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Thermal Processing of Vegetables Increases Cis Isomers of Lutein and Zeaxanthin

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Carotenoids, lutein and zeaxanthin, found in fruits and vegetables, comprise the macula pigment of the eye. These carotenoids exist in plants as the all-trans geometric form; however, in human plasma, cis isomers of these carotenoids have also been identified. Thermal processing can induce carotenoid trans to cis isomerization. The aim of this research was to determine if thermal processing induces isomerization of lutein and zeaxanthin and to quantify the extent of this reaction. High-performance liquid chromatography was used to separate and quantitate geometric isomers of lutein and zeaxanthin. Isomers were tentatively identified by UV−visible absorbance spectra, comparison of retention times to those of isomerized standards using C_{30} chromatography, and mass spectrometry. Thermal processing increased the percent cis isomers of lutein and zeaxanthin up to 22 and 17%, respectively. Further studies are needed to consider the physiological impact of consuming carotenoid isomers in processed vegetables.

KEYWORDS: C_{30} reversed-phase; HPLC; lutein; zeaxanthin; geometrical isomers

INTRODUCTION

Carotenoids, lutein and zeaxanthin, common in many fruits and vegetables, have been shown to be the primary components of the human macula pigment (*1*). The macula pigment is the center portion of the retina where its primary function is to protect the retina by filtering damaging blue light (*2*). Age-related macular degeneration and cataract formation are degenerative diseases of the eye that can lead to blindness. It has been shown that increased consumption of lutein and zeaxanthin and foods rich in these carotenoids decreases the risk of developing these diseases (*3*−*5*). With an increasing elderly population along with a more health conscious society, lutein has found its way into supplements, as well as being added as an ingredient in many food and beverage products in an attempt to combat these degenerative eye diseases (*6*, *7*).

The structural form of lutein and zeaxanthin found in the macula pigment consists primarily of the all-trans isomer; however, the cis isomers of lutein and zeaxanthin were detected at low concentrations (*8*). In nature, carotenoids are predominately present in the all-trans configuration (*9*). The long chain of conjugated carbon−carbon double bonds within carotenoids is susceptible to light, oxygen, heat, and acid degradations (*10*). When foods are thermally processed, the trans double bonds become susceptible to geometric isomerization, creating a cis configuration. The extent to which this occurs to provitamin A carotenoids and lycopene has been investigated in fruits and vegetables (*11*−*13*). However, there is little data dealing with the trans to cis isomerization of the carotenoids, lutein and zeaxanthin, resulting from thermal processing. To determine the extent to which the cis isomers of these carotenoids are produced, the various forms present in fresh and processed vegetables must be quantified.

Most carotenoid analytical data in fruits and vegetables (*14*, *15*) only quantify the all-trans form of lutein and zeaxanthin and provide no quantification of the geometrical isomers. Other data (*16*, *17*) report quantification of trans and cis isomers of lutein; however, each specific cis isomer is not individually quantified. Humphries and Khachik recently published a study that quantified the distribution of geometrical isomers of lutein and zeaxanthin in fruits, vegetables, wheat, and pasta products (*18*). The purpose of the present research is to (i) determine the effects of thermal processing on the isomerization of lutein and zeaxanthin in vegetables that contain significant quantities of these carotenoids and (ii) quantify the geometrical isomers of lutein and zeaxanthin present in these vegetables. Taken together, these results will provide a more accurate representation of the geometrical isomers present in vegetables that are commonly consumed.

MATERIALS AND METHODS

Materials. The lutein standard was purchased from Sigma Chemical Co. (St. Louis, MO). The zeaxanthin standard was purchased from Indofine Chemical Co. (Bellemeade, NJ). All extraction and high-performance liquid chromatography (HPLC) solvents (Fisher Scientific Co., Fair Lawn, NJ) were of certified HPLC or ACS grade. The vegetables evaluated were broccoli (*Brassica oleracea*), corn (*Zea mays*), kale (*Brassica oleracea*), green peas (*Pisum sativum*), and spinach (*Spinacia oleracea*). Approximately 10 lb of each vegetable was purchased from a local market in Columbus, OH, and subsequently

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divided into two lots. Lots were kept separate throughout processing, extraction, and chromatographic analysis. The quantitative data presented for each vegetable are based on the average of two extractions from each lot evaluated.

Processing. Within 24 h of purchase, samples were prepared as they are commonly consumed (e.g., stems and blemishes removed), and they were processed whole. One lot of each vegetable was used for processing. Canning was carried out at The Ohio State University pilot plant according to the National Canners Association (*19*) time and temperature requirements. Water was the canning medium. The broccoli was not canned but microwaved for 8 min using a Goldstar microwave oven, model ER-4010, with the addition of 10 mL of deionized water.

Extraction and Saponification. The following extraction procedures were carried out under UV filtered light to prevent isomerization and photodegradation. The entire lot of each product (fresh and processed) was pureed in a food processor (Cuisinart Mini Food Processor). All of the canned samples were drained prior to pureeing. Moisture analysis of the pureed tissue was performed in a vacuum oven at 70 °C and 20 in. Hg for 5.5 h. For each extraction, approximately 10 g of the pureed tissue, calcium carbonate (1 g), Celite (4 g), and acetone (50 mL) were mixed for 1 min and then homogenized using a PT 3100 Polytron homogenizer (Kimematica, Switzerland) for 1 min. This mixture was filtered through Nos. 1 and 42 (on the bottom) Whatman filter papers under vacuum. The filter cake was resuspended in 50 mL of acetone, homogenized for 1 min, and filtered through the same filter papers. This acetone extraction was repeated until the filter cake was colorless.

