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Lutein: A Valuable Ingredient of Fruit and Vegetables

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Lutein is a human serum carotenoid which is not synthesized by humans and thus must be obtained by the ingestion of food containing it such as fruits and vegetables. Lutein is present in different forms in those foods as all-trans-lutein, cis-lutein, epoxi-lutein, and lutein linked to proteins. It discusses if the intake of lutein or diets supplemented with lutein or diets rich in fruits and vegetables are important in the prevention of diseases like some cancers, cardiovascular diseases, etc., that may be affected by the antioxidant effect of lutein; or in the prevention of age-related macular degeneration and other eye diseases. The concentration of lutein in fruits and vegetables depends on the species. We've included the concentration of lutein in 74 species reported by different authors since 1990. Currently the quantification of lutein is mainly performed by HPLC, but more investigations into a quantification method for lutein, lutein isomers, and epoxi-lutein are necessary. Improvement of lutein extraction methods is important as well. Methods commonly used in the vegetable and fruit industry like heat treatment, storage conditions, etc. can change lutein concentrations; other factors depend on the plant, for instance the variety, the stage of maturity, etc.

Keywords lutein, health, fruit and vegetables concentration, quantification methods, lutein isomerization and degradation

INTRODUCTION

Lutein ((3*R*,3′*R*,6′*R*)β, ε-carotene-3-3′-diol) is a yellow carotenoid present in many commonly eaten fruits and vegetables. More than 600 carotenoids have been identified; lutein, β-carotene (β, β-carotene), violaxanthin (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β, β-carotene-3,3′-diol), and neoxanthin (5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β, β-carotene-3,5,3′-triol) are the major carotenoids accumulated in green leaves (Mercadante and Rodriguez-Amaya, 1991). They may be located in the chromoplast or chloroplast.

Due to the great importance of lutein to human health numerous investigations have been performed in recent years and numerous papers about them have been published. Some of those results have been reported in reviews about some specific points of lutein such as its health benefits, the presence of it and other carotenoids in some foods, etc. Some aspects of lutein that have been reviewed are: Bobrowska and Oledzka (2002) reviewed their natural occurrence in fruits and vegetables and in selected animal body tissues, the metabolism in animals and human and health aspects of dietary intake; however this particular review is in Polish. Astorg (1997) reviewed the food carotenoids and their relationship with cancer prevention. Handelman (2001) reviewed the role of carotenoids in human biochemistry, studying the role of lutein in eyes. Other reviews about the effects of lutein on eyes were written by Kinsky et al. (2003) and Mozaffariech et al. (2003). Johnson (2004) reviewed the biological role of lutein. The reports about lutein concentration in fruits and vegetables will be mentioned; most of them collected data about food frequently consumed in certain countries.

However a review about much of the information gathered about lutein has not been done recently. For this reason in this article I will comment on such aspects of lutein as: 1—occurrence of lutein in fruits and vegetables, 2—lutein extraction and quantification, 3—lutein concentrations in fruits and vegetables and factors that influence the concentration, 4—changes in lutein concentration during different types of fruit and vegetable industrial or home processing, 5—importance of lutein in human health and 6—other benefical effects of lutein. In general only papers after 1990 will be reported, since papers published before that are mentioned in them or can be found in reviews that focused on certain aspects of lutein.

OCCURRENCE OF LUTEIN IN FRUTIS AND VEGETABLES

This review is focussed on fruits and vegetables since the lutein is synthetised by them, whereas their presence in other food is due to their ingestion by animals.

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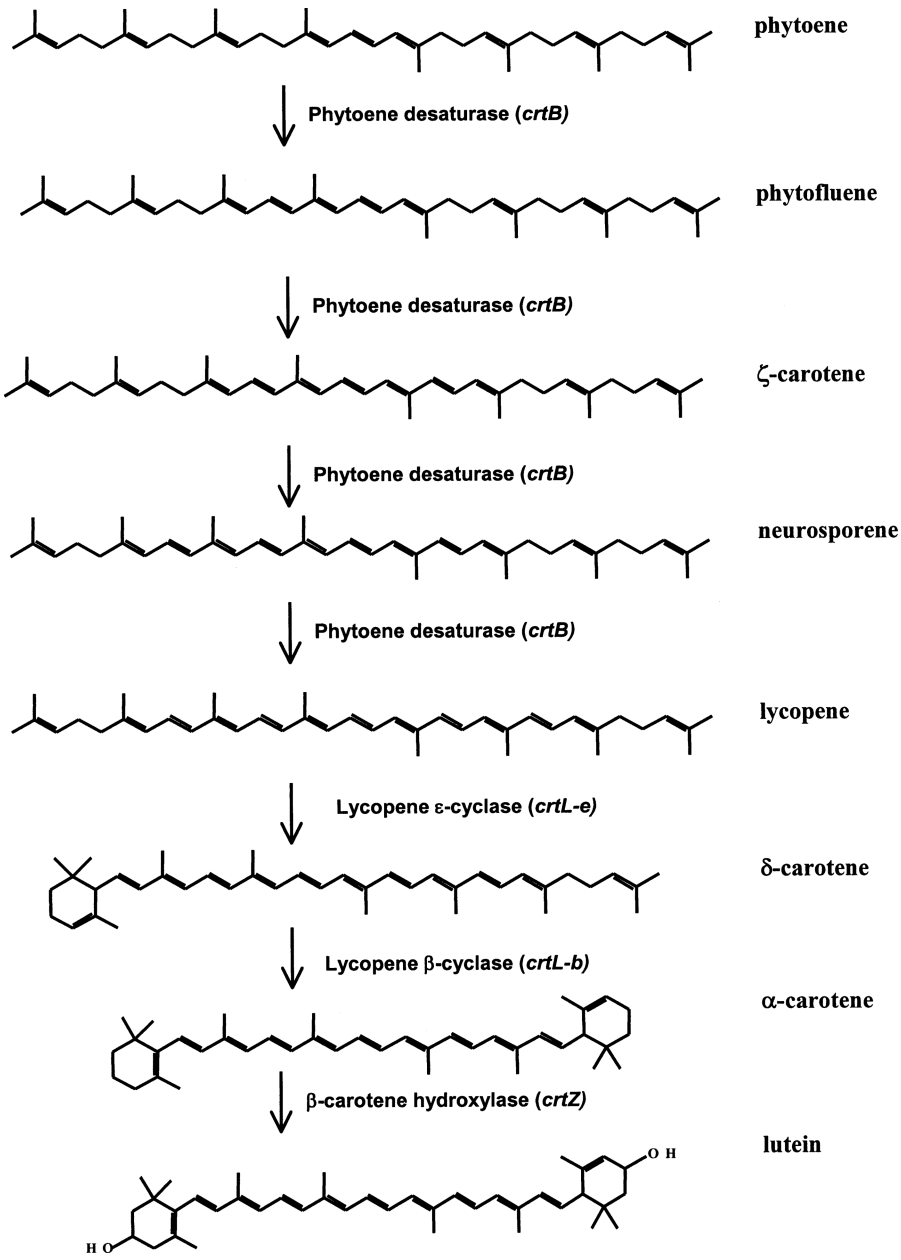


Figure 1 Biosysnthetic pathways for lutein production.

Forms of Lutein in Fruits and Vegetables

The biosynthetic pathway for lutein production is shown in Figure 1. This is important because there are researchers working to improve the lutein production of plants by the modification of genes responsible for lutein enzyme production.

Although it is generally accepted that vegetable carotenoids are in all-trans form, it is important to know if the isomers are present in fresh plants. Hyoungshin et al. (2002) reported the presence of a carotenoid isomerase in plants that catalyses the isomerization of poly-cis-carotenoids to all-trans-carotenoids.

It is known that all-trans-lutein is isomerised. The presence of geometrical cis-isomers 9-cis, 9′-cis, 13-cis and 13′-cis, the chemical structures shown in Figure 2, has been detected. Factors like light, temperature, etc. may induce all-trans-lutein isomerization during sample extraction and analysis; but taking into account those factors the presence of geometrical lutein isomers in fresh vegetables has been reported. Chen and Chen (1992) reported the presence of cis-lutein in fresh water convolvulus. Cano and Marin (1992) describe the presence of some isomers in fresh kiwi fruit extracts, and the same and others are found in canned fruit. Gandul-Rojas and Minguez-Mosquera (1996) reported the presence of cis-isomers in olive oil; what is still unknown is whether their presence is due to the treatment to obtain the oil or to the presence of those isomers in olives. Chen and Tang (1998) found 13-cis and 9-cis-lutein in carrot pulp waste.

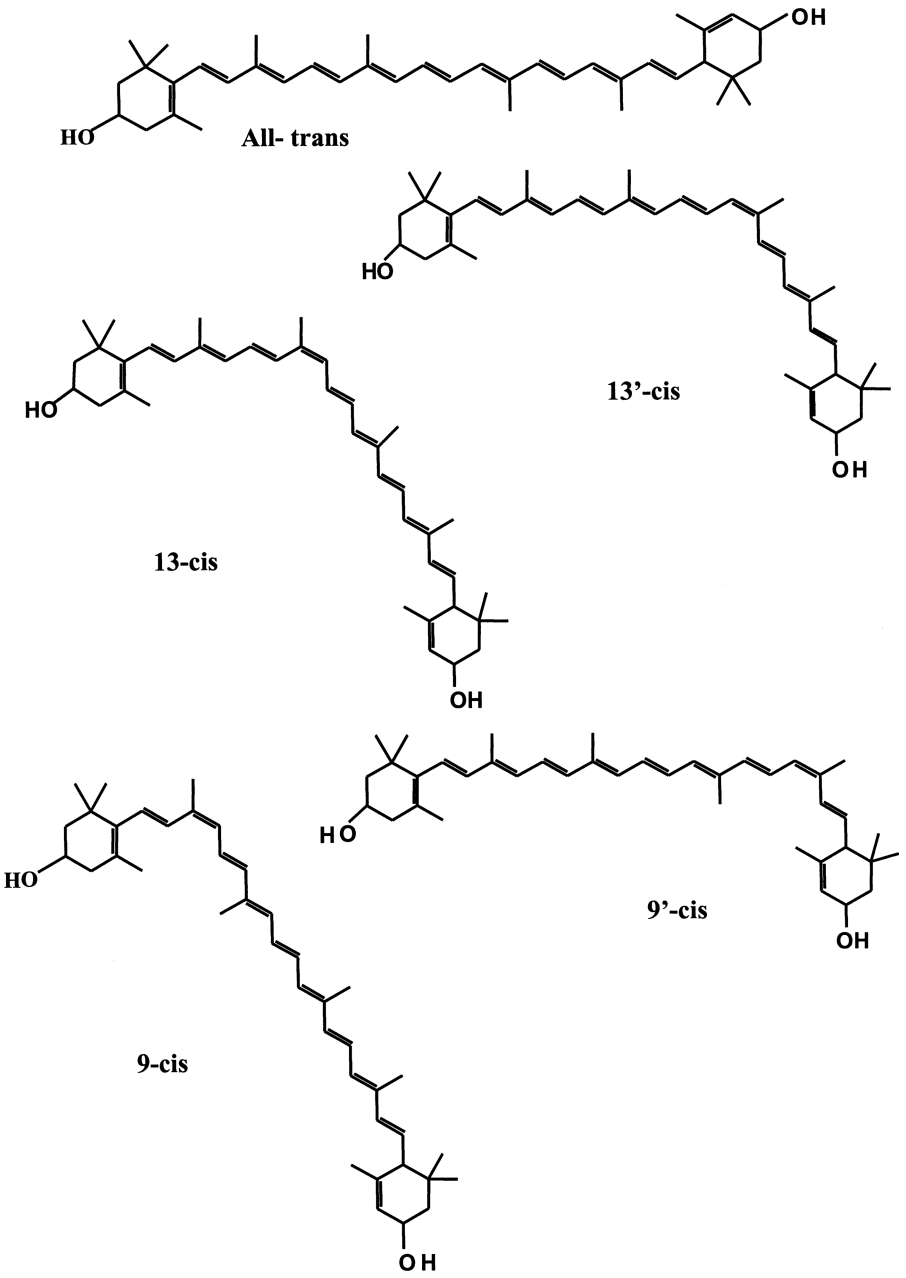


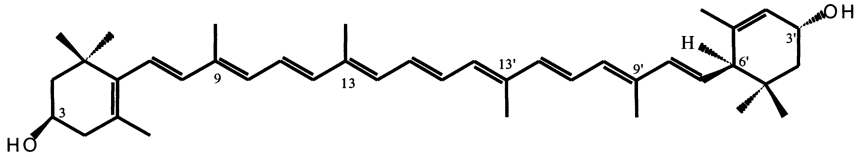
Figure 2 Chemical structure of lutein isomeric forms.

Monreal et al. (1999) found cis-isomers of lutein in fresh green beans. Humphries and Khachik (2003) analyzed some fruits and vegetables and found that in green and yellow-orange vegetables and fruits the concentration of the cis-isomer appears to be proportional to the concentration of their all-trans-isomer, with the all-trans-isomer predominating in most of them. Updike and Schwart (2003) reported the presence of the 13-cis-isomer of lutein in fresh broccoli, green peas, spinach, and sweet yellow corn but not in fresh kale.

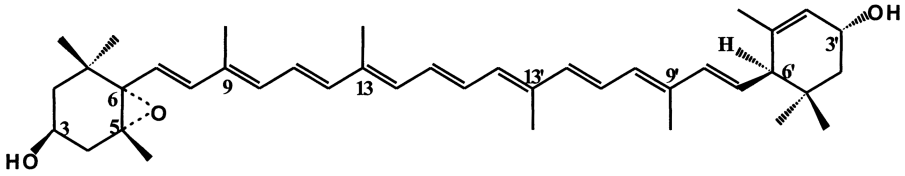
One possible explanation of the presence of cis-isomers in fruits and vegetables was postulated by O'Neil and Schwart (1995), who reported that the presence of cis-isomers in fresh vegetables likely results from chlorophyll derivatives acting as sensitizers that induce isomerization of all-trans-carotenoids to their respective cis form. However, the same authors found that although fresh kale tissues have chlorophyll, none contained cis-isomers or they were present only in very low quantities.

Investigation to determine whether there are differences between the all-trans and the cis-isomers of lutein regarding bioavailability, antioxidant properties, and other biological functions should be performed.

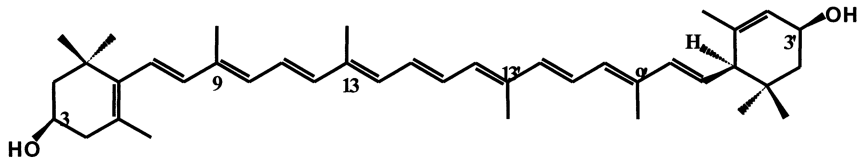
The presence of lutein epoxide (chemical structure shown in Figure 3) has also been reported in fresh vegetables: broccoli and spinach (Khachik et al., 1992b), sweet potato leaves (Chen



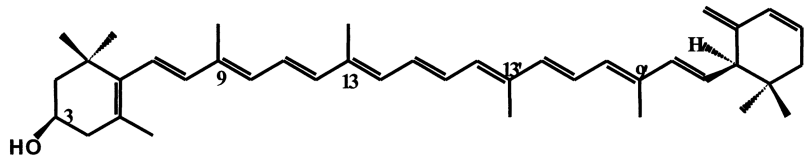
Lutein
(3*R*,3'*R*,6'*R*)-β,ε-carotene-3,3'-diol



Lutein epoxide
(3*S*,5*R*,6*S*,3'*R*,6'*R*)-5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol



3'-Epilutein
(3*R*,3'*S*,6*R*)-β,ε-carotene-3,3'-diol



Anhydrolutein I
(3*R*,6'*R*)-3-hydroxy-3,4-didehydro-β,γ-carotene

Figure 3 Chemical structure of lutein, lutein epoxide, 3′-epilutein and anhydrolutein I.

and Chen, 1993), chinese white cabbage (Wills and Rangga, 1996), olives (Gandul-Rojas et al., 1999), green peas (Edelenbos et al., 2001), tea (Ravichandra, 2002), etc. It is necessary to study their bioavailability and their possible influence on human health.

Because lutein bears one hydroxyl group on each ionone ring, it can be esterified with fatty acids in plant cells, resulting in mono- and di-acylated derivatives. Usually it is esterified with long chain fatty esters. Although not common in every food plant, small amounts of lutein diesters occur in the carotenoid fraction of several fruits and vegetables. Breithaupt et al. (2002), using liquid chromatography-mass spectrometry, described the following lutein-diesters: 1—in cape gooseberries (*Physalis peruviana*) dimyristol-lutein, myristolpalmitol-lutein and dipalmitol- lutein; 2—in kiwano (*Cucumis metuliferus*) dilauroyl-lutein, lauroylmyristol-lutein, dimyristol-lutein and myristolpalmitol-lutein and 3—in pumpkin (*Cucurbita pepo*) dimyristol-lutein, myristolpalmitol-lutein and dipalmitol-lutein.

There are few studies about the lutein esters present in fruits and vegetables. This could be because studies of lutein concentration usually also attempt to determine the presence of other carotenoids using the saponification method during sample preparation. This caused the hydrolyzation of all the ester links resulting in the removal of triglycerides, phospholipids, and

other fatty acid ester enlaces with alcohols. Although some reports about the lutein-epoxide cycle in some higher-plant species have been described (Foster et al., 2003), it is necessary to have a better insight into the biochemical reactions leading to carotenoid ester formation in plants, as well as knowing the enzymes responsible for lutein acylation and the consequences to the plant cell. A better knowledge of lutein esters present in fruit and vegetables would also help.

