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Carotenoid Extraction from Plants Using a Novel, Environmentally Friendly Solvent

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Few environmentally friendly solvents are available to extract carotenoids for use in foods. The most effective known solvents are products of the petroleum industry and toxic for human consumption. Yet carotenoid extracts are desirable for use in dietary supplements and as additives to enhance the health benefits of processed foods. Ethyl lactate is an excellent solvent to extract both *trans*- and *cis*-lycopene isomers from dried tomato powder, the extraction efficiency of which is enhanced by the addition of the antioxidants α-tocopherol and α-lipoic acid, both of which are known to benefit human health. It is also useful to extract lutein and β-carotene from dried powders prepared from white corn and carrots. Because of its low flammability and its origin as a byproduct of the corn and soybean industries, it is more advantageous than ethyl acetate, which is a petroleum product.

KEYWORDS: Carotenoids; extraction; ethyl lactate; lycopene; *trans*-lycopene; *cis*-lycopene; *tetra-cis* lycopene; β-carotene; lutein; isomerization; environmentally friendly

INTRODUCTION

Carotenoids are natural pigments that occur in bacteria, plants, fungi, and animals, comprising a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls). They cannot be synthesized by humans; hence, they must be obtained from dietary sources. For humans, the most important sources for carotenoids are plants. Carotenoids found in fruits and vegetables in relatively high concentrations are lycopene, β-carotene, lutein, zeaxanthin, β-cryptoxanthin, and α-carotene (*1*).

Carotenoid extracts are desirable for use in dietary supplements and as additions to processed foods. Carotenoids are important to human health. The essential role of β-carotene as the dietary source of vitamin A has been known for many years. More recently, the protective effects of carotenoids against serious disorders such as cancer, heart disease, and degenerative eye disease have been recognized (*1*). Many health benefits are attributed to the consumption of carotenoids and in particular to lycopene: (1) Lycopene is an important antioxidant and free radical scavenger. Free radicals can cause damage to both the structure and function of cell membranes, DNA, and proteins. This damage has been linked to the onset of many degenerative diseases such as cancer, atherosclerosis, cataracts, and age-related macular degeneration, as well as to premature aging. Lycopene is the most powerful biological antioxidant (*2*). The free radical quenching constant of lycopene was found to be more than twice that of β-carotene. (2) Lycopene is incorporated into lipoproteins, where it acts to decrease the oxidation of cholesterol, thereby helping to prevent vascular damage (*3, 4*). (3) Lycopene in the blood has been shown to be inversely proportional to the incidence of prostate tumors (*5*). (4) Research shows that carotenoids may provide protection against damage from ultraviolet radiation from sunlight, the major cause of sunburn, photodamage, and nonmelanoma skin cancer (*6*−*8*).

Lycopene is typically the carotenoid consumed in greatest amounts in Western diets. More than 85% of the lycopene in North American diets comes from tomato products, and more than 20 carotenoid pigments have been identified and quantified in tomatoes (*9*). Concentrations of lycopene in common tomatoes range from 20 to 200 ppm, on average 50 ppm on a fresh weight basis (*1*). Low amounts of other carotenoids such as α-, β-, γ-, and ε-carotenes, phytoene, phytofluene, neurosporene, and lutein are also present in tomatoes. Lycopene provides the richness and bright red color to the tomato, making it commercially important as a natural pigment. Processed foods are frequently fortified with carotenoids such as lycopene to increase nutritive value and/or enhance attractiveness.

Lycopene in nature occurs primarily in the trans-isomeric form. However, when tomatoes are processed, some of the lycopene is isomerized into cis forms. The Tangerine tomato contains almost 100% *tetra-cis*-lycopene (*10, 11*). In plasma, *cis*-lycopene isomers are predominant (*12*), apparently being isomerized in vivo (*13*). *cis*-Lycopene isomers are also found to be more bioavailable than the natural trans form (*12, 14*−*18*). Therefore, there is significant interest in methods to extract and measure the stereoisomeric forms present in samples.

Carotenoids are generally extracted from plant material, using organic solvents, such as hexane or methylene chloride (*19, 20*) [or by use of supercritical fluid extraction (*21*)], because of their hydrophobic nature and limited solubility in water. Inadequate extraction of many of the components may result when solvents

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are used to extract carotenoids, because extraction efficiency is determined by the structure of the individual carotenoids present. Carotenoids having hydroxyl groups, such as xanthophylls, are more soluble in hydrophilic solvents, and *cis*-lycopene isomers are also more hydrophilic than the straight-chain trans isomer (*22*).

Our research has been involved in carotenoids found in fruits and vegetables, notably tomato. Those of particular interest have been lycopene, β-carotene, phytoene, and phytofluene. Because we are working with products that are ingested by humans, the extraction of these carotenoids for use in food products has been of great interest. Commonly used solvents in most extraction processes have adverse effects on human health and cannot be removed completely. These solvents, therefore, are not acceptable. Most nonpolar solvents that have high extraction efficiencies are considered to be toxic.

Solvents used in various U.S. patents for extracting lycopene include hexane, ethyl acetate, methylene chloride, methanol, ethanol, propanol, and acetone (*23*−*25*). Currently, ethyl acetate is most commonly used for extracting carotenoids to be used in food products. Ethyl acetate, however, extracts β-carotene and lutein more effectively than the polar solvents mentioned, but is less efficient in extracting the all-trans isomer of lycopene. It is also not considered to be environmentally friendly and is highly flammable (explosive). Although it can be produced by a reaction of ethanol and acetic acid, its primary producer is the petroleum industry.

We selected ethyl lactate as a solvent for carotenoids because of its solubility in both aqueous and hydrocarbon solvents. It is an environmentally friendly solvent produced from fermentation of carbohydrate feedstocks available from the corn and soybean industries. The U.S. Food and Drug Administration has approved its use in food products. Ethyl lactate has a relatively high flashpoint and is colorless, environmentally benign, and completely biodegradable into CO_2 and water. Because of its miscibility with both hydrophilic and hydrophobic compounds, we considered it as a possible solvent for a diverse range of carotenoids and their stereoisomeric forms. Our purpose was to determine conditions for maximal extraction of commonly occurring carotenoids and to test the efficacy of added antioxidants in protecting them against oxidative degradation during the extraction process as they are separated from vegetable matter and exposed to light and O_2.