Because of the presence of chlorophylls in broccoli, kale, green peas, and spinach, these extracts were saponified with 75 mL of a 30% (w/v) methanolic potassium hydroxide solution at room temperature with constant stirring for 30 min. To this mixture, 50 mL of a hexane:diethyl ether (70:30 v/v) solution was added and then washed with deionized water until the layers separated. The organic layer containing the carotenoids was collected. This extraction procedure was repeated until the organic layer was colorless. The collected organic layers were passed through anhydrous sodium sulfate into a volumetric flask to remove any contaminating water. The flask was brought to volume with hexane. Samples were dried and stored with a nitrogen atmosphere in the dark at −20 °C. Within 24 h of extraction, all samples were filtered through nylon 0.2 μm pore syringe filters and analyzed using HPLC.

Chromatography. All extracts were analyzed in duplicate using reversed-phase HPLC employing a polymeric 3 μm C_{30} stationary phase (4.6 mm i.d. × 250 mm) carotenoid column (YMC/Waters, Milford, MA) and a Waters Symmetry C_{18} guard column (Waters). An isocratic solvent system consisting of 85 methanol containing 2% (v/v) 1 M ammonium acetate (pH 4.6):15 methyl-*tert*-butyl ether (MTBE) was delivered at 1 mL min^{-1}, using an Alliance 2695 Separations Module (Waters). Samples were often concentrated prior to injection so that the all-trans peak was in the range of the prepared standard curve. An automatic injector was used to inject 25 μL of sample resolublized in methanol. The column effluent was monitored using a Waters 996 photodiode array detector from 210 to 600 nm. Peaks were integrated using Empower chromatography software (Waters) on a Dell Optiplex GX240 Computer (Dell Computer Corporation, Round Rock, TX). Individual isomer concentrations were calculated using all-trans standard curves recorded at 444 and 450 nm for lutein and zeaxanthin, respectively.

Mass Spectrometry. HPLC-MS experiments were performed using a Micromass Quattro Ultima system with an APCI interface (Micromass, Manchester, U.K.). The mass spectrometer was connected to a Waters Alliance 2695 separations module (Waters) equipped with a photodiode array detector. Nitrogen was used as the cone gas at a flow rate of 127 L h^{-1} and as the desolvation gas at a flow rate of 214 L h^{-1}. The source temperature was 120 °C, and the desolvation temperature was 300 °C. The corona current was 14.5 μA, and the cone was 86 V. The chromatographic conditions were the same as those mentioned above.

Iodine Isomerization. The all-trans lutein and zeaxanthin standards were photoisomerized into an equilibrium mixture of various geometrical isomers according to a published procedure (*20*). Briefly, iodine catalyst was added at a concentration of 2% (w/w) of the carotenoid weight in hexane (determined spectrophotometrically at 444 and 450 nm using a specific molar absorptivity coefficient of 147,300 (*21*) and 132,900 L mol^{-1} cm^{-1} (*22*) for lutein and zeaxanthin, respectively). The solution was exposed to ambient fluorescent light for 1 h. The isomerized mixture was dried under a stream of nitrogen gas and resolublized in a suitable solvent for HPLC analysis as described above.

Table 1. Electronic Absorption Maxima and Mass Spectral Data of the Geometrical Isomers

isomer	absorption maxima[a] (nm)	molecular mass (*m*/*z*)
all-trans lutein	331, (444), 473	568
13-cis + 13′-cis lutein	330, (439), 466	568
9-cis lutein	330, (440), 467	568
9′-cis lutein	330, (440), 468	568
all-trans zeaxanthin	(450), 478	568
13-cis zeaxanthin	338, (444), 470	568
9-cis zeaxanthin	338, (445), 472	

[a] Measured in the LC mobile phase [methanol–MTBE (85:15)] using a photodiode array detector. Values in parentheses represent the main absorption maxima.

Peak Identification. All-trans lutein and zeaxanthin and their geometrical isomers (**Table 1**) were identified by (i) UV−visible absorbance spectra using a Waters 996 photodiode array detector with Empower chromatography software (Waters) on a Dell Optiplex GX240 personal computer (Dell Computer Corporation), (ii) comparison of retention times and absorbance spectra to those of isomerized standards, (iii) comparison to previous separations on C_{30} columns (*23*, *24*), and (iv) analysis using mass spectrometry.

Statistical Analysis. An analysis of variance was performed to test significance at $p < 0.05$. All statistical calculations were done using Statview version 5.0 (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION.

