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# Trolox Equivalent Antioxidant Capacity of Different Geometrical Isomers of α-Carotene, β-Carotene, Lycopene, and Zeaxanthin

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Isomerization of carotenoids, which is often encountered in food processing under the influence of temperature and light, may play a role in the observed protective effects of this group of secondary plant products. Investigation of in vitro antioxidant activity of prominent carotenoid geometrical isomers was undertaken in light of recent reports illustrating a large percentage of carotenoid (*Z*)-isomers in biological fluids and tissues. α-Carotene, β-carotene, lycopene, and zeaxanthin were isolated from foods or supplements and subsequently photoisomerized with iodine as a catalyst. Major *Z*-isomers of each carotenoid were fractionated by semipreparative $C_{30}$ HPLC. In vitro antioxidant activity of all isomers collected was measured photometrically using the Trolox equivalent antioxidant capacity (TEAC) assay. TEAC values of 17 geometrical isomers investigated ranged from 0.5 to 3.1 mmol/L. Three unidentified (*Z*)-isomers of lycopene showed the highest antioxidant activity, being significantly higher than the result for (all-*E*)-lycopene, which had approximately two times the activity of (all-*E*)-β-carotene. On the other hand, (9*Z*)-zeaxanthin had a more than 80% lower TEAC value compared to that of (all-*E*)-lycopene. These results allow for the in vivo relevance of (*Z*)-isomers of carotenoids to be considered.

**KEYWORDS: Trolox equivalent antioxidant capacity; α-carotene; β-carotene; lycopene; zeaxanthin; geometrical isomers**

## INTRODUCTION

Carotenoids as biological antioxidants are currently the focus of numerous investigations. Those carotenoids with nine or more conjugated double bonds are able to quench singlet oxygen with increasing activity depending on the number of conjugated double bonds (*1*) with lycopene being the most effective quencher of singlet oxygen (*2*). Miller et al. (*3*) investigated the antioxidant activity of different carotenoids, using the TEAC (Trolox equivalent antioxidant capacity) test. In this assay lycopene was the most effective radical scavenger, followed by β-cryptoxanthin and β-carotene, with the xanthophylls showing minimal activity. Using azo-initiated peroxyl radicals, α-carotene showed higher antioxidant potential than lutein. Both carotenoids had more efficacy than β-carotene and zeaxanthin, which where comparable (*4*).

Isomerization of carotenoids is often encountered in food processing and is influenced by both temperature and light exposure. It has become important to address the significance of carotenoid isomers and to decipher the role (*Z*)-isomers may play in light of recent reports highlighting a large percentage of carotenoid (*Z*)-isomers in biological fluids and tissues (*5*). For example, in tomatoes and tomato-based foods (all-*E*)-lycopene is predominant, accounting for 90−98% of total lycopene (*6*). In contrast, (all-*E*)-lycopene accounts for only 12−21%, and (*Z*)-isomers account for 79−88%, of total lycopene in benign or malignant prostate tissues. Lycopene in serum consists of 58−73% (*Z*)-isomers (*5*). Analyzing the formation of methyl linoleate hydroperoxides, (9*Z*)-β-carotene showed higher in vitro antioxidant activity compared to that of (all-*E*)-β-carotene (*7*). In rats *Dunaliella bardawil* powder (42% (all-*E*)-β-carotene, 43% (9*Z*)-β-carotene) inhibited formation of hepatic conjugated dienes after alcohol-induced oxidative stress significantly better than synthetic β-carotene (97% (all-*E*)-β-carotene, 3% (15*Z*)-β-carotene) (*8*). Exposure of *Dunaliella bardawil* to oxidative stress induced a sequential degradation of pigments in the order (9*Z*)-β-carotene > (all-*E*)-β-carotene, indicating that (9*Z*)-β-carotene is a more effective scavenger of reactive oxygen species (*9*). In another study, (9*Z*)-β-carotene did not show any significant effect on generation of reactive oxygen species by stimulated neutrophils. In contrast, (13*Z*)-β-carotene scavenged superoxide and hydroxyl radicals (*10*). Using the copper-induced LDL oxidation, synthetic β-carotene was twice as effective as the *Dunaliella* β-carotene in inhibiting