The study of lutein-epoxide and lutein esters may be of interest because they could have beneficial effects on human health. Thus more studies about their bioavailability and their effects on human health are of interest. Some studies have been performed; for example, it has been noted that once lutein is isolated from the plant, it is biologically active in either the ester or the free form (Clark and Bowen, 1998).

It has been reported that some carotenoids can be bound to proteins. Some proteins have been reported to contain carotenoids other than lutein, for example, Dietz Bryant et al. (1992) isolated a 54 kDa caroprotein containing α and β-carotene and Moros et al. (2002) reported that corn xanthophylls can be bound to a protein, probably zein. It is possible that those carotenoproteins contain lutein but in a low concentration, since Miluca et al. (1991) reported one protein-carotenoid complex containing mainly phytoene and small amounts of β- and α-carotene, lutein and lutein epoxide; also a 18 kDa protein containing lutein and other carotenoids such as α-carotene, β-carotene and in minor concentrations phytoene and ξ-carotene have been found by Zhou et al. (1994).

It has been proposed that carotenoproteins may increase the bioavailability of carotenoids, which would seem to make them quite important; however, the low concentration of carotenoids attached to proteins could indicate that this influence is low (Zhou et al., 1994). More studies about the linkage of lutein to proteins in vegetables and fruits and their influence on the bioavailability of lutein should be performed.

Determination of Lutein

Lutein extraction and quantification will be reviewed.

In general the vegetables or fruits analyzed are fresh or processed products (Koning and Roomans, 1997; Murkovic et al., 2002; Updike and Schwartz, 2003) that are treated immediately to avoid isomerization or degradation; when the sample was to be analzyed after a period more or less long it was lyophilized or frozen until extraction to avoid changes in lutein concentration.

Due to possible isomerization and/or degradation during the extraction and analysis process some authors described the methods used to avoid undesirable lutein changes. In some cases di *tert*-butil-methylphenol is added to the solvents to avoid carotenoid changes during the extraction. Most of the authors recommend the use of nitrogen atmosphere. Since another factor than can affect lutein is light, working under subdued or ultraviolet filtered light has been proposed (Granado et al., 1992; Updike and Schwartz, 2003). Working at low temperatures like 0° C (Khachik et al., 1992b) has also been proposed.

Different solvents such as acetone (Heinonen, 1990; Monreal et al., 1999; Updike and Schwartz, 2003), acetone + diethyl ether (Wills and Rangga, 1996; Ravichandra, 2002), or + hexane (Tang and Chen, 2000), or + petroleum ether (Lopez-Hernandez et al., 1996; De la Cruz-Garcia et al., 1997), or + a mixture of solvents such as hexane + ethanol + toluene (Mercadante and Rodriguez-Amaya, 1991; Yen and Chen, 1995); and other solvents like tetrahydrofuran (Granado et al., 1992), tetrahydrofuran + methanol (Ben-Amotz and Fisher, 1998), hexane (Guedes de Pinho et al., 2001), etc. are used in the extraction of lutein and other carotenoids.

The use of two different solvents sometimes can give similar results. Lopez-Hernandez et al. (1993) compared in green beans extraction with tethrahydrofurane stabilized with butylhydroxytoluene, and a 50/50 mixture of acetone/petroleum ether, observing that the extraction of lutein and β-carotene was similar using the two methods.

The election of solvent sometimes depends on the carotenoids and other compounds that are to be extracted like the chlorophylls and other lyposoluble compounds present in the matrix; if we want only to quantify lutein to discover the solubility, stability, and absorbtivity of this xanthophyll in different organic solvents the work of Craft and Soares (1992) can be consulted.

The relationship between sample/solvent is also variable. In general the authors specified that the extraction was realized more than one time until no colour was detected in the sample. This point is important because an incomplete extraction will give erroneous results. On the other hand as I will explain later some processes such as heating might or might not improve the breaking of cellular and chloroplast or chromoplast membranes.

There are also different ways to mix the sample with the solvent while trying to extract all the lutein. It must be taken into account that the lutein is in chloroplasts or chromoplasts that must be broken to obtain the maximal extraction. The extraction methods described are: the simple stirring of the sample with the dissolvent on a magnetic stirrer (Granado et al., 1992; Guedes de Pinho et al., 2001), the use of a homogenizator (Cano and Marin, 1992), an ultra-turax (Huck et al., 2000), a Waring blender (Khachik et al., 1992b) a Polytron blender (McGhie and Ainge, 2002) or another homogenizing system like ultrasonic (Edelenbos et al., 2001). The boiling of the extract with the matrix to be analyzed has also been reported (Moros et al., 2002).

Sometimes methods such as centrifugation are used to separate the lipid extract from the plant debris after the extraction (Koning and Roomans, 1997; Ben-Amotz and Fishler, 1998). Some compounds like the polypropylene of the tubes used in the centrifugation of serum for lutein determination could produce interference in HPLC quantification when electrochemical detection is used (Yen and Hsu, 2004).

Saponification is considered the best means of removing chlorophylls and degradation products, undesired lipids, and other interfering substances, and to hydrolyse carotenoid esters. Although this step may be carried out in the matrix to be

extracted, it is frequently performed after organic extraction. Depending on the conditions used (hydrolysis time, temperature, potassium hydroxide concentration, number and volume for partition, and washing, the particular carotenoid), saponification may produce destruction or structural transformation, giving an inaccurate reading of the total carotenoid concentration. Kimura et al. (1990) reported that lutein is degraded significantly when lutein extraction from kale is performed followed by saponification, also reporting that the losses could be reduced to insignificant levels by the use of a nitrogen atmosphere or an antioxidant such as pyrogallol.

Sometimes saponification is not necessary. This is the case when the chromatographic method separates the chorophylls from the carotenoids of interest, as is indicated by De Sio et al. (2001). According to this author, using a C_{30} column can separate the chlorophylls from the carotenoids in some vegetables; or when the matrix contains low amounts of chlorophylls or other compounds that are not eluted at the same time as lutein or the other carotenoids to be studied.

Granado et al. (2001) has proposed a fast, reliable, and low-cost saponification protocol for analysis of carotenoids in vegetables. The protocol also increases carotenoid recovery. Another protocol was described previously by Hart and Scott (1995) to obtain the maximum concentration of lutein and other carotenoids.

Some methods used to quantified lutein are:

1. The measurement of total absorption at maximum wavelength absorption, taking into account the extinction coefficient of this particular carotenoid in a particular solvent. This method sometimes does not allow the quantification of lutein, since other carotenoids like β-carotene have a similar maximum of absorbance, and as has been indicated, usually lutein is extracted with other carotenoids like β-carotene.
   To avoid the interference of other carotenoids, quantification can be performed by open column chromatography followed by spectrophotometric quantification.
2. Other methods which have been proposed are the colorimetric methods. One of those is the tristimulus colorimetry; this method applied to orange fruit allowed Melendez-Martinez et al. (2003a) to establish a significant correlation ($P < 0.05$) between tristimulus parameters and the content of β-cryptoxanthin, lutein + zeaxanthin and β-carotene The authors showed that it is possible to obtain equations, by means of multiple regression models, which allow the determination of the individual carotenoid levels from the color parameters.
3. HPLC is the most commonly used method to quantify lutein and other carotenoids. HPLC determinations have been performed using different eluyents, column phases and detectors. However the enormous range of carotenoid content reported in fruits and vegetables probably reflects the wide variability from both methodological variations and food-related factors. As can be observed in Table 1 numerous stationary and mobile phases have been used. The use of an isocratic or gradient elution process is not indicated in the table. Huck et al. (2000) studied different eluyents proposed by other authors to obtain a good separation of β-carotene, lutein, zeaxanthin and β-criptoxanthin; using a C18 column and working at low temperatures; however although they obtain good results they did not separate the isomers of those compounds, which could be important in human nutrition.

Some of the methods allow the separation of different carotenoids and their isomers. For example a good resolution of 13-cis-lutein, 13′-cis-lutein, all-trans-lutein and 9′-cis-lutein was reported by Guil-Guerrero et al. (2003) using a C_{30} column; separation of those isomers and 9-cis-lutein was reported by Updike and Schwartz (2003).

One of the used detectors is a diodo-array detector which allows possible identification of these compounds based on the maximum absorption peaks.

Scott et al. (1996) later did an inter-laboratory study and found a large discrepancy among results from the different laboratories, suggesting that the effect of chromatographic system and standardization of carotenoid solutions are probably not major variables, whereas the preparation of carotenoid extract may account for more than half of the total variance; these results should be taken into account to improve the extraction techniques for each matrix.

Lutein Levels in Vegetables and Fruits

Due to interest in the carotenoid content of foods and their repercussion in human health preservation, Mangels et al. (1993) elaborated a database where the authors compiled the concentration of β-carotene, α-carotene, lutein+zeaxanthin, lycopene, and β-cryptoxanthin of different foods, taking into account the results of 180 articles published from 1971 to 1991. The Mangels et al. database was reviewed by Holden et al. in 1999; however, in their revision the authors showed interest only in the reports of U.S. carotenoid references. They did not include 51 reports on data from other countries; on the other hand, they included the lutein and zeaxanthin concentrations together, maintaining the criteria of Mangels et al. (1993), even though there are methods to quantify the two compounds.

Pelz et al. (1998) reported carotenoid database values of some carotenoids to evaluate their intake by the German population. Murkovic et al. (2000) established an Austrian carotenoid database, analysing samples of 31 types of vegetables.

I collected data of lutein concentration in 74 species of fruits and vegetables from all over the world reported by 44 authors since 1990 (see Table 2). In some cases concentrations of lutein and violaxanhin are reported together. In most of the cases the samples were purchased in markets, and if they were submitted to different treatments those were performed in the laboratory; there are also data of samples from cultivars, from restaurants and from hydroponic cultivars. The data are of fresh or treated samples, with the type of treatment indicated in each case; I did

Table 1 HPLC columns and eluyents used in lutein and other carotenoids determination

Column	Eluyents	Eluted carotenoids	Source
Vydac 201 TP54 (250 × 4.6 mm), 5 μm (Hesperia, CA)	Methanol/methylene chloride (44/65)	Lutein (some isomers), α-carotene (some isomers),β-carotene (some isomers)	Chen et al., 1995
Vydac 201 TP, 5 μm (Vydac, Hesperia, CA, USA)	Methanol/tetrahydrofuran (95/5)	Lutein, zeaxanthin, α-carotene, β-carotene, lycopene	Koning and Roomans, 1997
Vydac 201TP54 (250 × 4.6 mm) (Photochem, Wesel, Germany)	Methanol/acetonitrile/2-propanol (54/44/2)	Lutein, zeaxanthin	Hentschel et al., 2002
Vydac 201 TP54 (250 × 4.6 mm), 5 μm (RP-18, 300A)	Methanol/acetonitrile/dichlorometane (85/10/5))	Lutein, zeaxantin, α-carotene, β-carotene, lycopene	Majchrzak et al., 2000
Zorbax ODS (25 × 0.46 cm i.d.), 5–6 μm (Du Pont)	Acetonitrile/dichloromethane/methanol (70/20/10)	α-carotene, β-carotene, γ-carotene, lutenin	Heinonen, 1990
Spheri-5-ODS (220 × 4.6 mm), 5 μm (Brownlee Labs, Kontron Analytic)	Acetonitrile/methanol (85/15)	Lutein, zeaxanthin, β-cryptoxanthin, lycopene, β-carotene (some isomers)	Granado et al., 1992
Hypersil ODS (10 cm × 4.4 mm i.d.), 5 μm (Hewlett-Packard)	Methanol, water, ethyl acetate	Violaxanthin, neoxanthin, lutein (some isomers, epoxide), anteraxanhin (some isomers), auroxanhin (some isomers)	Cano and Marin, 1992
Spherisorb ODS2 (250 × 4.6 mm), 5 μm (Merck, Germany)	Ethyl acetate, acetonitrile, water	Neoxanthin, violaxanthin, luteoxanthin, zeaxanthin, lutein, β-carotene	Guedes de Pinho et al., 2001
201 TP54 (250 × 4.6 mm), 5 μm (Hepseria, CA, USA)	Acetonitrile, methanol, dichloromethane, triethylamine	Lutein+β-cryptoxanthin, lycopene, α-carotene, β-carotene	Murkovic et al., 2002
PontoSil 200 silica gel and YMC silica gel (250 × 4.6 mm)	Acetone/water (85/15)	Lutein (some isomers), zeaxanthin (some isomers)	Dachtler et al., 1998
Silica-based nitrile bonded (25 cm × 4.6 mm i.d.), 5 μm	Hexane/dichloromethane/methanol/ *N*,*N*-diisopropylethulamine (75/25/0.3/0.1)	Lutein (some isomers), zeaxanthin (some isomers), violaxanthin.	Humphries and Khachik, 2003
HS-5-Silica column (4 × 125 mm) (Perkin-Elmer)	Heptane modified with 2-propanol (2 ml/l)	β-carotene, lutein	Grela et al., 1999
LiChrospher-Si (250 × 4 mm i.d.), 5 μm (MZ Analysentechnic, Mainz, Germany)	Hexane, 2-propanol	Lutein, β-carotene	Psomiadou and Tsimidou, 2001
LiChrospher 100 RP-18 (244 × 4 mm i.d.), 5 μm (Merk Kebo Lab, Denmark)	Methanol, water, ethyl acetate	Neoxanthin (some isomers), violaxanthin, lutein-epoxide, lutein (some isomers)	Edelenbos et al., 2001
C_{18} Bondapack (300 × 3.9 i.d. mm), 10 μm	Acetonitrile/metanol/ethyl acetate (88/10/2)	Lutein, cryptoxanthin, lycopene, γ-carotene, α-carotene, β-carotene	Tee and Lim, 1991
Microsorb C_{18} (25 cm × 4.6 mm i.d.), 5 μm (Raining Instrument, Co.)	Acetonitrile/methanol/dichloromethane/ hexane (85/10/2.5/2.5)	Neoxanthin (some isomers), violaxanthin, lutein (some isomers, lycopene (some isomers), neurosporene, β-carotene (some isomers), α-carotene (some isomers), phytofluene (some isomers), phytoene (some isomers)	Khachik et al., 1992a
C_{18} Spherisorb ODS2 (250 × 4.6 mm), 2.5 μm (Sugelabor, Spain)	Methanol, acetonitrile, dichloromethane, hexane	β-Carotene, lutein	Lopez-Hernandez et al., 1993
C_{18} Ultremex (25 cm × 4.6 mm i.d.), 5 μm (Torrance, CA)	Acetonitrile/methanol/chloroform/ hexane (75/12.5/7.5/7.5)	Violaxanthin, violexanthin, lutein (epoxides and isomers), β-carotene (some isomers)	Chen and Chen, 1993
C_{18} Vidac TP201 (Hesperia, CA)	Methanol/acetonitrile/water (88/9/3)	Lutein	Zhou et al., 1994
Phenomenex C_{18}, ultremex, 5 μm (Torrance, CA, USA)	Acetonitrile/metanol/ethyl acetate (80/10/10)	Sinesiaxanthin, neoxanthin, violaxanthin (some isomer and epimers), violexanthin (some epimers), lutein (epoxide), β-cryptoxanthin	Yen and Chen, 1995
C_{18} Spherisorb ODS-2 (25 × 0.4 cm i.d.), 5 μm (Teknokroma, Barcelona, Spain)	Water, tretrabulammonium acetate, methanol, acetone	Neoxanthin (some isomers), violaxanthin, luteoxanthin, antheraxanthin (some isomers), mutatoxanthin, lutein (some isomers), β-cryptoxanthin	Gandul-Rojas and Minguez-Mosquera, 1996

(Continued on next page)

Table 1 HPLC columns and eluyents used in lutein and other carotenoids determination *(Continued)*

