JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Distribution of Lutein, Zeaxanthin, and Related Geometrical Isomers in Fruit, Vegetables, Wheat, and Pasta Products†

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Quantitative data with regard to dietary (3*R*,3′*R*,6′*R*)-lutein, (3*R*,3′*R*)-zeaxanthin, and their (*E*/*Z*)-geometrical isomers are scarce, and in most cases, only the combined concentrations of these two carotenoids in foods are reported. Lutein and zeaxanthin accumulate in the human macula and have been implicated in the prevention of age-related macular degeneration (AMD). The qualitative and quantitative distributions of lutein, zeaxanthin, and their (*E*/*Z*)-isomers in the extracts from some of the most commonly consumed fruits, vegetables, and pasta products were determined by HPLC employing a silica-based nitrile-bonded column. Green vegetables had the highest concentration of lutein (L) and zeaxanthin (Z), and the ratios of these carotenoids (L/Z) were in the range 12−63. The yellow-orange fruits and vegetables, with the exception of squash (butternut variety), had much lower levels of lutein in comparison to greens but contained a higher concentration of zeaxanthin. The ratio of lutein to zeaxanthin (L/Z) in two North American bread varieties of wheat (Pioneer, Catoctin) was 11 and 7.6, respectively, while in a green-harvested wheat (Freekeh) imported from Australia, the ratio was 2.5. Between the two pasta products examined, lasagne and egg noodles, the latter had a much higher concentration of lutein and zeaxanthin. The levels of the (*E*/*Z*)-geometrical isomers of lutein and zeaxanthin in these foods were also determined.

KEYWORDS: Carotenoids; carotenoid epoxides; hydroxycarotenoids; lactucaxanthin; lutein epoxide; violaxanthin; green and yellow-orange fruits and vegetables; (E/Z)-isomers of lutein and zeaxanthin; carotenoid HPLC separations; age-related macular degeneration

INTRODUCTION

The beneficial effects of consuming a carotenoid-rich diet have been well-documented in the literature. In addition to their role as precursors of vitamin A, carotenoids are valuable nutritionally as antioxidants (*1*), in the prevention of atherosclerosis (*2*, *3*), and in the prevention of age-related macular degeneration (AMD) (*4*, *5*). Fruits and vegetables are the main sources of carotenoids, and because of their role in maintaining health and nutritional status, there have been numerous reports on the qualitative and quantitative distributions of these compounds in foods (*6*−*12*). However, most of these published reports have primarily focused on the provitamin A carotenoids, α- and β-carotene, and β-cryptoxanthin because of the perception that provitamin A activity may have been a contributing factor in the observed health benefit of carotenoids in epidemiological studies.

Provitamin A carotenoids have been detected in human plasma (*7*) and are essential as precursors to retinoids, which play a crucial role in the visual cycle. In addition, a wide range of non-vitamin-A active carotenoids, including lutein, zeaxanthin, and their oxidative metabolites, have also been detected in human plasma and breast milk (*13*, *14*). In a recent report, Mares-Perlman et al. have reviewed the body of evidence for a protective role of lutein and zeaxanthin in the prevention of chronic disease (*15*).

Although to date, a wide range of carotenoids have been identified in human ocular tissues, the major carotenoids in the human macula are lutein and zeaxanthin (*16*−*18*). Epidemiological and observational studies have shown that concentration of macular carotenoids can be manipulated by dietary intake of lutein and zeaxanthin (*19*−*21*) and that these carotenoids may play an important role in the prevention of AMD (*22*−*24*). The onset of AMD has been attributed to phototoxic damage and free radicals (*23*, *24*), and because carotenoids have been shown to be effective radical scavengers at the low partial pressure encountered in the eye, this has been proposed as one of the mechanisms by which lutein and zeaxanthin may protect the eye. Evidence for the oxidative−reductive pathways of lutein and zeaxanthin in the human retina was first illustrated by

† Presented in part at the 12th International Carotenoid Symposium, Cairns, Australia, July 19−23, 1999.