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**Table 1.** Absorptivity Values and Wavelength Maxima of Carotenoid Standards (*12*, *13*, *14*)

| standard | solvent | wavelength (nm) | absorptivity value (*E*(1%, 1 cm)) |
|---|---|---|---|
| (all-*E*)-α-carotene | *n*-hexane | 444 | 2800 |
| (all-*E*)-*β*-carotene | *n*-hexane | 453 | 2592 |
| (9*Z*)-*β*-carotene | *n*-hexane | 445 | 2550 |
| (13*Z*)-*β*-carotene | *n*-hexane | 443 | 2090 |
| (15*Z*)-*β*-carotene | *n*-hexane | 447 | 1820 |
| (all-*E*)-lycopene | *n*-hexane | 472 | 3450 |
| (all-*E*)-zeaxanthin | ethanol | 450 | 2540 |

LDL lipid peroxidation (*11*) demonstrating higher efficacy for (all-*E*)-*β*-carotene compared to the mixture of (all-*E*)- and (9*Z*)-*β*-carotene.

The aim of this study was to investigate potential antioxidant activity of prominent geometrical isomers of α-carotene, *β*-carotene, lycopene, and zeaxanthin, using the TEAC assay. Information gained from this study will enable estimation of the protective potential of processed foods rich in carotenoid geometrical isomers. In addition, the possible role of (*Z*)-isomers within the organism can be considered.

## MATERIALS AND METHODS

**Chemicals.** 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich Chemical Co (St. Louis, MO). Manganese dioxide was obtained from Merck (no. 805958). Buffer salts and iodine were of analytical grade; all solvents used were of HPLC grade and used without further purification. α-Carotene was isolated from carrots, *β*-carotene was isolated from Betatene (a gift from Cognis Corporation, Cincinnati, OH), and (15*Z*)-*β*-carotene was kindly supplied by Hoffmann-La Roche, Basel, Switzerland. Lycopene was isolated from tomato paste, and zeaxanthin was isolated from dry Chinese wolfberries (*Lycium chinense*). Each carotenoid standard was measured photometrically and calculated using the dilution solvents (1:10) and values of $\lambda$ and *E*(1%, 1 cm) shown in Table 1. Concentrations of the (*Z*)-isomers of α-carotene, lycopene, and zeaxanthin were calculated using the values of the relevant (all-*E*)-isomer. All isomers were separated and analyzed using HPLC. (all-*E*)-α-Carotene and (all-*E*)-*β*-carotene were 100% pure. Purity of the (*Z*)-isomers of α-carotene ranged from 76 to 93%, each containing other (*Z*)-isomers and (all-*E*)-α-carotene. (*Z*)-Isomers of *β*-carotene were 65−87% pure with impurities of (all-*E*)-*β*-carotene. All lycopene isomers investigated (purity 70−85%) contained impurities of other lycopene isomers, as well as oxidation products. Purity of the zeaxanthin isomers ranged from 88 to 100%, containing 0−12% impurities of other zeaxanthin isomers. Most isomers were not 100% pure because of partial coelution while fractionating.

**Isolation of Carotenoids.** All samples were homogenized in methanol with added calcium bicarbonate and Celite, and were subsequently filtered through Whatman No. 42 and No. 1 filter papers. Carotenoids were extracted using a mixture of hexane/acetone (1:1, v/v). In the case of zeaxanthin, the extract was further saponified in order to hydrolyze the xanthophyll esters prevalent in the wolfberries and extracted thereafter with diethyl ether. For *β*-carotene, Betatene was dissolved directly in methyl-*tert*-butyl ether (MTBE) and filtered through a 0.2-$\mu$m filter prior to fractionation.

**Iodine Isomerization.** Carotenoid standards (200−300 $\mu$g/mL) were dissolved in hexane/acetone (1:1, v/v). Iodine crystals were added to the standard at ∼1% of the carotenoids weight and photoisomerized (exposed to ambient light) according to the method of Zechmeister (*15*). The resulting mixtures of isomers were stored, after removal of the solvent under a stream of nitrogen, at −20 °C until analysis and fractionation.

**HPLC Analysis.** All mixtures of isomers were analyzed using a Hewlett-Packard model 1100 solvent delivery system, autosampler, and photodiode array detector. For separation, an analytical scale polymeric $C_{30}$ (250 × 4.6 mm, 3 $\mu$m) column (Waters/YMC, Milford, MA) was used. Mobile-phase conditions were variable consisting of different gradients of methanol and MTBE tailored for the optimal separation of major carotenoid isomers. The (*Z*)-isomers of α-carotene, *β*-carotene, and zeaxanthin were tentatively identified by their retention behavior on the $C_{30}$ column and according to their unique electronic absorption spectra (*16*, *17*). The electronic absorption maxima of all isomers investigated are shown in Table 2.