Column	Eluyents	Eluted carotenoids	Source
C_{18} Novapak, 5 μm (Waters, Milford, USA)	Acetonitrile, methanol, water	β-Carotene, ε-carotene, lycopene, β-cryptoxanthin, zeaxanthin, lutein, antheraxanthin, mutatoxanthin, flavoxanthin, auroxanthin, luteoxanthin, violaxanthin, neoxanthin	Wills and Rangga, 1996
C_{18} Spherisorb ODS2 (250 × 4.6 mm i.d.), 5 μm (Sugelabor, Spain)	Methanol, acetonitrile, dichloromethane, hexane	Lutein, β-carotene	De la Cruz-Garcia et al., 1997
C_{18} Vydac 201 TP54 (250 × 4.6 i.d. mm), 5 μm (Hysperia, CA)	Methanol/acetonitrile (9/1)	Neoxanthin, violaxanthin (and cis), lutein, zeaxanthin (and cis), α-cryptoxanthin, β-cryptoxanthin (and cis), α-carotene, β-carotene (some isomers), lycopene	Ben-Amotz and Fishler, 1998
C_{18} Hypersil ODS (25 cm × 4.6 mm i.d.), 5 μm (Hewlett-Packard)	Methanol, water, ethyl acetate	Lutein (some isoemers), violaxanthin, luteoxantin, auroxanthin, flavoxanthin, antheraxanthin (some isomers), neoxanthin (some isomers)	Monreal et al., 1999
C_{18} Ultrasphere (250 × 4.6 mm i.d.), 5 μm (Beckman)	Dichloromethane/methanol/acetonitrile/ water (5/85/5.5/4.5)	Astaxanthin (some isomers and esters), lutein, canthaxanthin, β-carotene	Yuang and Chen, 1999
C_{18} Lichrosorb (46 × 250 mm) 6 μm	Acetonitrile/2-propanol/methanol/water (39/52/5/4)	Lutein, β-carotene, lycopene (some isomers, epoxi)	Abushita et al., 2000
C_{18} Vydac 201TP54	Methanol/methylene chloride (99/1)	Lutein, α-carotene, β-carotene and their isomers	Tang and Chen, 2000
C_{18} Sperhisorb S3 ODS2 (4.6 × 150 mm), 2.3 μm	Acetonitrile, methanol, ethyl acetate, triethylamine	Neoxanthin, violaxanthin, lactucaxanthin, lutein, β-carotene	Kimura and Rodriguez-Amaya, 2003
C_{18} Kromasil (250 × 4.6 mm), 5 μm (Hichrom Ltd., Reading, UK)	Methanol, acetonitrile, methylene chloride, water, butylated hydroxytoluene, triethylamine	Neoxanthin, violaxanthin, anteraxanhin, lutein+zeaxanthin, lutein epoxide, α-cryptoxanthin, β-cryptoxanthin, α-carotene, β-carotene	Melendez-Martínez et al., 2003b
C_{18} Vydac 218TP54 (250 × 4.6 mm), 5 μm	Methanol	Lutein, zeioxanthin, β-cryptoxanthin (some isomers), α-carotene, β-carotene (some isomers), phytoene+phytofluene,	Hamano and Mercante, 2001
C_{18} Sphrisorb ODS (4.6 × 150 mm), 2.3 μm	Acetonitrile, methanol, ethyl acetate	Neoxanhin, violaxanthin, lutein, β-carotene	Sá and Rodriguez-Amaya, 2003
C_{30} RPLC (4.6 i.d. × 250 mm), 5 μm (Gaithersburg, MD)	Methyl *tert*-butyl ether, methanol	β-Carotene (some isomers), α-carotene (some isomers), lutein (tentatively some isomers)	Emenhiser et al., 1996
C_{30} (4.6 i.d. × 250 mm), 3 μm (NIST; Gaithersburg, MD)	Methyl *tert*-butyl ether, methanol	β-Carotene (some isomers), lycopene, lutein	Henry et al., 1998
C_{30}YMC (250 × 4.6 mm) (YMC, Schermbeck, Germany)	Methyl *tert*-butyl ether, methanol	Lutein, zeaxanthin	Hentschel et al., 2002
C_{30} (4.6 × 250 mm), 5 μm (YMC/Waters Inc., Wilmington, NC)	Methyl *tert*-butyl ether, methanol, water	Lutein, zeaxanthin, β-cryptoxanthin	Moros et al, 2002
C_{30} (4.6 × 250 mm), 5 μm	Methanol/methyl-*ter*-butyl ether (50/50)	Neoxantin, violaxantin, lutein (some isomers), zeaxantin, cryptoxantin, β-carotene (some isomers), lycopene (some isomers)	Guil-Guerrero et al., 2003
C_{30} (4.6 mm i.d. × 250 mm), 3 μm (YMC/Waters)	Methanol containing 2% (v/v) 1 M ammonium acetate/methyl *tert*-butyl ether (85/15)	Lutein (some isomers), zeaxanthin (some isomers)	Updike and Schwartz, 2003

not introduce data about cooked dishes since their composition and technological treatment is variable. Where a range of values is indicated, the different values may be due to data from different cultivars, or data from the same product purchased in different markets, or products collected at different times of the year, etc.

Some papers mentioned the presence of lutein but not its concentration, so this data are not included in the table; for example, Yen and Chen (1995) reported its presence in orange peels, Mercadante and Rodríguez-Amaya (1997) reported a low concentration in mangos, Tenorio et al. (2004) found it in asparagus.

As can be observed in the table the concentration reported by different authors sometimes differs considerably. This could be due to some of the factors mentioned above or to variabilities in the quantification process.

Table 2 Lutein composition (mg/100 g wet basis) of vegetables and fruits fresh or submitted to different treatments

Material	Treatment	Concentration	Sources
Apple (*Malus coronaria*)	Raw[1]	0.084	Hart and Scott, 1995
Appricot (*Prunus presica*)	Dried[1]	0.044	Hart and Scott, 1995
	Fresh[1]	0.101	
	Freeze-dried[1]	0.01[b]	Ben-Amotz and Fishler, 1998
Artichoke (*Cynara acolymus*)	Fresh[1]	0.16	Granado et al., 1992
	Boiled (30 min)[1]	0.28	
Asparagus (green) (*Asparragus officinallis*)	Fresh[1]	0.61	Granado et al., 1992
	Boiled (25 min)[1]	0.74	
	Fresh[1]	0.025	Müller, 1997
Banana (*Musa sapientum*)	Fresh[1]	0.033	Hart and Scott, 1995
	Freeze-dried[1]	0.04[b]	Ben-Amotz and Fishler, 1998
Bean (*Phaseolus vulgaricus*)	French. Fresh[1]	0.41	Tee and Lim, 1991
	French. Fresh[1]	0.47	Hart and Scott, 1995
	Cooked[1]	0.58	
	French. Fresh[1]	0.76	Müller, 1997
	Fresh[1]	0.36	Granado et al., 1992
	Boiled[1] (35 min)	0.49	
	Fresh[1]	0.59–0.69	Khachik et al., 1992b
	Microwaved[1] (4 min)	0.61	
	Boiled[1] (9 min)	0.71	
	Fresh[1]	0.42–0.65	Lopez-Hernandez et al., 1993
	Fresh[1]	0.62–0.03	Monreal et al., 1999
	Green. Fresh[1]	0.41	Humphries and Khachik, 2003
	Green. Fresh[1]	0.49	Hart and Scott, 1995
	Cooked	0.55	
	Boiled (10–20 min)[2]	0.02–0.03	Sá and Rodriguez-Amaya, 2003
	Stir-fried (10–30 min)[2]	0.03–0.04	
	Broad. Fresh[1]	0.50	Hart and Scott, 1995
	Cooked[1]	0.62	
	Runner. Fresh[1]	0.56	Hart and Scott, 1995
	Cooked[1]	0.63	
Bean long (*Vigna sinensis*)	Fresh[1]	0.41	Tee and Lim, 1991
Bean lima (*Phaselus lunatus*)	Canned[1]	0.36	Humphries and Khachik, 2003
Beet (*Beta vulgaris*)	Fresh[1]	1.50	Granado et al., 1992
	Boiled[1] (35 min)	1.96	
Broccoli *(Brassica oleracea*, variety *Botrytis)*	Fresh[1]	2.83	Khachik et al., 1992b
	Steamed[1] (5 min)	3.25	
	Microwaved[1] (5 min)	3.28	
	Fresh[1]	1.61	Hart and Scott, 1995
	Cooked[1]	1.95	
	Fresh[1]	2.36	Huck et al., 2000
	Fresh[1]	1.51	Humphries and Khachik, 2003
	Fresh[1]	8.36[b]	Updike and Schwartz, 2003
	Boiled [2] (5–15 min)	0.31–0.35	Sá and Rodriguez-Amaya, 2003
	Stir-fried[2] (5.20 min)	0.27–0.37	
Brussel aprout (*Brassica oleracea*)	Fresh[1]	0.18	Granado et al., 1992
	Boiled[1] (25 min)	0.47	
	Frozen. Raw[1]	0.61	Hart and Scott, 1995
	Frozen. Cooked[1]	0.62	
Cabbage (*Brassica oleracea*)	Red. Fresh[1]	0.08	Granado et al., 1992
	Boiled[1] (38 min)	0.23	
	Red. Fresh[1]	0.15	Müller, 1997
	Savoy. Fresh[1]	0.10	Hart and Scott, 1995
	Cooked[1]	0.34	
	White. Fresh[1]	0.08	Müller, 1997
	Fresh[1]	0.060	Granado et al., 1992
	Boiled[1] (25 min)	0.09	
	Fresh[1]	0.08	Hart and Scott, 1995
	Cooked[1]	0.11	
	Freeze-dried[1](leaves)	0.05[b]	Ben-Amotz and Fishler, 1998

(Continued on next page)

Table 2 Lutein composition (mg/100 g wet basis) of vegetables and fruits fresh or submitted to different treatments *(Continued)*

Material	Treatment	Concentration	Sources
Cashew (*Anacardium occidentale*)	Fresh[1]	0.77	Tee and Lim, 1991
	Frozen pulp[1]	0.004–0.004	Assuncao and Mercadante, 2003
	Concentrated juice[1]	0.0002	
	Ready to drink beverage[1]	0.0006	
Caja (*Spondias lutea*)	Pulp. Fresh[1]	0.20	Rodriguez-Amaya and Kimura, 1989
	Pulp. Frozen[1]	0.62	Hamano and Mercante, 2001
	Juice. Pasteurized[1]	0.35	
Carob been (*Ceratonia siligua*)	Freeze-dried[1]	0.02[b]	Ben-Amotz and Fishler, 1998
Carrot (*Daucus carota*)	Fresh[1]	0.11–0.56	Heinonen, 1990
	Jucice. Fresh[1]	0.29[c]	Granado et al., 1992
	Juice. Boiled[1] (33 min)	0.27[c]	
	Fresh[1]	0.6	Chen et al., 1995
	Fresh[1]	0.17–0.28	Hart and Scott, 1995
	Cooked[1]	0.15–0-31	
	Frozen. Raw[1]	0.27	
	Frozen. Cooked[1]	0.30	
	Fresh[1]	0.30	Konings and Roomans, 1997
	Fresh[1]	0.10–0.56	Müller, 1997
	Freeze-dried[1]	0.78	Ben-Amotz and Fishler, 1998
	Fresh[1]	0.28	Huck et al., 2000
	Freeze-dried[1]	0.72	Tang and Chen, 2000
Cassava (*Manihot esculenta*)	Fresh. Leaves[3]	29–86	Adewusi and Bradbury, 1993
	Tubers[3]	0.005–0.6	
Cauliflower (*Brassica oleracea*)	Fresh[1]	0.004	Granado et al., 1992
	Boiled[1] (30 min)	0.015	
	Fresh[1]	Trace	Hart and Scott, 1995
	Cooked[1]	Trace	
	Fresh[1]	0.015	Müller, 1997
Celery (*Apium graveolens*)	Fresh[1]	0.00	Huck et al., 2000
Ceylon spinach (*Brasella rubra*)	Fresh[1]	1.30	Tee and Lim, 1991
Cherry (*Prunus avium*)	Freeze-dried[1]	0.01[b]	Ben-Amotz and Fishler, 1998
Chicory (*Cichorium intybus*)	Fresh[1]	5.70	Kimura and Rodriguez-Amaya, 2003
Chinese chives (*Allium tuberosum*)	Fresh[1]	1.08	Tee and Lim, 1991
	Fresh[1]	0.0	Wills and Rangga, 1996
Chinese kale (*Brassica alboglabra*)	Fresh [1]	1.54	Tee and Lim, 1991
Chinese spinach (Amaranthus tricolor)	Fresh[1]	2.9	Wills and Rangga, 1996
Chinese white cabbage (*Brassica chinensis*)	Fresh[1]	0.96	Tee and Lim, 1991
	Fresh[1]	2.1	Wills and Rangga, 1996
Chinese flowering cabbage (*Brassica parachinensis*)	Fresh[1]	2.7	Wills and Rangga, 1996
Chinese cabagge (*Brassica pekinensis*)	Fresh[1]	2.7	Wills and Rangga, 1996
Chinese mustard (*Brassica juncea*)	Fresh[1] . Leaves	1.01	Tee and Lim, 1991
Collard greens *(Brassica oleracea)*	Fresh[1]	5,12	Humphries and Khachik, 2003
Coriander (*Coriandrum sativum*)	Fresh[1]. Leaves	1.33	Tee and Lim, 1991
Corn (*Zea mays*)	Canned[1]	0.199	Konings and Roomans, 1997
	Sweet. Frozen. Raw[1]	0.52	
	Sweet Frozen. Raw[1]	0.44	Hart and Scott, 1995
	Freeze-dried[1]	0.43	Ben-Amotz and Fishler, 1998
	Fresh[1]	1.47	Moros et al., 2002
	Canned[1]	0.20	Humphries and Khachik, 2003
	Fresh[1]	1.2[b]	Updike and Schwartz, 2003
	Canned[1]	1.2[b]	
Cucumber (*Cucumis sativus*)	Fresh[1]	0.16	Granado et al., 1992
	Freeze-dried[1]	0.05[b]	Ben-Amotz and Fishler, 1998
Curry (*Murray koenigii*)	Fresh[1]. Leaves	5.25	Tee and Lim, 1991
Dill (*Anethum graveolens*)	Freeze-dried[3]	3.41	Ben-Amotz and Fishler, 1998
Endive (*Cichorium endiva*)	Fresh[1]	2.08	Müller, 1997
	Stir-fried[1]	0.23	Sá and Rodriguez-Amaya, 2003
Eggplant (*Solanum melongena* variety *esculenta*)	Freeze-dried[1]	0.96	Ben-Amotz and Fishler, 1998

Table 2 Lutein composition (mg/100 g wet basis) of vegetables and fruits fresh or submitted to different treatments *(Continued)*

Material	Treatment	Concentration	Sources
Garland chrysanthemum (*Ipomea* spp.)	Fresh[1]	0.38	Chen, 1992
	Boiled[1] (16 min)	0.25	
	Microwave[1] (16 min)	0.21	
Grape (*Vitis vinifera*)	Freezer-dried[1]	0.01[b]	Ben-Amotz and Fishler, 1998
	Fresh[1]	6.6	Guedes de Pinho et al., 2001
	Must[1]	1.19	
Guava (*Psidium guajava*)	Freeze-dried[1]	0.27[b]	Ben-Amotz and Fishler, 1998
Kale *(Brassica oleracea* variety*Acephala*)	Fresh[1]	11.1–0.71[a]	Mercadante and Rodríguez-Amaya, 1991
	Fresh[1]	18.63	Müller, 1997
	Fresh[1]	6.39	Huck et al., 2000
	Fresh[1]	15	Humphries and Khachik, 2003
	Fresh[1]	51.5[b]	Updike and Schwartz, 2003
	Canned[2]	49	Sá and Rodriguez-Amaya, 2003
	Stir-fried[2] (10 min)	0.29–0.35	
Kiwifruit (*Actinida deliciosa*)	Fresh[1]	0.16	McGhie and Ainge, 2002
Leek (*Allium ampelloprasum* variety *porrum*)	Fresh[1]	0.16	Hart and Scott, 1995
	Cooked[1]	0.16	
Lettuce (*Lactuca sativa*)	Romaine. Fresh[1]	0.073	Tee and Lim, 1991
	Leaf. Fresh[1]	0.34	Granado et al., 1992
	Iceberg. Fresh[1]	0.14	
	Butterhead. Fresh[1]	1.61	Hart and Scott, 1995
	Iceberg. Fresh[1]	0.11	
	Fresh[1]	0.41	Drews et al., 1997
	Iceberg. Fresh[1]	0.64	Müller, 1997
	Lambs. Fresh[1]	9.65	
	Fresh[1]	2.92	
	Romaine. Fresh[1]	1.31	Ben-Amotz and Fishler, 1998
	Curly. Fresh[3]	1.54	Kimura and Rodriguez-Amaya, 2003
	French. Fresh[3]	2.29	
	Boston. Fresh[3]	2.14	
	Freelice. Fresh[3]	1.02	
	Freeze-dried[1]	0.17[b]	Humphries and Khachik, 2003
Mandarin (*Citrus reticulata*)	Fresh[1]	0.05	Hart and Scott, 1995
	Fresh[1]	0.07	Humphries and Khachik, 2003
Mango (*Mangifera indica*)	Fresh[1]	0.010	Humphries and Khachik, 2003
	Freezer-dried[1]	0.58[b]	Ben-Amotz and Fishler, 1998
Marrow (*Cucurbita pepo* subps. *pepo*)	Fresh[1]	0.13	Hart and Scott, 1995
	Cooked[1]	0.14	
Mint (*Mentha pipperita*)	Fresh[1]. Leaves	1.70	Tee and Lim, 1991
Nectarine (*Prunus persica*)	Fresh[1]	0.02	Humphries and Khachik, 2003
	Freezer-dried[1]	0.15[b]	Ben-Amotz and Fishler, 1998
Olive (*Olea europaea*)	Fresh[1]	0.71–0.23[b]	Gandul-Rojas et al., 1999
	Virgin oil. Raw[1]	19.1–93.4	Gandul-Rojas and Minguez-Mosquera, 1996
	Fresh[1]	2–39 5–16	Psomiadou and Tsimidou, 2001
Onion (*Allium cepa*)	Spring. Fresh[1]	0.33	Tee and Lim, 1991
	Fresh[1]	0.02	Granado et al., 1992
	Boiled[1] (38 min)	0.05	
	Fresh[1]	0.015	Müller, 1997
	Spring. Raw[1]	0.26	Hart and Scott, 1995
	Green, Freeze-dried[1]	0.3[b]	Ben-Amotz and Fishler, 1998
Opuntia (*Opuntia ficus-indica*)	Freeze-dried[1]	0.46[b]	Ben-Amotz and Fishler, 1998
Orange (*Citrus sinensis*)	Fresh[1]	0.064	Hart and Scott, 1995
	Fresh[1]	0.35	Humphries and Khachik, 2003
	Juice. Fresh[1]	0.67	Lee and Coates, 2003
	Juice. Pasteurised[1] (90°C 30s)	0.76	

(Continued on next page)

Table 2 Lutein composition (mg/100 g wet basis) of vegetables and fruits fresh or submitted to different treatments *(Continued)*

