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Comparative Study on Antioxidant Activity of Lycopene (*Z*)-Isomers in Different Assays

Lars Müller,[†] Pascale Goupy,[‡] Kati Fröhlich,[†] Olivier Dangles,[‡,§] Catherine Caris-Veyrat,[‡] and Volker Böhm*[,†]

[†]Institute of Nutrition, Friedrich Schiller University Jena, Dornburger Strasse 25-29, D-07743 Jena, Germany

[‡]UMR 408 Safety and Quality of Plant Products, INRA, F-84000 Avignon, France

[§]UMR 408, University of Avignon, F-84000 Avignon, France

ABSTRACT: Several studies have implicated the potent antioxidant properties of lycopene. However, most of the studies used only the (*all-E*)-isomer. (*Z*)-Isomers of lycopene were found in substantial amounts in processed foods and in human tissues. In the present study, we investigated *in vitro* the antioxidant activity of (5*Z*)-, (9*Z*)-, and (13*Z*)-lycopene compared to the (*all-E*)-isomer. Additionally, prolycopene, the (7*Z*,9*Z*,7′*Z*,9′*Z*)-isomer found in tangerine tomatoes, was analyzed. No significant differences were found between the isomers in ferric reducing antioxidant power assay and in bleaching the radical cation of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), both based on ET mechanisms. In contrast, scavenging activity against peroxyl radicals generated by thermal degradation of 2,2′-azobis(2-amidinopropane) (AAPH) was higher in the (*Z*)-isomers. (5*Z*)-Lycopene was most antioxidant in scavenging lipid peroxyl radicals, evaluated by analyzing the inhibition of $MbFe^{III}$ lipid peroxidation of linoleic acid in mildly acidic conditions (pH 5.8) in a micellar environment, modeling a possible antioxidant action in the gastric compartment.

KEYWORDS: FRAP, ABTS, peroxyl radical scavenging, lipid peroxidation, lycopene (*Z*)-isomers, structure−activity relationship

INTRODUCTION

Lycopene, β-carotene and lutein/zeaxanthin are the most abundant carotenoids detected in the blood of people from European countries.[1] The polyisoprenoid structure of carotenoids makes them susceptible to enzymatic[2] or chemical oxidative cleavage[3] and isomerization from (*E*)- to (*Z*)-forms. Both (*Z*)-isomers and cleavage products of carotenoids (apocarotenoids) can be regarded as potentially bioactive metabolites. Lycopene is an acyclic carotenoid having 11 conjugated double bonds in (*all-E*)-isomer and various (*Z*)-configurations (see Figure 1), as originally suggested by Zechmeister in 1938.[4] Lycopene in fruits and vegetables occurs mostly (80−97%) in the (*all-E*)-configuration.[5] Food processing and preparation of lycopene-containing meals increase the proportion of lycopene (*Z*)-isomers. The proportion of (*Z*)-isomers varied from 4% in preserved tomato paste up to 65% in long-term cooked spaghetti sauce prepared from canned tomatoes under household conditions (45−60 min at 85−110 °C). Additionally, the order of increasing proportion of lycopene isomers in this food sample was observed as (13*Z*+15*Z*) ≪ (9*Z*) < (5*Z*) < (*all-E*).[6] Lycopene (*Z*)-isomers also play a quantitatively important role in human plasma and tissues[6,7] where levels of (*Z*)-isomers range up to more than 50% of total lycopene. The most abundant (*Z*)-isomers of lycopene in human blood plasma are (5*Z*)-, (9*Z*)- and (13*Z*)-Lycopene,[6−8] possibly formed *in vivo* after absorption of (*all-E*)-lycopene.[7,9]

In contrast to red tomatoes, 90% of lycopene found in tangerine tomatoes (*Lycopersicon esculentum* var. Tangella) is the tetra-(*Z*)-isomer (7*Z*,9*Z*,7′*Z*,9′*Z*)-lycopene, commonly named prolycopene.[10] The lack of the enzyme carotenoid isomerase caused by the loss of the *tangerine* gene CRTISO[11] leads to accumulation of this poly-(*Z*)-isomer and consequently to the orange color. Tetra-(*Z*)-lycopene from tangerine tomatoes is known to be highly bioavailable.[12]

Physicochemical properties and bioavailability of (*all-E*)- and (*Z*)-isomers of lycopene are known to be different. Lycopene (*Z*)-isomers have been described to be more bioavailable than the naturally occurring (*all-E*)-form.[5,12]

Epidemiological studies have indicated a significant inverse correlation between the intake of lycopene or concentrations of lycopene in serum and the risk of several diseases.[5] In particular, there is evidence of a link between the consumption of lycopene-containing food or a high lycopene concentration in blood and a decreased risk of prostate cancer. The biological activity of lycopene is thought to be primarily due to its antioxidant properties.[13] Studies have shown that lycopene is irreversibly related to oxidation of lipids, proteins and DNA.[5]

Lycopene and other carotenoids were shown to prevent *in vitro* peroxidation processes caused by singlet oxygen ($^1O_2$)[14] and peroxyl radicals ($ROO^•$).[15,16] It is known that the carotenoid molecule reacts with a free radical with formation of a new, much more stable radical due to the conjugated double bond system, which allows the delocalization of the unpaired electron.[17] To date, most works on the antioxidant properties of lycopene have dealt with (*all-E*)-lycopene only. Hence, the influence of the (*Z*, *E*)-isomerism of lycopene on its antioxidant properties is not totally clarified yet. Improved antioxidant activity of (*Z*)-isomers compared to (*all-E*)-carotenoids has been observed by using