**Table 2.** Electronic Absorption Maxima of the Carotenoid Geometrical Isomers Investigated

| isomer | absorption maxima[a](nm) | | | | $[\epsilon_2/\epsilon_1]$ [b] |
|---|---|---|---|---|---|
| (all-*E*)-α-carotene | 336 | 424 | (446) | 473 | [0.052] |
| (9*Z*)-α-carotene | 330 | 418 | (441) | 467 | [0.102] |
| (9′*Z*)-α-carotene | 330 | 420 | (441) | 469 | [0.086] |
| (13*Z*)-α-carotene | 332 | 416 | (438) | 465 | [0.469] |
| (13′*Z*)-α-carotene | 332 | 416 | (438) | 465 | [0.410] |
| (all-*E*)-*β*-carotene | | 426 | (452) | 478 | [0.000] |
| (9*Z*)-*β*-carotene | 340 | 422 | (447) | 473 | [0.094] |
| (13*Z*)-*β*-carotene | 339 | 420 | (445) | 470 | [0.371] |
| (15*Z*)-*β*-carotene | 338 | 424 | (449) | 474 | [0.497] |
| (all-*E*)-lycopene | 363 | 446 | (472) | 504 | [0.098] |
| (*Z*)-lycopene no. 1 | 361 | 441 | (467) | 498 | [0.127] |
| (*Z*)-lycopene no. 2 | 361 | 441 | (467) | 498 | [0.131] |
| (*Z*)-lycopene no. 3 | 361 | 441 | (467) | 498 | [0.093] |
| (*Z*)-lycopene no. 4 | 363 | 446 | (472) | 504 | [0.109] |
| (all-*E*)-zeaxanthin | | 425 | (450) | 477 | [0.000] |
| (9*Z*)-zeaxanthin | 338 | 422 | (445) | 472 | [0.097] |
| (13*Z*)-zeaxanthin | 338 | 420 | (444) | 470 | [0.440] |

[a] Measured in the LC mobile phase (MTBE–methanol) using a photodiode array detector. Values in parentheses represent the main absorption maxima. [b] Ratio of absorption intensity ($\epsilon_2$) at the near-UV maximum to absorption intensity ($\epsilon_1$) at the main absorption maximum.

**Table 3.** Mobile-Phase Mixtures of Methanol and Methyl-*tert*-butyl Ether (MTBE) for Carotenoid Fractionation on a $C_{30}$ Column (250 × 10 mm, 3 $\mu$m)

| carotenoid | solvent | |
|---|---|---|
| | methanol | MTBE |
| α-carotene | 75% | 25% |
| *β*-carotene | 75% | 25% |
| lycopene | 50% | 50% |
| zeaxanthin | 90% | 10% |

**Fractionation.** All mixtures of isomers were fractionated using a Spectra Physics SP8800 solvent delivery system and a Waters 996 photodiode array detector. The column used was a semipreparative $C_{30}$ column (250 × 10 mm, 3 $\mu$m) prepared at the National Institute of Standards and Technology (Gaithersburg, MD). Mixtures of methanol and MTBE were used as mobile phases (for details see Table 3). The relevant fractions were sampled and analyzed for purity by analytical $C_{30}$ HPLC as described above. All solutions were dried down using a vacuum evaporator (35 °C). The residues were stored at −20 °C until further analysis.

**TEAC Assay.** Antioxidant activity was determined following a procedure similar to that of Miller et al. (*3*). $ABTS^{\bullet+}$ radical cation was prepared by passing a solution of ABTS through manganese dioxide on a filter paper. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2 $\mu$m syringe filter. This solution was diluted in 5 mM phosphate buffered saline (PBS) pH 7.4 to an absorbance of 0.70 at 734 nm and preincubated at room temperature for 2 h prior to use. Trolox was used as an antioxidant standard. 2.5 mM Trolox was prepared in PBS for use as a stock standard. Fresh working standards were prepared daily by diluting this stock solution with PBS.