Identification of the Geometrical Isomers. Figure 1 shows the chemical structures of the geometrical isomers of lutein and zeaxanthin. Zeaxanthin is a symmetrical carotenoid whose predominant cis isomers are 13-cis and 9-cis. However, lutein, an asymmetrical carotenoid, has more cis isomers as compared to zeaxanthin (**Figure 1**). Tentative identification of the isomers was achieved by comparing the UV−visible spectra data recorded by the Waters 996 photodiode array detector to absorption maxima and spectra previously reported (*23*−*25*). The cis isomers of lutein have a hypsochromatic shift in the absorption maxima as compared to the all-trans lutein isomer. As shown in **Table 1**, the absorption maxima of the 9-cis and 13-cis isomers of lutein differ from the all-trans lutein isomer by 4 and 5 nm, respectively The absorption maxima of 9-cis zeaxanthin and 13-cis zeaxanthin differ from the absorption maxima of all-trans zeaxanthin by 5 and 6 nm, respectively. These differences in the hypsochromatic shift of the lutein and zeaxanthin isomers are consistent with previous papers (*23*−*25*). Standards of lutein and zeaxanthin were iodine-isomerized and analyzed by HPLC as describe above. **Figure 2** shows the all-trans standards and the isomers of lutein and zeaxanthin that resulted from iodine isomerization. Both spectral and retention data of isomers resulting from thermal processing were analogous to data of the isomers resulting from iodine isomerization. Previous separations of lutein and zeaxanthin isomers on C_{30} columns were compared to the elution order of isomers found in thermally processed vegetables to aid in identification (*23*, *26*). α-Carotene has the same chromophore (number of conjugated double bonds) as that of lutein; however, in lutein, each end group contains a hydroxyl group where as in α-carotene these hydroxyl groups are absent. Emenhiser et al. isolated and elucidated the structure of the predominant isomers of α-carotene (*20*). The elution order of the lutein isomers resulting from thermal processing was similar to that obtained from separations

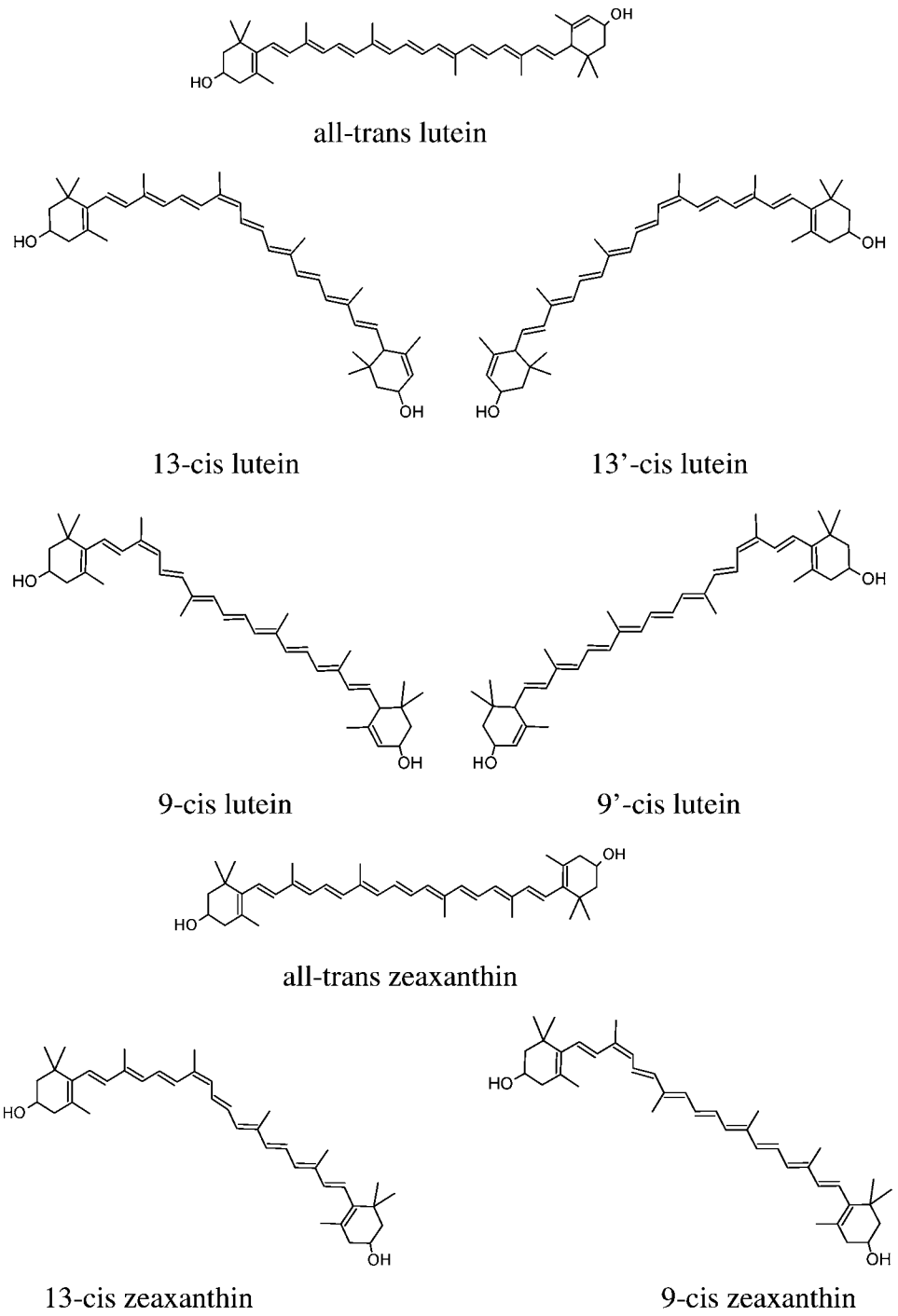

Figure 1. Chemical structures of the geometrical isomers of lutein and zeaxanthin.

of α-carotene isomers. Mass spectrometry was employed to confirm the identification of individual isomers. All isomers had molecular masses consistent with the all-trans compounds (**Table 1**). On the basis of the elution order, absorption maxima, and mass spectrometry for conformation of molecular mass, the cis isomers of lutein and zeaxanthin were tentatively identified. To confirm the position of the cis bond within the isomeric structure, large quantities of purified isomers are needed, and nuclear magnetic resonance spectroscopy should be employed.

