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Photoinduced Isomerization of Lycopene and Application to Tomato Cultivation

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Supporting Information

ABSTRACT: The present study aimed to investigate if growth conditions have an impact on the isomeric composition of lycopene in tomatoes. First a model system for photoinduced isomerization was established. Tomato extracts were irradiated with a halogen lamp, whose wavelength spectrum is close to the spectrum of daylight and thus mimics field-grown cultivation. Different optical filters were interposed between lamp and samples to simulate greenhouse conditions. 5-*cis*-Lycopene was formed preferentially while the concentration of 7-*cis*-lycopene decreased in field-grown model systems. The change of isomerization in greenhouse model systems led to a significantly different ratio. Consequently 5-*cis*- and 7-*cis*-lycopene were identified as potent markers for the differentiation of various lighting conditions during cultivation. This result was verified in biological samples. Authentic field-grown tomatoes (*var. Lycopersicon esculentum Mill. var. commune L. H. Bailey "Harzfeuer"*) showed a significantly higher content of 5-*cis*-lycopene 5.90 ± 0.45% compared to tomatoes of the same variety grown under electric lighting 4.11 ± 0.10%. Additionally, the ratio of 7-*cis*-lycopene was significantly lower under field-grown conditions.

KEYWORDS: *lycopene, isomerization, lighting, tomato cultivation, field-grown, greenhouse-grown*

INTRODUCTION

Tomatoes are one of the most important vegetables in the human diet. For example in Germany 24.9 kg, including 16.8 kg as processed products, were consumed per head in year 2010/11.[1] Hence tomatoes comprise the main source for lycopene in the human diet.[2] The importance of this carotenoid was shown by numerous investigations concerning its function and role for human health. Several studies reported that lycopene is closely associated with cardiovascular benefits and with prevention of various cancers, most notably prostate cancer.[3,4] These effects are most likely attributed to its structure consisting of 11 conjugated and 2 unconjugated double bonds which form the basis of the high antioxidant capacity and its function as the most effective singlet oxygen quencher of all carotenoids.[5] The *all-trans* configuration dominates in plant sources,[4] but the highly unsaturated system allows isomerization processes, so that a total of 1056 *cis*-isomers can be formed theoretically.[6] Isomerization of carotenoids is induced by thermal energy, light or chemical reactions.[7] The formed *cis*-isomers were characterized in the literature with a higher antioxidant activity and bioavailability that make processed food a better source for carotenoids in nutrition.[8,9] Consequently studies demonstrated an accumulation of *cis*-lycopene isomers in human serum and tissue and thus suggested potentially higher health benefits compared to *all-trans*-lycopene.[8,10] Nevertheless, the specific biological importance of *cis*-isomers has not been clarified so far.[10]

Thermal isomerization of lycopene and other carotenoids such as *β*-carotene are discussed contradictorily in the literature. Some studies showed no significant change in isomeric composition during heating of lycopene in model systems.[11,12] On the other hand degradation of *all-trans*-lycopene in hexane at room temperature with simultaneous formation of predominantly 13-*cis*-, 5-*cis*- and 9-*cis*-lycopene was reported by Lambelet et al.[13] Among others, studies of Colle et al. and Zhang et al. confirmed the isomerization process of *all-trans*- to various *cis*-isomers during heating, while no increase of 5-*cis*-lycopene was observed.[6,14] Isomerization of carotenoids is a very complex process and therefore leads to disparate results depending on the reaction conditions, e.g. *all-trans*-lycopene seems to be more stable in vegetable matrix while in model systems the used solvent has an enormous influence on the progress. This makes it difficult to compare various studies.

On the other hand studies of photoinduced isomerization processes are limited and lead to nonuniform results, too. Shi et al. showed that exposure of tomato purée to light caused no change in total and in *all-trans*-lycopene, but observed a loss of *cis*-isomers.[12] In contrast a total loss of 13.1% of *all-trans*-lycopene paralleled by a significant formation of 5-*cis*, 9-*cis*-, 13-*cis*- and 15-*cis*-lycopene was illustrated in studies of Lee et al. after 144 h of storage under light.[11] Furthermore the understanding of photoinduced isomerization has no practical relevance so far.

The focus of the present study was to characterize the photoinduced isomerization process of lycopene in model systems in detail. Different sources of light were used, because up to now there are no studies on the influence of the wavelength used in the progress of lighting. Results were then transferred to biological systems including consumer available

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tomatoes. It was possible to differentiate between authentic greenhouse-grown and field-grown tomatoes.