α-Carotene isomers, *β*-carotene isomers, and lycopene isomers were dissolved in hexane, and zeaxanthin isomers were dissolved in acetone. $ABTS^{\bullet+}$ solution (1.0 mL) and 200 $\mu$L of the solution of Trolox or the

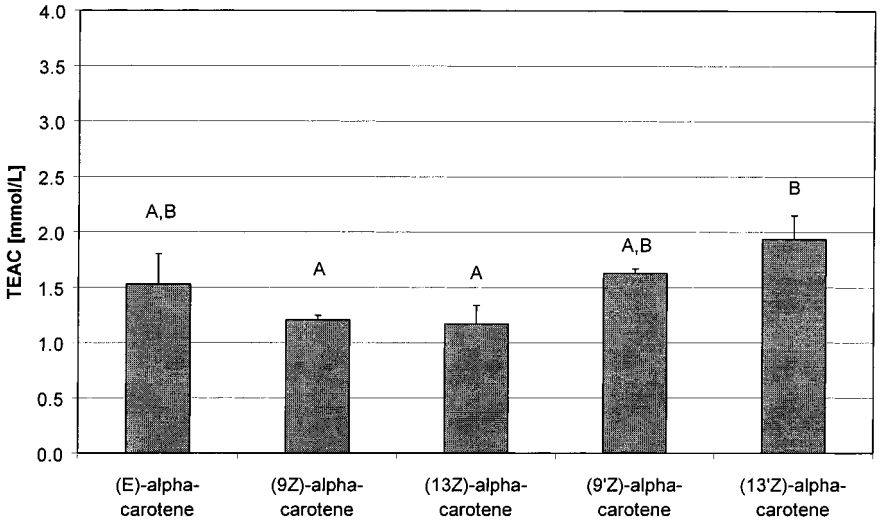


**Figure 1.** Trolox equivalent antioxidant capacities (TEAC) of (9*Z*)-α-carotene, (13*Z*)-α-carotene, (9′*Z*)-α-carotene, and (13′*Z*)-α-carotene compared to that of (all-*E*)-α-carotene. Bars showing the same index are not significantly different ($p > 0.05$).

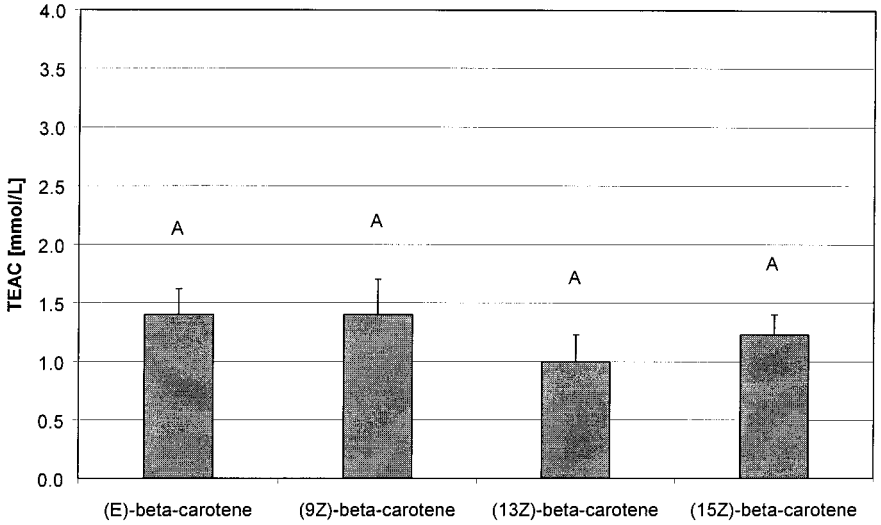


**Figure 2.** Trolox equivalent antioxidant capacities (TEAC) of (9*Z*)-β-carotene, (13*Z*)-β-carotene, and (15*Z*)-β-carotene compared to that of (all-*E*)-β-carotene. Bars showing the same index are not significantly different ($p > 0.05$).

carotenoids were vortexed for 30 s in reaction tubes, which were centrifuged for 60 s at 10000 rpm. The absorbance at 734 nm of the lower phase was taken in a Hewlett-Packard 8453 spectrophotometer exactly 2 min after initiation of mixing. PBS blanks and hexane blanks were run in each assay. The dose−response curve for Trolox (0−100 μM) was used to determine the TEAC values of all isomers investigated. Each isomer was analyzed at least in quadruplicate.

**Statistical Analysis.** All results (excluding purity) are given as mean ± standard deviation. Differences between variables were tested for significance by one way ANOVA procedure, Tukey−HSD (SPSS for Windows, Release 10.07; SPSS, Chicago, IL).

