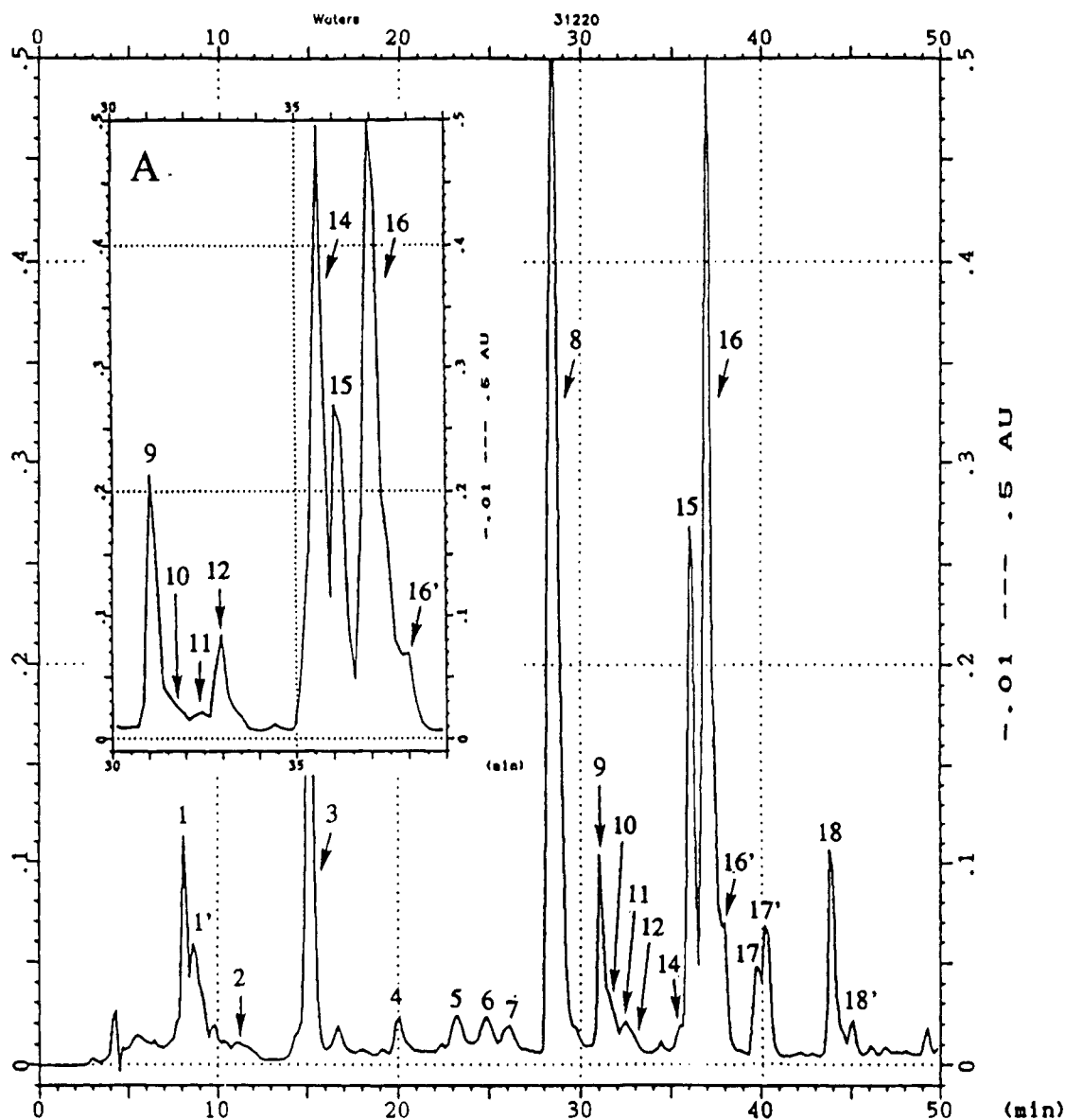


Figure 2. HPLC profile of carotenoids in an extract of vegetarian vegetable soup. For peak identification, see Table 4. HPLC conditions are described in the text. An expansion of the profile from 30 to 39 min is shown in the inset (A). Peak identifications are the same as in Table 4 except that the monitored wavelengths are 436, 440, 464, 409, and 409 nm for peaks 9, 10, 11, 12, and 14, respectively, in the inset (A).

identification of three carotenoids (neurosporene and γ - and ζ -carotene). The identification of the interfering HPLC peaks, which are believed to be chlorophyll degradation products of the green vegetable ingredients in these foods, will be described later in this paper.