C_{30} Reversed-Phase HPLC Separation of Cis/Trans Isomers. A C_{30} stationary phase for reversed-phase HPLC was employed to separate geometrical isomers of lutein and zeaxanthin in vegetable extracts. This stationary phase was capable of separating cis and trans isomers of lutein and zeaxanthin under isocratic conditions. **Figure 3** illustrates the resolution of three lutein isomers and two zeaxanthin isomers in representative samples of fresh and processed yellow sweet corn. As shown in **Figure 3B**, processing resulted in formation of 13-cis and 9-cis isomers of lutein and the 13-cis isomer of zeaxanthin in yellow sweet corn. **Figure 4** illustrates the resolution of three lutein isomers present in representative samples of fresh and processed kale. As shown in **Figure 4B**, processing resulted in the formation of the 13-cis, 9-cis, and 9′-cis isomers of lutein.

Qualitative Distribution of Cis/Trans Isomers. For the majority of samples analyzed, the all-trans isomer was lower in processed as compared to fresh samples on a percent basis

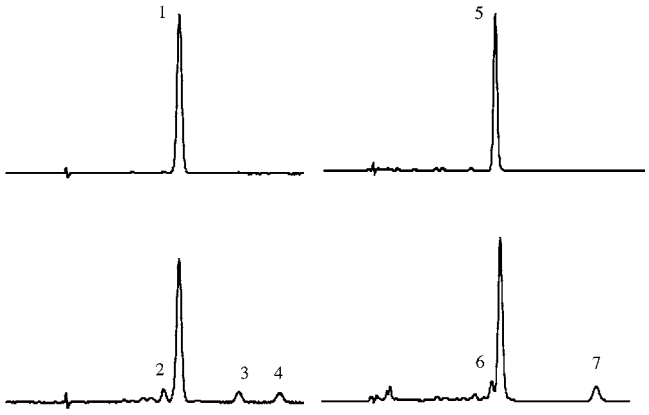

Figure 2. Chromatograms representing all-trans lutein and all-trans zeaxanthin standards and their respective iodine-isomerized standards. Peaks: (1) all-trans lutein, (2) 13-cis + 13′-cis lutein, (3) 9-cis lutein, (4) 9′-cis lutein, (5) all-trans zeaxanthin, (6) 13-cis zeaxanthin, and (7) 9-cis zeaxanthin.

(**Table 2**). This change in isomeric composition results from trans to cis isomerization, which occurred during thermal processing. Canning of kale caused the largest increase in total cis isomers of lutein on a percent basis (22%), followed by processing of corn (12%), spinach (11%), green peas (6%), and broccoli (3%). Thermal processing of corn resulted in a 17% increase of cis isomers of zeaxanthin (**Table 3**). In all vegetables, the increase in cis isomers in the processed product was significantly greater than the quantities of cis isomers in the fresh product. Therefore, the carotenoids in all vegetables investigated were susceptible to trans to cis isomerization as a result of thermal processing. Even though broccoli was not canned and thus underwent a less intense thermal process, lutein was still susceptible to trans to cis isomerization.

The predominant cis isomer of lutein in processed vegetables was 13-cis lutein followed by smaller quantities of 9-cis lutein and 9′-cis lutein. The 13-cis isomer of lutein was present in fresh broccoli, green peas, spinach, and to a lesser extent in sweet yellow corn. This 13-cis isomer was not present in fresh kale. The presence of these cis isomers in fresh vegetables likely results from chlorophyll derivatives acting as sensitizers that induce isomerization of all-trans carotenoids to their respective cis forms (*27*). However, we have no explanation for the absence of cis isomers in fresh kale tissue, which contains chlorophyll. The largest increase of the 13-cis isomer of lutein due to processing occurred in corn (11%) followed by the kale (9%). Only a 2−3% increase in the 13-cis isomer of lutein was observed during processing of broccoli, green peas, and spinach. The 9-cis isomer of lutein was found only in corn, kale, green peas, and spinach postprocessing. The 9-cis isomer of lutein was 1.4, 2.3, 4.8, and 6.8% of the total isomeric profile in corn, pea, spinach, and kale, respectively. The 9′-cis isomer occurred less frequently and was only observed in pea (1.3%), spinach (3.1%), and kale (5.7%) as a percentage of the total isomeric