## RESULTS

Figure 1 shows the TEAC values of the five different α-carotene isomers: (all-*E*)-α-carotene, (9*Z*)-α-carotene, (9′*Z*)-α-carotene, (13*Z*)-α-carotene, and (13′*Z*)-α-carotene. The (9′*Z*)- and (13′*Z*)-α-carotene isomers were found to have higher antioxidant activity than their (9*Z*)- and (13*Z*)-α-carotene counterparts. However, only the TEAC value of the (13′*Z*)-isomer was significantly higher than that of both (9*Z*)- and (13*Z*)-isomers ($p < 0.05$). In contrast, all β-carotene isomers investigated showed comparable antioxidant activities ($p > 0.05$) (Figure 2).

(all-*E*)-Lycopene and four prominent (*Z*)-isomers were investigated (Figure 3). Figure 4 shows a HPLC chromatogram of a tomato paste extract after isomerization with iodine. These (*Z*)-isomers were analyzed by LC−MS to ensure that each isolated compound possessed the same molecular weight as (all-*E*)-lycopene (unpublished observation). The electronic absorption spectra of these (*Z*)-isomers showed only small cis-absorption in the near UV region (Table 2), indicating the cis double bonds are located more decentrally (*18*). The exact configuration is being determined as part of a separate investigation. One (*Z*)-isomer (no. 4, tentatively identified as (5*Z*)-lycopene), eluting shortly after (all-*E*)-lycopene, showed the same efficacy as the (all-*E*)-isomer. In contrast, the other three (*Z*)-isomers had significantly higher ($p < 0.05$) antioxidant capacity than (*E*)-lycopene.

Besides (all-*E*)-zeaxanthin, the two (*Z*)-isomers (9*Z*)-zeaxanthin and (13*Z*)-zeaxanthin were investigated (Figure 5). The (all-*E*)-isomer and the (13*Z*)-isomer showed comparable TEAC values ($p > 0.05$), however we observed a significantly lower value ($p < 0.05$) for (9*Z*)-zeaxanthin.

Antioxidant activities of all carotenoid isomers investigated are listed in Table 4. All lycopene isomers showed the highest

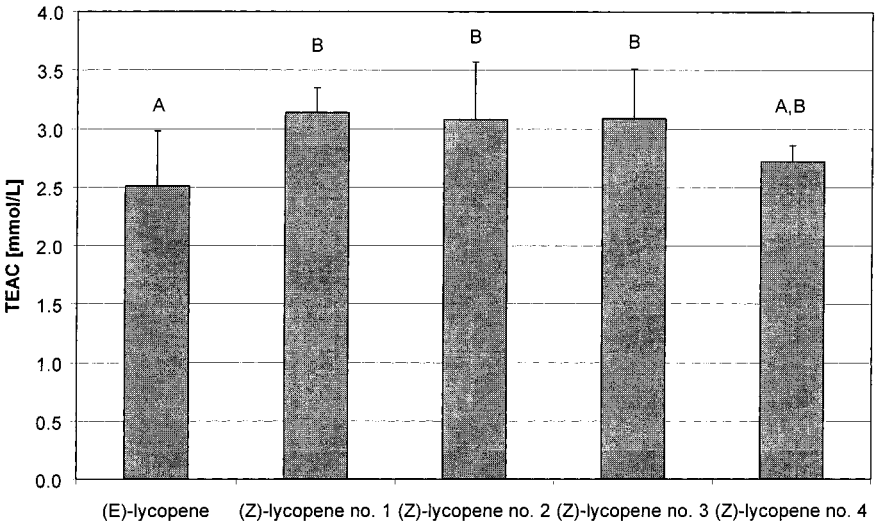


**Figure 3.** Trolox equivalent antioxidant capacities (TEAC) of four lycopene (*Z*)-isomers (nos. 1–4, see Figure 4) compared to (all-*E*)-lycopene. Bars showing the same index are not significantly different ($p > 0.05$).