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10.1021/jf026073e CCC: $25.00
Published on Web 00/00/0000 **PAGE EST: 5.9**

Khachik et al. in 1997 (*25*), who isolated and identified the oxidation products of these carotenoids in human and monkey retinas. In view of the implication of lutein and zeaxanthin in the prevention of AMD, an accurate account of the levels and relative distribution of these carotenoids in foods is essential. Although, the complete profiles of carotenoids in most common fruits and vegetables have been previously reported (*26*–*31*), only the combined concentration of lutein, zeaxanthin, and their (*E*/*Z*)-geometrical isomers were determined. This is due to the fact that separation of a diverse range of dietary carotenoids including lutein and zeaxanthin, their stereoisomers, and their metabolites cannot be achieved by high-performance liquid chromatography (HPLC) employing a single set of conditions. Consequently, the current food composition tables in most cases do not provide the much-needed data with regard to the individual concentration of lutein and zeaxanthin. Despite the fact that dietary sources of zeaxanthin are limited, this carotenoid is found in substantial concentrations in the human serum and retina, and as a result, its presence in foods cannot be overlooked (*13*, *14*, *16*–*18*, *25*). The present study aimed to determine the concentration of lutein, zeaxanthin, and their *E*/*Z*-isomers in commonly consumed fruits, vegetables, and pasta products that are considered as basic foods in Western diets.

MATERIALS AND METHODS

Source of Fruit and Vegetables. Fruit and vegetables were obtained from a local supermarket (Maryland) and included kale (*Brassica oleracea*), collard greens (*B. oleracea*), broccoli (*B. oleracea*), spinach (*Spinacia oleracea*), green beans (*Phaseolus vulgaris*), fresh parsley (*Petroselinum crispum*), pumpkin, butternut (*Cucurbita moschata*, var.), orange (*Citrus sinensis*), papaya (*Carica papaya*), mango (*Mangifera indica*), nectarine (*Prunus persica*), and lettuce (*Lactuca sativa*). Canned sweet corn (*Zea mays*), peas (*Pisum sativum*), and lima beans (*Phaseolus lunatus* L. (syn. *P. limensis*)) were also analyzed because these preparations are the most commonly consumed forms of these foods. Lasagne and egg noodles were purchased in the dehydrated form, and two varieties of wheat, catoctin and pioneer, were from the 1998 harvest in Maryland. Freekeh (a green-harvested wheat) was imported from Australia as a commercial product.

Nomenclature. The terms *all-E* (all-trans) and *Z* (cis) refer to the geometrical isomers of carotenoids. Throughout this manuscript, the common names of lutein and zeaxanthin have been used instead of their correct systematic names, and unless specified, these names refer to the combined *all-E* (all-trans) and *Z* (cis) isomers of these carotenoids. The chemical structures and the correct systematic names for the carotenoids discussed in the text are shown in **Figure 1**.

Extraction of Carotenoids from Foods. Green and yellow-orange fruits and vegetables were extracted and saponified according to the methods previously published by Khachik et al. (*6*, *26*–*29*). All extracts were subjected to saponification; this allowed the removal of chlorophylls in greens and in the case of yellow-orange fruits and vegetables resulted in the conversion of carotenol esters to their parent hydroxycarotenoids. The extraction and saponification of the pasta products were also performed similarly to remove the lipids.

Chromatographic System and HPLC Analysis of Carotenoids. The analyses were performed on an Agilent Technology model 1050 HPLC system equipped with a quaternary solvent delivery system, 1050 autosampler, thermostated column compartment, and 1050 diode array detector. The data were stored and processed on a compaq DeskPro 590 personal computing system, 17 in. color monitor, and a Hewlett-Packard Laserjet 4 Plus printer on Windows 98 employing the hp-ChemStation software (version A.05.01). Separations were carried out on a silica-based nitrile-bonded (25 cm length × 4.6 mm internal diameter, 5 μm spherical particle) column (Regis Chemical, Morton Grove, IL). The column was protected with a Brownlee nitrile-bonded guard cartridge (3 cm length × 4.6 mm i.d.; 5 μm particle size). Green and yellow-orange fruits and vegetables were analyzed by HPLC with eluent A, and Freekeh (green-harvested wheat), wheat, and pasta products were analyzed by HPLC with eluent B.