Material	Treatment	Concentration	Sources
Papaya (*Carica papaya*)	Fresh[1]	0.82	Tee and Lim, 1991
	Freeze-dried[1]	0.1[b]	Ben-Amotz and Fishler, 1998
	Fresh[1]	0.02	Humphries and Khachik, 2003
Parsley (*Petroselinum crispum*)	Fresh[1]	5.81	Hart and Scott, 1995
	Fresh[1]	13.78	Müller, 1997
	Freeze-dried[1]	0.15[b]	Ben-Amotz and Fishler, 1998
	Fresh[1]	10.82	Humphries and Khachik, 2003
Peach (*Prunus persica*)	Fresh[1]	0.078	Hart and Scott, 1995
	Freeze-dried[1]	0.01	Ben-Amotz and Fishler, 1998
	Fresh[1]	0.02	Humphries and Khachik, 2003
Pea, grass (*Lathyrus sativa*)	Fresh[1]	0.56	Grela et al., 1999
	Extruded[1] (90–120°C)	0.17	
Pea, green (*Pisum sativum* variety*sativum*)	Frozen[1]	1.63	Hart and Scott, 1995
	Cooked[1]	1.02	
	Fresh[1]	0.47–0.99	De la Cruz-Garcia, 1997
	Boiled[1] (30 min)	1.61–2.50	
	Fresh[1]	1.3	Edelenbos et al., 2001
	Boiled[1] (3min)	1.8	
	Canned[1]	0.7	Humphries and Khachik, 2003
	Fresh[1]	4.1[b]	Updike and Schwartz, 2003
	Canned[1]	5.8[b]	
Pear (*Pyrus communis*)	Freeze-dried[1]	0.12[b]	Ben-Amotz and Fishler, 1998
Pepper green (*Capsicum annuum*)	Fresh[1]	0.34	Granado et al., 1992
	Boiled[1] (25 min)	0.38	
	Raw[1]	0.66	
	Cooked[1]	1.02	
	Raw[1]	0.66	Hart and Scott, 1995
	Cooked[1]	1.02	
Pepper red (*Capsicum annuum lycopersiciforme rubrum*)	Fresh[1]	1.4–0.037	Minguez-Mosquera and Hornero-Mendez, 1994
	Fresh[1]	1.6	Lopez-Hernandez et al., 1996
Pepper red, Chilli (*Capsicum annuum*)	Freeze-dried[1]	0.32[b]	Ben-Amotz and Fishler, 1998
Pepper yellow (*Capsicum annuum lycopersiciforme flavum*)	Fresh[1]	0.22	Tee and Lim, 1991
Pepper orange	Fresh[1]	9.88–1.2	Matus et al., 1991
	Raw[1]	0.50	Hart and Scott, 1995
Pittango (*Stenocalix pitanga*)	Freeze-dried[1]	0.09[b]	Ben-Amotz and Fishler, 1998
Potato (Solanum tuberosum)	Fresh[1]	0.012	Granado et al., 1992
	Boiled[1] (20 min)	0.044	
	Fresh[1]	20.96	Chen and Chen, 1993
	Microwaved[1] (8 min)	9.23	
	Fresh[1]	0.012	Müller, 1997
	Sweet leaves. Freeze-dried[1]	0.05[b]	Ben-Amotz and Fishler, 1998
	Freeze-dried[1]	0.02[b]	
	Fresh[1]	0.19–19.9	Nesterenko and Sink, 2003
Prune (*Prunus domestica*)	Freeze-dried[1]	0.22[b]	Ben-Amotz and Fishler, 1998
	Yellow. Freeze-dried[1]	0.23[b]	
Pumpkin (*Cucurbita pepo*)	Fresh[1]	0.94	Tee and Lim, 1991
	Fresh[1]	0.10	Granado et al., 1992
	Boiled[1] (15 min)	0.12	
	Fresh[1]	17–0.14[a]	Murkovic et al., 2002
Roquete (*Boletus edulis*)	Fresh[3]	5.22	Kimura and Rodriguez-Amaya, 2003
Soybean (*Glycine hispida*)	Green. Seeds[1]	0.09–0.14	Momma et al., 1994
	Yellow. Seeds[1]	0.044–0.068	
	Black. Seeds[1]	0.013	
Spinach (*Spinacia oleracea*)	Fresh[1]	4.18	Tee and Lim, 1991
	Fresh[1]	4.22	Granado et al., 1992
	Boiled[1] (10 min)	6.42	
	Fresh[1]	3.92	Huck et al., 1992
	Fresh[1]	9.50	Khachik et al., 1992b

Table 2 Lutein composition (mg/100 g wet basis) of vegetables and fruits fresh or submitted to different treatments *(Continued)*

Material	Treatment	Concentration	Sources
	Steamed[1] (3 min)	0.61	
	Microwaved[1] (1.5 min)	0.71	
	Raw[1]	5.87	Hart and Scott, 1995
	Cooked[1]	7.41	
	Canned[1]	92.3[b]	Konings and Roomans, 1997
	Fresh[1]	9.54	Müller, 1997
	Fresh[1]	5.0[b]	Humphries and Khachik, 2003
	Fresh[1]	88.1[b]	Updike and Schwartz, 2003
Stining Nettle (*Urtica dioica*)	Fresh[1]	4.5–3.3[b]	Guil-Guerrero et al., 2003
Strawberry (*Fragaria vesca*)	Fresh[1]	0.006	Hart and Scott, 1995
Tapioca (*Manihot utilissima*)	Fresh shoot[1]	1.67	Tee and Lim, 1991
Tea (*Thea synensis*)	Fresh[1], leaves	10–211[b]	Ravichandra, 2002
	Orthodox tea[4]	16.8	
Tomato (*Solanum lycopersicum*)	Fresh[1]	0.13	Tee and Lim, 1991
	Fresh[1]	0.04–0.07	Granado et al., 1992
	Raw[1]	0.078	Hart and Scott, 1995
	Cooked[1]	0.12	
	Fresh[1]	0.05[b]	Konings and Roomans, 1997
	Fresh[1]	0.09	Müller, 1997
	Freeze-dried[1]	1.43[b]	Ben-Amotz and Fishler, 1998
	Fresh[1]	0.028–0.338	Abushita et al., 2000
Water spinach (*Ipomoea aquatica*)	Fresh[1]	0.6	Wills and Rangga, 1996
Water cress (*Nasturtium aquaticum*)	Raw[1]	10.73	Hart and Scott, 1995
	Fresh[1]	2.6	Wills and Rangga, 1996
	Fresh[1]	7.54	Kimura and Rodriguez-Amaya, 2003
Wheat durum (*Triticum durum*)	Raw[1]	1.5–4.0	Hentschel et al., 2002

[1]market, [2]restaurant, [3]cultivar, [4]hydroponic cultivar. [a]lutein + violaxanthin, [b]mg/100 mg of dry weight, [c]mg/100 mL.

Some authors established a difference among the vegetables according to the color of the edible portion on green, red-orange, and yellowish-white in agreement with the associations used in epidemiological studies. In general, as can be observed in the Table 1; the lutein concentration is higher in green vegetables (lettuce, broccoli, water cress, parsley, pea, spinach) than in yellowish-white vegetables (cucumber, potato, onion, cabbage) and the concentration in yellowish vegetables is, in general, higher than in red-orange vegetables (tomato, carrot).

Although in some cases, such as potatoes, where the concentration of lutein is lower than in other vegetables, this compound is one of the predominant carotenoids present in that vegetable (Nesterenko and Sink, 2003).

Factors Affecting the Lutein Concentration in Fruits and Vegetables

In addition to the fruit or vegetable species (see Table 2), other naturally-occurring factors which can influence lutein concentrations are the variety, type, stage of maturity, part of the plant, kind of cultivar, etc.

There are differences in a vegetable among species and varieties. For example Murkovic et al. (2002) reported different lutein + zeaxantin concentrations in three species of pumpkins (*Cucurbita pepo, C. maxima* and *C. moschata*), finding that there was a relationship between the orange or yellow color of the fresh pumpkin and the predominant carotenoids. The species and varieties studied by those authors showed a higher lutein content corresponded to a yellower colour. Differences among 12 tomato varieties has been reported by Abushita et al. (2000), who found concentrations of 0.77 mg/100 g in the Delfine variety and 0.338 in the Petula one. Differences among 11 wheat varieties have been reported by Adom et al. (2003).

Maturing causes changes in color in some fruits and vegetables. For example there are pepper varieties that start out green and become red by the final stage of maturity. These color changes are due to changes in some components such as the carotenoids. Changes in photosynthetic activity during maturation may influence changes in lutein concentration. Changes in lutein concentrations during the maturation process differ depending on the vegetable; in some cases an increase in lutein concentration has been reported, whereas in other cases a decrease has been described.

A decrease in lutein concentrations during the maturation process has been reported by different authors. Minguez-Mosquera and Hornero-Mendez (1994) found that the concentrations of lutein decrease in peppers with an increase in maturity; lutein is found to be present when the pepper is green but with maturity lutein concentrations decrease and the red pepper does not contain lutein in appreciable concentrations. Moma et al. (1994) reported a decrease in yellow soybeans. Razungles et al. (1996) found a decrease in grapes. Gandul-Rojas et al. (1999) reported

a decrease in olive fruit. The different stages of leaf senescence of *Pistacia lentiscus* in adverse climate conditions also influence lutein concentrations, which decreased in the last stages (Munne-Bosch and Penuelas, 2003).

Other authors reported an increase of lutein during maturity in some vegetables. Guil-Guerrero et al. (2003) found that the concentration of all-trans-lutein was higher in the mature leaves of stinging nettles than in young ones. Adewusi and Bradbury (1993) reported the same behavior in cassava leaves.

Differences among parts of the plant have also been reported in olives (Gandul-Rojas et al., 1999). Hart and Scott (1995) reported that in general the outer leaves, skin, etc. of fruits or vegetables contain higher levels of carotenoids than inner parts of the plants; for instance the outer leaves of a Savoy cabbage contain 150 times more lutein than the average of the inner portion. However no differences between the skin and pulp of grapes was reported by Guedes de Pinho et al. (2001).

Differences between the same species grown in different countries have also been reported. Differences between olive oils from Spain and Greece were reported by Psomiadou and Tsimidou (2001). These authors indicated that the differences could be due to a variety differences or to different extraction methods. It is very difficult to compare results obtained from olives from the two countries taking into account all the variables that can influence lutein quantification.

Working with kale, differences in lutein + violaxanthin concentrations among cultivars were found, while seasons also influence the concentration. Differences among cultivars of green peas were reported by Edelenbos et al. (2001), in olive oil (Psomiadou and Tsimidou, 2001), in carrots (Heinonen, 1990) and in cassava (Adewusi and Bradbury, 1993).

The kind of cultivar also matters; in conventionally produced lettuce the concentration is higher than in hydroponic cultivars (Kimura and Rodriguez-Amaya, 2003).

Green leaves taken from cabbage, leek, and lettuce show that the major carotenoid present was β-carotene in summer and lutein in the other seasons; seasonal variations can be due to both environmental factors, i.e. temperature and light intensity, and physiological factors (Takagi et al., 1990)

The influence of some pesticides and fertilizers on carotenoid content has been reported. Kopsell et al. (2003) did not find an influence in the use of sulphur fertilizer on the lutein concentration of kale. However, the herbicide norflurazon, while not influencing the growth of the duckweed plant (*Lemna minor*), significantly reduced the lutein content (Tkalec et al., 2003); it has been reported that this herbicide reduces peroxidase activity.

As can be observed there are numerous factors that can influence lutein and other carotenoid concentrations in each fruit and vegetable. For this reason it is important to increase the studies of the concentration in each fruit and vegetable commonly consumed in the world to obtain an average concentration of those compounds.

EFFECTS OF PROCESSING AND STORAGE

Many vegetables and some fruits are cooked before being consumed. Heat treatments in industry or at home along with other treatments commonly used in the food industry to increase food shelf-life or to manufacture food products can affect lutein concentrations. Some of them are mentioned below.

Heat Treatments

There are contradictory data about the influence of heat treatments on lutein concentration. Some authors report an increase in lutein concentrations after heating whereas others found a decrease, and still others found no changes due to heating. Some of these results can be observed in Table 2. Some of each type of finding will be commented on here; however it is my opinion that a decrease in lutein concentrations could occur in most cases during heating. I believe this hypothesis to be supported by the isomerization, epimerization, oxidation, or other degradation processes that have been reported to occur during heating.

The influence of the thermal process in lutein isomerization has been reported. 9-Cis-lutein and 13-cis-lutein were found in liquid carrots and their concentration increased after some heat treatments and consequently concentrations of all-trans-lutein decreased during heating (Chen et al., 1995). Canned carrots treated at 116°C for 75 min. resulted in isomerization; however the formed isomers were not identified, and it should be taken into account that the treatment applied is very high (Emenhiser et al., 1996). Updike and Schwartz (2003) found that the canning of kale caused the largest increase in total cis isomers (13-cis, 13′-cis, 9-cis and 9′-cis-lutein isomers) on a percentage basis (22%), followed by processing corn (12%), spinach (11%), green peas (6%) and broccoli (3%); in Table 1 the total lutein amount of those vegetables is reported. Guil-Guerrero et al. (2003) found in a lipid extract of stinging nettle 13-cis-lutein, 13′-cis-lutein and 9′-cis-lutein; however the authors do not indicate whether the isomers were in the fresh vegetables, formed during the extraction process, or formed during heating.

Chen and Chen (1993) reported the presence of 9-cis-lutein and 13-cis-lutein 5,6 epoxide in fresh sweet potato leaves. As the time of microwave treatment increased, their concentration decreased; the authors also report the formation of two dehydration lutein compounds during microwave treatment for 8 min, the formed compounds being the 3,4-didehydro-β, ε-carotene-3′-ol and 3′,4′-didehydro-β, β-caroten-3-ol; the possible implication of chlorophylls in lutein isomerization is discussed by the authors.

Food processing can also result in the partial dehydratation of lutein; Yokota et al. (2003) reported the presence of anhydrolutein I [(3*R*,6′*R*)-3-hydroxy-3′,4′-didehydro-β, γ-carotene] in tomato puree. The epimerization of lutein to 3′-lutein [(3*R*,3′*R*,6′*R*)-$\beta-\varepsilon$-carotene-3-3′-diol] and its dehydratation to anhydrolutein I (their chemical structure is shown in Figure 3) has also been reported by Deli et al. (2004) in treated

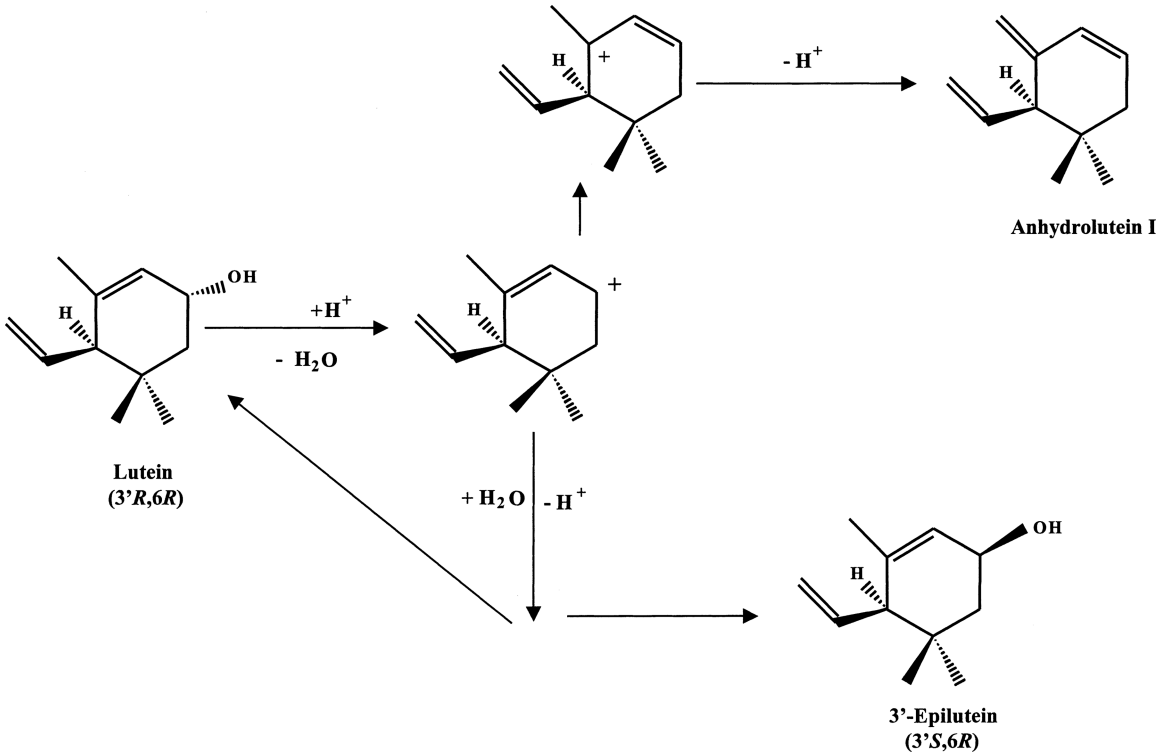


Figure 4 Possible mechanism of epimerization and dehydratation of all-trans-lutein.

paprika, blackberries, and other fruits and vegetables, and they proposed a mechanism for epimerization and dehydration of all-trans- lutein that is shown in Figure 4.