The percent recovery of carotenoids after extraction and chromatography was determined by adding a known amount of the internal standard, β -apo-8'-carotenal, to each sample before extraction. Recovery of the internal standard was at least 90% for all extractions. This suggests that the losses of carotenoids during the extraction procedure were minimal.

The coefficients of variation (CV) of carotenoid concentrations for the vegetable juice quality control sample (bottom of Table 2) indicated the reproducibility of the laboratory technique throughout this study. The coefficient of variation ranged from 4% for phytoene to 13% for lycopene-5,6-diol.

The significance of the effects of product brand or geographic region from which samples were obtained was determined using statistical analysis. At the 95% confidence level, there was no significant effect on

carotenoid (lycopene and β -carotene) values because of brand or geographic region. Therefore, mean carotenoid concentration values that appear in Tables 2 and 3 were calculated to include data for samples from the three cities and for both name-brand and store-brand products. The only product for which analysis of the effect of brand or geographic region was not possible was vegetable beef soup. In this case store brands were available from only one city (New York). In addition, only one name brand of vegetable beef soup was analyzed from each of the three cities. As a result, insufficient data were available to perform statistical analysis of variance for this product.

β -Cryptoxanthin, a vitamin A active carotenoid that was of interest to USDA and NCI scientists who developed the CAREX data base, was not detected in any of the foods which were analyzed. This is consistent with the literature dealing with tomatoes or tomato-based foods (Curl, 1961; Zakaria et al., 1979; Daood et al., 1987; Tan, 1988; Heinonen et al., 1989; Khachik et al., 1992b).

Carotenoid Values of Tomato-Based Foods. Val-

Table 2. Carotenoids (Milligrams per 100 g) in Tomato-Based Soups, Tomato Juice, Vegetable Juice, and the Quality Control Sample

sample	α -carotene	β -carotene	γ -carotene	ζ -carotene	lutein	lycopene	neurosporene	phytoene	phytofluene	lycopene-5,6-diol
tomato soup										
mean \pm SD ^a	nd ^b	0.23 \pm 0.047	1.95 \pm 0.41	0.17 \pm 0.016	0.09 \pm 0.02	10.92 \pm 2.92	1.53 \pm 0.20	1.72 \pm 0.172	0.72 \pm 0.176	0.11 \pm 0.03
CV, %		20	21	9.5	21	27	13	10	25	29
N ^d	6	6	4	6	6	6	4	6	6	6
vegetable beef soup										
mean \pm SD	0.46 \pm 0.05	1.51 \pm 0.29	C ^e	C	0.11 \pm 0.03	0.31 \pm 0.034	C	0.35 \pm 0.06	0.19 \pm 0.02	tr ^f
CV, %	11	19			28	11		16	10	
N	4	4	4	4	4	4	4	4	4	4
minestrone soup										
mean \pm SD	0.21 \pm 0.12	0.92 \pm 0.39	C	C	0.15 \pm 0.08	1.48 \pm 0.83	C	0.28 \pm 0.11	0.17 \pm 0.09	nd
CV, %	56	43			53	56		38	52	
N	5	5	5	5	5	5	5	4	4	5
vegetarian veg soup										
mean \pm SD	0.41 \pm 0.18	1.50 \pm 0.37	C	C	0.16 \pm 0.04	1.93 \pm 0.51	C	0.60 \pm 0.07	0.31 \pm 0.04	tr
CV, %	44	25			26	26		12	13	
N	5	5	5	5	5	5	5	4	5	5
tomato juice										
mean \pm SD	nd	0.27 \pm 0.04	1.74 \pm 0.20	0.18 \pm 0.03	0.06 \pm 0.02	10.77 \pm 1.07	1.23 \pm 0.27	1.90 \pm 0.19	0.83 \pm 0.14	0.11 \pm 0.03
CV, %		13	11	19	24	10	22	10	17	29
N	6	6	6	6	6	6	6	6	6	6
vegetable juice										
mean \pm SD	0.21 \pm 0.03	0.83 \pm 0.14	C	C	0.08 \pm 0.02	9.66 \pm 0.12	C	1.71 \pm 0.16	0.69 \pm 0.07	0.05 \pm 0.01
CV, %	14	17			21	1		9	10	10
N	3	3	3	3	3	3	3	3	3	3
quality control (vegetable juice)										
mean \pm SD	0.25 \pm 0.01	0.91 \pm 0.11	C	C	0.09 \pm 0.01	8.56 \pm 0.53	C	1.69 \pm 0.07	0.73 \pm 0.037	0.08 \pm 0.01
CV, %	5	12			9	6		4	5	12
N	9	9	9	9	9	9	9	9	9	9