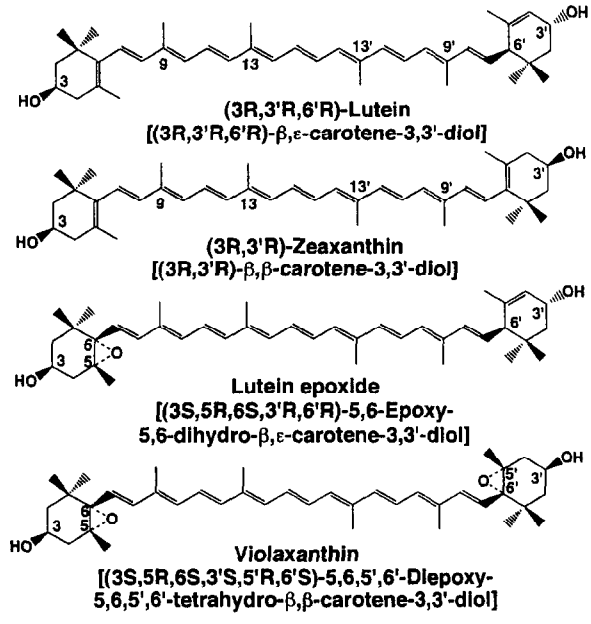

Figure 1. Chemical structure of the major hydroxycarotenoids and carotenoid epoxides in fruits and vegetables.

Eluent A. A combination of isocratic and gradient HPLC employing a two-pump solvent module was used with this eluent. Pump A pumped a mixture of hexanes (75%), dichloromethane (25%), methanol (0.30%), and *N*,*N*-diisopropylethylamine (DIPEA, 0.1%), and pump B pumped a mixture of hexanes (75%), dichloromethane (25%), methanol (1%), and DIPEA (0.1%). At time zero, 95% pump A and 5% pump B were pumped isocratically for 25 min. At 25 min, a linear gradient was run for 20 min by increasing the composition of pump B from 5% to 65% at the expense of the composition of pump A, which was reduced from 95% to 35%. Once the gradient was completed (45 min), the HPLC eluent was maintained at this composition (35% pump A and 65% pump B) for 80 min to ensure the elution of carotenoid epoxides. At the end of each run, the column was reequilibrated under the original isocratic conditions for 25 min.

Eluent B. This eluent consisted of an isocratic mixture of hexanes (75%), dichloromethane (25%), methanol (0.3%), and DIPEA (0.1%). The flow rate with eluents A and B was 0.7 mL/min, and the HPLC runs were monitored at $\lambda = 450$ nm. Eluent B was employed as the HPLC injection solvent in all separations.

Source of Carotenoid Standards. (3*R*,3′*R*,6′*R*)-Lutein (85% pure) and 13*Z*,13′*Z*-lutein were isolated and purified from a saponified extract of marigold flowers (Kemin Foods, LC, Des Moines, IA) by crystallization and preparative HPLC according to a procedure published by Khachik et al. (*32*). Reference samples of 9*Z*-lutein, 9′*Z*-lutein, 13*Z*-lutein, 13′*Z*-lutein, 9*Z*-zeaxanthin, and 13*Z*-zeaxanthin were prepared by isomerization of lutein and zeaxanthin as published previously by Khachik et al. (*33*). Lutein epoxide and violaxanthin were isolated from green vegetables according to a published procedure (*26*). (3*R*,3′*R*)-Zeaxanthin was a gift from Hoffmann-La Roche (Basel, Switzerland).

RESULTS

The qualitative and quantitative distributions of lutein and zeaxanthin in fruits, vegetables, wheat, and pasta products were determined by normal-phase HPLC using a silica-based nitrile-bonded column (spherical particles). While this HPLC column has been successfully employed for the separation of lutein, zeaxanthin, and their geometrical isomers as well as their metabolic byproducts, it fails to separate other dietary carotenoids (*13*, *14*, *32*, *33*). However, since the objective of the present study was to measure the concentrations of lutein, zeaxanthin, and their geometrical isomers in foods, no attempt was made to determine the detailed profiles of other dietary