MATERIALS AND METHODS

Chemicals. The following chemicals of analytical grade were commercially available: hexane, ethanol, acetone (Carl Roth GmbH, Karlsruhe, Germany), potassium hydroxide (Grüssig GmbH, Filsum, Germany), butylated hydroxyltoluene (Fluka/Sigma-Aldrich, Steinheim, Germany), sodium chloride (Carl Roth GmbH, Karlsruhe, Germany), β-ionone (Sigma-Aldrich, Steinheim, Germany), pseudo-ionone (Alfa Aesar, Karlsruhe, Germany), NMR solvents (ARMAR Chemicals, Döttingen, Switzerland). Methanol and methyl *tert*-butyl ether as HPLC solvents were purchased from Sigma-Aldrich (HPLC grade ≥99.8%).

Tomato Samples. In this study three different groups of tomato samples were considered. In cooperation with the Leibniz Institute of Plant Biochemistry (Halle, Germany) a first set was cultivated under greenhouse conditions. Tomato plants of the variety *"Harzfeuer"* (*Lycopersicon esculentum Mill. var. commune L. H. Bailey "Harzfeuer"*) were sown in winter and grown under irradiation by sodium vapor lamps. Only ripe red tomatoes were harvested during the period of March to April. Until analysis whole tomatoes were stored at −32 °C. In a second set tomatoes of the same variety were cultivated from April under field-grown conditions at two different locations near Halle, Germany. These tomato plants were exposed to sunlight during the complete period of growing and ripening without use of foils. From July to September only ripe and full red tomatoes were harvested. The third set of tomatoes were samples available from local markets and food stores with different origins and varieties. Altogether 74 samples of six countries (Netherlands, Germany, Spain, Belgium, Italy and Morocco) were collected and stored at −32 °C until processing. Investigations proved stability of isomeric composition during storage of intact tomatoes at −32 °C until preparation for analyses.

Sample Preparation. Tomato slices, defrosted or fresh material, were homogenized at 0 °C with a mixer for 30 s under reduced light. Samples of 25 to 30 g of the resulting paste were filled into Falcon tubes and stored at −20 °C for at least 3 to 4 days. The frozen samples were lyophilized afterward for 3 days. Extraction of carotenoids was performed according to Sadler et al.[15] 200 mL of hexane/ethanol/acetone (50/25/25) containing 0.01% BHT (butylated hydroxyltoluene) was added to every sample. The extraction was performed by shaking the samples on an orbital shaker (170 to 180 rpm) at room temperature and under exclusion of light for 18 h. Afterward solids were separated by a filtration step. An additional saponification step was performed to separate carotenoids from simultaneously extracted fatty compounds like triglycerides. The alkaline hydrolysis also improved separation on the HPLC system. 20 mL of a 10% methanolic potassium hydroxide solution was added to the filtrate, and the mixture was shaken at room temperature for 20 h. To exclude effects induced by oxygen the organic layer was saturated with argon before. Subsequently the alkaline extract was washed 5 times each with 80 mL of a 2% sodium chloride solution to neutralize the organic layer and to remove saponification products from the carotenoid extract. The resulting orange colored organic layer was dried using a rotary evaporator (Laborota 4000, Heidolph) at 20 °C. Resulting solids were dissolved in 4 mL of methyl *tert*-butyl ether and diluted accordingly 1:10 in mobile phase (methanol/MTBE, 50/40).

To exclude thermal or photoinduced isomerization processes the whole preparation was performed under reduced light using aluminum foil or dark glassware and the temperature never exceeded 25 °C. Further investigations confirmed that the native isomeric composition was not influenced during storage and all steps of preparation.

Analytical HPLC−UV and HPLC-DAD. A Jasco PU-2089 Plus quaternary gradient unit pump with degasser and a Jasco AS-2055 Plus autosampler (Gross-Umstadt, Germany) were used. Elution of materials was monitored by a Jasco UV-2075 Plus UV detector (Gross-Umstadt, Germany) operating at 450 nm. For DAD analyses a Jasco MD-2015 Plus detector was used. Chromatographic separations were performed on a RP-C30 column (5 μm, 250 × 4.0 mm, YMC Europe, Dinslaken, Germany) connected to a C30 guard column (5 μm, 10 × 4.0 mm, YMC Europe, Dinslaken, Germany) using a flow rate of 0.65 mL min^{-1}. The chosen column temperature was 20 °C. The mobile phase used consisted of methanol (solvent A) and methyl *tert*-butyl ether (solvent B). Samples were injected at 40% B (held 5 min), and the gradient then changed to 83% B in 50 min. Then the gradient changed to 100% B in 5 min (held 10 min) and to 40% B in 5 min (held 10 min).