^a SD, standard deviation. ^b nd, not detected. ^c CV, coefficient of variation. ^d N, number of values used to calculate the mean and SD. ^e C, coeluted with pheophytins. ^f tr, trace, below 0.005 mg/100 g.

Table 3. Carotenoids (Milligrams per 100 g) in Whole Tomatoes, Catsup, Spaghetti Sauce, Tomato Paste, Tomato Puree, and Tomato Sauce

sample	β -carotene	γ -carotene	ζ -carotene	lutein	lycopene	neurosporene	phytoene	phytofluene	lycopene-5,6-diol
whole tomatoes									
mean \pm SD ^a	0.23 \pm 0.04	1.50 \pm 0.28	0.21 \pm 0.05	0.08 \pm 0.02	9.27 \pm 1.02	1.11 \pm 0.13	1.86 \pm 0.29	0.82 \pm 0.14	0.11 \pm 0.01
CV, ^b %	20	19	25	32	11	11	16	17	11
N ^c	6	6	6	4	6	6	6	6	6
catsup									
mean \pm SD	0.59 \pm 0.12	3.03 \pm 1.25	0.33 \pm 0.05	nd ^d	17.23 \pm 2.18	2.63 \pm 0.73	3.39 \pm 0.38	1.54 \pm 0.18	0.18 \pm 0.05
CV, ^b %	21	41	17		13	28	11	12	28
N	9	8	8	9	9	8	9	9	8
spaghetti sauce									
mean \pm SD	0.44 \pm 0.07	3.02 \pm 0.42	0.34 \pm 0.07	0.16 \pm 0.04	15.99 \pm 0.90	3.15 \pm 0.90	2.77 \pm 0.66	1.56 \pm 0.53	0.17 \pm 0.03
CV, ^b %	16	14	19	23	26	29	24	34	19
N	9	9	9	9	9	9	9	9	9
tomato paste									
mean \pm SD	1.27 \pm 0.24	9.98 \pm 1.15	0.84 \pm 0.08	0.34 \pm 0.11	55.45 \pm 4.33	6.95 \pm 0.84	8.36 \pm 0.80	3.63 \pm 0.38	0.44 \pm 0.08
CV, ^b %	19	12	9	32	8	12	10	10	18
N	9	9	9	9	9	9	9	9	9
tomato puree									
mean	0.41	2.94	0.25	0.09	16.67	2.11	2.40	1.08	0.17
tomato sauce									
mean \pm SD	0.45 \pm 0.13	3.17 \pm 0.64	0.29 \pm 0.03	tr ^e	17.98 \pm 1.47	2.48 \pm 0.54	2.95 \pm 0.43	1.27 \pm 0.2	0.16 \pm 0.02
CV, ^b %	29	20	12		8	22	15	16	14
N	9	9	9	9	9	9	9	9	9

^a SD, standard deviation. ^b CV, coefficient of variation. ^c N, number of values used to calculate the mean and SD. ^d nd, not detected. ^e tr, trace, below 0.005 mg/100 g.

ues indicating carotenoid concentration [mean \pm standard deviation (SD)] in extracts of tomato-based soups, tomato juice, and vegetable juice are listed in Table 2. Compared to other carotenoids, lycopene was detected in the highest concentration (1.48–10.92 mg/100 g) in all of the products except vegetable beef soup, which contained high levels of β -carotene (1.51 mg/100 g). High concentrations of lycopene were expected (Klaui and Bauernfeind, 1981; Daood et al., 1987; Tan, 1988) because tomatoes or tomato paste was among the primary ingredients. Among the carotenoids that were identified, lycopene is the most efficient quencher of singlet oxygen (Di Mascio et al., 1990), which makes its presence in the diet of considerable interest. In the case of vegetable beef soup, according to the label, there is a greater amount of carrots compared to tomatoes. This probably accounts for the higher concentration of β -carotene compared to lycopene in vegetable beef soup. β -Carotene values were also high in products such as vegetarian vegetable soup because of the presence of green, yellow, and orange vegetables, which are high in β -carotene.