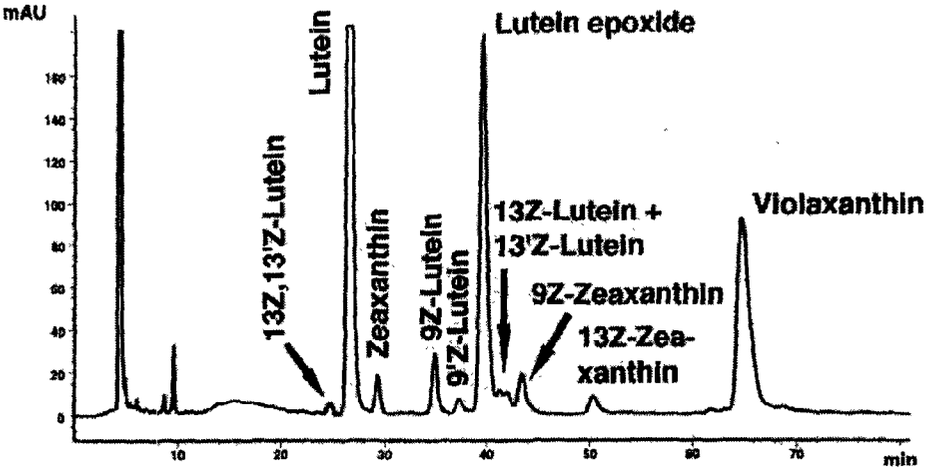

Figure 2. Carotenoid HPLC profile of an extract from green beans on a silica-based nitrile-bonded column employing eluent A. Conditions are described in the text.

carotenoids by an alternative HPLC method. Because the extracts from foods were saponified in all cases, ethyl β-apo-8′-carotenoate or other carotenoid esters could not be employed as an internal standard. The HPLC peaks of other potentially useful carotenoid internal standards either eluted too early in the chromatogram or interfered with the HPLC peaks of the carotenoids of interest. Therefore, no internal standard in the extraction of the various samples was used. To monitor the accuracy and reproducibility of the HPLC analysis of carotenoids, a solution containing known concentrations of (3*R*,3′*R*,6′R)-lutein and (3*R*,3′*R*)-zeaxanthin was routinely analyzed by HPLC with eluents A and B. The recovery and reproducibility of the HPLC analysis for carotenoids with these eluents were greater than 95%.

Carotenoids in various extracts from foods were identified by comparison of their HPLC retention times, UV−visible absorption spectra, and co-injection with those of synthetic or isolated standards. Details regarding the UV−visible absorption maxima of carotenoids have been previously published by Khachik et al. (*13*, *14*, *26*, *28*, *29*, *32*, *33*).

Lutein, Zeaxanthin, and Their Geometrical Isomers in Fruits and Vegetables. The qualitative HPLC profiles of the extracts from the green vegetables examined in this report were nearly identical, and the major differences appeared to be the concentration of the individual carotenoids. The only exception was romaine lettuce that contained a significant level of lactucaxanthin (148 μg/100 g of edible food), which is a rare dietary dihydroxycarotenoid. In all cases, the chlorophylls were removed from the extracts of green vegetables by saponification to prevent their HPLC peak interference with carotenoids. As an example, the HPLC profile (eluent A) of a saponified extract from green beans is shown in **Figure 2**. The early eluting unmarked HPLC peaks (2−10 min) in this chromatogram are most likely α-carotene, β-carotene, α-cryptoxanthin, and β-cryptoxanthin, which do not interfere with the HPLC peaks of lutein, zeaxanthin, and their related *Z*-isomers. However, two carotenoid epoxides, lutein epoxide and violaxanthin, that are abundant in greens and certain yellow-orange fruits and vegetables interfere with the separation of lutein and zeaxanthin under the isocratic HPLC conditions employing eluent B (*13*, *14*, *32*, *33*). As shown in **Figure 1**, this problem can be solved by subjecting the extracts from fruits and vegetables to gradient HPLC with eluent A. The concentrations of lutein, zeaxanthin, and their geometrical isomers in fruits and vegetables are shown in **Table 1**. Green vegetables were found to be an excellent source of lutein and zeaxanthin. The *Z*-isomers of lutein and zeaxanthin were also present in raw fruits and vegetables as well as several canned foods such as lima beans, green peas, and sweet corn. In greens and yellow-orange fruits and vegetables, the concentrations of the *Z*-isomers of lutein appear to be proportional to the concentrations of their *all-E*-isomers. While in green and yellow-orange fruits and vegetables, the *all-E*-isomer of lutein predominates, this was not always the case for zeaxanthin. The concentration of 9*Z*-zeaxanthin, which was the only geometrical isomer of this carotenoid detected in foods, was in several cases higher than that of *all-E*-zeaxanthin. The ratio of lutein to zeaxanthin in greens ranged from 12 to 63. The yellow-orange fruits and vegetables contained a higher concentration of zeaxanthin, but the level of lutein was somewhat lower than those found in greens. The yellow-orange foods with significant concentrations of zeaxanthin were corn, squash (butternut variety), oranges, and nectarine.