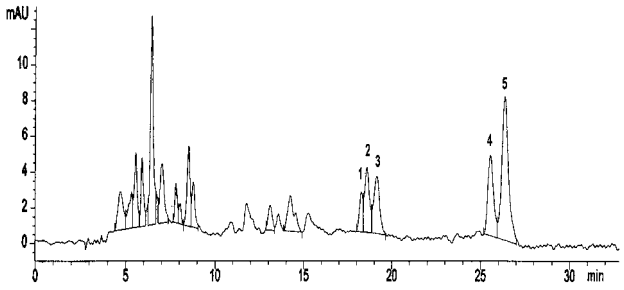


**Figure 4.** HPLC chromatogram of a tomato paste extract after iodine isomerization. Chromatographic conditions: YMC $C_{30}$ (250 × 4.6 mm, 3 $\mu$m); 1.0 mL/min methanol (A)/MTBE (B) gradient (0 min 60% A/40% B, 20 min 50% A/50% B, 30 min 50% A/50% B, 40 min 60% A/40% B); column temperature ambient; detection UV 450 nm; **1** = lycopene (*Z*)-isomer no. 1; **2** = lycopene (*Z*)-isomer no. 2; **3** = lycopene (*Z*)-isomer no. 3; **4** = (all-*E*)-lycopene; **5** = lycopene (*Z*)-isomer no. 4.

relative antioxidant activities being twice as effective as (all-*E*)-$\beta$-carotene. In contrast, (9*Z*)-zeaxanthin had only less than half the antioxidant activity of (all-*E*)-$\beta$-carotene.

## DISCUSSION

Measures of antioxidant activity for carotenoids can be determined by their ability to be involved in electron-transfer reactions, the stability of the antioxidant-derived free radicals after their action as an antioxidant, their interaction with other antioxidants, and their reactivity with active oxygen. The different modes of action are electron donation, $H^{\bullet}$ donation, or adduct formation. These differ among the various carotenoids (*19*). The TEAC assay, first described by Miller et al. (*20*), was originally used to determine the antioxidant activity of hydrophilic compounds. Radicals are generated through the peroxidase activity of metmyoglobin in the presence of hydrogen peroxide. This early version of the assay determines the ability of antioxidants to delay the formation of radicals as well as the capability to scavenge radicals. For carotenoids, another version of the TEAC assay was published (*3*) using preformed radical cations after oxidation of the ABTS by manganese dioxide, which was used with slight modifications for our study.

Geometrical isomers of $\alpha$-carotene, $\beta$-carotene, lycopene, and zeaxanthin showed TEAC values over a wide range of 0.5−3.1 mmol/L. Lycopene isomers demonstrated the highest

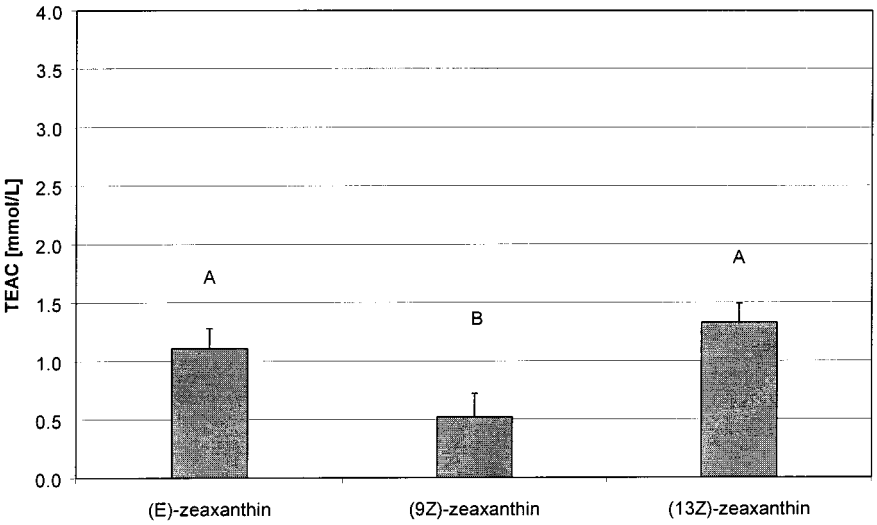


**Figure 5.** Trolox equivalent antioxidant capacities (TEAC) of (9*Z*)-zeaxanthin and (13*Z*)-zeaxanthin compared to that of (all-*E*)-zeaxanthin. Bars showing the same index are not significantly different ($p > 0.05$).