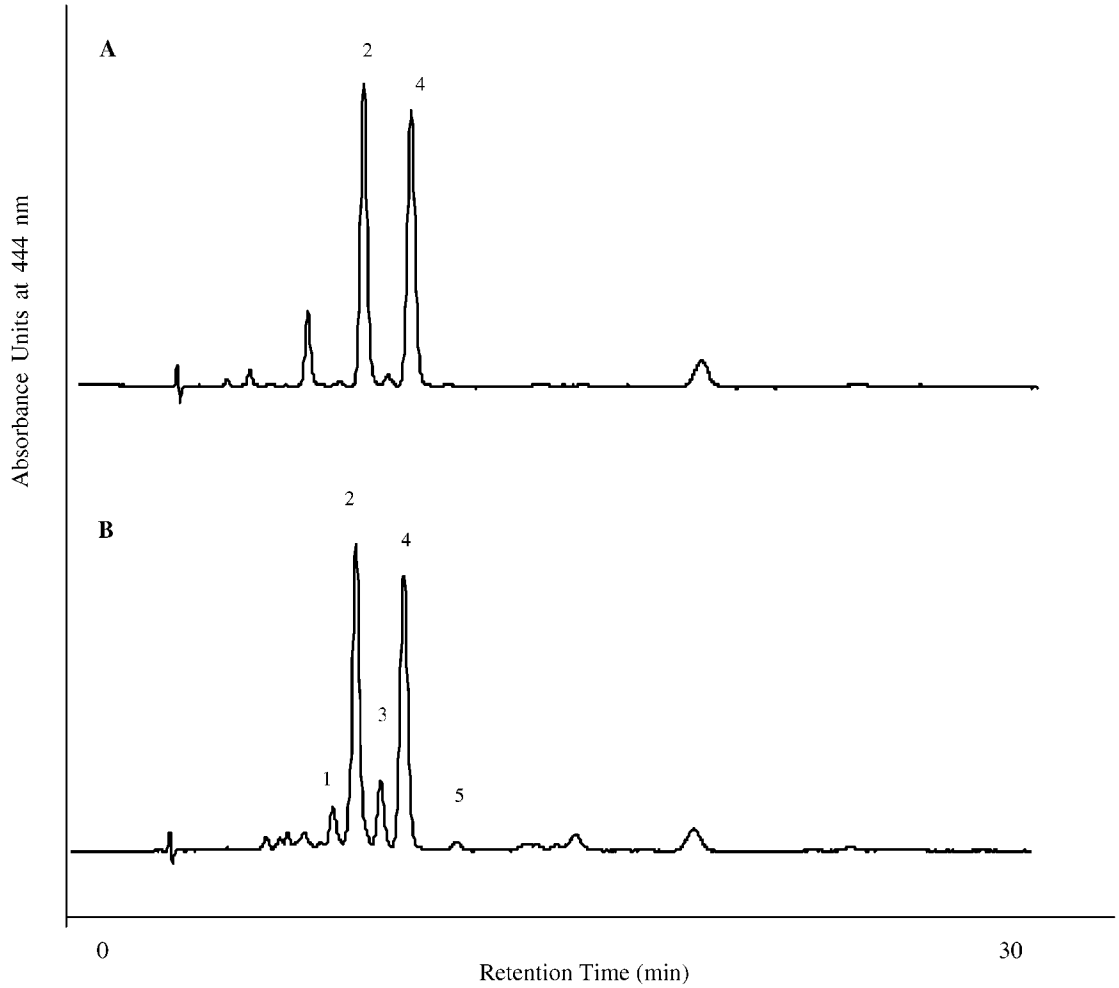

Figure 3. Reversed-phase HPLC separation of lutein and zeaxanthin isomers in (**A**) fresh and (**B**) canned yellow sweet corn using a 3 μm C_{30} stationary phase column and 85:15 methanol/MTBE mobile phase. Peaks: (1) 13-cis lutein, (2) all-trans lutein, (3) 13-cis zeaxanthin, (4) all-trans zeaxanthin, and (5) 9-cis lutein.