MATERIALS AND METHODS

Plant Material and Sample Preparation. Red tomatoes (*Solanum lycopersicum* cv. VFNT Cherry) were harvested from our rooftop greenhouse. Heirloom Tangerine tomatoes were purchased during the months of August through October from River Dog Farm (Guinda, CA). Frozen white corn and fresh carrots were purchased from a local supermarket. All vegetables were coarsely chopped and lyophilized. The resultant dried material was ground into a fine powder, using a mortar, pestle, and liquid N_2, and passed through a no. 60 mesh screen (250 mm). The solids content of the powder was 95−98%. Tomato homogenate that had been treated to obtain a high *cis*-lycopene isomer content according to a proprietary method was lyophilized and treated similarly to the other vegetable material.

Chemicals. Dichloromethane, 99.9% HPLC-grade, DL-α-lipoic acid, anhydrous tetrahydrofuran, and, for standard solutions, β-carotene (type IV from carrots), mixed isomer carotene (from carrots), and lutein (from alfalfa) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Ethyl lactate, DL-α-tocopherol, ethanol (EtOH), HPLC-grade methanol (MeOH), methyl-*tert*-butyl ether (MTBE), and ethyl acetate (EtOAc) were purchased from Fisher Scientific (Fair Lawn, NJ). Lycopene for standard solutions was extracted and purified from berries of autumn olive (*Elaeagnus umbellate* Thunberg) plants, which were a gift from Beverly A. Clevidence (Beltsville Human Nutrition Research Center, USDA, ARS, Beltsville, MD).

Carotenoid Extraction and Analysis. Dry weights of vegetable samples were determined using a model AVC-80 microwave moisture/solids analyzer (CEM Corp, Mathews, NC). Samples of tissue (2−4 g) were placed between two tared fiberglass pads and heated at 50% power for 4.5 min, which was sufficient time for water loss from the sample to be complete. Moisture content (or percent solids) was determined by difference in weight after drying.

Three replicates of lyophilized vegetable powder, each weighing 0.25−1.0 g, were placed in 20 mL glass vials along with antioxidant where specified, and 10 mL of ethyl lactate or ethyl lactate/ethanol mixture (0−100%) was added. In subdued light, vials were capped, mixed vigorously with the aid of a vortex mixer, and placed in a temperature-controlled water bath. After specified times, 100 μL of mixture was removed, passed through a 0.2 μm nylon filter, and injected directly into the HPLC, or the absorbance peak of the carotenoid of interest was measured, using a Beckman DU 640 spectrophotometer. Absorbance of *trans*-lycopene was measured at 474, that of *tetra-cis*-lycopene at 427, and that of β-carotene and lutein at 450 nm. For comparison, carotenoids were also extracted from vegetable tissues (in the case of tomatoes, the whole fruit minus the seeds) using various specified organic solvents according to the method described by Ishida et al. with modifications to maximize extraction of both polar and nonpolar species (*22*).

HPLC analysis was carried out using a Waters HPLC equipped with a model 2690 separations module, a model 996 photodiode array detector, and a C_{30} carotenoid column (4.6 × 240 mm i.d., 3 μm particle diameter, polymeric) (Waters Corp., Milford MA). Carotenoids were analyzed essentially according the method described by Ishida and Chapman (*26*), except that a mobile phase of MTBE/MeOH/EtOAc (45:40:15) was used. *cis*- and *trans*-lycopene isomers were identified by their absorption spectra and retention times (*26*, *27*). Lycopene used for standard solutions (see above, Chemicals) was analyzed by HPLC and found to be 97% *trans*-lycopene. No standards were available for the various *cis*-lycopene stereoisomers. Only *tetra-cis*-lycopene could be identified because of its distinctive absorption spectrum, but other specific cis isomers of lycopene could not be distinguished from one another. However, because we used a similar mobile phase and the same column as those used by Fröhlich et al. (*28*), we can assume that the order of elution of the *cis*-lycopene isomers was also similar. Both trans and cis isomers of lycopene were quantified by the *all-trans*-lycopene calibration as in other studies (*19*, *27*, *28*). *cis*-Lycopene isomers, other than *tetra-cis*-lycopene, were grouped together for quantification.

RESULTS AND DISCUSSION

As examples of carotenoid-containing vegetables, we chose species that are high in the particular carotenoids of interest (**Figure 1**): carrots for their high β-carotene content, white corn for its almost exclusive carotenoid content of lutein, red tomatoes for their high *trans*-lycopene isomer content and widespread consumption in Western diets, and Tangerine tomatoes for their high *tetra-cis*-lycopene isomer content. Differences in physical properties of these carotenoids and their isomers change their solubilities in solvents. Therefore, efficient extraction of these compounds requires changes in solvents/solvent ratios.

Optimal Extraction Time and Temperature. Data on the amounts of carotenoids extracted at 30, 45, and 60 °C for periods up to 5 h of incubation and measured, using a spectrophotometer at appropriate wavelengths, are depicted in **Figure 2**. Lutein is less stable at higher temperatures, so the concentrations extracted from white corn decrease above 30 °C (**Figure 2A**). However, at 2 h, the amount extracted begins to plateau so that increasing extraction time is not warranted. On the other hand, we found the optimal temperature and time for extracting β-carotene from carrot to be 60 °C and 0.5 h (**Figure 2B**). β-Carotene is degraded at this temperature after 0.5 h.