**Table 4.** TEAC Values of Five α-Carotene Isomers, Four β-Carotene Isomers, Five Lycopene Isomers, and Three Zeaxanthin Isomers

| carotenoid | Trolox equivalent antioxidant capacity[a] |
|---|---|
| (*Z*)-lycopene isomer 1 | $3.1 \pm 0.2^{A}$ |
| (*Z*)-lycopene isomer 2 | $3.1 \pm 0.5^{A}$ |
| (*Z*)-lycopene isomer 3 | $3.1 \pm 0.4^{A}$ |
| (*Z*)-lycopene isomer 4 | $2.7 \pm 0.1^{AB}$ |
| (all-*E*)-lycopene | $2.5 \pm 0.5^{B}$ |
| (13′*Z*)-α-carotene | $1.9 \pm 0.2^{C}$ |
| (9′*Z*)-α-carotene | $1.6 \pm 0.04^{C,D}$ |
| (all-*E*)-α-carotene | $1.5 \pm 0.3^{C,D,E}$ |
| (all-*E*)-β-carotene | $1.4 \pm 0.2^{C,D,E}$ |
| (9*Z*)-β-carotene | $1.4 \pm 0.3^{C,D,E}$ |
| (13*Z*)-zeaxanthin | $1.3 \pm 0.2^{D,E}$ |
| (15*Z*)-β-carotene | $1.2 \pm 0.2^{D,E}$ |
| (9*Z*)-α-carotene | $1.2 \pm 0.04^{D,E}$ |
| (13*Z*)-α-carotene | $1.2 \pm 0.2^{D,E}$ |
| (all-*E*)-zeaxanthin | $1.1 \pm 0.2^{D,E}$ |
| (13*Z*)-β-carotene | $1.0 \pm 0.2^{E,F}$ |
| (9*Z*)-zeaxanthin | $0.5 \pm 0.2^{F}$ |

[a] Values showing the same index are not significantly different ($p > 0.05$).

antioxidant potential with approximately twice the activity of (all-*E*)-β-carotene. To date, published results for the antioxidant activity of β-carotene isomers differ widely due to the various test systems employed. Often (9*Z*)-β-carotene (used as mixture with (all-*E*)-β-carotene) was more effective than (all-*E*)-β-carotene (*7*, *8*, *9*). However, there are also investigations showing higher efficacy for (9*Z*)-β-carotene compared to the mixture of (9*Z*)-β-carotene and (all-*E*)-β-carotene (*11*). In our investigations, the four β-carotene isomers (all-*E*)-, (9*Z*)-, (13*Z*)-, and (15*Z*)-β-carotene showed comparable TEAC values ($p > 0.05$). This observation could depend on the experimental conditions used. All measurements were made under atmospheric pressure, which led to the loss of the antioxidant activity of β-carotene in an earlier study (*21*). However, as long as identical conditions for all geometrical isomers investigated were employed, it is reasonable to compare relative results. Another important point is the choice of solvent used. In a recent study, the observed TEAC of β-carotene strongly depended on the solvent type (*22*). However, these authors used only solvents compatible with water and therefore investigated only low concentrations because of the limited solubility.

According to our knowledge this is the first study to present antioxidant activity data regarding geometrical isomers of α-carotene, lycopene, and zeaxanthin. Selected (*Z*)-isomers such as (13′*Z*)-α-carotene and the lycopene (*Z*)-isomers no. 1−3 showed significantly higher TEAC values when compared to their corresponding (all-*E*)-isomers. This observation is important when one considers that in contrast to tomatoes and tomato based foods with 90−98% (all-*E*)-lycopene (*6*), serum lycopene may be 58−73% in the (*Z*)-conformation (*5*). On the other end of the ranged TEAC values, (9*Z*)-zeaxanthin had less than half the antioxidant activity compared to that of (all-*E*)-zeaxanthin. It therefore becomes paramount to determine the geometrical configurations of major carotenoids during routine analyses that pertain to potential biological activity. Information gained from this study, together with reports on biological isomer levels, allows for further consideration of the biological relevance of circulating carotenoid (*Z*)-isomers.

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## LITERATURE CITED

(1) Yang, W. T.; Min, D. B. Chemistry of singlet oxygen oxidation of foods. In *Lipids in Food Flavors*; Ho, C.-T., Ed; American Chemical Society: Washington, DC, 1994; pp 15−29.

(2) Di Mascio, P.; Kaiser, S.; Sies, H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* **1989**, *274*, 532−538.

(3) Miller, N. J.; Sampson, J.; Candeias, L. P.; Bramley, P. M.; Rice-Evans, C. A. Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.* **1996**, *384*, 240−242.