Analytical HPLC−MS^2. A Jasco PU-2080 Plus quaternary gradient pump with degasser and a Jasco AS-2057 Plus autosampler (Jasco, Gross-Umstadt, Germany) were used. PR-C30 column and HPLC gradient program used were the same as for HPLC−UV measurements. Elution of carotenoids was monitored by mass detection. The mass analyses were performed using an Applied Biosystems API 4000 quadrupole instrument (Applied Biosystems, Foster City, CA, USA) equipped with an API source using an atmospheric pressure chemical ionization (APCI, negative mode) interface. The LC system was connected directly to the probe of the mass spectrometer. Nitrogen was used as sheath and auxiliary gas. The optimized parameters for mass spectrometry of lycopene were Q1 536.7 amu, Q3 467.6 amu, DP −75 V, CE −27 eV, CXP −12 V. β-Carotene showed no fragment in Q3 (Q1 536.7 amu, DP −60 V).

Isolation and Syntheses of Authentic Carotenoid Standards. *all-trans*-Lycopene was isolated from tomatoes in the above-described extraction method. After extraction the dry residue was taken up in dichloromethane and precipitated with methanol. The formed precipitate was filtered off, washed with methanol and dried under argon stream. The resulting *all-trans*-lycopene standard was verified via 1D- and 2D-NMR spectra with an isomeric purity of 81% detected by HPLC-UV analyses. 5-*cis*-Lycopene was synthesized according to Hengartner et al. applying a C_{15}+C_{10}+C_{15}-Wittig strategy.[16] The central component was provided by synthesis on the basis of Frederico et al. and Ernst et al.[17,18] After the final Wittig condensation 150 mg of 5-*cis*-lycopene with 92% isomeric purity was obtained. The structure of the isomer was established by one- and two-dimensional NMR analyses. In contrast to Isler et al. 15-*cis*-lycopene and 15-*cis*-β-carotene were also synthesized via a C_{15}+C_{10}+C_{15} route instead of the described C_{10}+C_{20}+C_{10} scheme.[19] The central compound 2,7-dimethylocta-2,6-dien-4-yne-1,8-dial was assembled according to Isler et al.[19] This compound, without the suggested chain elongation to a C_{20}-compound, was then directly coupled to the respective C_{15}-Wittig salts. To obtain 15-*cis*-lycopene the same C_{15}-salt as for synthesis of the 5-*cis*-isomer was used for condensation. Instead of pseudo-ionone as the starting compound β-ionone was used to generate the corresponding 15-*cis*-β-carotene. The applied Wittig condensation led to 15,15′-dehydrolycopene and -β-carotene. The final hydrogenation step by Lindlar was performed as described by Islar et al. resulting in 15-*cis*-lycopene (60% isomeric purity) and 15-*cis*-β-carotene (90% isomeric purity).[19] This modified synthesis route allowed for a comparatively rapid generation of standards, because almost the same Wittig compound was used to generate 3 different carotenoid isomers. In contrast to Isler et al., a synthesis of C_{10}-Wittig salts was not required.

Irradiation of Tomato Extracts as Model System. To simulate different light conditions during the cultivation of tomatoes various model systems were established. Tomato extracts were irradiated with a halogen lamp, to mimic the wavelength spectrum of daylight. Thus, the irradiation with the halogen lamp was used to simulate field-grown conditions. To investigate the cultivation in greenhouses, different optical filters, which are impermeable for wavelengths below a defined value, were interposed between lamp and standard solution. A 550 nm filter was used to simulate lighting with a sodium vapor lamp, which is typical for cultivation of tomatoes in greenhouses in countries such as The Netherlands or Germany, where natural sunlight does not suffice to harvest ripe tomatoes during winter or spring months. The wavelength spectrum of these lamps is optimized for photosynthesis, i.e. they emit almost no light in the range of 250 to 550 nm. Growing tomatoes under greenhouse conditions without the use of additional sources of light was simulated by interposing a 420 nm filter between lamp and sample solution. Typical materials that are used for

cultivation in greenhouses are PE foils, PMMA sheets or glass. These materials cause a light permeability of less than 90%, which in cultivation depends additionally on the level of contamination, roof pitch and deterioration. Light with wavelengths below 400 nm is efficiently blocked by these materials.[20]