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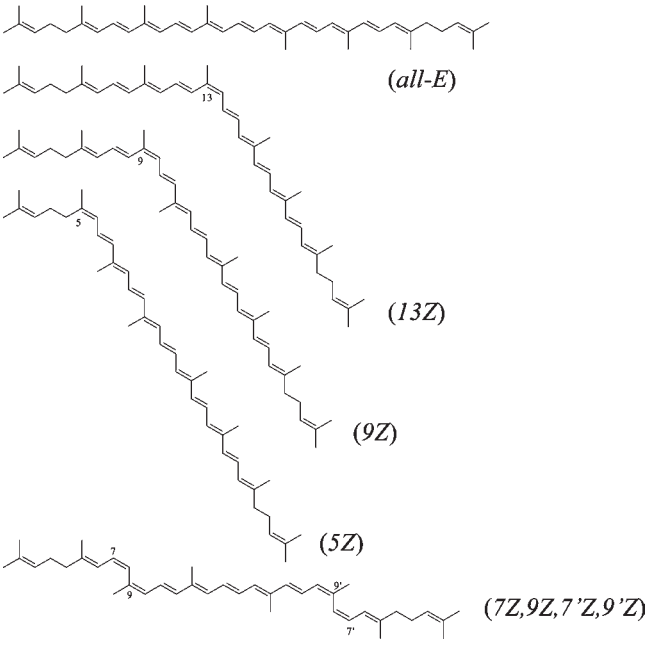

Figure 1. Structures of lycopene isomers.

different tests, e.g. scavenging free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$),[18] peroxidation of methyl linoleate induced by diazo compounds like 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN),[19] 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP/AAPH) or *tert*-butyl hydroperoxide.[18] However, other research groups reported opposite results, e.g. in oxidation of low density lipoprotein (LDL) and in quenching singlet oxygen.[20,21]

Based on this literature, we assessed the antioxidant activity of the main (*Z*)-isomers of lycopene compared to its (*all-E*)-form using four different *in vitro* assays: the ferric reducing antioxidant power (FRAP assay),[16] the activity to reduce radical cations of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (αTEAC assay),[16] the activity to scavenge peroxyl radicals (LPSC assay)[16] and the heme-induced peroxidation of linoleic acid in mildly acidic emulsions mimicking postprandial lipid oxidation in the gastric compartment (MbFe^{III}-LP assay).[22,23] The DPPH$^{\bullet}$-scavenging assay, frequently used to assess the antioxidant activity of phenolic compounds, was not considered because carotenoids are not reactive enough toward this radical.[16]

MATERIALS AND METHODS

Chemicals. Methanol (MeOH), dimethyl sulfoxide (DMSO), methyl *tert*-butyl ether (MTBE) and all others solvents used were of HPLC grade. HPLC grade water (18 MΩ) was prepared using a Millipore Milli-Q purification system (Millipore GmbH, Schwalbach, Germany). Buffer salts and all other chemicals were of analytical grade. The phosphate buffered salines (PBS; 10 mM, pH 5.8 and 6.8) were prepared by using Millipore Q-plus water and passed through a column of Chelex-100 chelating resin (Bio-Rad, Marnes-la-Coquette, France) to remove contaminating free metal ion traces. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), $K_2S_2O_8$, 2,4,6-tripyridyl-*s*-triazine (TPTZ), horse heart metmyoglobin (MbFe^{III}, type II, MW ≈ 17,600 g mol^{-1}), linoleic acid and Tween-20 (polyoxyethylenesorbitan monolaurate) were obtained from Sigma-Aldrich (Taufkirchen, Germany). All these products of the highest quality available (95−99%) were used without purification. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Acros Organics (Schwerte, Germany). Manganese dioxide and iodine were obtained from Merck (Darmstadt, Germany). Luminol was purchased from Fluka (Buchs, Switzerland).

α-Tocopherol (α-T) was purchased from Calbiochem (Darmstadt, Germany) with a purity of 100% shown by GC. (*all-E*)-Lycopene was a gift from BASF (Ludwigshafen, Germany). (7*Z*,9*Z*,7′*Z*,9′*Z*)-Lycopene was isolated from tangerine tomato. Other lycopene (*Z*)-isomers were prepared by using iodine or heat isomerization and fractionation using preparative C_{30}-HPLC.[24] The dried lycopene isomers were sealed in ampules under argon atmosphere and stored not longer than 2 months at −80 ± 5 °C. Purities of all isolated lycopene isomers were controlled by an analytical HPLC before sealing in ampules, immediately after storage as well as at the same time as the analysis of the antioxidant activities (isomers several hours in solution). All isolated lycopene isomers showed a purity of 97−99% before and immediately after storage. Concentrations of the lycopene isomers and α-T in solution were determined by using the molar absorption coefficients at the specific wavelengths listed in Table 1. Due to the low stability of the isolated isomers of lycopene, all steps had to be completed rapidly and under subdued light.

Heat and Iodine Isomerization of Lycopene. (*all-E*)-Lycopene was dissolved in mixtures of organic solvents. A lycopene isomer mixture with high amounts of (*all-E*)- and (13*Z*)-lycopene (about 50% (*all-E*), 30% (*13Z*), 20% other lycopene isomers and degradation products) was prepared by heating (5 h, 50 ± 2 °C) of an (*all-E*)-lycopene solution (MeOH:MTBE 50:50, v/v). Lycopene isomer mixtures with high amounts of other interesting (*Z*)-isomers (e.g., (5*Z*)-, (9*Z*)-lycopene) were prepared by using iodine isomerization according to Zechmeister.[25] Iodine dissolved in *n*-hexane (5% iodine relative to initial amount of (*all-E*)-lycopene, w/w) was added to a saturated solution of (*all-E*)-lycopene in cyclohexane:toluene (80:20, v/v), and this mixture was incubated for 30−60 min (room temperature, darkness). The resulting mixture of lycopene isomers was dried under a high flow of nitrogen at room temperature, sealed in ampules (argon atmosphere) and stored at −20 ± 5 °C until fractionation.[24]