(4) Farombi, E. O.; Britton, G. Antioxidant activity of palm oil carotenes in organic solution: effects of structure and chemical reactivity. *Food Chem.* **1999**, *64*, 315−321.

(5) Clinton, S. K.; Emenhiser, C.; Schwartz, S. J.; Bostwick, D. G.; Williams A. W.; Moore, B. J.; Erdman, J. W. cis−trans lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiol. Biomarkers Prev.* **1996**, *5*, 823−833.

(6) Nguyen, M. L.; Schwartz, S. J. Lycopene stability during food processing. *Proc. Soc. Exp. Biol. Med.* **1998**, *218*, 101−105.

(7) Levin, G.; Mokady, S. Antioxidant activity of 9-cis compared to all-trans β-carotene in vitro. *Free Radical Biol. Med.* **1994**, *17*, 77−82.

(8) Werman, M. J.; Ben-Amotz, A.; Mokady, S. Availability and antiperoxidative effects of β-carotene from *Dunaliella bardawil* in alcohol-drinking rats. *J. Nutr. Biochem.* **1999**, *10*, 449−454.

(9) Jiménez, C.; Pick, U. Differential reactivity of β-carotene isomers from *Dunaliella bardawil* toward oxygen radicals. *Plant Physiol.* **1993**, *101*, 385−390.

(10) Bohne, M.; Struy, H.; Gerber, A.; Gollnick, H. Protection against UVA damage and effects on neutrophil-derived reactive oxygen species by β-carotene. *Inflammation Res.* **1997**, *46*, 425−426.

(11) Lavy, A.; Ben-Amotz, A.; Aviram, M. Preferential inhibition of LDL oxidation by the all-trans isomer of β-carotene in comparison with 9-cis β-carotene. *Eur. J. Clin. Chem. Clin. Biochem.* **1993**, *31*, 83−90.

(12) Craft, N. E.; Brown, E. D.; Smith, J. C. Effects of storage and handling conditions on concentrations of individual carotenoids, retinal, and tocopherol in plasma. *Clin. Chem.* **1988**, *34*, 44−48.

(13) Olmedilla, B.; Granado, F. Rojas-Hidalgo, E.; Blanco, I. A rapid separation of 10 carotenoids, three retinoids, alpha-tocopherol and d-alpha-tocopherol acetate by high performance liquid chromatography and its application to serum and vegetable samples. *J. Liquid Chromatogr.* **1990**, *13*, 1455−1483.

(14) Schierle, J.; Härdi, W.; Faccin, N.; Bühler, I.; Schüep, W. Geometrical isomers of β,β-carotene. In *Carotenoids Volume 1a: Isolation and Analysis*; Britton, G., Liaaen-Jensen, S., Pfander, H. Eds.; Birkhäuser: Basel, Switzerland, 1995; pp 265−272.

(15) Zechmeister, L. Cis−trans isomerization and stereochemistry of carotenoids and diphenylpolyenes. *Chem. Rev.* **1947**, *34*, 267−344.

(16) Emenhiser, C.; Sander, C. S.; Schwartz, S. J. Capability of a polymeric $C_{30}$ stationary phase to resolve cis−trans carotenoid isomers in reversed-phase liquid chromatography. *J. Chromatogr. A* **1995**, *707*, 205−216.

(17) Emenhiser, C.; Englert, G.; Sander, L. C.; Ludwig, B.; Schwartz, S. J. Isolation and structural elucidation of the predominant geometrical isomers of alpha-carotene. *J. Chromatogr. A* **1996**, *719*, 333−343.

(18) Stahl, W.; Sundquist, A. R.; Hanusch, M.; Sies, H. Separation of β-carotene and lycopene geometrical isomers in biological samples. *Clin. Chem.* **1993**, *39*, 810−814.

(19) Bast, A.; Haenen, G. R. M. M.; van den Berg, R.; van den Berg, H. Antioxidant effects of carotenoids. *Int. J. Vitam. Nutr. Res*. **1998**, *68*, 399−403.
(20) Miller, N. J.; Rice-Evans, C.; Davies, M. J.; Gopinathan, V.; Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* **1993**, *84*, 407−412.
(21) Burton, G. W.; Ingold, K. U. $\beta$-carotene: An unusual type of lipid antioxidant. *Science* **1984**, *224*, 569−573.
(22) van den Berg, R.; Haenen, G. R. M. M.; van den Berg, H.; Bast, A. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* **1999**, *66*, 511−517.

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