Dry tomato extracts were dissolved in hexane to a final lycopene concentration of about 10 to 15 μmol/L. Aliquots of 400 μL were transferred into a silica bulb and filled up to 4 mL using hexane. The resulting solutions were irradiated for defined periods of time (5, 10, 15, 30, 60, 120, 180, and 240 min) and dried afterward under argon stream. Residues were taken up in 400 μL of mobile phase (methanol/MTBE, 50/50) followed by HPLC−UV analyses. The isomeric composition was determined by integration of all identified lycopene peaks first. Then the areas of the separate peaks were divided by the sum of all lycopene values to express the percentage of the respective lycopene concentration.

Thermal Treatment of Tomato Extracts. Above described aliquots of tomato extract solution were also used for thermal treatment experiments. Tomato extracts in hexane were stirred at 65 °C in dark glass vials for 5, 10, 15, 30, 60, 120, 180, and 240 min, followed by HPLC−UV analyses. These experiments showed the differences between thermal and photoinduced isomerization processes. Moreover this model system was used to exclude artifacts based on thermal isomerization processes during the extraction method.

Nuclear Magnetic Resonance Spectroscopy (NMR). NMR spectra were recorded on a Varian VXR 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C or on a Varian Unity Inova 500 instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively.

Statistical Analysis. Analyses of carotenoid concentrations were performed in duplicate for each model system and resulted in coefficients of variation less than 5%. All significance tests were performed by two-sample *t*-test with a probability value of 99%. WELCH test was used alternatively in the case of differing standard deviations. Same probability value was selected. Confidence intervals were calculated with a probability value of 95%.

RESULTS AND DISCUSSION

Identification of Carotenoid Isomers. Extraction of tomatoes and analyses via HPLC−UV led to the typical chromatogram shown in Figure 1. For initial identification of different carotene isomers LC/APCI-MS was performed. During negative ion APCI a pseudo molecular ion of *m*/*z* 536.7 was formed for peaks **2** to **16**, to verify these 15 structures as carotenes. A proximate study by MS^2 analyses allowed differentiation between β-carotene and lycopene isomers, because only the molecular ion of lycopene formed the unique fragment of *m*/*z* 467.6, caused by elimination of one terminal isoprene group (−69.1), which is in line with data from Fang et al. in 2003.[21] In contrast β-carotene showed no fragmentation during negative ion APCI, due to its cyclic ionone end groups. Thus peaks **2** to **7** were identified as β-carotene isomers while signals **8** to **16** were characterized as lycopene isomers. Absolute configuration of the different isomers was verified by various approaches. Comparison of retention times using commercially available references led to the identification of *all-trans*-lutein (**1**), *all-trans*-β-carotene (**6**) and *all-trans*-lycopene (**15**). Moreover 15-*cis*-β-carotene (**2**), 15-*cis*-lycopene (**8**) and 5-*cis*-lycopene (**16**) were verified unequivocally by independently synthesized authentic reference compounds. The remaining isomers were assigned based on their spectral characteristics by HPLC-DAD analysis. According to the literature the *Q*-ratio (absorption at the subsidiary "*cis*-peak" (ca. 360 nm) divided by the absorption at λ_{max} (ca. 465 nm)) was used to characterize the other peaks. A more