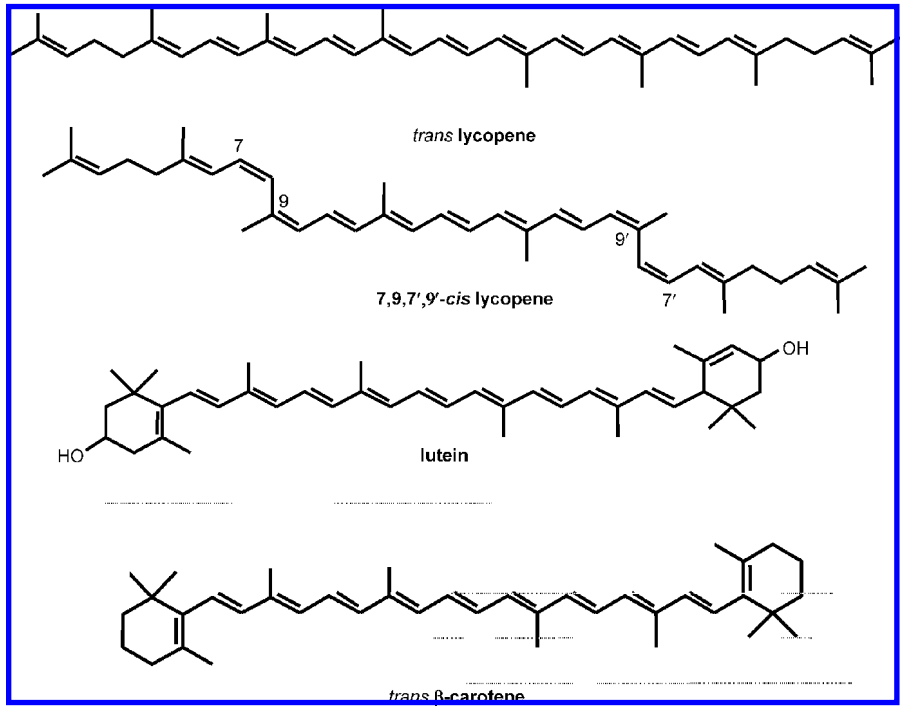

Figure 1. Structures of carotenoids extracted from vegetable powders.

A large percentage of *tetra-cis*-lycopene from Tangerine tomatoes is extracted at 45 °C after 0.5 h (**Figure 2C**). This amount increases slowly with additional extraction time up to 4 h. A similar pattern is seen in the extraction of *trans*-lycopene from red tomato at 30 °C (**Figure 2D**), which increases up to 5 h, after which time no more samples were taken. The increase in the amount extracted seems to reach a plateau at 4 h. At 60 °C, *trans*-lycopene concentration decreases after 1 h, indicating oxidative degradation. However, at 45 °C, close to the maximum amount of lycopene is extracted after 1 h of incubation. The amount extracted increased gradually with prolonged incubation at this temperature, so both *tetra-cis*- and *trans*-lycopene isomers are extracted most efficiently and close to maximum concentrations at 45 °C after 0.5 h, but the possibility exists that accessibility of both isomers increases slowly with prolonged incubation.

Using ethyl lactate as solvent, our data then show that the optimal temperature and extraction time for both trans and tetra-cis isomers of lycopene are 45 °C and 1 h. On the other hand, both β-carotene and lutein are less stable at higher temperatures and therefore most efficiently extracted at 30 °C. β-Carotene is maximally extracted after 0.5 h, but lutein requires 2 h.

Effect of Ethanol as Cosolvent on Carotenoid Extraction. Data obtained on carotenoid extraction using various ratios of ethanol and ethyl lactate as solvents are shown in **Figure 3**. As depicted in **Figure 2A**, lutein is less stable at temperatures higher than 30 °C. **Figure 3A** shows that it is extracted at 30 °C more efficiently with increasing percentages of ethanol and incubation. The largest amount of lutein was extracted from white corn using100% ethanol and increases with incubation times up to 5 h. However, after 2 h of extraction, the amount plateaus and increases slowly thereafter. β-Carotene from carrot, on the other hand, is more stable than lutein at higher temperatures (**Figure 2B**) and, therefore, more is extracted at higher temperatures. One should note in **Figure 3B** that, although β-carotene degradation is indicated during the first 2 h in **Figure 2B** at 60 °C, the amount extracted is larger than at lower temperatures, and higher ethanol concentrations increase the amounts extracted, so that 100% ethanol extracts the highest amounts of β-carotene. Maximum β-carotene is extracted at 60 °C after 2 h. Our data indicate that β-carotene in the carrot powder matrix is quite accessible, because increased extraction times do not yield larger amounts.

Addition of ethanol to ethyl lactate at all temperatures increases extraction of *tetra-cis*-lycopene from Tangerine tomato powder. However, at 60 °C, at which extraction periods of >2 h seem to result in gradual degradation, the addition of ethanol seems to stabilize the tetra-cis isomer, and higher concentrations of *tetra-cis*-lycopene are extracted. Maximum yields are attained between 60 and 100% ethanol (**Figure 3C**). At 45 °C, at which the greatest yield of the isomer is obtained in the absence of ethanol (**Figure 2C**), the optimal ethanol concentration and extraction time are 80% ethanol and 3 h, but the amount extracted is within experimental error of the amount obtained at 60 °C at 80% ethanol after 1 h. We see no significant differences in the amounts extracted at 60, 80, and 100% ethanol. Therefore, the shorter time of 1 h at 80% ethanol and a slightly higher temperature of 60 °C seem to be more expedient.

A more complex situation appears in the case of ethanol addition to ethyl lactate to extract *trans*-lycopene from red tomato (**Figure 4**). At 30 °C, it is obvious that ethyl lactate with no added ethanol is most efficient in extracting *trans*-lycopene (**Figure 4A**). Less and less *trans*-lycopene is extracted with increasing concentrations of ethanol (0 > 40 > 60 > 80 > 100% ethanol). At 45 °C, however, the activation of another phenomenon becomes evident (**Figure 4B**). After 0.5 h of extraction, 40 > 60 > 80 > 100% ethanol, but at 5 h 80 = 60 > 40 > 100 > 0%. At 60 °C extraction temperature (**Figure 4C**) after 0.5 h, 40 = 80 > 60 > 100 = 0%, but after 5 h, 100 = 80 > 60 = 40 > 0% ethanol. Therefore, a complete reversal in the order of yield versus percent ethanol occurs between 30 and 60 °C after 5 h. These apparently peculiar data can be explained by examining the HPLC data of red tomato powder extracted using 100% ethanol after 4 h of incubation at 60 °C (see Extraction of *cis*- and *trans*-Lycopene Isomers and **Table**