α -Carotene was detected in vegetable beef, minestrone, and vegetarian vegetable soup as well as vegetable juice at concentrations of 0.46, 0.21, 0.41, and 0.21 mg/100 g, respectively. Carrots contain approximately 3.6 mg/100 g (Mangels et al., 1993) of α -carotene and were the likely contributors. Reconstituted carrot juice is the most likely contributor of α -carotene in vegetable juice.

Phytoene, phytofluene, ζ -carotene, and neurosporene are each part of a stepwise dehydrogenation in the conversion of phytoene into lycopene in higher plants (Goodwin, 1971) and therefore were expected to be present in extracts of tomato products. Phytoene, phytofluene, and lycopene were detected in extracts of all of the tomato-based products (Tables 2 and 3).

Neurosporene, γ -carotene, and ζ -carotene are present in all of the soups and in vegetable juice; however, they

were not quantified from extracts of products containing vegetables (vegetable beef soup, minestrone soup, vegetarian vegetable soup, and vegetable juice) because of the presence of pheophytins (degradation products of chlorophylls) in extracts of these products. Pheophytins from the green vegetable ingredients were extracted with the carotenoids and interfered with HPLC detection of neurosporene, γ -carotene, and ζ -carotene (Figure 2, peaks 9–14). Pheophytins were identified from their absorption spectra (Goedheer, 1966). Under the chromatographic conditions employed, these three carotenoids and pheophytins appeared as unresolved HPLC peaks which resulted in overlapping absorption spectra between 400 and 460 nm as determined by the photodiode array detector. The HPLC peaks of pheophytins and the three carotenoids coeluted, making quantification of the carotenoids by peak integration inaccurate under these HPLC conditions. It has been shown that saponification of food extracts can remove the chlorophylls, resulting in a much simpler chromatographic profile (Khachik et al., 1986). However, due to the partial loss of carotenoids as a result of this procedure (Khachik et al., 1986), saponification was not employed.

In most cases ingredients listed on food labels were the same for similar name-brand and store-brand products. In the case of minestrone soup, however, ingredients were not identical and carotenoid values varied for the two types of products. Although analysis of variance did not detect any significant difference between carotenoid values for name-brand and store-brand minestrone soups, the coefficients of variation were quite high, ranging from 38% for phytoene to 56% for lycopene and α -carotene. Differences in ingredients for the two types of soups (name brand and store brand) could explain the variability in the data.

Table 3 contains carotenoid concentrations for whole tomatoes, tomato catsup, spaghetti sauce, tomato paste, tomato puree, and tomato sauce. α -Carotene was not detected in any of these products, nor was it detected

Table 4. Absorption Maxima and HPLC Peak Identification of Carotenoids in Extracts of Tomato Paste and Vegetarian Vegetable Soup

HPLC peak	carotenoid or chlorophyll	monitored wavelength, nm [λ_{\max} , nm] ^{a,b}
1 + 1'	<i>all-trans</i> -lutein and <i>cis</i> -luteins	450 [424, 446, 473]
2	5,6-dihydroxy-5,6-dihydrolycopene (lycopene-5,6-diol)	450 [432, 453, 483]
3	β -apo-8'-carotenal (int std)	450 [457]
4	lycopene 1,2-epoxide	450 [445, 472, 504]
5	lycopene 5,6-epoxide	450 [430, 455, (484)]
6	1,2-dimethoxyprolycopene (tentative identification)	450 [413, 435, 463]
7	5,6-dimethoxy-5,6-dihydrolycopene	450 [430, 455, (484)]
8	lycopene	470 [446, 473, 504]
9	pheophytin <i>b</i>	450 [436]
10	neurosporene	450 [(416), 440, 470]
11	γ -carotene	450 [(440), 464, 494]
12	pheophytin <i>a</i>	450 [409]
13	ζ -carotene	400 [380, 400, 427]
14	pheophytin <i>a</i> isomer and ζ -carotene	450 [409]
15	α -carotene	450 [380, 400, 427]
16 + 16'	<i>all-trans</i> - β -carotene	450 [425, 448, 476]
	<i>cis</i> - β -carotene	450 [(430), 454, 480]
17 + 17'	<i>all-trans</i> - or <i>cis</i> -phytofluene	450 [341, 448, 474]
18 + 18'	<i>all-trans</i> - or <i>cis</i> -phytoene	350 [333, 350, 368] 290 [287]