Extraction and Isolation of (7*Z*,9*Z*,7′*Z*,9′*Z*)-Lycopene. (7*Z*,9*Z*,7′*Z*,9′*Z*)-Lycopene ("prolycopene, tetra-(*Z*)-lycopene") was isolated from a specific yellow tomato variety (tangerine tomato; *Lycopersicum esculentum Mill. AILSA CRAIG T.*), grown by using seeds from Kulturpflanzenbank Gatersleben der Stiftung Institut für Pflanzengenetik und Kulturpflanzenforschung (Gatersleben, Germany). Homogenized tomatoes were extracted with a mixture of MeOH:MTBE (50:50, v/v) by homogenization for 5 min at 15,000 rpm using an ultraturrax (IKA, Staufen, Germany). The extraction was repeated until the extract was colorless, and the combined organic phases were rotary evaporated under reduced pressure at room temperature. The residue was redissolved in MeOH:MTBE (50:50, v/v) using an ultrasonic bath, the resulting concentrated tomato extract was filtered and used for the fractionation. The prefractionation and purification were carried out at room temperature on a preparative C_{30}-HPLC-column (300 × 10.0 mm, 5 μm; YMC Europe, Schermbeck, Germany) using a mobile phase consisting of a mixture of MeOH and MTBE, a flow of 4.0 mL/min, a detection wavelength of 450 nm and an injection volume of 1 mL. HPLC pump model L-7100 (Merck, Darmstadt, Germany), UV−vis detector model variable wavelength monitor (Knauer, Berlin, Germany) and integrator model Chromatopac C-R6A (Shimadzu, Duisburg, Germany) were used. A prefractionation (MeOH:MTBE 50:50, v/v) was necessary to provide the isomer at adequate concentration. The preisolated extract contained prolycopene and a small percentage (3−5%) of β-carotene. Prolycopene was purified using MeOH:MTBE (90:10, v/v). The eluates with the separated isomer were concentrated under vacuum in a rotary evaporator and the residue of the solvent was dried under a nitrogen flow at room temperature.

Analytical HPLC Apparatus and Conditions. All isolated isomers were analyzed for purity using an isocratic and/or a gradient

Table 1. Absorption Coefficient and Maximum Absorption Wavelengths of Lycopene Isomers and α-Tocopherol

compd	solvent	wavelength (nm)	abs coeff ($E_{1\%,1cm}$)	ref	molar abs coeff (ε), calcd from $E_{1\%,1cm}$
(*all-E*)-lycopene	*n*-hexane, 2% CH_2Cl_2	472	3450	Schierle et al., 1997[6]	185213
(5*Z*)-lycopene	*n*-hexane, 2% CH_2Cl_2	470	3466	Aebischer et al., 1999[39]	186072
(9*Z*)-lycopene	*n*-hexane	464	3241	Müller et al., 1997[40]	173993
(13*Z*)-lycopene	*n*-hexane, 2% CH_2Cl_2	463	2533	Müller et al., 1997[40]	135984
(7*Z*,9*Z*,7′*Z*,9′*Z*)-lycopene	*n*-hexane, 2% CH_2Cl_2	437	1956	Hengartner et al., 1992[41]	105007
α-tocopherol	ethanol	492.5	75.8	Franke et al., 2007[42]	3265

C_{30}-HPLC method using a Merck-Hitachi (Darmstadt, Germany) HPLC system (pump L-7100, degasser L-7614, autosampler L-7200, diode array detector L-7450, interface L-7000) and a Jetstream plus column oven (JASCO, Gross-Umstadt, Germany). For isocratic separation, an analytical polymeric C_{30}-column (YMC Carotenoid S 5 μm 250 × 4.6 mm (YMC Europe, Dinslaken, Germany)), preceded by a C_{18} ProntoSil 120-5-C18 H (10 × 4.0 mm, 5 μm) column (Bischoff, Leonberg, Germany), was used. The mobile phase (0.45 mL/min) was a mixture of MTBE, MeOH and ethyl acetate (5:4:1, v/v/v) with a runtime of 40 min, detection wavelength of 470 nm and column temperature of 25 ± 1 °C. For the gradient method, another analytical C_{30}-column (Trentec Stability 100 C30 PEEK, 250 × 4.6 mm, 5 μm (Trentec, Rutesheim, Germany)), preceded by the above-described precolumn, was used. As mobile phase (1.0 mL/min) the following gradient of MeOH (A) and MTBE (B) was used: 0 min, 90:10 A:B; 40 min, 50:50 A:B; 60 min, 50:50 A:B; 70 min, 90:10 A:B; 75 min, 90:10 A:B. Column temperature was 10 ± 1 °C and detection wavelength 470 nm. Lycopene isomers were identified assuming the same order of elution as in Fröhlich et al.[24]

Antioxidant Activity. In all four assays standards of α-tocopherol (α-T) were analyzed first for calibration. An α-T stock solution (ca. 2.3 μM) was prepared in ethanol and stored in the freezer (−24 ± 2 °C). Fresh working standards were prepared daily by blowing off the ethanol under nitrogen at 30 ± 2 °C, dissolving the residue in *n*-hexane (for FRAP and αTEAC assay) or MTBE:DMSO (20:80, v/v) for LPSC assay and reconstituting these solutions with the respective solvent to concentrations of approximately 5−250 μM. Blanks of *n*-hexane and MTBE:DMSO (20:80, v/v) were analyzed. Isomers of lycopene (*all-E*-, 5*Z*-, 9*Z*-, 13*Z*-, 7*Z*,9*Z*,7′*Z*,9′*Z*-, respectively) were dissolved daily fresh in *n*-hexane to defined concentrations by spectrophotometric determination using the molar absorption coefficients listed in Table 1. The antioxidant activities of the lycopene isomers in FRAP, αTEAC, and LPSC assay were expressed as moles of α-tocopherol equivalents (α-TE)/mole of lycopene isomer. Results of $MbFe^{III}$-LP method were expressed as $IC_{50\alpha\text{-}T}$.

FRAP Assay. Lycopene isomer solutions (100 μL, 1−20 μM in *n*-hexane) were mixed with acidic FRAP reagent (1000 μL, pH 3.6, containing 1.67 mM $FeCl_3$ and 0.83 mM TPTZ), following a procedure recently published.[16] After mixing (6 min, 25 ± 1 °C, thermo shaker, 1400 rpm), the solutions were transferred completely into half-micro cuvettes (1.5 mL, polystyrene) and centrifuged for 30 s at 1000*g*. Exactly 8 min after starting of shaking, the absorbance at 595 nm of the aqueous layer was detected in a JASCO V-530 spectrophotometer (Gross-Umstadt, Germany). Each experiment was repeated three times at different concentrations of the lycopene isomers (1−20 μM). Blanks of *n*-hexane were analyzed.