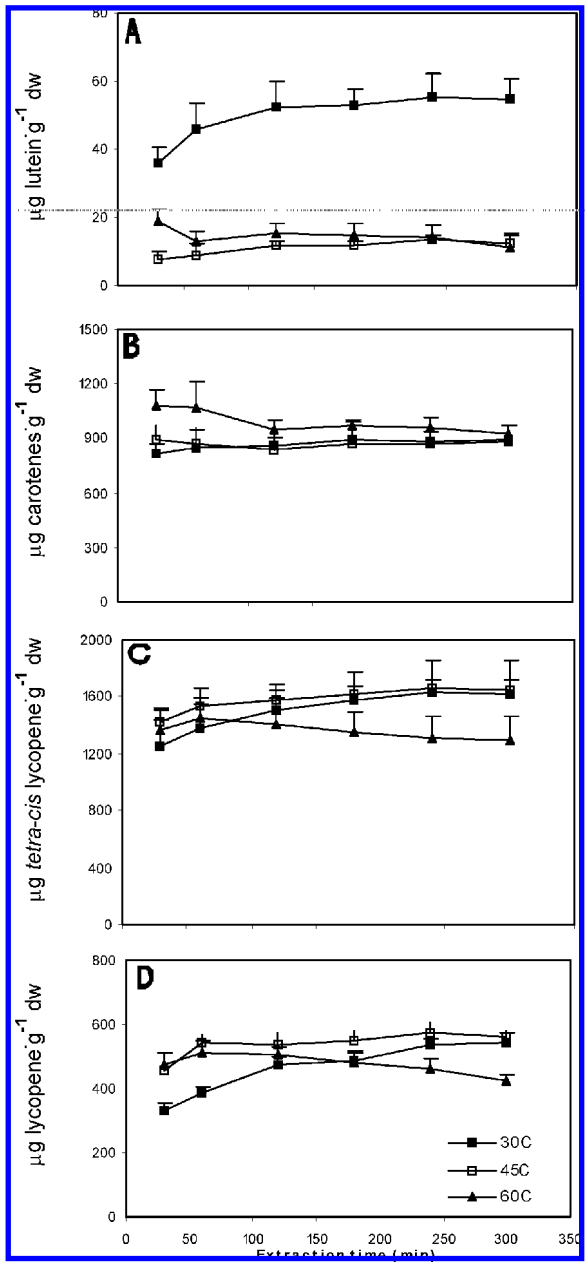

Figure 2. Effects of temperature and extraction time on yields of carotenoids obtained using ethyl lactate: (**A**) lutein from white corn; (**B**) carotenes (all carotenes, predominantly β-) from carrots; (**C**) *tetra-cis*-lycopene from Tangerine tomatoes; (**D**) *trans*-lycopene from red tomatoes.

2). *cis*-Lycopene isomers are barely detectable in the unheated red tomato powder. However, after 4 h of heating at 60 °C in 100% ethyl lactate, 50% of the lycopene is present as *cis*-lycopene isomers, and, in 100% ethanol, 90% of the lycopene has been converted from trans to cis. In other words, isomerization occurs in both solvents, but to a greater extent in ethanol. The data used to assess the effect of ethanol as a cosolvent for lycopene extraction in this part of the investigation was obtained using spectrophotometer readings at 470 nm, which provides a total concentration of lycopene but does not distinguish between *cis*- and *trans*-lycopene isomers.

Addition of Antioxidants. We picked two antioxidants, α-tocopherol (TOC) and α-lipoic acid (LA), because of their benefits to human health. α-Tocopherol, or vitamin E, is a fat-soluble vitamin and an important antioxidant. It has a hydroxyl group, which can donate a hydrogen atom to reduce free

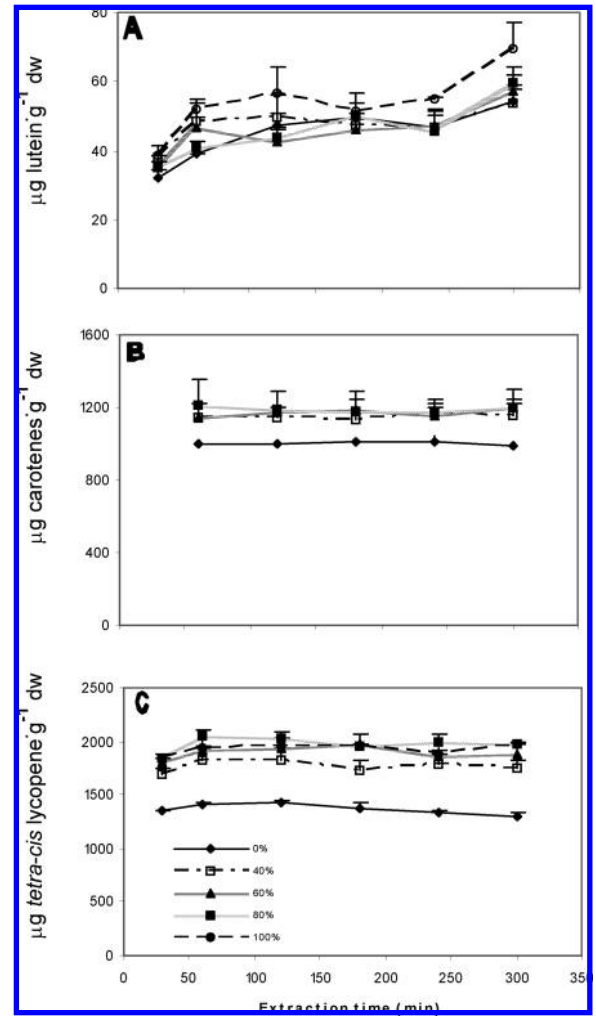

Figure 3. Effects of ethyl alcohol added to ethyl lactate, temperature, and extraction time on carotenoid yields from vegetable powders: (**A**) lutein from white corn at 30 °C; (**B**) carotenes (all carotenes, predominantly β-) from carrots at 60 °C; (**C**) *tetra-cis*-lycopene from Tangerine tomatoes at 60 °C.

radicals, and a hydrophobic side chain, which allows it to interact and penetrate biological membranes. TOC is abundant in vegetable oils, nuts, seeds, and wheat germ. It is also found in whole grains, fish, peanut butter, and green leafy vegetables (*29*). Epidemiological studies have suggested an inverse relationship between vitamin E intake and the risk of cardiovascular disease (*30, 31*), presumably by protecting against oxidation of low-density lipoproteins (*32*). α-Lipoic acid, or thioctic acid, is also a lipophilic antioxidant and an essential cofactor for mitochondrial respiratory enzymes. It is believed to have therapeutic potential against damage resulting from oxidative stress, for example, ischemia, diabetes, neurodegeneration, and radiation (*33*, *34*).