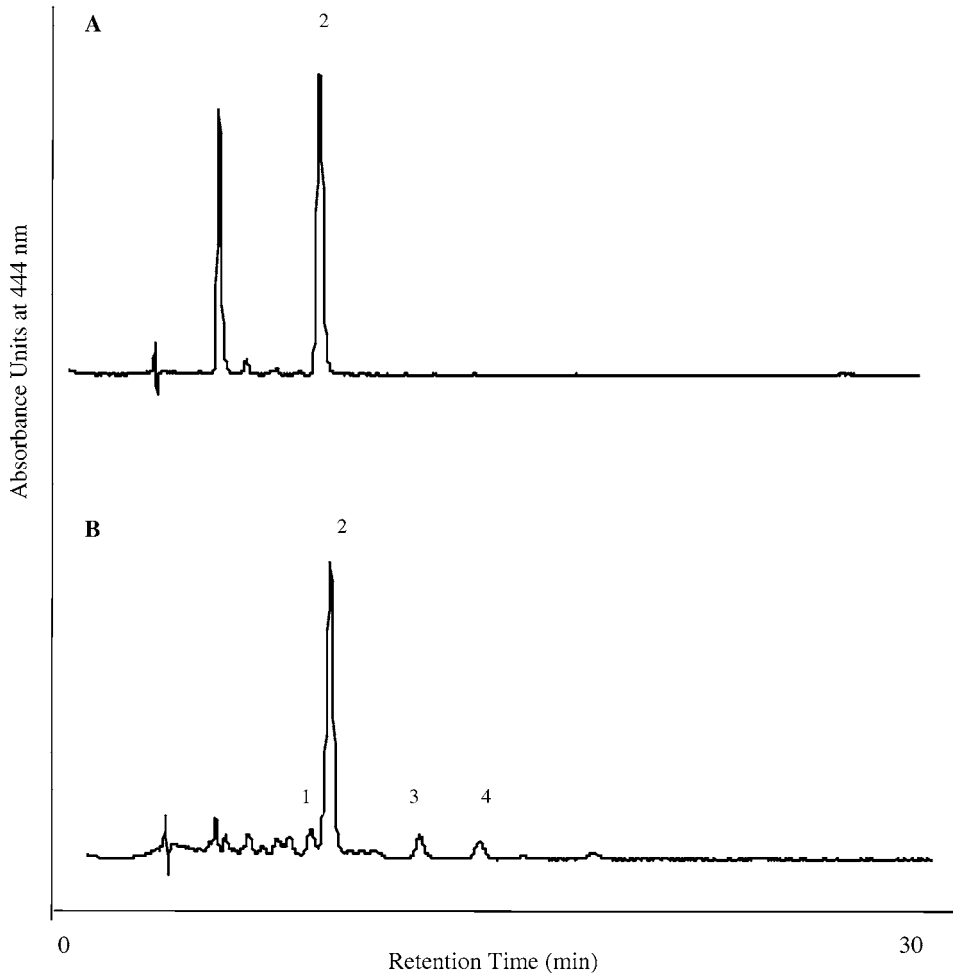

Figure 4. Reversed-phase HPLC separation of lutein isomers in (**A**) fresh and (**B**) canned kale using a 3 μm C_{30} stationary phase column and 85:15 methanol/MTBE mobile phase. Peaks: (1) 13-cis, (2) all-trans, (3) 9-cis, and (4) 9′-cis.

Table 2. Quantitative Distribution of Lutein Isomers in Fresh and Processed Vegetables[a,b] and Percent Moisture of Tissue Purees

extract	all-trans	13-cis	9-cis	9′-cis	total cis	total	moisture[c]
broccoli							
fresh	83.0	0.6			0.6	83.6	87.8
microwaved	101.8	3.9			3.9	105.7	87.1
corn							
fresh	12.0	tr[d]			tr	12.0	77.2
canned	11.0	1.4	0.2		1.6	12.6	82.2
kale							
fresh	515.4					515.4	85.9
canned	489.8	58.6	42.7	35.9	137.2	627.0	87.5
pea							
fresh	40.7	0.7			0.7	41.4	76.2
canned	54.3	2.4	1.4	0.8	4.6	58.9	83.1
spinach							
fresh	853.9	27.1			27.1	881.0	93.5
canned	793.6	56.2	44.4	28.9	129.5	923.1	90.6

[a] Data are based on an average of two extractions from each lot. Each extraction was analyzed by HPLC in duplicate. [b] Concentrations are in μg g^{-1} dry weight. [c] Moistures are based on duplicate determinations of each lot. [d] tr, trace.

Table 3. Quantitative Distribution of Zeaxanthin Isomers in Fresh and Processed Corn[a,b] and Percent Moisture of Tissue

corn	all-trans	13-cis	total cis	total	moisture[c]
fresh	23.8	0.1	0.1	23.9	77.2
canned	19.8	4.2	4.2	24.0	82.2

[a] Data are based on an average of two extractions from each lot. Each extraction was analyzed by HPLC in duplicate. [b] Concentrations are in μg g^{-1} dry weight. [c] Moistures are based on duplicate determinations of each lot.

profile. Only these three cis isomers of lutein were observed in these vegetables. In a survey of major carotenoid and chlorophyll constituents of several green vegetables, Khachik et al. (*16*) found that the 13-cis isomer of lutein was more prevalent than the 9-cis isomer of lutein in broccoli, spinach, and kale. Edelenbos et al. (*28*) also found the 13-cis isomer of lutein to be more prevalent than the 9-cis isomer in processed green peas.

Quantitative Distribution of Cis/Trans Isomers. On a quantitative basis (μg g^{-1} dry weight), processing resulted in 42, 26, 22, 5, and 4% increases in total lutein content of green peas, broccoli, kale, spinach, and corn, respectively, relative to total lutein content in fresh samples (**Table 2**). It has been observed that lutein has greater stability to degradation, as compared to its hydrocarbon carotenoid counterparts, when heated (*29*); therefore, the increased content of lutein in processed vegetables was most likely a result of a loss of soluble solids into the canning medium (*30*, *31*), inactivation of carotenoid-oxidizing enzymes (*32*), and/or increased extraction efficiency due to disruption of carotenoid−protein complexes (*33*). All of the vegetables that demonstrated an increase in total lutein concentration relative to fresh samples had had the

canning medium drained prior to analysis. Because the broccoli was not canned and had no substantial liquid to drain, the increase in lutein most likely resulted from an increase in extraction efficiency due to softening and disruption of the tissue releasing lutein from within the chloroplast (*34*).

Carotenoid concentrations of vegetables vary with plant variety, maturity, growing conditions, season of the year, and the part of the plant consumed (*14*). Many papers that have analyzed vegetables containing lutein have not analyzed specific cis isomers of lutein but rather quantified the total amount of lutein present, presumably only the all-trans form (*14*, *15*, *35*). Recently, Humphries and Khachik analyzed several fruit, vegetable, wheat, and pasta products for lutein and zeaxanthin and their relative geometrical isomers (*18*). In all of the vegetables that were analyzed, cis isomers of both xanthophylls were found and quantified separately. Their work along with our present findings will help create a database describing specific isomers of lutein and zeaxanthin and their concentrations found in foods. In addition, few studies have analyzed fresh and processed foods originating from the same starting material processed under controlled conditions, and consequently the true effects of thermal processing were not determined.