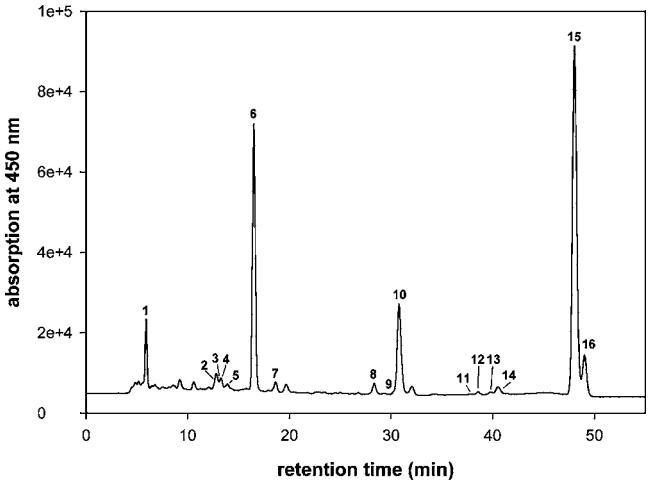

Figure 1. HPLC with UV detection at a wavelength of 450 nm showing separation of carotenoid isomers of a tomato extract. Peak identification: (1) lutein, (2) 15-*cis*-β-carotene, (3) *cis*-β-carotene, (4) 13-*cis*-β-carotene, (5) *cis*-β-carotene, (6) *all-trans*-β-carotene, (7) 9-*cis*-β-carotene, (8) 15-*cis*-lycopene, (9) lycopene *cis*-isomer 9, (10) 13-*cis*-lycopene, (11) lycopene *cis*-isomer 11, (12) 9-*cis*-lycopene, (13) lycopene *cis*-isomer 13, (14) 7-*cis*-lycopene, (15) *all-trans*-lycopene, (16) 5-*cis*-lycopene.

central *cis*-bond leads to a higher *cis*-peak to give a higher *Q*-ratio.[22,23] Consequently in comparison to the literature data 4 more isomers (**4**, 13-*cis*-β-carotene; **7**, 9-*cis*-β-carotene; **9**, 13-*cis*-lycopene; **12**, 9-*cis*-lycopene) were identified.[23−26] Our experiments gave the highest *Q*-ratio for 15-*cis*-lycopene (0.57), followed by 13-*cis* (0.48), 9-*cis* (0.26), 5-*cis* (0.11) and *all-trans* (0.06). This also suggested isomer **14** to be 7-*cis*-lycopene, because just a weak absorption was observed in the range of 360 nm, which points to a more terminal *cis*-bond (*Q*-ratio 0.19). Beside of its low *Q*-ratio the HPLC retention pattern was in line with data from the literature. The configuration of isomers **9**, **11** and **13** could not be clearly attributed due to the lack of authentic standards, but compared to the literature these signals were suggested to be di- or poly-*cis*-isomers. The presented identification of isomers based on a combination of authentic standards, MS and UV/vis data fully complies with the literature and therefore can be used for the following analyses.[16,26,27] However, it must be mentioned that the authentic synthesized 15-*cis*-lycopene elutes as the first lycopene isomer using a RP-C30 column, which is in contrast to some of the literature.[23,28]

Photoinduced Isomerization of Tomato Extracts as Model System. Transformation of *all-trans*-lycopene during irradiation using a halogen lamp was observed over a period of four hours to get a better understanding of photoinduced isomerization processes. Different optical filters were used to trigger differences in isomerization due to irradiation at diverse wavelengths (Figures 2A to 2C).

Model system A (Figure 2A) simulated tomato cultivation under field-grown conditions. The used light source was similar to sunlight, as the applied halogen lamp emitted light of wavelengths between 300 and 850 nm with a wide maximum range from 450 to 700 nm. During irradiation a rapid degradation of *all-trans*-lycopene of initially 75.59% to 59.48% was observed. The loss of *all-trans*-lycopene seemed to be almost linear (degradation rate ca. 4.0%/h) during 4 h of treatment. In parallel various *cis*-isomers were formed, most