Both of these powerful antioxidants are naturally occurring and essential to living organisms. Therefore, their choice as protectants against oxidation of substances to be consumed by humans is certainly preferable to petroleum-based compounds, for example, β-hydroxytoluene (BHT). To date, LA has not been used as an antioxidant for the purpose of protecting carotenoids or other compounds that are susceptible to oxidative degradation during extraction procedures.

Both TOC and LA were tested for their ability to protect lutein, β-carotene, and the trans and tetra-cis isomers of lycopene against degradation during extraction using 100% ethyl lactate.

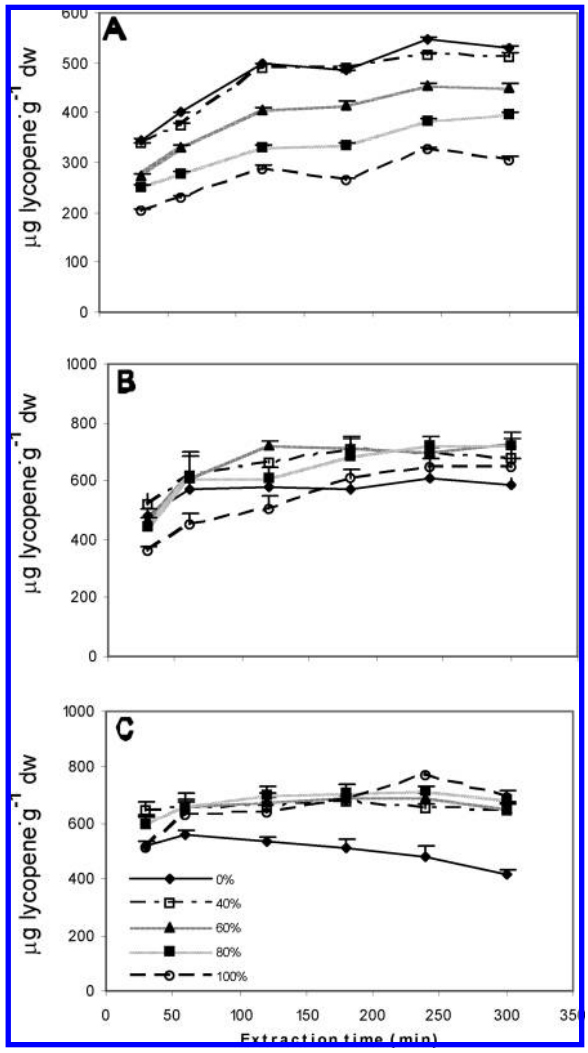

Figure 4. Effects of ethyl alcohol added to ethyl lactate, temperature, and extraction time on lycopene yields from red tomato powder: (**A**) 30 °C; (**B**) 45 °C; (**C**) 60 °C.

Specified amounts of antioxidant were added along with the solvent at the beginning of extraction. Concentrations of TOC and LA ranged from 0 to 200 mg/10 mL of solvent. Data obtained using TOC are shown in **Figure 5**. All concentrations tested (50−200 mg/10 mL of solvent) prevented loss of lutein to the same extent during extraction for up to 5 h and resulted in higher yields (**Figure 5A**). As mentioned earlier, extractions at higher temperatures caused greater losses, and therefore the effects of the antioxidant at temperatures higher than 30 °C are not included in the data. Similarly, extraction of β-carotene from carrot gave greatest yields at 60 °C extraction temperature, and therefore only data at this temperature are shown. TOC at 50 mg/10 mL of solvent gave optimal yields of β-carotene from carrot powder after 1 h. The amount extracted was higher than after 5 h of extraction, indicating degradation during longer exposures to this high temperature (**Figure 5B**). As shown in **Figure 2C**,**D**, extraction of both trans and tetra-cis isomers of lycopene is most efficient at 45 °C because of degradation at 60 °C. At 45 °C, increasing extraction time results in increasing lycopene yields up to 4 h. However, with the addition of TOC, both lycopene isomers seem to be protected against degradation, and yields are increased with prolonged incubation for up to 4 h at all three temperatures. At all temperatures, all levels of added TOC seemed to give similar protection. Maximal concentrations of both *trans*- and *tetra-cis*-lycopene were