^a Determined in HPLC eluent employing the photodiode array detector (described in the text). ^b Values in brackets represent points of inflection.

in tomato soup or tomato juice (Table 2). This is consistent with results reported in at least five previous studies in which α -carotene was investigated in tomatoes or tomato products (Zakaria et al., 1979; Daood et al., 1987; Tan, 1988; Heinonen et al., 1989; Khachik et al., 1992b). There are, however, at least two reports of the presence of α -carotene in tomatoes. Padmavati et al. (1992) studied the effect of different cooking methods on the β -carotene content of vegetables. Their results show that α -carotene was detected at 2.78 mg/100 g in tomatoes. The method used to detect α -carotene is not clearly stated and can only be assumed to be the same as the method used to detect β -carotene (column chromatography followed by color intensity measurement at 436 nm). At 436 nm it is possible that ζ -carotene was mistakenly identified as α -carotene because both carotenoids absorb in this region of the visible spectrum. Bushway et al. (1986) reported a level of 258.9 μ g/100 g of α -carotene in tomatoes using HPLC and detection at 470 nm. At 470 nm ζ -carotene has minimal if any absorption; therefore, it is unlikely that the authors were detecting ζ -carotene at this wavelength and mistaking it for α -carotene. However, the detection of α -carotene in tomatoes, in both of these examples, may be due to the cultivar of tomatoes that was analyzed.

Tomato paste, compared to the other tomato products, contained the highest concentration of total carotenoids (87 mg/100 g). The higher concentration was due to the removal of water from tomatoes during processing to form the paste.

Summary. This study provides essential data, not previously available, on carotenoid concentrations of single component and multicomponent tomato-based foods that are frequently consumed in the United States. The concentrations of four carotenoids (α - and β -carotenes, lycopene, and lutein), which were entered into the USDA/NCI database, in conjunction with data from food consumption surveys, will allow the calculation of intake levels of individual carotenoids for nutrition and health-related dietary studies. This information in conjunction with the identification and quantification of other carotenoids (γ - and ζ -carotenes, neurosporene, phytoene, phytofluene, and lycopene-5,6-diol) may prove to be important in the study of antioxidants and their role, acting individually or in concert, in diet and health.

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LITERATURE CITED

- Bushway, R. J.; Yang, A.; Yamani, A. M. Comparison of alpha- and beta-carotene content of supermarket versus roadside stand produce. *J. Food Qual.* **1986**, *9*, 437-443.
- Chug-Ahuja, J. K.; Holden, J. M.; Forman, M. R.; Mangels, A. R.; Beecher, G. R.; Lanza, E. The development and application of a carotenoid database for fruits, vegetables, and selected multicomponent foods. *J. Am. Diet. Assoc.* **1993**, *93*, 318-323.
- Colditz, G.; Branch, L.; Lipnick, R.; Willett, W. C.; Rosner, B.; Posner, B. M.; Hennekens, C. H. Increased green and yellow vegetable intake and lowered cancer deaths in an elderly population. *Am. J. Clin. Nutr.* **1985**, *41*, 32-36.
- Curl, A. L. The xanthophylls of tomatoes. *J. Food Sci.* **1961**, *26*, 106-111.
- Daood, H. G.; Biacs, P. A.; Hoschke, A.; Harkay-Vinkler, M.; Hajdu, F. Separation and identification of tomato fruit pigments by TLC and HPLC. *Acta Aliment.* **1987**, *16*, 339-350.
- Di Mascio, P.; Devasagayam, T. P.; Kaiser, S.; Sies, H. Carotenoids, tocopherols and thiols as biological singlet molecular oxygen quenchers. *Biochem. Soc. Trans.* **1990**, *18*, 1054-1056.
- Goedheer, J. C. Visible absorption and fluorescence of chlorophyll and its aggregates in solution. In *The Chlorophylls*; Vernon, L. P., Seelley, G. R., Eds.; Academic Press: New York, 1966; pp 147-184.
- Goodwin, T. W. Biosynthesis. In *Carotenoids*; Isler, O., Ed.; Birkhauser Verlag: Basel, Switzerland, 1971; pp 589-592.
- Graham, S.; Haughey, B.; Marshall, J.; Brasure, J.; Zielezny, M.; Freudenheim, J.; West, D.; Nolan, J.; Wilkinson, G. Diet in the epidemiology of gastric cancer. *Nutr. Cancer* **1990**, *13*, 19-34.
- Heinonen, M. I.; Ollilainen, V.; Linkola, E. K.; Varo, P. T.; Koivistoinen, P. E. Carotenoids in Finnish foods: Vegetables, fruits, and berries. *J. Agric. Food Chem.* **1989**, *37*, 655-659.
- Khachik, F.; Beecher, G. R.; Whittaker, N. F. Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green