The kinetics of carotenoid degradation have not been extensively studied and the majority of the studies to date have focused on β-carotene in low-moisture and solvent-based systems (Henry et al., 1998). The kinetics of lutein degradation during the heating of sunflower oil has been reported by Henry et al. (1988) to be adjusted to a first order reaction kinetic; in the same conditions lutein is more stable than lycopene and β-carotene; the degradation compounds formed during lutein degradation were not described in this study, the authors indicating that they are presumably a combination of epoxides, apo-carotenals and isomers.

Although lutein resists degradation more than other carotenoids in model systems the data can not be extrapolated to foods. Food systems are complex because of the numerous other compounds present. Compounds such as proteins, carbohydrates, and lipids may act as co-oxidant and promote oxidation or may have a protective effect (Henry et al., 1998).

Some authors have reported that as a consequence of changes in all-trans-lutein during heating their concentration decreases. Micozzi et al. (1990) reported that cooking reduced the lutein content in green, leafy vegetables. A reduction in lutein concentration was detected in garland chrysanthemum (a green vegetable grown in Taiwan) when it underwent boiling or microwave treatments. The loss was higher in conventional than in microwave treatment (Chen, 1992).

However, as I have indicated, some authors report an increase in lutein concentrations during cooking: Granado et al. (1992) after boiling fresh vegetables, De la Cruz-Garcia et al. (1997) after 30 min. of boiling, 40 min. of steaming, 5 min. pressure-cooking after the first issue of vapour, or 5 min. microwaving of green peas, Edelenbos et al. (2001) after boiling fresh green peas for 3 minutes. Lee and Coats (2003) after treating fresh orange juice at 90°C for 30 s.

Some authors point to an increase in chemical extractability of carotenoids due to the heat treatments in order to explain the increase in lutein concentrations after cooking (Granado et al., 1992; De la Cruz-Garcia et al., 1997; Hart and Scott, 1995).

Updike and Shwart (2003) reported that the total lutein concentrations in canned products is higher than in fresh ones; the authors explain that this could be due to: 1—a loss of soluble solids into the canning medium (Ogunlesy and Lee, 1979; Weckel et al., 1962); 2—an inactivation of carotenoid-oxidizing enzymes (Baloch et al. 1977) 3—and/or an increased extraction efficiency due to disruption of carotenoid-protein complexes.

A good stability of lutein during different heat treatments has been reported by different authors: Khachik et al. (1992b) observed similar effects on all-trans-lutein and cis-lutein after submitting fresh broccoli, spinach, and green beans to steamed or microwave treatments for short periods; they also observed that the cis/trans lutein isomerization was not influenced by the cooking conditions employed. Abushita et al. (2000) found no loss in tomato that was evaporated under vacuum at 60–70° C for approximately 4 h and sterilized at 100°C for 30 min.

during canning. Sá and Rodriguez-Amaya (2003) reported that lutein has good stability during cooking; however those authors analysed only cooked vegetables from restaurants, not the composition of the fresh material.

The results reported by Khachik et al. (1992b) could be due to the low heat treatments; however the authors did not find lutein changes after boiling green beans for one hour; those results are in opposition to the data found by other authors that reported an increase during heating. This is explained by those authors as a result of an incomplete extraction of carotenoids from the chloroplast in the fresh materials, as occur in the results when an increase of lutein is reported, as heat increases the accessibility of the solvent to the carotenoids; this implies that usually extraction in fresh vegetables is incomplete and is improved by heating. If this theory is correct, discovering if there really are changes in lutein concentration during heating it will be necessary to find a good method to completely extract the carotenoids from fresh fruits and vegetables.

In conclusion it is necessary to analyze the same vegetable before and after heat treatments commonly used in industry or at home, extracting the lipid fraction in conditions that allow the total extraction of lutein (this could be done by heating the fresh vegetables after the extraction and performing a new extraction to see if lutein or some lutein compounds are present), and analyzing the samples in the same conditions for the possible presence of isomers in fresh and treated vegetable and the possible compounds formed from lutein oxidation, epimerization or degradation.

Other Industrial Treatments

In the food industry the storage of fresh or treated fruits and vegetables is important. Storage conditions can influence the concentration of lutein. As has been reported previously different factors such as light, temperature, etc. can influence carotenoid isomerization and degradation, which process can occur when fruits or vegetables are stored.

The influence of light and temperature on pure lutein during storage has been reported by Shi and Chen (1997). The studies performed by those authors proved that illumination and high temperature promote lutein degradation, while an ordinary freezer could satisfy the requirements for maintaining lutein solutions and lutein-containing products. However, it must be taken into account that these studies have been performed using pure lutein, and the compounds present in foods could influence the behavior of lutein when the food is submitted to storage.

Influence of temperature storage has been reported by Monreal et al. (1999) on green beans kept at 12, 8 or 4°C for 26 days; they found that green beans stored at 12°C didn't show a decrease of lutein during storage, while at lower temperatures a decrease of lutein concentration was seen with increased storage time. Light can also influence lutein degradation during storage. Kopas-Lane and Warthesen (1995) reported a loss of 25% in lutein concentrations when fresh spinach was kept under light for 8 days, whereas no changes in lutein concentrations were found when the spinach was stored in the dark. Light does not influence lutein concentrations in all vegetables in the same way. Kopas-Lane and Warthesen (1995) reported no influence by light during the storage of carrots. The different behavior among vegetables such as spinach and carrots could be due to the presence of chlorophylls, since it has been reported that chlorophylls are destructive to isolated carotenoids during light exposure.

The kinetics of lutein loss during storage have been reported. Chen and Tang (1998) observed that all-trans-lutein, 9-cis-lutein, and 13-cis-lutein from powder obtained from carrot pulp are lost during storage in either dark or light, although the loss was higher in samples kept in the light, and follows a first order reaction. All trans-lutein decreased while increasing the 13-cis and to a lesser extent the 9-cis-lutein isomers during storage, following a first order reaction, these results were obtained during storage of freeze-dried carrot powder at different temperatures between 4 and 45°C for 12 weeks (Tang and Chen, 2000). As can be observed the two authors reported different results, which could be due to the matrix, storage conditions or other undetermined factors.

Powder samples can be obtained by different methods such as by spray-drying or by freeze-drying. Tang and Chen (2000) compared these two methods in powdered carrots stored for some months, and found that spray-dried powder is more susceptible to lutein isomerization than freeze-dried powder. The authors explain this data by pointing out that during spray drying the powder granule may undergo shrinkage because of hot air penetration, which in turn results in the formation of numerous tiny pores on the surface of the powder, while in contrast during freeze-drying, no shrinkage or deformation of powder granules occurrs because of the sublimation of water from ice crystals at freezing temperature. The concentration of lutein+zeaxanthin in red pepper powder obtained by spray drying or air-fountain drying at different temperatures decreases following a first order reaction when the temperature of treatment is not very high (105 or 115°C).

A fermentation process is used in some foods such as in olive processing to produce green table olives. During fermentation there is a reduction in pH but this reduction has no affect on lutein concentration (Minguez-Mosquera and Gallardo-Guerrero, 1995). Other fermentation processes as well as a high temperature drying process occur during the manufacturing of tea, which according to some authors causes carotenoid degradation (see Ravichandra, 2002). Loss of lutein in tea manufacturing has been quantified by Ravichandra (2002) to be 21% when fresh tea leaves were processed to obtain "orthodox tea."

Another factor than can affect lutein concentration is the curing stage of vegetables. Fan et al. (1993) detected a decrease of approximately one-third of the concentration of lutein after 50 days of curing in green mustard.

Industrial treatment of food such as extrusion can also affect lutein concentrations. Green peas submitted to extrusion-cooking temperatures of 90/100/120/100°C suffered a reduction in lutein concentrations (Grela et al.,1999).

As has been observed the results obtained by different authors vary. In any case, it is important to take into account that industrial or home processing can influence lutein concentrations in foods. This is important when lutein concentrations in prepared foods are determined because results may differ even among different brands of the same product depending on the matrix, the type of treatment used, the conservation treatment, etc.

IMPORTANCE OF LUTEIN IN HUMAN HEALTH

More of the studies of carotenoids until 1990 were on their total carotenoid content or those that have activity as provitamin A. However, in recent years different authors claim to know the composition and concentration of the main carotenoids important to human health present in each fruit and vegetable. Another side of the story is the study of carotenoids present in human serum; it is important to remember that carotenoids are not biosynthetized by animals, which have to obtain them from food. Fruits and vegetables are the most important contributors of carotenoids in the typical human diet (Heinonen, 1990).

The five carotenoids with the highest known blood concentrations in human populations are: α-carotene(β, ε-carotene), β-carotene, lycopene (ψ, ψ-carotene), lutein and β-cryptoxanthin ((3*R*)-β, β.carotene-3-ol). However, other carotenoids like: zeaxanthin ((3*R*,3′*R*)-β, β-carotene-3,3′-diol), epilutein ((3*R*,3′*R*,6S)-β, ε-carotene-3,3′-diol), α-cryptoxanthin ((3′*R*,6′*R*)-β, ε-caroten-3-ol), neurosporene (7,8-dihydro-ψ, ψ-carotene), phytofluene (hexahydro-ψ, ψ-carotene), phytoene (octahydro-ψ, ψ-carotene), γ-carotene (β, ψ-carotene) and ξ-carotene (tetrahydro-ψ, ψ-carotene) as also present in human serum; Khachik et al. (1997b) reported the presence in serum of carotenoid metabolites. To highlight the importance of lutein in serum, we would like to draw attention to a study performed by Tzeng et al. (2004) which found that in a total of 62 human serums β-carotene was present in the highest amount followed by lutein and lycopene.

Bioavailability

The bioavailability of lutein is important since carotenoids are not synthesized by humans, the concentration of those compounds in humans depend on their intake and their bioavailability. Contradictory results about bioavailability have been reported; however most of them reported a low bioavailability.

Most of the studies are performed analyzing the concentration of lutein in serum some time after the intake of matrix containing this compound. These studies could be improved by the use of isotopic labelled lutein, which method has been employed by Kelm et al. (2001), Kurilich et al. (2003) and Lienau et al. (2003). Kurilich et al., (2003) found isotopic labeled lutein in plasma 11 hours after kale intake containing labeled lutein.

Studies have been performed from intragastrical feeding. Studies working with intragastrical feeding of tomato puree, chopped spinach, and carrot puree showed that: 1—the stomach initiates the transfer of carotenoids from the vegetable matrix to the fat phase of the meal, 2—lycopene is less efficiently transferred to micelles than β-carotene and lutein, and 3—the very small transfer of carotenoids from their vegetable matrices to micelles explains the poor bioavailability of these phytomicroconstituents (Tyssandier et al., 2003).

Some factors that can influence the bioavailability have been commented upon by Zaripheh and Erdmand (2002). It has been assumed that the highly lipophilic food micronutrients (fat-soluble vitamins and phytochemicals with potential health benefits, the carotenoids and phytosterols) have the same metabolism in the human upper gastrointestinal tract and that they follow the same pathway as lipids (Borel, 2003). Although some factors such as molecular species, fat, and food matrix affect absorption efficiency of most of those compounds, other factors such as fiber and micronutrients apparently affect absorption of only some of them.

Other factors can influence lutein absorption. One important factor is solubility. Free or esterified lutein is practically insoluble in aqueous systems, and their solubility in food grade solvents (oils) is very limited (Amar et al., 2003). To improve the absorption of lutein added to food or intake as different formula present in the market, the substance could be encapsulated or microencapsulated. Microencapsulation has been proposed by Amar et al. (2003).

Other factors including the source of lutein, their interaction with other carotenoids, etc. can influence their absorption. With respect to the lutein source, Hof et al. (1999b) and Erdman (1999) found that the bioavailabilities of β-carotene and lutein vary substantially among different vegetables. Berg and Vliet (1998) reported that the availability of lycopene from tomato paste was high but availability of carotenoids from carrots and spinach was low. The low availability of lutein from carrots was reported by Thuermann et al. (2002). Other vegetables appear to have a better bioavailability. Riso et al. (2003) found that the ingestion of dark green leafy vegetables such as spinach or broccoli increase the levels of lutein in serum a few hours after eating, and concentration remains high until 80 hours after ingestion; these results are not in agreement with those reported previously by Berg and Vliet (1998).

The relative bioavailability of carotenoids can be different. The relative bioavailability of lutein from vegetables is higher than that of β-carotene (Erdman, 1999; Hof, 1999a). Some carotenoids can affect the absorption of others, as shown by Berg and Vliet (1998), who observed that lutein negatively affected β-carotene absorption when given simultaneously, but lycopene had no effect. Studies on other carotenoids influencing lutein bioavailability should be performed.

Also the form lutein is in can affect their bioavailability. It has been indicated that their binding with protein should increase their bioaviability (Zhou et al., 1994). Lutein-esters before being absorbed require de-esterification by intestinal enzymes; Bowen

et al. (2002) reported that the lutein-ester dissolution was a greater limitation to bioavailability than lutein-ester hydrolysis. Barua and Olson (2001) found no absorption of lutein-epoxide dissolved in corn oil. Several research groups have suggested that cis-isomers of lycopene are better absorbed than the all-trans form because of the shorter length of the cis-isomer, the greater solubility of cis-isomers in mixed micelles and/or the lower tendency of cis-isomers to aggregate (Ferreira et al., 2000; Boilean et al., 2002); however, I could not find data about lutein isomers bioavailability.

Due to the low bioavailability of lutein, some authors proposed diet supplementation of lutein by their addition to foods to create antioxidant formulas (Challem, 2002; Krurger et al., 2002). Currently there are formula containing lutein in different concentrations in the market, with commercials indicating that their consumption could aid in the prevention of some diseases. Most of the lutein used in commercial products is extracted from marigold flowers (*Tagetes erecta*).

Some of the carotenoids present in serum are secreted by the mammary gland, the amount secreted being dependent on some factors that influence their concentration in serum. Comparing the carotenoid intake of the major carotenoids of women from different countries from all over the world Pramuk et al. (2003) found that patterns of breast milk carotenoids were unique to each country and qualitative patterns reflected the dietary carotenoid supply. The secretion by the mammary gland must be important for lactating babies. It must be taken into account that the amount of lutein and other carotenoids usually decreased with the duration of the lactation (Jewell et al., 2004).

Antioxidant Effect

When the antioxidant effect of lutein is studied two aspects should be taken into account:

1. The antioxidant effect of carotenoids can reduce the risk of chronic disease by protecting against free-radical mediated damage. Due to their structure not all the carotenoids act in the same way. For instance, lycopene is an efficient quencher of singlet oxygen and lutein is more efficient in quenching lipid peroxide radicals (Southon, 2000).
2. The possible degradation of carotenoids can lower their effectiveness. For example the antioxidant capacity of lutein gradrually decreased during storage, probably because of its degradation after prolonged exposure to light (Yen and Chen, 1995).
3. Some of the studies of antioxidant activity have been performed using model systems. It must be taken into account that the results obtained showing the antioxidant effect of carotenoids using model systems could not be translated to their influence on food or on humans. A great number of studies reported the antioxidant activity of carotenoids in organic solutions; for example, the inhibition of oxidative rancidity in soybean oils, salmon muscle and methyl linolea lutein and other carotenoids was reviewed in 1993 by Maestro-Duran and Borja-Padilla. On the other hand, there are contradictory results obtained in studies of carotenoids in oxidation of oils, as has been commented on by Haila et al. (1996).

It is important to remember the antioxidant activity of carotenoids and the possible influence of this characteristic on human health. Not all the carotenoids have the same antioxidant effectiveness; Lee and Min (1990) compared the effects of lutein, zeaxanthin, lycopene, isozeaxanthin, and astaxanthin on the photo-oxidation of soybean oil, finding that the antioxidant effectiveness of carotenoids increased with the number of conjugated double bonds. Buratti et al. (2001) compared the antioxidant activity of different lipophilic compounds obtained from vegetables by an electrochemical method, and observed that under those conditions lutein showed lower activity than lycopene, β-carotene, zeaxanthin, α-carotene and cryptoxanthin.

Prevention of Cancer

The antioxidant is associated with lower DNA damage and higher repair activity. Agents capable of reacting with and chemically modifying DNA are known potential carcinogens, so any substance which protects DNA or increases repair activity in the case of damage has health benefits.

There is little doubt that oxidant stress can cause DNA damage. Pool-Zobel et al. (1997) analyzed the influence of the intake of lycopene, β-carotene, α-carotene and lutein on DNA damage, and reported that carotenoid-containing plant products exert a cancer-protective effect via a decrease in oxidative and other damage to DNA in humans.

Recently there has been some controversy over whether the health benefits of some carotenoids are due to the carotenoids themselves, or if it is necessary to ingest the vegetable containing the carotenoid. The second point of view indicates that not only the carotenoid but also other compounds in the vegetables can have a beneficial effect; perhaps the action of those compounds enhances or is necessary for the effect of the carotenoids, or vice versa.

As can be observed in the studies that I will report there is some controversy about the possible beneficial effects of lutein or other carotenoids in lung, laryngeal, breast, prostate, colon, and skin cancer. However, it must be taken into account: 1—that the results present were in general obtained working with different amount of people with or without knowledge of cancer in other family members. 2—Some studies have been performed with animals, and it may be impossible to translate the obtained results to humans. 3—The ingestion of lutein has been performed in different forms for different time periods. 4—Some works have been performed with the intake of a carotenoid rather than with the intake of vegetables or the intake of carotenoids with other compounds; the results obtained could differ, for

example the bioavailibility of lutein supplementation could be different to that of lutein from food. 5- Only some of the numerous reports about this item have been reported in this paper.