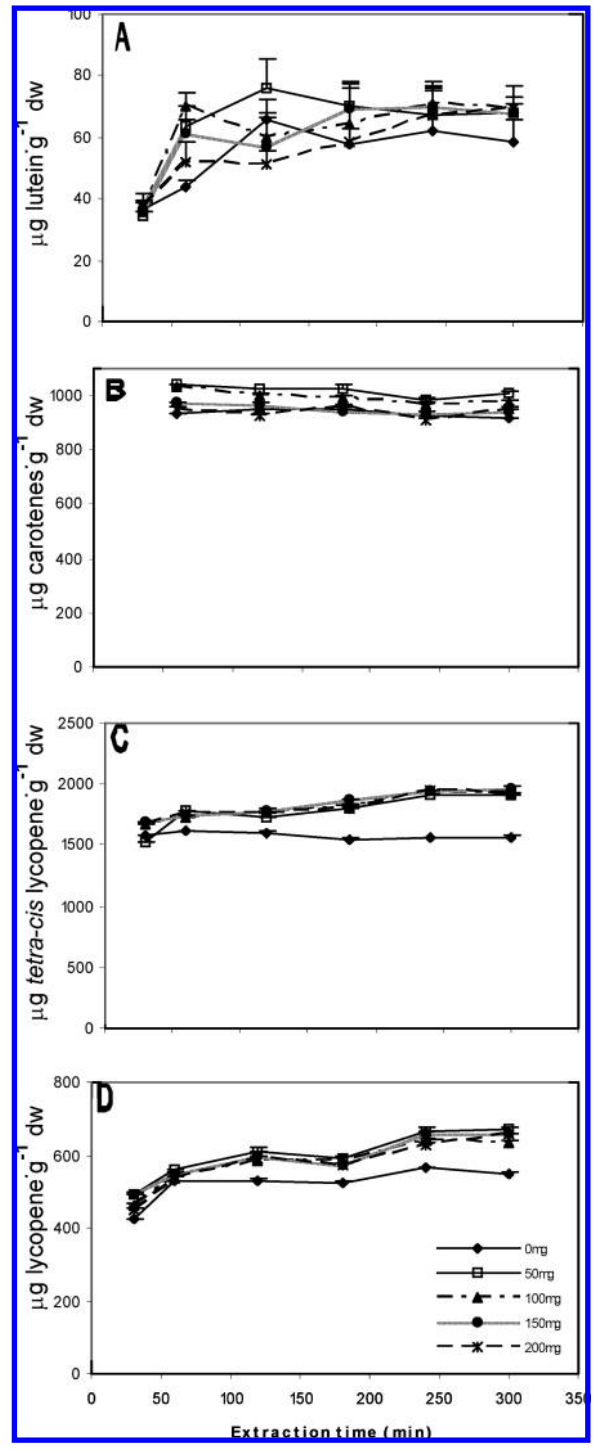

Figure 5. Effects of α-tocopherol concentrations and extraction time on carotenoid yields from vegetable powders: (**A**) lutein from white corn at 30 °C; (**B**) carotenes (all carotenes, predominantly β-) from carrots at 60 °C; (**C**) *tetra-cis*-lycopene from Tangerine tomatoes at 60 °C; (**D**) lycopene from red tomatoes at 45 °C.

extracted after 4 h. However, the optimal conditions for extracting *tetra-cis*-lycopene are 60 °C with 50 mg of TOC added/10 mL (**Figure 5C**) and for *trans*-lycopene are 45 °C with 50 mg of TOC added/10 mL (**Figure 5D**). We chose 45 °C for *trans*-lycopene from red tomato powder because yields at 60 °C extraction temperature showed losses after extraction times greater than 4 h. *tetra-cis*-Lycopene then is more stable at 60 °C than the *trans*-lycopene isomer.

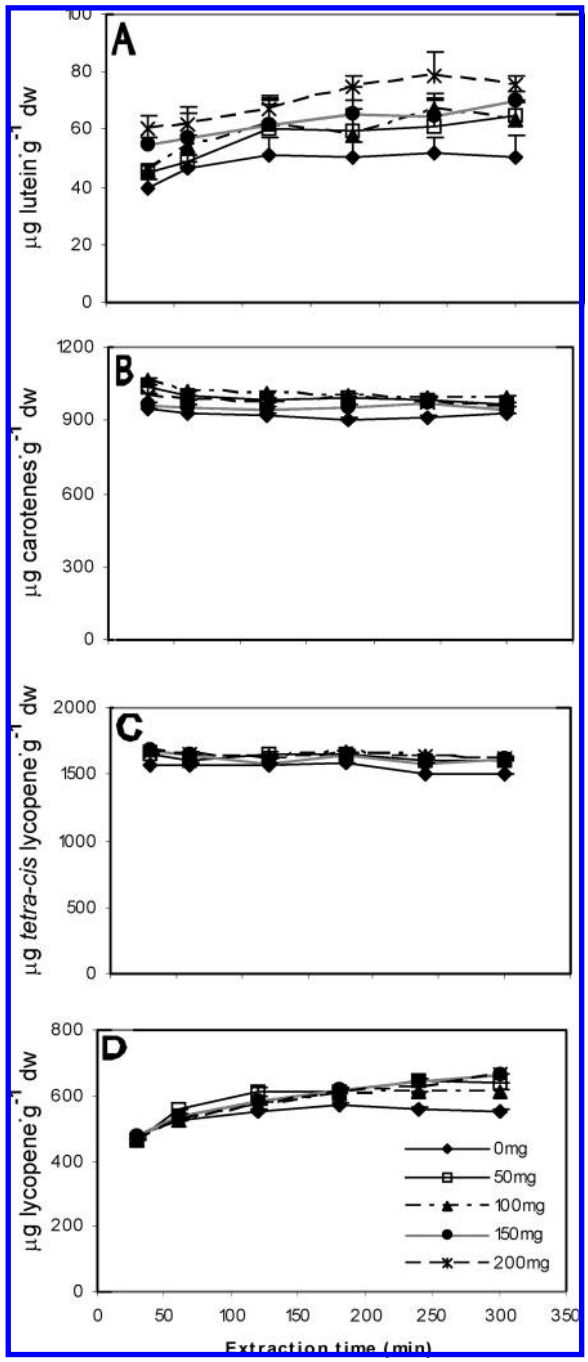

Figure 6. Effects of α-lipoic acid concentrations and extraction time on carotenoid yields from vegetable powders: (**A**) lutein from white corn at 30 °C; (**B**) carotenes (all carotenes, predominantly β-) from carrots at 60 °C; (**C**) *tetra-cis*-lycopene from Tangerine tomatoes at 45 °C; (**D**) lycopene from red tomatoes at 45 °C.

LA seems to protect carotenoids similarly to TOC, although the data obtained on lutein extraction indicate greater stability using LA. The data are less variable (smaller SDs) than those obtained using TOC. (Extraction with added TOC was repeated in an attempt to reduce variability in the data; however, no improvement was achieved.) The maximum amount of extracted lutein is obtained at 30 °C using 200 mg of LA/10 mL of solvent for 3−4 h. Lower concentrations of LA improve lutein yield, which increases with the amount of LA added (**Figure 6A**). As with TOC, the highest yield of β-carotene is obtained after 0.5 h at 60 °C and 50 mg of LA/10 mL of solvent (**Figure 6B**). Lower temperatures are less effective, and higher concentrations of