Many studies report data on a fresh weight basis, and other studies report data on a dry weight basis with no moisture content information included, thus making comparisons of individual studies difficult. To minimize this confusion, Mangels et al. (*36*) surveyed many papers, evaluated their data, and subsequently created a carotenoid database that compiled acceptable values for carotenoid content of fruits and vegetables including median, minimum, and maximum values of five carotenoids on a fresh weight basis. Values obtained for lutein in the vegetables investigated in this research were both within and outside (data not shown) the range of values given by Mangels et al. (*36*). This most likely results from factors affecting composition as mentioned above.

The processing of vegetable products has been found to enhance the bioavailability of carotenoids. Gartner et al. (*37*) found that lycopene bioavailability from tomato paste (a thermally processed product) was greater than that from fresh tomatoes. Dewanto et al. (*38*) also found thermal processing to significantly increase the bioacessibility of lycopene from processed tomatoes. Dewanto et al. reasoned that this occurred primarily because of an increase in release of carotenoids from the food matrix resulting from thermal processing. This increase of bioaccessibility from thermally processed vegetable products may occur with lutein. However, further studies must determine if this is indeed the case.

The implication of consuming cis isomers in these products has not been considered extensively. Additionally, we investigated the lutein isomeric profile of a commercially available meal replacement containing lutein and a commercially available supplement containing lutein. In both products, detectable levels of cis isomers were found in addition to the all-trans isomer. The geometrical isomers of lutein and zeaxanthin have been isolated from human plasma (*39*) and human retinas (*8*). This evidence indicates that cis isomers of xanthophylls can either be absorbed directly from foods or result from a trans to cis isomerization after absorption. Questions remain as to whether cis isomers could participate in photoprotection of the retina as effectively as that proposed for their all-trans counterparts. Further studies must be conducted to follow cis isomers during digestion and absorption and determine what physiological impact, if any, these cis isomers may have.

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LITERATURE CITED

(1) Bone, R. A.; Landrum, J. T.; Tarsis, S. L. Preliminary Identification of the Human Macular Pigment. *Vision Res.* **1985**, *25*, 1531−1535.

(2) Landrum, J. T.; Bone, R. A. Lutein, Zeaxanthin, and the Macular Pigment. *Arch. Biochem. Biophys.* **2001**, *385*, 28−40.

(3) Seddon, J. M.; Ajani, U. A.; Sperduto, R. D.; Hiller, R.; Blair, N.; Burton, T. C.; Farber, M. D.; Gragoudas, E. S.; Haller, J.; Miller, D. T.; Yannuzzi, L. A.; Willett, W. Dietary Carotenoids, Vitamins A, C, and E, and Advanced Age-Related Macular Degeneration. *JAMA* **1994**, *272*, 1413−1420.

(4) Chasan-Taber, L.; Willett, W. C.; Seddon, J. M.; Stampfer, M. J.; Rosner, B.; Colditz, G. A.; Speizer, F. E.; Hankinson, S. E. A Prospective Study of Carotenoid and Vitamin A Intakes and Risk of Cataract Extraction in US Women. *Am. J. Clin. Nutr.* **1999**, *70*, 509−516.

(5) Brown, L.; Rimm, E. B.; Seddon, J. M.; Giovannucci, E. L.; Chasan-Taber, L.; Spiegelman, D.; Willett, W. C.; Hankinson, S. E. A Prospective Study of Carotenoid Intake and Risk of Cataract Extraction in US Men. *Am. J. Clin. Nutr.* **1999**, *70*, 517−524.

(6) Fullmer, L. A.; Shao, A. The Role of Lutein in Eye Health and Nutrition. *Cereal Foods World* **2001**, *46*, 408−413.

(7) Silva, S. Lutein in Food and Beverage Applications. *Innovations Food Technol.* **2002**, *February*, 63−65.

(8) Khachik, F.; Bernstein, P. S.; Garland, D. L. Identification of Lutein and Zeaxanthin Oxidation Products in Human and Monkey Retinas. *Invest. Ophthalmol. Visual Sci.* **1997**, *38*, 1802−1811.

(9) Zechmeister, L. *Cis*−*Trans Isomeric Carotenoids, Vitamins A and Arylpolyenes*; Academic Press: New York, 1962.

(10) Chen, B. H.; Chen, T. M.; Chien, J. T. Kinetic Model for Studying the Isomerization of α- and β-Carotene During Heating and Illumination. *J. Agric. Food Chem.* **1994**, *42*, 2391−2397.

(11) Lessin, W. J.; Catigani, G. L.; Schwartz, S. J. Quantification of *cis*−*trans* Isomers of Provitamin A Carotenoids in Fresh and Processed Fruits and Vegetables. *J. Agric. Food Chem.* **1997**, *45*, 3728−3732.

(12) Nguyen, M.; Francis, D.; Schwartz, S. Thermal Isomerization Susceptibility of Carotenoids in Different Tomato Varieties. *J. Sci. Food Agric.* **2001**, *81*, 910−917.

(13) Chandler, L. A.; Schwartz, S. J. HPLC Separation of Cis−Trans Carotene Isomers in Fresh and Processed Fruits and Vegetables. *J. Food Sci.* **1987**, *52*, 669−672.

(14) Hart, D. J.; Scott, K. J. Development and Evaluation of an HPLC Method for the Analysis of Carotenoids in Foods, and the Measurement of the Carotenoid Content of Vegetables and Fruits Commonly Consumed in the UK. *Food Chem.* **1995**, *54*, 101−111.

(15) 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.

(16) 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.

(17) 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.* **1992**, *40*, 390−398.