αTEAC Assay. The procedure to assess the α-tocopherol equivalent antioxidant activity followed a procedure recently described.[16] One milliliter of $ABTS^{\bullet+}$ solution (in PBS buffer, 75 mM, pH 7.4, $E_{734\ nm}$ = 0.70 ± 0.02), which was preformed by using manganese dioxide, was added to lycopene isomer solution (100 μL, 1−20 μM in *n*-hexane). After shaking of the tube for 30 s, the mixtures were transferred into cuvettes and centrifuged as described in the FRAP assay above. At exactly 2 min after the start of mixing, the absorbance of the lower phase ($ABTS^{\bullet+}$ solution) was taken at 734 nm in the spectrophotometer. Each experiment was repeated three times at different concentrations of the lycopene isomers (1−20 μM). Blanks of *n*-hexane were analyzed.

LPSC Assay. A chemiluminescence based assay using luminol as chemiluminescent dye and AAPH as radical generator under atmospheric conditions was used to determine the peroxyl radical scavenging activity of the different lycopene isomers. The procedures followed the descriptions published recently.[16] One milliliter of each lycopene isomer in *n*-hexane was dried under a stream of nitrogen at 30 ± 2 °C. The residues were dissolved stepwise, first in 200 μL of MTBE and followed by 800 μL of DMSO. Fifty microliters of α-T standard (5−250 μM), blank or lycopene isomer solution, 50 μL of luminol (10 mM in DMSO:borax buffer, 80:20 v/v, pH 9.5), 100 μL of DMSO:borax buffer (80:20 v/v, pH 9.5) and 150 μL of AAPH (60 mM in DMSO:PBS buffer 80:20 v/v, pH 7.4) were combined into the wells of a white 96-well microplate (Greiner Bio-One, Frickenhausen, Germany). The final concentrations of the isomers on the microplate were 0.2−2 μM. The chemiluminescence signal from the microplate at 37 ± 1 °C was taken for the luminol oxidation every minute up to 2 h in a FluoStar Optima microplate reader (BMG Labtech, Offenburg, Germany). Each experiment was repeated three times at different concentrations of the lycopene isomers (1−20 μM). MTBE:DMSO (20:80, v/v) acted as blank.

Inhibition of the $MbFe^{III}$-LP of Linoleic Acid. The experimental conditions used were adapted from already published procedures.[22,23] $MbFe^{III}$ (17.6 mg) was dissolved in 50 mL of phosphate buffer (10 mM, pH 6.8). Its concentration was standardized at 10 μM using $\varepsilon = 7700\ M^{-1}\ cm^{-1}$ at 525 nm. Isomers of lycopene were dissolved in *n*-hexane:dichloromethane (98:2), and the concentration was adjusted to 1 mM by spectrophotometrical determination analysis using the molar absorption coefficients (Table 1). Defined volumes of daily prepared solutions of linoleic acid (7 mM), Tween 20 (40 mM) and (*all-E*)-lycopene or its (*Z*)-isomers and α-tocopherol (0.5 mM) in chloroform were mixed, and the solvents were removed under reduced pressure in darkness at 25 ± 2 °C. The residue was immediately dissolved in 10 mL of 10 mM phosphate buffer at pH 5.8 with gentle stirring. The final concentrations in the solution were linoleic acid, 0.7 mM; Tween 20, 2 mM; (*all-E*)-lycopene and its (*Z*)-isomers, 0.5−2.5 μM; α-tocopherol, 0.2−1 μM. Two milliliters of the freshly prepared solution was transferred to the temperature controlled spectrometer cells (37 ± 1 °C) containing magnetic stir bars for continuous mixing and sealed with Teflon stoppers. At time zero, lipid oxidation was initiated by adding 20 μL of a 10 μM solution of $MbFe^{III}$ to a final concentration of 100 nM. UV−vis absorbance was recorded in a Specord diode-array spectrometer (Analytik Jena GmbH, Germany) (optical path length: 1 cm) with periodic monitoring every 26 s at 234 nm and at the maximum absorption wavelengths of the lycopene isomers (Table 1). Each experiment was repeated three or four times at different concentrations of the lycopene isomer.

Statistical Analysis. All results (excluding purity analysis of the isomers) are given as mean ± standard deviation (SD). Differences of antioxidant activity between the lycopene isomers within one assay were tested for significance by one-way ANOVA procedure, using the

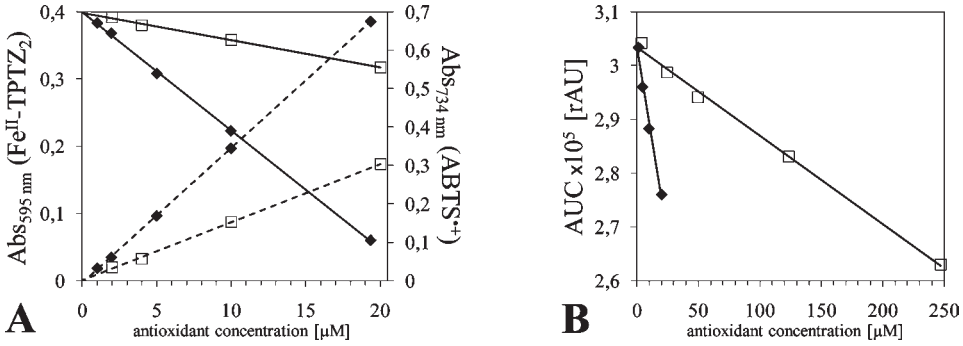

Figure 2. Change of the absorbance (A) at 595 nm (Fe^{II}−$TPTZ_2$ in FRAP assay, dotted lines) and 734 nm ($ABTS^{\bullet+}$ in αTEAC assay, full lines), and increase of the area under the curve (AUC, LPSC assay, B) after reaction with different concentrations of α-tocopherol (□) and (5*Z*)-lycopene (◆).