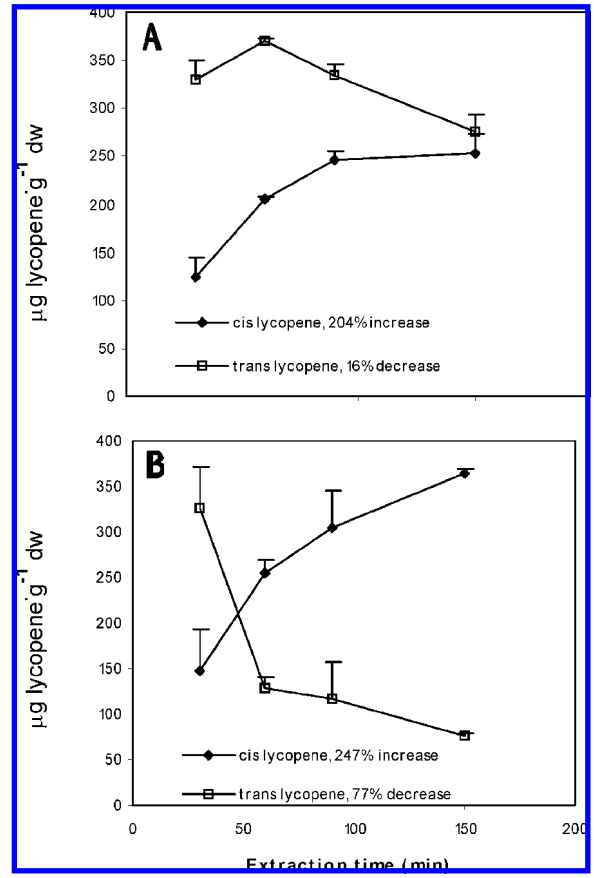

Figure 7. Changes in lycopene isomer concentrations during extraction from red tomato powder, using (**A**) 100% ethyl lactate and (**B**) 100% ethanol as solvents.

added LA do not improve β-carotene extraction. Both *tetra-cis*- and *trans*-lycopene show signs of degradation at 60 °C (see **Figure 2C**,**D**). Maximal amounts of *tetra-cis*-lycopene are extracted from Tangerine tomato powder at 45 °C in the presence of 50 mg of LA/10 mL of solvent after 0.5 h. Increasing LA concentration above 50 mg/10 mL does not benefit extraction, nor do increased extraction periods (**Figure 6C**). At 60 °C, extracted *tetra-cis*-lycopene concentrations were higher at all LA concentrations (50−200 mg of LA/10 mL). *trans*-Lycopene from red tomato powder is stable at 45 °C, and the data seem to indicate that after 3 h, more lycopene is being extracted in the presence of LA, whereas without its protection, lycopene concentrations decrease after 3 h (**Figure 6D**). The data at 60 °C show possibly small increases in lycopene concentrations after 3 h with the addition of 50−200 mg of LA/10 mL of solvent. However, these increases are not statistically significant and therefore do not warrant the extra time and energy expended in longer extractions times at higher temperatures. α-Lipoic acid concentrations of >50 mg/10 mL did not seem to increase protection against lycopene degradation; therefore, concentrations of >50 mg/10 mL do not seem to be warranted.

Extraction of *cis*- and *trans*-Lycopene Isomers. In our laboratory, we have been particularly interested in extracting both *cis*- and *trans*-lycopene isomers from tomato and tomato products. Therefore, we extended our investigations on the use of ethyl lactate for this purpose. Lycopene was extracted using either methylene chloride/methanol/H_2O (MMH) (40:40:20) (*27*), ethyl lactate, or ethyl alcohol from red and Tangerine tomato powders for 2 h at 60 °C. Solutions containing extracted carotenoids were centrifuged to remove any particulate material,

Table 1. Concentrations of Carotenoids Extracted from Vegetable Powders by Various Solvents and Determined Using Spectrophotometric Measurements[a]

	trans-lycopene		*tetra-cis*-lycopene		β-carotene		lutein	
solvent	μg g^{-1} DW	SD	μg g^{-1} DW	SD	μg g^{-1} DW	SD	μg g^{-1} DW	SD
ethyl acetate	295.62	18.08	962.85	60.00	1017.87	56.60	6.89	0.52
ethyl lactate	415.77	31.80	1174.57	138.52	898.99	14.30	6.53	1.11
MMH	558.48	58.44	1817.09	124.99	876.99	98.98	6.17	0.13
ethanol	489.35	47.82	1609.91	76.83	1140.54	49.44	8.38	0.18

[a] Vegetable powders prepared from white corn, carrots, Tangerine tomatoes, and red tomatoes were extracted in triplicate, using the solvents indicated at 60 °C for 2 h. Carotenoid concentrations were measured spectrophotometrically at specific wavelengths as described under Materials and Methods. (The percentage of maximum extractable carotenoid was determined experimentally, using the amount extracted by each solvent and by comparison with the amount extracted by the most efficient solvent determined for that compound: methylene chloride/methanol/ H_2O (MMH) for lycopene, ethanol for carotenes (all carotenes, predominantly β-), and ethyl lactate for lutein.)

Table 2. Concentrations of Lycopene Isomers Extracted from Red and Tangerine Tomato Powders Using Various Solvents and Measured Using HPLC[a]

		carotenes[b]		*tetra-cis* lycopene		*cis*-lycopenes		*trans*-lycopene		total lycopene		% *cis*	
sample	solvent[c]	μg g^{-1} of DW	SD	μg g^{-1} of DW	SD	μg g^{-1} of DW	SD	μg g^{-1} of DW	SD	μg g^{-1} of DW	SD	μg g^{-1} of DW	SD
red tomato	ethyl lactate	425.44	86.46	0.00	0.00	270.59	16.73	260.26	10.04	538.58	27.46	50.24	0.81
	ethyl lactate + TOC	403.15	32.83	0.00	0.00	508.99	23.77	298.57	2.85	807.56	25.43	63.03	0.99
	ethyl lactate + LA	389.88	5.16	0.00	0.00	471.29	13.46	439.01	8.57	910.29	21.67	51.77	0.32
	ethanol	1160.47	46.30	0.00	0.00	446.67	17.19	54.19	5.17	500.86	22.14	89.18	0.60
Tangerine tomato	ethyl lactate	250.61	3.22	1479.57	60.24	1.90	0.28	0.42	0.07	1481.89	60.14	99.84	0.01
	ethyl lactate + TOC	748.38	18.76	2408.17	40.22	20.86	7.47	2.44	0.93	2431.47	34.01	99.04	0.04
	ethyl lactate + LA	346.43	17.40	2050.69	40.71	1.60	0.77	1.60	0.92	2053.89	41.13	99.84	0.04
	ethanol	356.66	13.92	3819.11	57.76	1.02	1.76	0.00	0.00	3820.12	58.91	99.97	0.00

[a] Tomato powders were extracted in triplicate, using the solvents indicated (± antioxidants) at 60 °C for 4 h. Carotenoids were separated using the HPLC and measuring areas under peaks at specified wavelengths (see Materials and Methods). [b] Carotenes = total carotenes (all carotenes, primarily β-carotene). [c] TOC and LA: 200 mg/10 mL of solvent.

and the supernatants were analyzed using a spectrophotometer as described under Materials and Methods. The data obtained are shown in **Table 1**. The maximum amount of extractable lycopene (see footnote, **Table 1**) is set as that extracted by MMH. As indicated in **Figure 3A**,**B**, 100% ethanol is the most effective solvent for extracting both lutein and β-carotene. The relative effectiveness of ethyl lactate, ethyl acetate, and MMH is shown in **Table 1**.