Lutein, Zeaxanthin, and Their Geometrical Isomers in Wheat and Pasta Products. Because of the absence of carotenoid epoxides in wheat and pasta products, the extracts from these foods were analyzed by HPLC under isocratic conditions employing eluent B. A typical HPLC profile of a green-harvested wheat (Freekeh) is shown in **Figure 3**. The concentrations of lutein, zeaxanthin, and their geometrical isomers in three varieties of wheat and two common pasta products are shown in **Table 2**. In these wheat and pasta products, the levels of lutein were found to be higher than that of zeaxanthin. The ratio of lutein to zeaxanthin in these foods ranged from 2.5 to 12. The *all-E*-isomers of lutein and zeaxanthin in wheat and pasta products were also higher than their corresponding *Z*-isomers; the only exception was lasagne in which nearly equal amounts of *all-E*- and 9*Z*-zeaxanhin were present. The concentration of lutein and zeaxanthin in Freekeh, a green-harvested wheat imported from Australia, was much higher than the two North American bread wheat, Catoctin and Pioneer.

DISCUSSION

As shown in **Table 1**, the concentration of lutein in green vegetables is much higher than that of zeaxanthin. The abundance of lutein over zeaxanthin in nature has been attributed to the dominant role of lutein in photosynthesis (*34*). The qualitative profile of carotenoids in green vegetables is generally the same with the exception of romaine lettuce in which a

Table 1. Quantitative Distribution of Lutein, Zeaxanthin, and Related Geometrical Isomers in Selected Fruits and Vegetables[a]

	concentration of lutein and zeaxanthin (μg/100 g)										
	lutein (L)						zeaxanthin (Z)				
foods	*all-E*	9*Z*	9′*Z*	13*Z* + 13′*Z*	13*Z*,13′*Z*	total *E* + *Z*	*all-E*	9*Z*	13*Z*	total *E* + *Z*	L/Z ratio
greens											
beans, green	390.0	19.5	5.8	1.6	1.2	418.1	23.0	12.0	*b*	35.0	12
beans, lima (canned)	275.5	27.5	27.5	19.6	6 0	356.1	16.0	*b*	*b*	16 0	22
broccoli	1343	65.0	16.4	81.7	4.5	1510.6	9.4	33.4	*b*	42.8	35
collards	4940	72.0	77.0	11.0	20.0	5120.0	128.0	12.0	*b*	140.0	37
kale	13053	390.0	815.0	678.0	64.0	15000.0	50.2	189.8	*b*	240.0	63
lettuce, romaine[c]	148.0	12.0	3.0	5.0	2.0	170.0	2.5	5.5	*b*	8 0	21
parsley	9924.0	351.7	53.1	481.2	10.0	10820.0	134.0	368.0	*b*	502.0	22
peas (canned)	661.8	21.1	8.0	26.1	2.0	719.0	40.1	10.9	*b*	51.0	14
spinach	8447.0	223.7	38.0	442.3	6.0	9157.0	130.8	394.3	*b*	525.1	17
yellow-orange											
corn (canned)	163.8	21.0	3.0	10.2	*b*	198.0	310.0	22.7	*b*	332.7	0.6
mango	10.0	*b*	*b*	*b*	*b*	10.0	10.0	*b*	*b*	10.0	1.0
nectarine	12.2	4.5	3.3	*b*	*b*	20.0	62.0	108.0	*b*	170.0	0.1
oranges	350.0	*b*	*b*	*b*	*b*	350.0	250.0	*b*	*b*	250.0	1.4
oranges, mandarine	48.3	14.2	2.0	6.0	*b*	70.5	52.0	8.0	*b*	60.0	1.2
papaya	23.1	*b*	*b*	*b*	*b*	22.1	22.1	*b*	*b*	22.1	1.0
peaches	20.0	*b*	*b*	*b*	*b*	20.0	20.0	*b*	*b*	20.0	1.0
plum, red	40.0	*b*	*b*	*b*	*b*	40.0	*b*	*b*	*b*	*b*	
squash, acorn	50.0	*b*	*b*	*b*	*b*	50.0	*b*	*b*	*b*	*b*	
squash, butternut	1793.0	146.0	334.0	127.0	*b*	2400.0	280.0	*b*	*b*	280.0	8.6

[a] With the exception of the canned foods, all fruits and vegetables were analyzed in the raw form. The detection limit for HPLC analysis of carotenoids was 0.1 ng. [b] Not detected. [c] Romaine lettuce also contained lactucaxanthin, 148 μg/100 g of edible food.