- vegetables by liquid chromatography. *J. Agric. Food Chem.* **1986**, *34*, 603–616.
- Khachik, F.; Beecher, G. R.; Goli, M. B.; Lusby, W. R.; Daitch, C. E. Separation and quantification of carotenoids in human plasma. *Methods Enzymol.* **1992a**, *213*, 205–219.
- Khachik, F.; Goli, M. B.; Beecher, G. R.; Holden, J.; Lusby, W. R.; Tenorio, M. D.; Barrera, M. R. Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *J. Agric. Food Chem.* **1992b**, *40*, 390–398.
- Klaur, H.; Bauernfeind, J. C. Carotenoids as food colors. In *Carotenoids as colorants and vitamin A precursors*; Bauernfeind, J. C., Ed.; Academic Press: New York, 1981; p 146.
- Kvale, G.; Bjelke, E.; Gart, J. J. Dietary habits and lung cancer risk. *Int. J. Cancer.* **1983**, *31*, 397–405.
- Le Marchand, L.; Yoshizawa, C. N.; Kolonel, L. N.; Hankin, J. H.; Goodman, M. T. Vegetable consumption and lung cancer risk: a population-based case-control study in Hawaii. *J. Natl. Cancer Inst.* **1989**, *81*, 1158–1164.
- Mangels, A. R.; Holden, J. M.; Beecher, G. R.; Forman, M. R.; Lanza, E. Carotenoid Content of fruits and vegetables: An evaluation of analytic data. *J. Am. Diet. Assoc.* **1993**, *93*, 284–296.
- Micozzi, M. S. Foods, micronutrients, and reduction of human cancer. In *Nutrition and cancer prevention: Investigating the role of micronutrients*; Moon, T. E., Micozzi, M. S., Eds.; Dekker: New York, 1989; pp 213–241.
- Micozzi, M. S.; Beecher, G. R.; Taylor, P. R.; Khachik, F. Carotenoid analyses of selected raw and cooked foods associated with a lower risk for cancer. *J. Natl. Cancer Inst.* **1990**, *82*, 282–285.
- Padmavati, K.; Udipi, S. A.; Rao, M. Effect of different cooking methods on β -carotene content of vegetables. *J. Food Sci. Technol.* **1992**, *29*, 137–140.
- Shekelle, R. B.; Liu, S.; Raynor, W. J., Jr.; Lepper, M.; Maliza, C.; Rossof, A. M. Dietary vitamin A and risk of cancer in the Western Electric study. *Lancet* **1981**, *2*, 1185–1189.
- Sies, H.; Stahl, W.; Sundquist, A. R. Antioxidant functions of vitamins. Vitamins E and C, β -carotene, and other carotenoids. *Ann. N. Y. Acad. Sci.* **1992**, *669*, 7–20.
- Tan, B. Analytical and preparative chromatography of tomato paste carotenoids. *J. Food Sci.* **1988**, *53*, 954–959.
- Zakaria, M.; Simpson, K.; Brown, P. R.; Krstulovic, A. Use of reversed-phase high performance liquid chromatographic analysis for the determination of provitamin A carotenoids in tomatoes. *J. Chromatogr.* **1979**, *176*, 109–117.
- Ziegler, R. G. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. *J. Nutr.* **1989**, *119*, 116–122.
- Ziegler, R. G.; Mason, T. J.; Stemhagen, A.; Hoover, R.; Schoenberg, J. B.; Gridley, G.; Virgo, P. W.; Fraumeni, J. R., Jr. Carotenoid intake, vegetables, and the risk of lung cancer among white men in New Jersey. *Am. J. Epidemiol.* **1986**, *123*, 1080–1093.

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