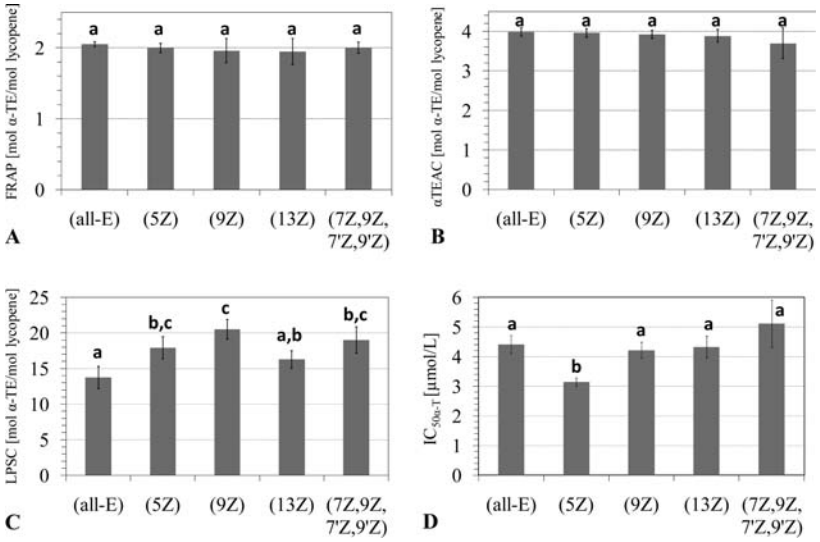

Figure 3. Antioxidant activities of lycopene isomers compared to α-tocopherol (α-T) using FRAP (A), αTEAC (B), LPSC (C) and $MbFe^{III}$-LP assay (D). Bars with different letters within one chart differ significantly (ANOVA, post hoc S−N−K, $p < 0.05$).

Student−Newman−Keuls (S−N−K) post hoc test (SPSS for Windows, release 17.0, SPSS Inc., Chicago, IL). Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

In general, carotenoids are able to act as antioxidants by three different mechanisms: (I) electron transfer (ET), (II) hydrogen atom transfer (HAT), and (III) formation of radical−carotenoid adducts. The activities of carotenoids differ among the various carotenoids.[16,26] The ability of lycopene and its isomers to undergo ET was assessed in the FRAP and αTEAC assays, by reduction of $Fe^{III}TPTZ_2$ and $ABTS^{\bullet+}$, respectively. The LPSC assay measured the ability of lycopene to scavenge peroxyl radicals based on the formation of radical−carotenoid adducts or HAT mechanisms. The assay based on the $MbFe^{III}$-LP of linoleic acid assesses the ability of lycopene isomers to scavenge lipid peroxyl radicals in mildly acidic micellar solution. In the latter two reaction mechanisms, ET and HAT from the carotenoid to the radicals as well as radical addition on the carotenoid skeleton can operate. Different isomers of lycopene were tested on their antioxidant activity in the *in vitro* assays with α-tocopherol as reference. (15*Z*)-Lycopene, another isomer found in processed tomato products, as well as (5*Z*,9*Z*)- and (5*Z*,9′*Z*)-lycopene, which were recently identified in human plasma (unpublished data), could not be tested because they were produced in too low yields by iodine-induced isomerization of (*all-E*)-lycopene.

FRAP Assay. In the FRAP assay, the ability of the lycopene isomers to reduce the Fe^{III}−$TPTZ_2$ complex into the highly blue colored Fe^{II}−$TPTZ_2$ complex was measured. The original protocol of Benzie and Strain,[27] which was used for the determination of the ferric reducing activity of water-soluble antioxidants, was slightly adapted to permit the investigation of lipophilic antioxidants by application of a biphasic test system as described.[16] Figure 2A shows representative data for the linear increase in concentration of the reduced species, Fe^{II}−$TPTZ_2$ (absorbance at 595 nm), with increasing concentration of the (5*Z*)-lycopene and α-tocopherol. The stronger linear increase of the absorption at the specific wavelength of the Fe^{II}−$TPTZ_2$ complex compared to α-tocopherol illustrates the multiple higher ferric reducing activity of lycopene, represented in Figure 2A by the (5*Z*)-isomer. Figure 3A shows the activity of the investigated lycopene isomers, which was two times higher than that of α-tocopherol, but not significantly different between the isomers ($p > 0.05$). Recently, our group showed that β-carotene did not have any significant ability to reduce ferric ions in this assay.[16] In the gastric compartments, dietary iron (e.g.,

ferric iron from plant food) can create oxidizing conditions for fast lipid oxidation processes.[28,29] (*all-E*)-Lycopene and its (*Z*)-isomers from tomatoes and tomato products can provide protection by their activity to reduce ferric iron and therefore hinder the initiation of lipid peroxidation processes. Furthermore, the group of Matos et al. showed, in studies with Wistar rats, that *in vivo* lycopene provides protection of prostate tissue against oxidative damage induced by ferric nitrilotriacetate.[30] Thus, it is tempting to speculate that lycopene (*Z*,*E*)-isomerization would not alter this protection.