Le-Marchand et al. (1993) found evidence of a protective effect by certain carotenoids such as lutein against lung cancer and of the greater protection afforded by consuming a variety of vegetables compared to only foods rich in a particular carotenoid. Michaud et al. (2000) reported that diets rich in a variety of carotenoids from fruits and vegetables may reduce risk for lung cancer. Rohan et al. (2002) found that the intake of some carotenoids like lutein is not associated with lung cancer risk, at least for the sample used by those authors. Wright et al. (2003) reported not only the importance of whole carotenoids but also of any specific carotenoid in preventing lung cancer.

Bidoli et al. (2003) found that consumption of lutein, other carotenoids, and micronutrients is inversely related to laryngeal cancer risk.

Zhang et al. (1997) suggested that higher breast adipose concentrations of retinoids and some carotenoids such as lutein may be associated with decreased risk of breast cancer. Dorgan et al. (1998) reported that the carotenoids β-cryptoxanthin, lycopene, and lutein/zeaxanthin may protect against breast cancer. Strong inverse associations were found for increasing quantities of α-carotene, β-carotene, lutein/zeaxanthin, total vitamin C from foods and total vitamin among pre-menopausal women with a positive family history of breast cancer (Zhang et al., 1999). Toniolo et al. (2001) reported that a low intake of carotenoids like lutein, through poor diet and/or lack of vitamin supplementation, may be associated with increased risk of breast cancer and may have public health relevance for people with markedly low intakes. Hulten et al. (2001) found a significant trend of an inverse association between lutein and breast cancer risk in pre-menopausal women. Chew et al. (2003) reported that dietary lutein, especially at 0.002%, inhibited mammary tumor in mouse growth by selectively modulating apoptosis, and by inhibiting angiogenesis.

The results obtained by Cohen et al. (2000) suggest that high consumption of vegetables, especially cruciferous vegetables, may be associated with reduced prostate cancer risk.

Lutein and lycopene in small doses may potentially prevent colon carcinogenesis on rats (Narisawa et al., 1996). Muhlhofer et al. (2003) analyzed the carotenoid concentration in the colons of patintes with colorectal cancer and found that all carotenoids investigated, including lutein, are reduced in colorectal adenomas, suggesting that mucosal carotenoids could serve as biomarkers for predisposition to colorectal cancer. Moreover, anti-tumor activity exerted by carotenoids is limited due to mucosal depletion; the obtained results induced the authors to speculate that supplementation of a larger array of carotenoids might be beneficial for patients with colorectal adenoma.

Fung et al. (2003) found no evidence that vitamins A, C, and E, folate, or carotenoids such as lutein play an important protective role against incidents of squamous cell carcinoma of the skin.

Carotenoids like lutein may not only prevent the cancer apparition; Molnar et al. (2004) reported that the multidrug resistant protein that belongs to the ATP-binding superfamily are present in a majority of human tumors and are an important final cause of therapeutic failure. Therefore compounds, including some carotenoids such as lutein which inhibit the function of the multidrug resistant-efflux proteins may improve the cytotoxic action of anticancer chemotherapy.

Prevention of Eyes Diseases

Although the importance of vitamin A to vision has been recognized for years, it has been recently discovered that other vitamins, carotenoids, and trace elements, found particularly in foods such as fruits and vegetables, are of significance in eye nutrition.

The yellow color of the macula lutea of the primate retina is due to the presence of macular pigment, composed of two dietary xanthophylls, lutein, and zeaxanthin, and another xanthophyll, meso-zeaxanthin. The latter is presumably formed from either lutein or zeaxanthin in the retina; we will also see that degradation products of lutein and geometrical isomers of lutein and zeaxanthin have been found in the retina.

It has been suggested that these macula carotenoids play a role in protection against light-dependent damage. Age-related macular degeneration (AMD) is thought to be the result of a lifetime of oxidative insult that results in photoreceptor death within the macula. It is an irreversible process that is the major cause of blindness of, and happens with increasing incidence in, the elderly. By absorbing blue-light, the macular pigment protects the underlying photoreceptor cell layer from light damage, possibly initiated by the formation of reactive oxygen species during a photosensitized reaction. Increased risk of AMD may result from low levels of lutein and zeaxanthin (macular pigment) in the diet, serum or retina, and excessive exposure to blue light.

To know the possible importance of lutein in macula protection it is necessary to know its concentration in plasma and, of course, in the retina where it is transported by lipoproteins from the plasma. To evaluate the levels of lutein and zeaxanthin in the retina different methods have been used. A heterochromatic flicker photometry, which determines the optical density of macular pigment, can be used (which partly depends on retina lutein concentration); however the results found by Wenzel et al. (2003) indicate that multiple optical density measurements of macular pigment may be necessary to evaluate lutein and zeaxanthin levels in the retina accurately. The optical density of macular pigment can also be determined by reflectometry (Cardinault et al., 2003). However there is a more direct method, called resonance Raman spectroscopy, which is a novel objective non-invasive in vivo laser-optical technique that can be use to measure the concentration of the macular carotenoid pigments lutein and zeaxanthin in the living human retina of young and elderly adults (Gellermann et al., 2002; Zhao et al.,

2003). The Raman technique is quantitative as well as objective and may lead to a new method for rapid screening of carotenoid pigment levels in large populations at risk for vision loss from age-related macular degeneration.

Nutritional supplementation with lutein and their influence in the prevention of AMD has been reviewed by Bartlett and Eperjesi (2003), who reported a possible therapeutic role of lutein in AMD. Blum (2000) reported that epidemiological data suggest that individuals with higher intakes of lutein, zeaxanthin and vitamin E are better protected from age related (and non-age related) macular degeneration. Protection from cataracts can also result from consumption of foods containing antioxidants such as vitamins C and E and lutein, while supplementation with riboflavin and niacin also reduces risk. Landrum and Bone (2001) showed that an increased dietary intake of lutein or zeaxantin increases the macular carotenoid levels. In 2003 Bone et al. reported that it remains to be demonstrated whether lutein or zeaxanthin dietary supplements reduce the incidence of AMD, whereas Sies and Stahl (2003) reported that epidemiological studies provide evidence that an increased consumption of lutein is associated with a lowered risk for age-related macular degeneration, and Krinsky et al. (2003) reported that the macular pigments can be increased in primates by either increasing the intake of foods that are rich in lutein and zeaxanthin or by diet supplementation with lutein or zeaxanthin.

Although increasing the intake of lutein or zeaxanthin could be protective against the development of age-related macular degeneration, a causative relationship has yet to be experimentally demonstrated.

Compounds from the isomerization, oxidation, and degradation of lutein have been detected in retina. Khachik et al. (1997a) analyzed extracts from human retina by HPLC and found in addition to lutein and zeaxantin a major carotenoid resulting from direct oxidation of lutein that was identified as 3-hydroxy-β, ε-caroten-3′-one, several of the geometric isomers of lutein and zeaxanthin were also detected at low concentrations.

The presence of the direct oxidation product of lutein and 3′-epilutein ((3*R*,3′S,6*R*)-β, ε-carotene-3,3′-diol), a metabolite of lutein (chemical structure shown in Figure 3), in human retina suggests that lutein and zeaxanthin may act as antioxidants to protect the macula against short-wavelength visible light; the proposed oxidative-reductive pathways for lutein and zeaxanthin in human retina may therefore play an important role in the prevention of AMD degeneration and cataracts. Khachik et al. (2002) also try to explain the presence of the isomers in the retina, indicating that the transformation of carotenoids in the human eye involves a series of oxidation-reduction and double-bond isomerization reactions to render some isomers found in human eyes.

Research about the possible protective effect of lutein isomers in AMD was not found. As has been indicated geometric isomers can be found in the retina, but they could have arrived to the retina by the same pathway as all-trans-lutein and could also have a beneficial effect in macula protection.

Some products of lutein metabolism such as 3-hydroxy-β, ε-caroten-3′-one, $\varepsilon\varepsilon$-carotene-3,3′-dione, 3-hydroxy-β, ε-caroten-3-one and cis-3-hydroxy-β, ε-caroten-3-one and the geometric isomers have been described in human serum and milk (Khachik et al., 1997b). This could indicate that the oxidation-reduction and double-bond isomerization not only occur in the retina but can also occur in other parts of the organisms, giving a protective effect.

Lutein intake could be beneficial in other eye diseases such as choroideremia. This is an incurable X-linked retinal degeneration caused by mutations in the gene encoding rab escort protein-1. Duncan et al. (2002) performed a study of the effect of supplementation of oral lutein in a subset of patients, concluding that macular pigment density can be augmented by oral intake of lutein in patients with choroideremia; there was no short-term change in the central vision of the patients on the supplement, but long-term influences of lutein supplementation on disease natural history warrant further study.

Visual function in patients with age-related cataracts who received lutein supplements improved, suggesting that a higher intake of lutein, through lutein-rich fruits and vegetables or supplements, may have beneficial effects on the visual performance of people with age-related cataracts (Moeller et al., 2000 and Olmedilla et al., 2003).

Prevention of Cardiovascular Diseases

The antioxidant activity can increase the LDL (low density-lipoprotein) oxidation resistance, the protective effect is known to be beneficial by aiding in the prevention of some cardiovascular diseases.

Some studies have also been performed on the relationship of the influence of lutein in LDL oxidation and the possible influence on atheroma formation. Siemensma (1997) discussed the antioxidation activity of dietary carotenoids with respect to their potential role in the prevention of degenerative diseases such as atherosclerosis. Southon (2000) reported that the carotenoid supplementation diet did not increase low density lipoprotein oxidation resistance; however consumption of carotenoid-rich fruits and vegetables did. The possible effect of lutein dietary intake on the prevention of early atherosclerosis has been reported by Dwyer et al. (2001) based in epidemiological, in vitro, and mouse model findings.

Nicolle et al. (2003) reported in studies performed on rats that the administration of a diet with carrots modifies cholesterol absorption and bile acid excretion and increases antioxidant levels, which effects could be also interesting for cardiovascular protection.

Effects on Other Diseasses

The antioxidant effect of carotenoids can be important in the prevention of other diseases as well, notably Alzheimer's

disease (AD). A large body of experimental evidence suggests that in AD pathogenesis an important role is played by oxidative stress, but there is still a lack of data on in vivo markers of free radical-induced damage. Mecocci et al. (2002) studied the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative damage to DNA, in peripheral lymphocytes and of some carotenoids such as lutein in serum; they observed that in patients with AD there is a increase of markers showing oxidative damage (8-OHdG), which is correlated with decreased levels of plasma antioxidants. These findings suggest that lymphocyte DNA 8-OHdG content in patients with AD reflects a condition of increased oxidative stress related to a poor antioxidant status. As mild cognitive impairment may represent a prodromal stage of Alzheimer's disease, and oxidative damage appears to occur as one of the earliest pathophysiological events in Alzheimer's disease, an increased intake of antioxidants like lutein, zeaxanthin, and/or α- and β-carotene in patients with mild cognitive impairment could be helpful in lowering the risk of conversion to dementia (Rinaldi et al., 2003).

Khachik et al. (1997b) reported that carotenoids such as lutein may exert their potential biological activity in the prevention of certain diseases by other mechanisms different to the antioxidant one; these biological activities are: 1—enhancement of the activity of cellular communication, 2—stimulation of the activity of phase II enzymes (detoxification enzymes), and 3—anti-inflammatory/immune-related properties.

An EC funded research project (AIR2-CT93-0888) (Flair-flow Europe, 2001) examined the beneficial health effects of fruit and vegetable carotenoids in a large number of human volunteers. No evidence of antioxidant activity was found for isolated carotenoids (β-carotene, lutein or lycopene) consumed as supplements. However, a high consumption of carotenoid-rich fruits and vegetables appeared to have a protective effect by decreasing low-density cholesterol sensitivity to oxidation, and by reducing oxidative damage to DNA. The obtained results suggest that other elements of fruits and vegetables may possibly take part in these protective processes, and that it is the natural balance of carotenoids achieved through a diet rich in fruits and vegetables which provides the most effective protection against diseases such as cancer, cardiovascular diseases and age-related eye disorders.

In conclusion the overall body of evidence is insufficient to conclude that increasing levels of lutein and zeaxanthin, specifically, will confer an important health benefit. Future advances in scientific research are required to gain a better understanding of the biologic mechanisms of their possible role in preventing disease. Additional research is also required to understand the effect of their consumption, independent of other nutrients in fruits and vegetables, on human health.

As can be observed by the data shown previously some recent findings about the protective effect of whole fruits and vegetables rather than isolated carotenoids are reported; however the results obtained do not invalidate the hypothesis that isolated carotenoids can also have a beneficial effect.

OTHER BENEFICIOUS EFFECTS OF LUTEIN

Some examples of other uses for lutein, in addition to their beneficial health effects, are indicated.

Some of the carotenoids are used as pigments in the food industry. Lutein use is permitted by the European Union as food coloring (European Parliament and Council Directive, 1944). Lutein can be used as a food additive for enhancing the color of egg yolk and chicken skin and for imparting yellow to orange colors to vegetable oils, mayonnaise, dairy products, etc. (Antony and Shankaranarayana, 2001). Breithaupt (2004) has developed a HPLC method for the determination of carotenoid food additives in processed foods.

Another interesting characteristic of lutein and other carotenoids like β-carotene could be their possible use to distinguish among different single-variety oils; Gandul-Rojas and Minguez-Mosquera (1996) found different ratios of lutein/β-carotene in oils obtained from different single olive varieties.

Taylor et al. (1992) established, using a step-wise multiple regression analysis, a linear relationship between the levels of six pigments, one of which is an isomer of lutein and another is lutein-epoxide, and the quality of manufactured black tea.

The so-called "yellow pigment" content of durum wheat has been used for a long time as an indicator of the color quality of durum wheat and pasta products. For decades the chemical nature of these pigments has been associated with carotenoids, namely lutein and its fatty acid esters (reviewed by Hentschel et al., 2002). However, these authors found that analysing different durum wheat cultivars showed that the fraction of carotenoids in the complete "yellow pigment" amounted to only 30–50% of the yellow pigment quantities indicating that other unidentified compounds influenced the color as well. On the other hand no fatty acid ester of lutein was detected by those authors.

CONCLUSION

Lutein is one of the carotenoids present in plasma and other organic fluids which has an antioxidant effect that can be beneficial to human health. Lutein is not synthesized by humans, and for this reason it must be ingested via foods that contain it, such as fruits, vegetables, egg yolk, etc. Lutein can be present in vegetables as all-trans-lutein, but some geometrical isomers, epoxi-lutein, esterified-lutein and lutein linked to proteins has also been reported to be present. The possible influence of these other forms on human health should be studied.

Some years ago nobody doubted the beneficial effects of carotenoids like lutein in the prevention of diseases in which the oxidative factor is important, for instance some types of cancer. However, in recent years there has been some controversy over whether it is the consumption of carotenoids or the fruits and vegetables that are important to human health, the second idea indicating that there are other compounds in fruits and that

are necessary for the beneficial effects of carotenoids such as lutein to be made evident. More studies should be performed regarding this question.

Although the importance of fruit and vegetable consumption may be more important than that of plain lutein, in my opinion it is important to know what the concentration of this carotenoid is in the different foods to try to keep a balanced diet with a adequate intake of this and other carotenoids. Lutein is synthesized in fruits and vegetables but their concentration is variable depending on a lot of factors such as the species, variety, stage of maturity, cultivar, climate, part of the fruit or vegetable, etc; for this reason it is necessary to do numerous studies if we want to know how much lutein may be in a given fruit or vegetable.

It is necessary to remember that some fruits and vegetables are consumed after being submitted to treatments such as heat treatments, extrusion, etc and some are stored before or after those treatments for varying periods of time. More studies are necessary to improve our knowledge of changes in isomerization, epimerization, oxidation and degradation of lutein that can occur during heat treatments or other industrial treatments of fruits and vegetables.

It has been described that there are different methods to analyze all-trans-lutein, their geometrical isomers, lutein-epoxide, etc. It has also been reported that depending on the methodology used the obtained lutein concentrations vary considerably. It will be necessary to perform inter-laboratory studies to try to find a consistent and accurate method to determine lutein concentrations, as well as to quantify the geometrical isomers of lutein, lutein epoxide and degradation products.

Lutein is important to human health and for this and all previously expressed reasons an effort to improve the knowledge that we have at the moment is necessary, with the additional benefit that this same criteria could be applied to other carotenoids of interest like lycopene, β-carotene, etc.

REFERENCES

Abushita D.A., Daood, H.G., and Biacs, P.A. 2000. Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. J. *Agric. Food Chem*., **48**:2075–2081.

Adewusi, S.R.A., and Bradbury, J.H. 1993. Carotenoid in cassava: Comparison of open column and HPLC methods of analysis. *J. Sc. Food Agric*., **62**:375–383.

Adom, K.K., Sorrells, M.E., and Liu, R.H. 2003. Phytochemical profiles and antioxidant ac tivity of wheat varieties. *J. Agric. Food Chem*., **51**:7825–7843.

Amar, I., Aserin, A., and Garti, N. 2003. Solubilization patterns of lutein and lutein esters in food grade nonionic microemulsions. *J. Agric. Food Chem*., **51**:4775–4781.

Antony, J.I.X., and Shankaranarayana, M.L. 2001. Lutein. *Rld Food Ingred*., **(April/May)**: 64–67.

Assunsao, R.B., and Mercadante, A.Z. 2003. Carotenoid and ascorbic acid composition from commercial products of cashwe apple (*Anacardium occidentale* L.). *J. Food Com. Anal*., **16**:647–654.

Astorg, P. 1997. Food carotenoids and cancer prevention: and overview of current research. *Trends Food Sci. Technol*., **8**:406:413.