(18) Humphries, J. M.; Khachik, F. Distribution of Lutein, Zeaxanthin, and Related Geometrical Isomers in Fruit, Vegetables, Wheat, and Pasta Products. *J. Agric. Food Chem.* **2003**, *51*, 1322−1327.

(19) National Canners Association. *Processes for Low-Acid Canned Foods in Metal Containers*, 11th ed.; Bulletin 26-L; National Canners Association: Washington, DC, 1976.

(20) Emenhiser, C.; Englert, G.; Sander, L. C.; Ludwig, B.; Schwartz, S. J. Isolation and Structural Elucidation of the Predominant Geometrical Isomers of α-Carotene. *J. Chromatogr. A* **1996**, *719*, 333−343.

(21) Craft, N. E.; Soares, J. H. Relative Solubility, Stability and Absorptivity of Lutein and β-Carotene in Organic Solvents. *J. Agric. Food Chem.* **1992**, *40*, 431−434.

(22) Britton, G. UV/Visible Spectroscopy. *Carotenoids Volume 1B: Spectroscopy*; Birkhauser Verlag: Switzerland, 1995; pp 13−62.

(23) Emenhiser, C.; Sander, L. C.; Schwartz, S. J. Capability of a Polymeric C_{30} Stationary Phase to Resolve *cis*−*trans* Carotenoid Isomers in Reversed-Phase Liquid Chromatography. *J. Chromatogr. A* **1995**, *707*, 205−216.

(24) Bohm, V.; Puspitasari-Nienaber, N. L.; Ferruzzi, M. G.; Schwartz, S. J. Trolox Equivalent Antioxidant Capacity of Different Geometrical Isomers of α-Carotene, β-Carotene, Lycopene, and Zeaxanthin. *J. Agric. Food Chem.* **2002**, *50*, 221−226.

(25) Hadden, W. L.; Watkins, R. H.; Levy, L. W.; Regalado, E.; Rivadeneira, D. M.; van Breemen, R. B.; Schwartz, S. J. Carotenoid Composition of Marigold (*Tagetes erecta*) Flower Extract Used As Nutritional Supplement. *J. Agric. Food Chem.* **1999**, *47*, 4189−4194.

(26) Dachtler, M.; Glaser, T.; Kohler, K.; Albert, K. Combined HPLC-MS and HPLC NMR On-Line Coupling for the Separation and Determination of Lutein and Zeaxanthin Stereoisomers in Spinach and in Retina. *Anal. Chem.* **2001**, *73*, 667−674.

(27) O'Neil, C. A.; Schwartz, S. J. Photoisomerization of β-Carotene by Photosensitization with Chlorophyll Derivatives as Sensitizers. *J. Agric. Food Chem.* **1995**, *43*, 631−635.

(28) Edelenbos, M.; Christensen, L. P.; Grevsen, K. HPLC Determination of Chlorophyll and Carotenoid Pigments in Processed Green Pea Cultivars (*Pisum sativum L.*). *J. Agric. Food Chem.* **2001**, *49*, 4768−4774.

(29) Henry, L. K.; Catignani, G. L.; Schwartz, S. J. Oxidative Degradation Kinetics of Lycopene, Lutein, and 9-cis and All-trans β-Carotene. *J. Am. Oil Chem. Soc.* **1998**, *75*, 823−829.

(30) Ogunlesi, A. T.; Lee, C. Y. Effect of Thermal Processing On The Stereoisomerisation Of Major Carotenoids And Vitamin A Value of Carrots. *Food Chem.* **1979**, *4*, 311−318.

(31) Weckel, K. G.; Santos, B.; Hernan, E.; Laferriere, L.; Gabelman, W. H. Carotene Components of Frozen and Processed Carrots. *Food Technol.* **1962**, *16*, 91−94.

(32) Baloch, A. K.; Buckle, K. A.; Edwards, R. A. Effect of Processing Variables on the Quality of Dehydrated Carrot. I. Leaching Losses and Carotenoid Content. *J. Food Technol.* **1977**, *12*, 285−293.

(33) Kirk, J. T. O.; Tilney-Basset, R. A. E. Carotenoids and phycobiliproteins. *The Plastids: Their Chemistry, Structure, Growth and Inheritance*, 2nd ed.; Elsevier/North-Holland Biomedical Press: Amsterdam, 1978; pp 90−97.

(34) Gross, J. *Pigments in Vegetables: Chorophylls and Carotenoids*; Van Nostrand Reinhold: New York, 1991; pp 105−107.

(35) Sommerburg, O.; Keunen, J. E. E.; Bird, A. C.; van Kuijk, F. J. G. M. Fruits and Vegetables that are Sources for Lutein and Zeaxanthin: the Macular Pigment in Human Eyes. *Br. J. Ophthalmol.* **1998**, *82*, 907−910.

(36) 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.

(37) Gartner, C.; Stahl, W.; Sies, H. Lycopene is More Bioavailable from Tomato Paste than from Fresh Tomatoes. *Am. J. Clin. Nutr.* **1997**, *66*, 116−122.

(38) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. *J. Agric. Food Chem.* **2002**, *50*, 3010−3014.

(39) Khachik, F.; Englert, G.; Daitch, C. E.; Beecher, G. R.; Tonucci, L. H.; Lusby, W. R. Isolation and Structural Elucidation of the Geometrical Isomers of Lutein and Zeaxanthin in Extracts from Human Plasma. *J. Chromatogr.* **1992**, *582*, 153−166.

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