αTEAC Assay. The bleaching of $ABTS^{\bullet+}$ is a widely used assay to determine the antioxidant activity of single compounds as well as of food and biological extracts. In this study, the lycopene isomers demonstrated a high antioxidant potential. In particular, the lycopene isomers were 4 times more potent than α-tocopherol (Figure 3B). The high activity of (*all-E*)-lycopene in $ABTS^{\bullet+}$ bleaching was recently confirmed.[16] Figure 2A shows representative data for the linear decrease in concentration of the oxidizing species, $ABTS^{\bullet+}$ (absorbance at 734 nm), with increasing concentration of the (5*Z*)-lycopene and α-tocopherol. The stronger decrease of absorption at the specific wavelength (734 nm) of the oxidizing agent $ABTS^{\bullet+}$ after reaction with the (5*Z*)-lycopene illustrates the multiple higher antioxidant activity of lycopene compared to α-tocopherol. For the first time, the TEAC assay for lipophilic antioxidants using preformed $ABTS^{\bullet+}$[26] was used in 2002 by Böhm et al.[31] to determine the antioxidant activity of lycopene and its geometrical isomers. Our previous studies suggested that lycopene (*Z*)-isomers had improved antioxidant activity compared to the (*all-E*)-form.[31] In the present work, no significant differences ($p > 0.05$) of the activity of bleaching $ABTS^{\bullet+}$ between the lycopene isomers investigated were observed (Figure 3B). But concentrations of the isomers used were not the same.[31] For this study, all isomer solutions were adjusted to the same concentrations (1−20 μM) by using the molar absorption coefficients of each isomer listed in Table 1. Our results indicate that position and number of (*Z*)-double bonds in the lycopene molecule have no influence on its ability to reduce the $ABTS^{\bullet+}$ radical cation (Figure 3B). In comparison, (*all-E*)-lycopene and its (*Z*)-isomers are 25−50% more potent antioxidants in this assay than β-carotene.[16,26]

LPSC Assay. The activity of the lycopene isomers to protect luminol from oxidation by peroxyl radicals generated by thermal degradation of AAPH under atmospheric conditions was determined in a monophasic mixture of buffers and DMSO, while the FRAP and αTEAC assays were carried out in biphasic water/*n*-hexane mixtures. The peroxyl radicals ($ROO^{\bullet}$) were formed by thermal decomposition of the diazo compound AAPH at a fixed rate in the presence of oxygen. These $ROO^{\bullet}$ can add at every part across the polyene chain of the carotenoid (CAR), resulting in the formation of a resonance-stabilized carbon-centered radical ($CAR\text{-}ROO^{\bullet}$). But in the reactions of carotenoids with lipid peroxyl radicals a HAT mechanism leading to the formation of carotenoid radicals ($CAR^{\bullet}$) and a hydroperoxyl compound is possible, as mentioned by Krinsky and Yeum.[17]

Figure 2B shows representative data for the linear decrease of chemiluminescence (CL) intensity induced by oxidative degradation of luminol (expressed as area under the curve, AUC) with increasing concentrations of (5*Z*)-lycopene and α-tocopherol. The faster the decrease of the CL signal (shown as AUC in the figure) while increasing the concentration, the more antioxidant the compound. The figure illustrates a multiple higher activity of the lycopene isomers compared to α-tocopherol, represented in this case by (5*Z*)-lycopene. After calculation using the AUC of the CL signal of each lycopene isomer and α-tocopherol as reference, the investigated lycopene isomers displayed peroxyl radical scavenging activities that were 13−20 times higher than that of α-tocopherol (Figure 3C). As also observed for β-carotene isomers in the studies of Levin et al.,[19] most of the (*Z*)-isomers of lycopene were found to be more potent than the (*all-E*)-isomer in our studies, too. The (5*Z*)-, (9*Z*)-, and (7*Z*,9*Z*,7′*Z*,9′*Z*)-lycopene isomers were significantly more efficient ($p < 0.05$) than the (*all-E*)-form in preventing luminol oxidation. By isomerization from (*all-E*)-lycopene (LPSC = 13.7 ± 1.6 mol of α-TE/mol) to its (9*Z*)-isomer (LPSC = 20.5 ± 1.4 mol of α-TE/mol), the antioxidant potential rose up significantly ($p < 0.05$) by approximately 30%. Theoretical studies based on ionization potentials and related antioxidant activity by Chasse et al.[32] suggested an improved peroxyl radical scavenging activity of (*Z*)-isomers in the order (5*Z*) > (9*Z*) > (13*Z*) > (*all-E*). Our results are in partial agreement with these predictions. For instance, the LPSC value of (5*Z*)-lycopene (LPSC = 17.9 ± 1.6 mol of α-TE/mol) was slightly but not significantly different ($p > 0.05$) from those of (13*Z*)-lycopene (LPSC = 16.3 ± 1.3 mol of α-TE/mol) and (9*Z*)-lycopene (LPSC = 20.5 ± 1.4 mol of α-TE/mol) in the present study (Figure 3C). The following order of decreasing activity can be stated as (9*Z*) ≥ (5*Z*) ≥ (13*Z*) ≥ (*all-E*). It has to be considered that, after dissolving the lycopene isomers in *n*-hexane, drying this solution under nitrogen and redissolving the isomers in MTBE + DMSO for LPSC measurements, oxidative degradation and isomerization can occur. The purity of the (*Z*)-isomers of lycopene, controlled using analytical HPLC method as describe above, decreased by isomerization back to the more stable (*all-E*)-lycopene (3−35%) and by minor degradation (1−2%) (data not shown). In an *ab initio* computational modeling study, (5*Z*)-lycopene was found to be the most stable isomer followed by the (*all-E*)-form.[32] Consistently, the purity of (*all-E*)- and (5*Z*)-lycopene decreased only slightly during the described procedures (each from 99% to 96%). The lycopene isomers (9*Z*)- and especially (13*Z*)-lycopene are known to be less stable in solution.[32] The purity actually decreased from 99% and 97% to 93% and 63%, respectively, by forming the more stable (*all-E*)- and (5*Z*)-isomers, both in equal amounts. (7*Z*,9*Z*,7′*Z*,9′*Z*)-Lycopene was more stable than the (13*Z*)-isomer, but less stable than the (9*Z*)-isomer, with a purity of 88% after dissolution in the solvent mixture. This major lycopene isomer isolated from tangerine tomatoes was partly isomerized into (13*Z*)-lycopene or oxidatively degraded, both routes contributing to approximately 6% loss in purity. (7*Z*,9*Z*,7′*Z*,9′*Z*)-Lycopene, with a LPSC value of 19.0 ± 1.9 mol of α-TE/mol of lycopene, showed a peroxyl radical scavenging activity equal to that of the (5*Z*)-isomer. However, the activity of this tetra-(*Z*)-isomer was lower than that of the (9*Z*)-isomer and higher than that of the (13*Z*)-isomer, even by trend and statistically not significant ($p > 0.05$).