Baloch, A.K., Bucle, K.A., and Edwards, R.A. 1977. Effect of processing variables on the quality of dehydrated carrot. I: Leaching losses and carotenoid content. *J. Food Technol*., **12**:285–293.

Barua, A.B., and Olson, J.A. 2001. Xanthophyll epoxides, unlike β-carotene monoepoxides, are not detectible absorbed by humans. *J. Nutr.*, **131**:3212–3215.

Bartlett, H., and Eperjesi, F. 2003. Age-related macular degeneration and nutritional supplementation: A review of randomised controlled trials. *Ophthal. Physiol. Optics J. British Coll. Ophthal. Optic. Optomet*., **23**:383–399.

Ben-Amotz, A., and Fishler, R. 1998. Analysis of carotenoids with enphasis on 9-*cis*-β-carotene in vegetables and fruits commonly consumed in Israel. *Food Chem*., **62**:515–520.

Berg, H. van den, and Vliet, T. van. 1998. Effect of simultaneous, single oral doses of β-carotene with lutein or lycopene on the β-carotene and retinyl ester responses in the triacylglycerol-rich lipoprotein fraction in men. *Am. J. Cli. Nutr.*, **68**:82–89.

Bidoli, E., Bosetti, C., et al. 2003. Micronutrients and laryngeal cancer risk in Italy and Switzerland: A case-control study. *Cancer Causes Control CCC*., **14**:477–484.

Blum, M. 2000. The hidden secrets of greens. *Int. Food Ingred*., **6**:7–8, 10.

Bobrowska, B., and Oledzka, R. 2002. Current views on the nutritional role of lutein and lycopene. *Bromatollogia I Chemia Toksykologiczna*, **35**:289–296.

Boilean, T.W., Boilean, A.C., and Erdman, J.W. Jr. 2002. Bioavailability of all-trans and cis-isomers of lycopene. *Eur. Biol. Med. Maywood*., **227**:914–919.

Bone, R.A., Landrum, J.T., Guerra, L.H., and Ruiz, C.A. 2003. Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans. *J. Nutr*., **133**:992–998.

Borel, P. 2003. Factors affecting intestinal absorption of highly lipophylic micronutrients (fat-soluble vitamins, carotenoids amd phytoesterols). *Clin. Chem. Lab. Med*., **41**:979–994.

Bowen, P.E., Espinosa, S.M., Hussain, E.A., and Stacewicz-Sapuntzakis, M. 2002. Esterification does not impair lutein bioavailability in humans does not impair lutein bioavailability in humans. *J. Nutr*., **132**:3668–3673.

Breithaupt, D.E. 2004. Simultaneous HPLC determination of carotenoids used as food coloring additives: Applicability of accelerated solvent extraction. *Food Chem*., **83**:449–456.

Breithaupt, D.E., Wirt, U., and Bamedi, A. 2002. Differentiation between lutein monoester regioisomers and detection of lutein diesters from marigold flowers (*Tagetes erecta* L.) and several fruits by liquid chromatography-mass spectroscopy. *J. Agric. Food Chem*., **50**:66–70.

Buratti, S., Pellegrini, N., Brenna, O.V., and Mannino, S. 2001. Rapid electrochemical method for the evaluation of the antioxidant power of some lipophilic food extracts. *J .Agric. Food Chem*., **49**:5136–5141.

Cano, P.M., and Marin, M.A. 1992. Pigment composition and colour of frozen and canned kiwi fruit slices. *J. Agric. Food Chem*., **40**:2141–2146.

Cardinault, N., Gorrand, J.M., Tyssandier, V., Grolier, P., Rock, E., and Borel, P. 2003. Short-term supplementation with lutein affects biomarkers of lutein status similarly in young and elderly subjects. *Experimental Gerontology*, **38**:573–582.

Challem, J. 2002. Putting antioxidants to use in functional formulas. *Functional Foods Nutraceuticals*, **(May)**:28–30, 32.

Chen, B.H. 1992. Studies on the stability of carotenoids in *Garland Chrysanthemun* (Ipomoea ssp.) as affected by microwave and conventional heating. *J. Food Protec*., **55**:296–300.

Chen, B.H., and Chen, Y.Y. 1992. Determination of carotenoids and chlorophylls in water convolvulus (*Ipomoea aquatica*) by liquid chromatography. *Food Chem*., **45**:129–134.

Chen, B.H., and Chen, Y.Y. 1993. Stability of chlorophylls and carotenoids in sweet potato leaves during microwave cooking. *J. Agric. Food Chem*., **41**:1315–1320.

Chen, B.H., Peng, H.Y., and Chen, E. 1995. Changes of carotenoids color, and vitamin A contents during processing of carrot juice. *J. Agric. Food Chem*., **43**:1912–1918.

Chen, B.H., and Tang, Y.C. 1998. Processing and stability of carotenoid powder from carrot pulp waste. *J. Agric. Food Chem*., **46**:2312–2318.

Chew, B.P., Brown, C.M., Park, J.S., and Mixter, P.F. 2003. Dietary lutein inhibits mouse mammary tumor growth by regulating angiogenesis and apoptosis. *Anticancer Res*., **23**:3333–3339.

Clark, J.P., and Bowen, P.E. 1998. Lutein esters having high bioavailability. Patent. WO9845241.

Cohen, J.H., Kristal, A.R., and Stanford, J.L. 2000. Fruit and vegetable intakes and prostate cancer risk. *J. Nat. Cancer Inst*., **92**:61–68.

Craft, N.E., and Soares, J.H. Jr. 1992. Relative solubility, stability and absrobtivity of lutein and β-carotene in organic solvents. *J. Agric. Food Chem*., **40**:431–434.

Dachtler, M., Kohler, K., and Alber, K. 1998. Reversed-phase high-performance liquid chromatographic identification of lutein and zeaxanthin steroisomers in bovine retina using a C30 bonded phase. *J. Chrom. B* **720**:211–216.

De La Cruz-Garcia, C., Gonzalez-Castro, et al. 1997. The effects of various culinary treatments on the pigment content of green beans *Phaseolus vulgaris*, L.). *Food Res. Int*., **30**:787–791.

Deli, J., Moln, R.P., Osz, E., Toth, G., and Zsila, F. 2004. Epimerization of lutein to 3′-epilutein in processed foods. *Bioorganic Med. Chem. Lett*., **14**:925–928.

Dietz-Bryant, J., McCord, J.D., Knigh-Unlu, L., and Erdman, J.W. Jr. 1992. Isolation and partial characterization of α- and β-carotene-containing carotenoprotein from carrot (*Daucus carota*) L. root chromoplasts. *J. Agric. Food Chem*., **40**:545–549.

De Sio, F., Servillo, L., Loiuduce, R., Laratta, B., and Castaldo, D. 2001. A chromatographic procedure for the determination of carotenoids and chlorophylls in vegetable products. *Acta Alimentaria*, **30**:395–405.

Dorgan, J.F., Sowell, A., Swanson, et al. 1998. Relationships of serum carotenoids, retinol, α-tocopherol, and selenium with breast cancer risk: Results from a prospective study in Columbia, Missouri (United States). *Cance rCauses Control CCC*, **9**:89–97.

Drews, M., Schonhof, I., and Krumbei, A. 1997. Content of minerals, vitamins, and sugras in iceberg lettuce (*Latuca sativa* var. *capitata* L) grown in the greenhouse dependent on cultivar and development stage. *Gartennauwissenschaft*, **62**:65–72.

Duncan, J.L., Aleman, T.S., Gardner, L.M., De-Castro, E., Marks, D.A., Emmons, J.M., Bieber, M.L., Steinberg, J.D. Bennett, J., Stone, E.M., MacDonald, I.M., Cideciyan, A.V., Maguire, M.G., and Jacobson, S.G. 2002. Macular pigment and lutein supplementation in choroideremia. *Experimental Eye Res*., **74**:371–381.

Dwyer, J.H., Navab, M., Dwyer, K.M., et al. 2001. Oxygenated carotenoid lutein and progression of early atherosclerosis. *Los Angeles Atherosclerosis Study Circulation*, **103**:2922–2927.

Edelenbos, M., Chritensen, L.P., and Grevsen, K. 2001. HPLC determination of chlorophyll and carotenoid pigments in processed green pea cultivars (*Pisum sativum*). *J. Agric. Food Chem*., **49**:4768–4774.

Emenhiser, C., Simunovic, N., Sandey, L.C., and Shwartz, S.J. 1996. Separation of geometrical carotenoid isomers in biological extracts using a polymeric C30 column in reversed-phase liquid chromatography. *J. Agric. Food Chem*., **44**:3887–3883.

Erdman, J.W. Jr 1999. Variable bioavailability of carotenoids from vegetables. *Am. J. Clin. Nut*., **70**:179–180.

European Parliament and Council Directive 94/36/EC 30.6. 1994. On colours for use in foodstuffs. *Official Journal of European Communities*, **237**:13–29.

Fan, J.J., Lee, H.C., and Yen, G.C. 1993. Quantification and identification of carotenoids and chlorophylls in raw and pickled mustard. *J. Chin. Agric. Chem. Soc*., **31**:765–775.

Flair-Flow Europe. 2001. Beyond fruit and vegetables: Antioxidants. *Flair-Flow Reports. FFE* 436/01/CG18, 1p.

Ferreira, A.L., Yeum, K.J., Lin, C., Smith, D., Kinsky, N.E., Wang, W.D., and Russell, R.M. 2000. Tissue distribution of lycopene in ferrets and rats after lycopene supplementation. *J. Nutr*., **130**:1256–1260.

Foster, B., Pogson, B.J., and Osmond, C.B. 2003. Occurrence of the lutein-epoxide cycle in mistletoes of the Loranthaceae and Viscaceae. *Planta*, **217**:868–879.

Fung, T.T., Spiegelman, D., Egan K.M., Giovannucci, E., Hunter, D.J., and Willett, W.C. 2003. Vitamin and carotenoid intake and risk of cancer. *Int. J. Cancer*, **103**:110–115.

Gandul-Rojas, B., and Minguez-Mosquera, M.I. 1996. Chlorophyll and carotenoid composition in virgin oils from various spanish olive varieties. *J. Food Sci. Agric*., **72**:31–39.

Gandul-Rojas, B., Roca, L., Cepero M., and Minguez-Mosquera, M.I. 1999. Chlorophyll and carotenoid patterns in olive fruits, *Olea europeaa* Cv. Arbequina. *J. Agric. Food Chem*., **47**:2207–2212.

Gellermann, W., Ermakov, I.V., Ermakova, M.R., McClane, R.W., Zhao, D.Y., and Bernstein, P.S. 2002. In vivo resonant Raman measurement of macular carotenoid pigments in the young and the aging human retina. *J. Opt. Soc. Am*. **19**:1172–1186.

Granado, F., Olmedilla, B., Blanco, I., and Rojas-Hidalgo, E. 1992. Carotenoid composition in raw and cooked Spanish vegetables. *J. Agric. Food Chem*., **40**:2135–2140.

Granado, F., Olmedilla, B., Gil-Martínez, E., and Blanco, I. 2001. A fast, reliable and low-cost saponification protocol for analysis of carotenoids in vegetables. *J. Food Comp. Anal*. **14**:479–489.

Grela, E.R., Jensen, S.K., and Jakobsen, K. 1999. Fatty acid composition and content of tocopherols and carotenoids in raw and extruded grass pea (*Latyrus sativus* L). *J. Sci. Food Agric*., **79**:2075–2078.

Guedes de Pinho, P., Silva Ferreira, A.C., Mendes Pinto, M., Gomez Benitez, J., and Hogg, T.A. 2001. Determination of carotenoid profiles in grapes, musts, and fortified wines from douro varieties of *vitis vinifera*. *J. Agric.Food Chem*., **49**:5484–5488.

Guil-Guerrero J.L., Rebollos-Fuentes M.M., and Torija Isasa M.E. 2003. Fatty acids and carotenoids from stinging nettle (*Urtica dioica* L.). *J. Food Comp. Anal*., **16**:111–119.

Haila, K.M., Lievonen, S.M., and Heinonen, M.I. 1996. Effects of lutein, lycopen, annatto and γ-tocopherol on autooxidation of triglycerides. *J. Agric. Food Chem*. **44**:2096–2100.

Handelman, G.J. 2001. The evolving role of carotenoids in human biochemistry. *Nutrition*, **17**:818–822.

Hamano, P.S., and Mercadante, A.Z. 2001. Composition of carotenoids from comemercial products of Caja (*Spondias lutea*). *J. Food Comp. Anal*., **14**:335–343.

Hart, D.J., and Scott, K.J. 1995. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content in vegetables and fruits commed in the UK. *Food Chem*., **54**:101–11.

Heinonen, M.I. 1990. Carotenoids and provitamin A activity of carrot (*Daucus carota*) cultivars. *J. Agric. Food Chem*., **38**:609–612.

Henry, L.K., Catignari, G .L., and Shwartz, S.J. 1998. Oxidative degradation kinetics of lycopene, lutein, and 9-cis and all-trans-β-carotene. *J. Am. Oil Chem. Soc*., **75**:823–829.

Hentschel, V., Kranl, K., Hollman, J., Lindhauer, M.G., Böhm, V., and Bitsch, R. 2002. Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. *J. Agric. Food Chem*., **50**:6663–6668.

Holden, J.M., Eldrigge, A.L., Beecher, G.R., et al. 1999. Carotenoid content of U.S. foods: An update of the database. *J. Food Comp. Anal*., **12**:129–196.

Hof, K.H., Van-het, Brouwer, I.A., West, C.E., et al. 1999a. Bioavailability of lutein from vegetables is 5 times higher than that of β-carotene. *Am. J. Clin. Nutr*., **70**:261–268.

Hof, K.H., Van-het, Tijburg, L.B.M., Pietrzik, K., and Weststrate, J.A. 1999b. Influence of feeding different vegetables on plasma levels of carotenoids, folate and vitamin C. Effect of disruption of the vegetable matrix. *Brit. J. Nutr*., **82**:203–212.

Huck, C.W., Popp, M., Scherz, H., and Bonn, G.K. 2000. Development and evaluation of a new method for the determination of the carotenoid content in selected vegetables by HPLC and HPLC-MS-MS. *J. Chrom. Sci*., **38**:441–449.

Hulten, K., Van-Kappel, A.L., Winkvist, A., Kaaks, R., Hallmans, G., Lenner, P., and Riboli, E. 2003. Carotenoids, α-tocopherols, and retinol in plasma and breast cancer risk in northern Sweden. *Cancer Causes Control CCC*, **12**:529–537.

Humphries, J.M., and Khachik, F. 2003. Distribution of lutein, zeaxanthin and related geometrical isomers in fruit, vegetables, wheat and pasta products. *J. Agric. Food Chem*. **51**:1322–1327.

Hyoungshin, P., Kreumen, S.S., Cuttriss, A.J., DellaPenna, D., and Pogson, B.J. 2002. Identification of carotenoid isomerase porvides insight into carotenoid biosyntehsis, prolamellar body formation and photomorphogenesis. *Plant Cell*, **14**:321–332

Jewell, V.V.C., Mayes, C.B.D., Tubman, T.R.J., Northrp-Clewes, C.A., and Thunham, D.I. 2004. A comparison of lutein and zeaxanthin concentration in formula and human milk samples from Northern Irland mothers. *Eur. J. Clin .Nutr.*, **58**:90–97.

Johnson, E.J. 2004. A biological role of lutein. *Food Rev. Int.*, **20**:1–16.

Kelm, M.A., Flanagan, V.P., Pawlosky, R.J., Novotny, J.A., Clevidence, B.A., and Britz, S.J. 2001. Quantitative determination of ^{13}C-labelled and endogenous β-carotene, lutein and vitamin A in human plasma. *Lipids*, **36**:1277–1282.

Khachik, F, Beecher, G.R., Goli, M.B., and Lusby, W.R. 1992a. Separation and quantification of carotenoids in foods. *Meth. Enzy.*, **213**:867–877.

Khachik, F., Goli, M.B., Beecher, G.R., et al. 1992b. Effect of food preparation on qualitative distribution of major carotenoids constituents of tomatoes and several green vegetables. *J. Agric. Food Chem.*, **40**:390–398.

Khachik, F., Bernstein, P.S., and Garland, D.L. 1997a. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Inv. Ophtal. Vis. Sci.*, **38**:1802–1811.

Khachik, F., Spangler, C.J., and Smith, J.C. Jr. 1997b. Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. *Anal. Chem.*, **69**:1873–1881.

Khachik, F., de-Moura. F.F., Zhao, D.Y., Aebischer, C.P., and Bernstein, P.S. 2002. Transformations of selected carotenoids in plasma, liver, and ocular tissues of humans and in nonprimate animal models. *Inv. Ophthal. Vis. Sci.*, **43**:3383–3392.

Kimura, M., Rodriguez-Amaya, D.B., and Godoy, H.T. 1990. Assessment of the saponification setp in the quantitative determination of carotenoids and provitamins A. *Food Chem.*, **35**:187–195.

Kimura, M., and Rodriguez-Amaya, D.B. 2003. Carotenoid composition of hydorponic leafy vegetables. *J. Agric. Food Chem.*, **51**:2603–2607.

Kinsky, N.I., Landrum, J.T., and Bone, R.A. 2003. Biological mechanisms of the protective role of lutein and zeaxanthin in the eye. *An. Rev. Nutrit.*, **23**:171–201.

Koning, E.J., and Roomans, H.H.S. 1997. Evaluation and validation of an LC method for the analysis of carotenoids in vegetables and fruits. *Food Chem.*, **59**:599–603.