In view of the data presented earlier in this study, the results obtained on lycopene extraction could possibly be explained by (1) isomerization occurring in both ethyl lactate and in ethyl alcohol, the degree of isomerization being greater in the latter solvent, or (2) increased accessibility of lycopene from tomato tissues during incubation with both ethyl lactate and ethyl alcohol, or (3) both. We therefore conducted further experiments, using HPLC analyses, to determine the concentrations of lycopene isomers after each extraction period. Red tomato powder was extracted at 60 °C for 2 h, using 100% ethyl lactate or 100% ethyl alcohol. Samples from each treatment were taken at 0.5 h intervals for 1.5 h and analyzed for *cis*- and *trans*-lycopene isomers, using HPLC. The data are depicted in **Figure 7**. **Figure 7A** shows the changes in isomeric concentrations during extraction using 100% ethyl lactate, and **Figure 7B** shows the changes occurring in 100% ethanol. Before treatment, we know from other analyses that the lycopene in our red tomato powder is almost all in the trans-isomeric form. In ethyl lactate at 60 °C, a considerable amount of isomerization has already occurred after 0.5 h and continues for the length of the experiment. The concentration of the *trans*-lycopene isomer increases up to 1 h and then declines, indicating degradation. The total amount of lycopene (cis + trans isomers) increases until 1.5 h, despite the fact that the concentration of the trans isomer decreases. This must then indicate that, during incubation of the tomato powder with ethyl lactate at 60 °C, accessibility to *cis*-lycopene isomers is increasing. This increased accessibility is not evident in the data obtained using 100% ethanol for extraction (**Figure 7B**). *trans*-Lycopene is isomerized in ethanol to about the same extent as in ethyl lactate, but more degradation seems to be occurring, so that the total concentration of lycopene is lower at all times after the first 30 min. The formation of cis isomers is higher in ethanol than in ethyl lactate, but the total concentration of lycopene extracted is lower (see also **Table 2**). One should note the greater stability of the *tetra-cis*-lycopene isomer in both ethyl lactate and in ethyl acetate compared with the *trans*-lycopene isomer at this temperature (**Table 2**). In previous studies on tomato processing by Hackett et al. (*35*), *tetra-cis*-lycopene from Tangerine tomato oleoresin was shown to isomerize along with degradation at temperatures between 75 and 100 °C and to degrade without isomerization between 25 and 50 °C. Our own studies on tomato processing (*28*) are also consistent with their data. These differences in our present investigation may reflect different effects of solvents upon the isomerization and degradation of lycopene. Ethyl lactate and ethyl alcohol, to a lesser extent, might have a protective effect on the integrity of *tetra-cis*-lycopene.

Advantages of Ethyl Lactate as a Solvent for Carotenoid Extraction. The extraction of carotenoids, notably lycopene, has been restricted by the lack of appropriate solvents that are compatible with food production. The primary solvent used for food purposes is ethyl acetate, and its use is restricted by a patent (*23*). Our data show that ethyl lactate has distinct advantages as a solvent for the extraction of lycopene isomers for human consumption. Methylene chloride, of course, is the most efficient of the solvents used; however, because of its toxicity, it cannot be used for ingested products. To extract lycopene isomers, 100% ethanol is 87−88% as effective as methylene chloride, but is more costly and more flammable. In addition, industrial ethanol is traditionally manufactured from ethylene, a petrochemical.

Table 1 shows that ethyl lactate is 74% as efficient as MMH in extracting *trans*-lycopene and 65% as efficient as MMH

compared with ethyl acetate (54 and 53%, respectively), which is the primary solvent currently used for these purposes. If red tomato powder is extracted at 45 °C for 1 h, then the amount extracted is almost equal (541 mg/g of dry weight) to that obtained using MMH for 2 h. Or, red tomato powder can be extracted at 45 °C for 2 h using 60% ethanol in ethyl lactate, and the extracted amount goes up to 724 mg/g of dry weight, which is much larger than that extracted using MMH (558 mg/g of dry weight). **Figure 7** shows that, if red tomato powder is extracted in ethyl lactate for 1 h at 60 °C, the total lycopene extracted increases to 586 mg/g of dry weight compared to 558 and 489 mg/g of dry weight in MMH and 100% ethanol (panels **A** and **B**, respectively). On the other hand, we note that considerable isomerization occurs. However, for food purposes this may represent an advantage. Considerable evidence has accumulated showing that *cis*-lycopene isomers are more bioavailable than *trans*-lycopene (*14*, *16*−*18*). If this is indeed the case, an increase in *cis*-lycopene isomer content would be beneficial to human health because of its associated protection against prostate cancer and other degenerative diseases resulting from free radical damage (*2*−*9*, *12*−*14*).

Our data, then, show that ethyl lactate is an excellent solvent for extracting food-grade lycopene. The maximum amount of total lycopene extracted from red tomato was obtained in our experiments at 60 °C, using ethyl lactate with added antioxidant. α-Tocopherol was more effective than α-lipoic acid. Considerable isomerization of *trans*-lycopene to its cis isomers occurred, but this may prove advantageous because of increased bioavailability of *cis*-lycopene isomers. The *tetra*-*cis*-lycopene isomer was resistant to isomerization, and its extraction was maximized using added antioxidant, TOC being more effective than LA. For the extraction of lutein and β-carotene, ethanol is the most efficient solvent of those tested. However, ethyl lactate is almost as efficient as ethyl acetate for these purposes (78 vs 82% for lutein; 79 vs 89% for β-carotene) compared to ethanol.

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