(*Z*)-Isomers are known to be better soluble due to their higher steric demand. Consequently, the higher antioxidant activity of the (*Z*)-isomers in this assay compared to (*all-E*)-lycopene could be a consequence of a better solubility of the (*Z*)-isomers in the solvent mixture used and their to their lower tendencies to self-aggregate.

The observation that lycopene (*Z*)-isomers displayed a significantly higher peroxyl radical scavenging activity than (*all-E*)-lycopene is of biological relevance. For peroxyl radicals no specific enzymatic detoxification system exists in human metabolism.[33] There is a controversial discussion concerning the high ratio of (*Z*)-isomers in human plasma and tissues, which could be the result

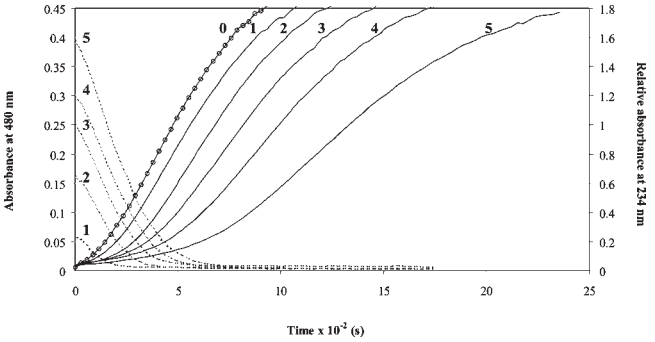

Figure 4. Accumulation of conjugated dienes (full lines, UV detection 234 nm) during the (5*Z*)-lycopene-inhibited $MbFe^{III}$–LP of linoleic acid and simultaneous consumption of the antioxidant at 480 nm (dotted lines) for different (5*Z*)-lycopene concentrations (0–5 μM). Linoleic acid (0.7 mM), $MbFe^{III}$ (100 nM), pH 5.8 phosphate buffer containing 2 mM Tween 20, 37 °C.

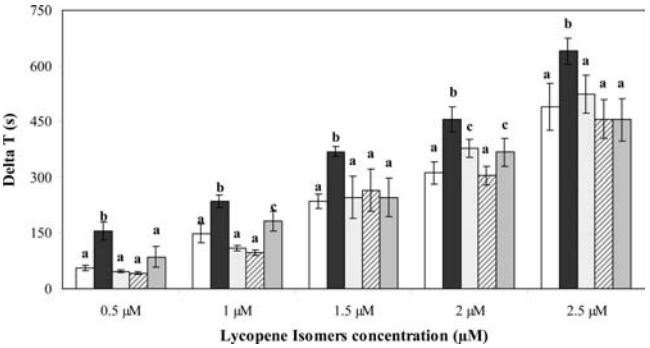

Figure 5. ΔT values for the inhibited $MbFe^{III}$-LP of linoleic acid in the presence of various lycopene isomers at different concentrations. Linoleic acid (0.7 mM), $MbFe^{III}$ (100 nM), pH 5.8 phosphate buffer containing 2 mM Tween 20, 37 °C. Lycopene isomers concentrations are 0.5–2.5 μM. Lycopene isomers investigated are (*all-E*) (blank), (5*Z*) (black), (9*Z*) (dotted), (13*Z*) (hatched gray) and (7*Z*,9*Z*,7′*Z*,9′*Z*) (gray). Bars with different letters within one concentration differ significantly (ANOVA, post hoc S–N–K, $p < 0.05$). Statistical analysis was performed for each concentration.

of preferential uptake into enterocytes during digestion, isomerization of ingested (*all-E*)-lycopene *in vivo*, or reduced catabolism of the (*Z*)-isomers compared to (*all-E*)-lycopene.[7] Nonetheless, it has to be considered that the high ratio of (*Z*)-isomers in human plasma and tissues may lead to an increase of the peroxyl radical antioxidant capacity and consequently to an increase of the lycopene contribution to the antioxidant defense system. The possibility of metabolic activation of lycopene by (*E*)- to (*Z*)-conversion was recently proposed following a postprandial human study using Caco-2 cells under gastric and duodenal conditions.[7]

Inhibition of the $MbFe^{III}$-LP of Linoleic Acid in Mildly Acidic Emulsions. The ability of (*all-E*)-lycopene and its (*Z*)-isomers to inhibit the $MbFe^{III}$-LP of linoleic acid (LH) was assayed in a pH 5.8 micellar solution by following the accumulation of conjugated dienes (CDs, mainly LOOH) and the concomitant consumption of the antioxidant.

For (*all-E*)-lycopene and its (*Z*)-isomers, CD accumulation proceeds with a lag phase followed by a propagation phase (Figure 4). During the lag phase, CD accumulation is slow and the carotenoid is preferentially oxidized as evidenced by its almost total consumption at the end of the lag period. The duration of the lag phase increases with the antioxidant concentration. After the lag phase, accumulation of CDs accelerates (propagation phase) to reach a maximal rate that is close to that in the absence of antioxidants. This behavior is similar to that of α-tocopherol in the same model[22,23] and suggests that the lipophilic lycopene isomers act in the lipid phase by reducing the lipid-derived peroxyl radicals ($LOO^{\bullet}$) and that their oxidation products do not possess a significant residual antioxidant activity. For each antioxidant concentration *C*, *T* is defined as the time needed to accumulate a given CD concentration after addition of $MbFe^{III}$ (e.g., that corresponding to a 0.7 increase in the absorbance at 234 nm from its value at time zero). In the absence of antioxidants, *T* becomes T_0. For a fixed antioxidant concentration, a larger $\Delta T = T - T_0$ means a higher antioxidant activity. From the ΔT values (Figure 5), it is clear that the antioxidant activities of the investigated lycopene isomers are not significantly ($p > 0.05$) different between them, except for (5*Z*)-lycopene ($p < 0.05$), which appears to be more potent at all concentrations studied.