Kopas-Lane, L.M., and Warthesen, J.J. 1995. Carotenoid photostability in raw spinach and carrots during cold storage. *J. Food Sci.*, **60**:773–776.

Kopsell, D.E., Kopsell, D.A., Randle, W.M., Coolong, T.W., Sams, C.E., and Curran-Celentano, J. 2003. Kale carotenoids remain stable while flavor compounds respond to changes in sulfur fertility. *J. Agric. Food Chem.* **51**:5319–5325.

Krinsky, N.L., Landrum, J.T., and Bone, R.A. 2003. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Ann. Rev. Nutr.*, **23**:171–201.

Kruger, C.L., Murphy, M., DeFreitas, Z., Pfannkuch, F., and Heimbach, J. 2002. An innovative approach to the determination of safety for a dietary ingredient derived from a new source: Case study using a crystalline lutein product. *Food Chem.Tox.*, **40**:1535–1549.

Kurilich, A.C., Britz, S.J., Clevidence, B.A., and Novotny, J.A. 2003. Isotopic labeling and LC-APCI-MS quantification for investigating absorption of carotenoids and phylloquinone from kale (*Brassica oleracea*). *J. Agric. Food Chem.* **51**:4877–4883.

Landrum, J.T., and Bone, R.A. 2001. Lutein, zeaxanthin, and the macular pigment. *Arch. Biochem. Biophys.* 385:28–40.

Le-Marchand, L., Hankin, J.H., Kolonel, L.N., et al. 1993. Intake of specific carotenoids and lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **2**:183–187.

Lee, H.S., and Min, D.B. 1990. Effects of quenching mechanisms, and kinetics of carotenoids in chlorophyll-sensitized photooxidation of soybean oil. *J. Agric. Food Chem.*, **38**:1630–1634.

Lee, H.S., and Coates, G.A. 2003. Effect of thermal pasteurisation on Valencia orange juice color and pigments. *Lebesm. Wiss. Technol.*, **36**:153–156.

Lienau, A., Glaser, T., Tang, G.W., Dolnilowski, G.C., Grusak, M.A., and Albert, K. 2003. Bioavailability of lutein in human from intrinsically labeled vegetables determined by LC-APCI-MS. *J. Nutrit. Biochem.***14**:663–670.

Lopez-Hernandez, J., Váquez-Oderiz, L., Vázquez-Blanco, M.E., Romero-Rodríguez, A., and Simal-Lozano, J. 1993. HPLC determination of major pigments in the bean *Phaseolus vulgaris*. *J. Agric. Food Chem.*, **41**:1613–1651.

Lopez-Hernandez, J., Oruña-Concha. M.J., Simal-Lozano, J., Vázquez-Blanco, M.E., and González-Castro, M.J. 1996. Chemical composition of Padron peppers (*Capsicum annuum* L.) grown in Galicia (N.W. Spain). *Food Chem.*, **57**:557–559.

Maestro-Duran, R., and Borja-Padilla, R. 1993. Antioxidant activity of vitamin C, E, and provitamin A. *Grasas Aceites*, **44**:107–111.

Majchrzak, D., Frank, U., and Elmadfa, I. 2000. Carotenoid profile and retinal content of baby food. *Eur. Food Res. Technol.* **210**:407–413.

Mangels, A.R., Holden, J.M., Beecher, G.R., et al. 1993 Carotenoid content of fruits and vegetables: An evaluation of analytical data. *J. Am. Diet. Ass.*, **93**:284–296.

Matus, Z., Deli, J., and Szabolcx, J. 1991. Carotenoid composition of yellow pepper during ripening: Isolation of b-cryptoxanthin 5,6-epoxide. *J. Agric. Food Chem.*, **39**:1907–1914.

McGhie, T.K., and Ainge, G.D. 2002. Color in fruit of Genus *Actinidia*: Carotenoid and chlorophyll composition. *J. Agric. Food Chem.* **50**:117–121.

Mecocci, P., Polidori, M.C., Cherubini, A., et al. 2002. Lymphocyte oxidative DNA damage and plasma antioxidants in Alzheimer disease. *Arch. Neurol.*, **59**:794–798.

Melendez-Martinez, A.J., Vicario, I.M., and Heredia, F.G. 2003a. Application of tristimulus colorimetry to estimate the carotenoids content in ultrafrozen orange juices *J. Agric. Food Chem.*, **51**:4219–4224.

Melendez-Martinez, A.J., Vicario, I.M., and Heredia, F.G. 2003b. A routine high-performance liquid chromatography method for carotenoid determination in ultrafrozen orange juice. *J. Agric. Food Chem..*, **51**:7266–7270.

Mercadante, A.Z., and Rodríguez-Amaya, D.B. 1991. Carotenoid composition of a leafy vegetable in relation to some agricultural variables. *J. Agric. Food Chem.*, **39**:1094–1097.

Mercadante, A.Z., and Rodríguez-Amaya, D.B. 1997. HPLC and mass spectophotometry analysis of carotenoids from mango. *J. Agric. Food Chem.*, **45**:120–123.

Michaud, D.S., Feskanich, D., Rimm E.B., et al. 2000. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am. J. Clin. Nutr.*, **72**:990–997.

Micozzi, M.S., Beecher, G.R., Taylor, P.R., and Khachik, F. 1990. Carotenoid analyses of selected raw and cooked foods associated with a lower risk for cancer. *J. Nat. Cancer Inst.*, **82**:282–285.

Miluca J.C.G., Juarros, J.L., Delas Rivas, J., Ibarrondo, J., and Gomez, R. 1991. Isolation of a yellow carotenoprotein from carrot. *Phytochem.*, **30**:1535–1539.

Minguez-Mosquera, M.I., and Gallardo-Guerrero, L. 1995. Anomalous transformation of chloroplastic pigments in gordal variety olives during processing for table olives. *J. Food Protec.*, **58**:1241–1248.

Minguez-Mosquera, M.I., and Hornero-Méndez, D. 1994. Formation and transformation of pigments during the fruti ripening of *Capsicum annuum* Cv. Bola and Agridulce. *J. Agric. Food Chem.* **42**:38–44.

Moeller, S.M., Jaques, P.F., and Blumberg, J.B. 2000. The potencial role of dietary xanthophylls in cataract and age-related macular degeneration. *J. Am. Coll. Nutr.*, **19**:522S–527S.

Molnar, J., Gyemant, N., Mucsi, I., et al. 2004. Modulation of multidrug resistance and apoptosis of cancer cells by selected carotenoids. *In Vivo*, **18**:237–244.

Moma, M., Terao, J., Ito, M., Saito, M., and Chikuni, K. 1994. Carotenoid components in soybean seeds varying with sedd color and maturation stage. *Biosci. Biotechnol. Biochem.* **58**:926–930.

Monreal, M., Ancos de, B., and Cano, P. 1999. Influence of critical storage temperatures on degradative pathways of pigments in green beans (*Phaseolus vulgaris* Cvs. Perona and Boby). *J. Agric. Food Chem.*, **47**:19–24.

Moros, E.E., Darnoko, D., Cheryan, M., et al. 2002. Analysis of xantophylls in corn by HPLC. *J. Agric. Food Chem.*, **50**:5787–5790.

Mozaffariech, M., Sacus, S., and Wedrich, A. 2003. The role of carotenoids, lutein and zeaxanthin, in protecting against age-related macula degeneration: A review based on controversial evidence. *Nutr. J*., **2**:20–24.

Muhlhofer, A., Buhler-Ritter, B., Frank, J., et al. 2003. Carotenoids are decreased in biopsies from colorectal adenomas. *Clin. Nutr.*, **22**:65–70.

Müller, H. 1997. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Z. Lebensm. Unters Forsch. A*, **204**:88–94.

Munne-Bosch, S., and Penuelas, J. 2003. Photo- and antioxidative protection during summer leaf senescence in *Pistacia lentiscus* L. grown under Mediterranean field conditions. *Annals Botany*, **92**:385–391.

Murkovic, M., Gams, K., Draxl, S., and Pfannhauser, W. 2000. Carotenoids in Austrian vegetables. *Ernaehrung*, **24**:116–118.

Murkovic, M., Mülleder, U., and Neunteuff, H. 2002. Carotenoid content in different varieties of pumpkins. *J. Food Comp. Anal*., **15**:633–638.

Narisawa, T., Fukaura, Y., Hasebe, M., et al. 1996. Inhibitory effects of natural carotenoids, α-carotene, β-carotene, lycopene and lutein, on colonic aberrant crypt foci formation in rats. *Cancer Lett*. **107**:137–142.

Nesterenko, S., and Sink, K.C. 2003. Carotenoid profiles of potato breeding lines and selected cultivars. *Hortoscience*, **38**:1173–1177.

Nicolle, C., Cardinalult, N., Aprikian, O., et al. 2003. Effect of carrot intake on cholesterol metabolism and on antioxidant status in cholesterol rat. *Eur. J. Nutr*., **45**:254–261.

Ogunlesy, A.T., and Lee, C.Y. 1979. Effect of thermal processing on the stereoisomerization of major carotenoids and vitamin A value of carrots. *Food Chem*., **4**:311–318.

Olmedilla, B., Granado, F., Blanco, I., and Vaquero, M. 2003. Lutein, but not α-tocopherol, supplementation improves visual function in patients with age-related cataracts: A 2-y double-blind, placebo-controlled pilot study. *Nutr. Burbank*, **19**:21–24.

O'Neil C.A., and Schwart, S.J. 1995. Photoisomerization of β-carotene by photosensibilization of chlorophyll with chlorophyll derivatives as sensitizers. *J. Agric. Food Chem*., **43**:631–635.

Pelz, R., Schmidt-Faber, B., and Heseker, H. 1998. Carotenoid intake in the German National Food Consumption Survey. *Zeitsch. Ernaehruns*, **37**:319–327.

Pool-Zobel, B.L., Bub, A., Mueller, H., Wollowski, I., and Rechkemmer, G. 1997. Consumption of vegetables reduces genetic damage in humans. First results of a human intervention trial with carotenoid-rich foods. *Carcinogenesis*, **18**:1847–1850.

Psomiadou, E., and Tsimidou, M. 2001. Pigment in Greek origin olive oils: Occurrence and levels. *J. Sci. Food Agric*., **81**:640–647.

Ravichandra, R. 2002. Carotenoid composition, distribution and degradation to flavour volatiles during black tea manufacture and the effect of carotenoid supplementation on tea quality and aroma. *Food Chem*., **78**:23–28.

Razungles, A., Babic, I., Sapins, J.C., and Bayonove, C. 1996. Particular behaviour of epoxy xanthophylls during veraison and maturation of grape. *J. Agric. Food Chem*., **44**:3821–3825.

Riso, P., Brusamolino, A., Ciappellano, S., and Porrini, M. 2003. Comparison of lutein bioavailability from vegetables and supplement. *Int. J. Vitam. Nutr. Res*., **73**:201–205.

Rinaldi, P., Polidori, M.C., Metastasio, A., et al. 2003. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. *Neurobiol. Aging*., **24**:915–919.

Rodriguez-Amaya, D.B., and Kimura, M. 1989. Carotenoids and valor vitamin A in Caja (*Spondias lutea*) *Cien. Tecnol. Alim*., **9**:148–162.

Rohan, T.E., Jain, M., Howe, G.R., and Miller, A.B. 2002. A cohort study of dietary carotenoids and lung cancer risk in women (Canada). *Cancer Causes Control CCC*, **13**:231–237.

Sá, M.D. de, and Rodríguez-Amaya, D.B. 2003. Carotenoid composition of cooked green vegetables from restaurants. *Food Chem*., **83**:595–600.

Scott, K.J., Finglas, P.M., Seale, R., et al. 1996. Interlaboratory studies of HPLC procedures for the analysis of carotenoids in foods. *Food Chem*., **57**:85–90.

Shi, X.M., and Chen, F. 1997. Stability of lutein under various storage conditions. *Nahrung*, **41**:38–41.

Sies, H., and Stahl, W. 2003. Non-nutritive bioactive constituents of plants: Lycopene, lutein and zeaxanthin. *Inter. J. Vitam. Nutri. Res*., **73**:95–100.

Siemensma, A.D. 1997. Functional carotenoids. II. *Int. Food Ingred*., **2**:15–16, 18, 20

Southon, S. 2000. Increased fruit and vegetable consumption within the EU: Potential health benefits. *Food Res. Int*., **33**:211–217.

Takagi, S., Kishi, F., Nakajima, K., Kimura, Y., and Nakano, M. 1990. A seasonal variation of carotenoid composition in green leaves and effect of environmental factors on it. *Scientific Reports of the Faculty of Agriculture, Okayama University*, **75**:1–7.

Tang, Y.C., and Chen, B.H. 2000. Pigment change of freeze-dried carotenoid powder during storage. *Food Chem*., **69**:11–17.

Taylor, S., Baker, D., Owour, P., Orchard, J., Othiens, C., and Gay, C. 1992. A model for predicting black tea quality from the carotenoid and chlorophyll composition of frehs green tea leaf. *J. Sci. Food Agric*., **58**:185–191.

Tee, E.S., and Lim, C.L. 1991. Carotenoid composition and content of Malaysian vegetables and fruits by the AOAC and HPLC methods. *Food Chem*., **41**:309–339.

Tenorio, M.D., Villanueva, M.J., and Sagardoy, M. 2004. Changes in carotenoids and chlorophylls in fresh green asparagus (*Asparagus officinalis* L) stored under modified athmosphere packaging. *J. Sci. Food Agric*. **84**:357–365.

Thuermann, P.A., Steffen, J., Zwernemann, C., et al. 2002. Plasma concentration response to drinks containing β-carotene as carrot juice or formulated as a water dispersible powder. *Eur. J. Nutr.*, **41**:228–235.

Tkalec, M., Perinic, Z., Vidakovic-Cifrek, Z., and Regula, I. 2003. Physiological response of duckweed (*Lemna minor* L.) to herbicide norflurazon. *Period. Biol*., **105**:269–274.

Toniolo, P., Van-Kappel, A.L., Akhmedkhanov, A., et al. 2001. Serum carotenoids and breast cancer. *Am. J. Epid*., **153**:1142–1147.

Tyssandier, V., Reboul, E. Dumas, J.F., et al. 2003. Processing of vegetable-borne carotenoids in the human stomach and duodenum. *Am. J. Physiol. Gastrointest. Liver Physiol*., **284**:G913–G923.

Tzeng, M.S., Yang, F.L., Wang-Hsu, G.S., and Chen, B.H. 2004. Determination of major carotenoids in human serum by liquid chromatography. *J. Food Drugs Anal*., **12**:79.83.

Updike, A.A., and Schwartz, S.J. 2003. Thermal processing of vegetables increases cis isomers of lutein and zeaxantin. *J. Agric. Food Chem*., **51**:6184–6190.

Weckel, K.G., Santos, B., Hernan, E., Laferrriere, L., and Gabelman, W.H. 1962. Carotene components of frozen and processed carrots. *Food Technol*., **16**:91–94.

Wenzel, A.J., Fuld, K., and Stringham, J.M. 2003. Light exposure and macular pigment optical density. *Invest. Ophthal. Vis. Sci*., **44**:306–309.

Wills, R.B.H., and Rangga, A. 1996. Determination of carotenoids in Chinese vegetables. *Food Chem*., **56**:451–455.

Wright, M.E., Mayne, S.T., Swanson, C.A., Sinha, R., and Alavanja, M.C. 2003. Dietary carotenoids, vegetables, and lung cancer risk in women: The Missouri women's health study (United States). *Cancer Causes Control CCC*, **14**:85–96.

Yen, W.J., and Chen, B.H. 1995. Isolation of xanthophylls from Taiwanese orange peels and their effects on the oxidation stability of soybean oil. *Food Chem*., **53**:417–425.

Yen, H.C., and Hsu, Y.T. 2004. Impurities from polypropylen microcentrifuge tubes as a potential source of interference in simultaneous analysis of multiple lipid-soluble antioxidant by HPLC with electrochemical detection. *Clinical Chem. Lab. Med*., **42**:390–395.

Yokota, T., Etoh, H., Oshima, S., Hayakawa, K., and Ishiguro, Y. 2003. Oxygenated lycopene and dehydrated lutein in tomato puree. *Biosc. Biotechnol. Biochem*., **67**:2644–2647.

Yuang, J.P., and Chen, F. 1999. Hydrolysis kinetics of astaxanthin esters and stability of astaxanthin of *Haematococcus pluvialis* during saponification. *J. Agric. Food Chem*., **47**:31–35.

Zaripheh, S., and Erdman, J.W. 2002. Factors that influence the bioavailability of xanthopylls. *J. Nutr*., **132**:531S–534S.

Zhao, D.Y., Wintch, S.W., Ermakov, I.V., Gellermann, W., and Bernstein, P.S. 2003. Resonance Raman measurement of macular carotenoids in retinal, choroidal, and macular dystrophies. *Arch. Ophthal*., **121**:967–972.

Zhang, S., Tang, G., Russell, R.M., Mayzel, K.A., Stampfer, M.J., Willett, W.C., and Hunter, D.J. 1997. Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects. *Am. J. Clin. Nutr.*, **66**:626–632.

Zhang, S., Hunter, D.J., Forman, M.R., Rosner, et al. 1999. Dietary carotenoids and vitamins A, C, and E and risk of breast cancer. *J. Nat. Cancer Inst*., **91**:547–556.

Zhou, J.R., Gugger, E.T., Erdman, E., and J.W. Jr. 1994. Isolation and partial characterization of an 18 kDa carotenoid-protein complex from carrot roots. *J. Agric. Food Chem*., **42**:2386–2390.