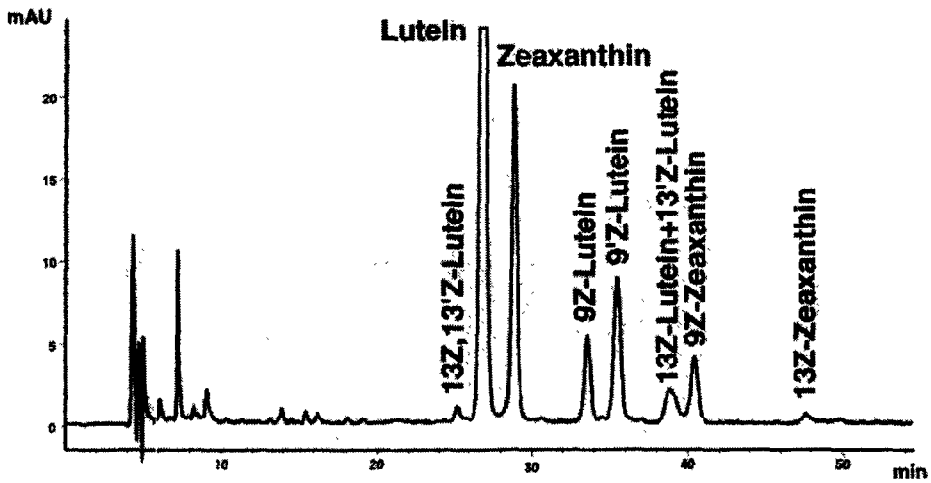

Figure 3. Carotenoid HPLC profile of an extract from a green-harvested wheat (Freekeh) on a silica-based nitrile-bonded column employing eluent B. Conditions are described in the text.

Table 2. Quantitative Distribution of Lutein, Zeaxanthin, and Related Geometrical Isomers in Selected Wheat and Pasta Products[a]

	concentration of lutein and zeaxanthin (ng/g)										
	lutein (L)						zeaxanthin (Z)				
foods	*all-E*	9*Z*	9′*Z*	13*Z* + 13′*Z*	13*Z*,13′*Z*	total *E* + *Z*	*all-E*	9*Z*	13*Z*	total *E* + *Z*	L/Z ratio
wheat											
Catoctin	28.7	1.0	0.9	1.8	*b*	32 4	1.9	1.2	*b*	3.1	11
Pioneer	195.8	7.6	7.6	13.1	*b*	224.1	19.0	10.3	*b*	29.3	7 6
Freekeh	624.5	44.1	81.5	32.7	9.1	791.9	242 5	59.9	12.9	315.3	2.5
pasta											
egg noddles	1095	77.0	74.1	117.6	28.4	1391 6	352.7	140.7	51.5	544 9	2.6
lasagne	226 6	14 9	15.2	25.7	6 3	288 7	11.3	12.5	*b*	23.8	12.1

[a] Detection limit for HPLC analysis of carotenoids was 0.1 ng in extracts from foods [b] Not detected.

substantial level of another dihydroxycarotenoid, lactucaxanthin (ε,ε-carotene-3,3′-diol) is present. The reduced concentration of lutein in lettuce in comparison to the other green vegetables has been partially attributed to the presence of lactucaxanthin (*35*). Lactucaxanthin is only found in a taxonomically restricted group of plants (*36*, *37*) and can replace lutein in the xanthophyll cycle of light harvesting complex II of the photosynthetic pathway (*35*). The nutritional value of lactucaxanthin is not

known at present, although this carotenoid has been identified in human serum and retina at low concentrations (*14*, *25*).