For a quantitative comparison between antioxidants, taking into account all the concentrations studied, an IC_{50} parameter was calculated as previously described.[34] Parameter *T* is approximately a quadratic function of *C*: $T/T_0 = 1 + aC + bC^2$. If IC_{50} is defined as the antioxidant concentration leading to $T = 2T_0$, one has the following: $IC_{50} = (\sqrt{(a^2 + 4b)} - a)/2b$. By fitting the T/T_0 vs C plots against the quadratic law, we obtained parameters *a* and *b*, from which the IC_{50} parameter can be calculated. In this work, we have defined an $IC_{50\alpha\text{-}T}$ relative to α-tocopherol. The lower the $IC_{50\alpha\text{-}T}$ value, the more effective the antioxidant. This quantitative analysis confirmed that (5*Z*)-lycopene with $IC_{50\alpha\text{-}T} = 3.14 \pm 0.14$ mol of α-TE/mol was the most efficient isomer in scavenging of the lipid peroxyl radicals, with a significant difference ($p < 0.05$) compared to the other isomers investigated (Figure 3D). (7*Z*,7′*Z*,9*Z*,9′*Z*)-Lycopene with $IC_{50\alpha\text{-}T} = 5.12 \pm 0.84$ mol of α-TE/mol was the weakest but not significantly different ($p > 0.05$) to (9*Z*)-lycopene ($IC_{50\alpha\text{-}T} = 4.21 \pm 0.27$ mol of α-TE/mol), (13*Z*)-lycopene ($IC_{50\alpha\text{-}T} = 4.32 \pm 0.38$ mol of α-TE/mol) and (all-E)-lycopene ($IC_{50\alpha\text{-}T} = 4.41 \pm 0.31$ mol of α-TE/mol). β-Carotene with $IC_{50\alpha\text{-}T} = 5.86 \pm 0.45$ mol of α-TE/mol (unpublished results) was significantly ($p < 0.05$) less antioxidant than (*all-E*)-lycopene and its (*Z*)-isomers. The lower inhibition afforded by carotenoids compared to α-tocopherol in our model may be attributed to (a) their slower reaction with the lipid peroxyl radicals in the micelles, (b) their scavenging of a smaller number of peroxyl radicals per antioxidant molecule (stoichiometry), (c) their formation of propagating peroxyl radicals by O_2 addition on the carbon centered radicals formed by addition of $LOO^{\bullet}$ on the conjugated polyene skeleton and (d) their competitive degradation by autoxidation and/or photooxidation.[22] In previous works[22,23,35] with the same model, we demonstrated that, unlike the flavonol quercetin, α-tocopherol and β-carotene are unable to prevent the formation of hypervalent heme iron species during activation of $MbFe^{III}$ by hydrogen peroxide and are thus unlikely to inhibit the onset of the peroxidation. Both rather react as chain-breaking antioxidants by reducing lipid peroxyl radicals (inhibition of propagation) in the lipid phase. The similar behavior of (*all-E*)-lycopene and its (*Z*)-isomers suggests that they probably act by the same mechanism. Several authors also demonstrated that carotenoids actually exhibit chain-breaking activity with the peroxyl radical, thus terminating the propagation of peroxidation.[36]

As mentioned above, the higher antioxidant efficiency of the (*Z*)-isomers in this assay compared to (*all-E*)-lycopene could be due to a better dispersion in the lipid/Tween micelles in relation

to their lower tendency to self-aggregate. A similar hypothesis was put forward to explain the higher solubility of (*Z*)-isomers in the mixed micelles formed in the intestine by combination of lipid digestion products and bile acids.[37]

In the acidic gastric compartment, the presence of O_2, dietary iron (e.g., $MbFe^{III}$ from red meat) and polyunsaturated fatty acids containing lipids can create favorable conditions for fast lipid oxidation processes[28] with the subsequent formation of potentially toxic lipid hydroperoxides and carbonyl compounds.[29] This postprandial lipid oxidation could be inhibited by lycopene and its (*Z*)-isomers via the consumption of tomato and tomato products.

In conclusion, according to our knowledge, this is the first study reporting on the antioxidant activity of geometrical isomers of lycopene in a diversity of assays. As mentioned by many authors working on antioxidant activity of compounds and complex matrices, the use of a minimum of three assays is recommended. Independent of the different mechanisms on which the various assays are based, all analyzed lycopene isomers were more antioxidant than α-tocopherol and *β*-carotene. Consequently, the contribution of lycopene to the antioxidant capacity of lycopene-rich food and in plasma might be higher than supposed until now. However, the activity of the various isomers varied depending on assay conditions. Our data might be of relevance in food processing. We could show that *in vitro* lycopene retains its high reducing potential despite changing its configuration to (*Z*)-isomers. Even more, the isomerization of (*all-E*)-lycopene to its (*Z*)-isomers, which occurs during tomato processing, improves the peroxyl radical scavenging activity of the containing lycopene. Furthermore, (*Z*)-isomers of lycopene are quantitatively important in human tissues with contribution to more than 50% of total lycopene. The regions of isomerization of lycopene are still discussed. The change of the conformation could happen during digestion in the gastrointestinal tract or after absorption into enterocytes or during lipoprotein metabolism in the liver. Nevertheless, we can conclude that the high content of (*Z*)-isomers might not have any disadvantageous effect on the contribution of lycopene to the antioxidant defense system.

AUTHOR INFORMATION

Corresponding Author

*Phone: +49(0)3641-949633. Fax: +49(0)3641-949702. E-mail: volker.boehm@uni-jena.de.

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ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); αTEAC, α-tocopherol equivalent antioxidant capacity; α-TE, α-tocopherol equivalents; AUC, area under the curve; FRAP, ferric reducing antioxidant power; DPPH, 2,2-diphenyl-1-picrylhydrazyl; HPLC, high-performance liquid chromatography; $IC_{50\alpha\text{-}T}$, half maximal inhibitory concentration compared to α-tocopherol; LPSC, luminol-chemiluminescence based peroxyl radical scavenging capacity; $MbFe^{III}$, metmyoglobin; $MbFe^{III}$-LP, metmyoglobin-induced lipid peroxidation

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