Among commonly consumed green vegetables, kale, parsley, spinach, and collards have the highest concentration of lutein and zeaxanthin (**Table 1**). The levels of zeaxanthin in green vegetables do not seem to be proportional to that of lutein; this is evident from the wide ranging ratios of these carotenoids as shown in **Table 1**. Certain yellow-orange fruits and vegetables such as corn, squash (butternut variety), oranges, and nectarine also serve as excellent sources of zeaxanthin. In most yellow-orange fruits and vegetables, the ratio of lutein to zeaxanthin is nearly 1 with the exception of corn and nectarine, which contain a higher concentration of zeaxanthin relative to lutein. Another exception is butternut squash in which the ratio of lutein to zeaxanthin is approximately 9. It must be noted that the green-harvested wheat, Freekeh, has a higher concentration of lutein and zeaxanthin relative to the North American bread wheats, Catoctin and Pioneer. The levels of lutein and zeaxanthin in wheat and pasta products are much lower than the levels in fruits and vegetables. The origin of lutein and zeaxanthin in pasta products is due to the presence of these carotenoids in egg yolk. The *Z*-isomers of lutein and zeaxanthin in raw fruits and vegetables presumably originate from photochemical isomerization of their *all-E*-isomers. In cooked and processed foods, these geometrical isomers are more likely to form from their *all-E* isomers as a result of heat treatment. The concentrations of *Z*-isomers of lutein and zeaxanthin in most fruits and vegetables appear to be proportional to the concentrations of their *all-E*-isomers (**Table 1**); this is also the case for wheat and pasta products (**Table 2**).

The abundance of lutein in commonly consumed fruits and vegetables is also reflected in the levels of this carotenoid in human serum (*7*, *13*, *14*) and ocular tissues (*17*, *18*, *25*, *38*). On the other hand, the dietary levels of zeaxanthin are much lower than that of lutein, and the range of concentration of zeaxanthin in fruits and vegetables is 8−50 μg/100 g of edible food (**Table 1**). The levels of lutein and zeaxanthin in human serum are largely dependent on dietary habits and carotenoid intake of individuals. However, in the absence of carotenoid supplementation, the ratio of lutein to zeaxanthin in the serum of healthy humans can vary in the range 2−12 (*7*, *13*, *14*, *38*). In human ocular tissues, the ratio of dietary (3*R*,3′*R*,6′*R*)-lutein to dietary (3*R*,3′*R*)-zeaxanthin is in the range 2−9 (*38*). It is interesting to note that in the macular region of the human eye, the ratio of lutein to zeaxanthin does not consistently reflect the ratio of these carotenoid found in foods and human serum. For example, it has been shown that the concentration of lutein is greater than that of zeaxanthin in the peripheral region of the macula, while zeaxanthin is more abundant in the central region (*39*−*41*). Meanwhile, a number of epidemiological studies in the early 1990s suggested a beneficial role for carotenoids, particularly lutein and zeaxanthin, and for antioxidant vitamins in the prevention of neovascular age-related macular degeneration (AMD) (*42*−*44*). The evidence for protection against AMD by carotenoids and antioxidant vitamins has been reviewed by Snodderly and Schalch (*45*, *46*). Therefore, on the basis of these observational and experimental studies, it appears that an increase in dietary intake of both lutein and zeaxanthin may turn out to be an effective strategy for the prevention of AMD.

In conclusion, the distribution of carotenoids and their related geometrical isomers in commonly consumed fruit and vegetables has important implications for the health of Western populations in addition to the basic nutritional needs of developing countries. The significance of lutein, zeaxanthin, and their *E*/*Z*-isomers is becoming increasingly evident in eye health and more specifically in relation to the prevention of AMD. Although the mechanism of uptake and accumulation of lutein and zeaxanthin in the eye is not known, a relationship between reduced plasma concentration of these carotenoids and an increase in the incidence of AMD has been established (*42*−*46*). Future studies should determine the optimum intake of lutein and zeaxanthin from foods and supplements that can effectively increase the levels of these carotenoids in human serum and ocular tissues. These studies will provide valuable information that can be used in clinical trials that investigate the efficacy of lutein and zeaxanthin in the prevention of AMD and other blinding disorders.

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Received for review October 28, 2002. Accepted December 20, 2002. J. M. Humphries acknowledges the support of a University of Adelaide Research Abroad Scholarship and a University of Adelaide Postgraduate Scholarship. F. Khachik acknowledges support from the Joint Institute for Food Safety and Applied Nutrition (JIFSAN), University of Maryland−Food and Drug Administration (UM−FDA).

JF026073E