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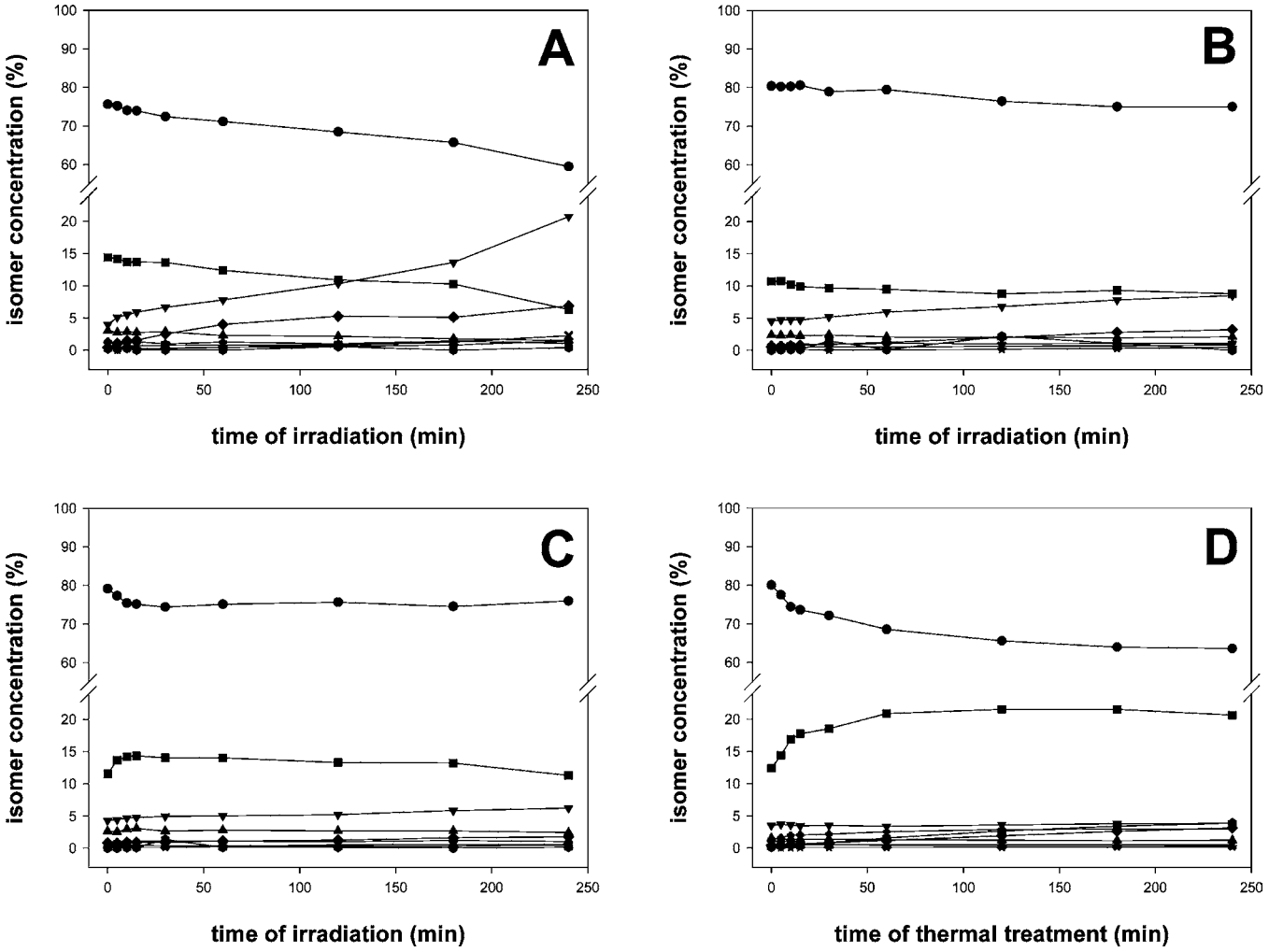

Figure 2. Concentrations of lycopene isomers during irradiation with a halogen lamp over a period of 4 h using no filter (A), 420 nm filter (B), 550 nm filter (C) and thermal treatment at 65 °C (D), expressed as percentage of total lycopene concentration (%). 15-*cis*-Lycopene (+), lycopene *cis*-isomer 9 (⬢), 13-*cis*-lycopene (■), lycopene *cis*-isomer 11 (★), 9-*cis*-lycopene (◆), lycopene *cis*-isomer 13 (×), 7-*cis*-lycopene (▲), *all-trans*-lycopene (●), 5-*cis*-lycopene (▼).

dominantly 5-*cis*-lycopene. The starting isomer content of 3.97% rose up to 20.74% in an almost linear fashion, too. The rate of formation (ca. 4.2%/h) was nearly the same as the degradation rate of *all-trans*-lycopene. A considerable change was observed for 9-*cis*-lycopene, too. Its concentration reached 6.88% starting from 0.44%. 13-*cis*- (from 14.42% to 6.30%), 15-*cis*- (from 1.37% to 1.04%) and 7-*cis*-lycopene (from 3.00% to 1.49%) degraded during irradiation. Remaining monitored isomers showed no relevant changes. Our studies demonstrate that the formation of 5-*cis*- and 9-*cis*-lycopene during lighting was preferred, while a simultaneous decrease of *all-trans*-, 15-*cis*-, 13-*cis*- and 7-*cis*-lycopene was observed. A theoretical approach to *cis*−*trans* isomerization published by Guo et al. can explain the present findings.[29] According to their work especially 5-*cis*-lycopene ($\Delta E_r^{\ddagger}$ = 35.2 kcal/mol) and also 9-*cis*-lycopene ($\Delta E_r^{\ddagger}$ = 23.1 kcal/mol) have a higher rotational barrier to reisomerize to the *all-trans* configuration than all other isomers ($\Delta E_r^{\ddagger}$ = 16.8 to 19.9 kcal/mol), which makes these two isomers more stable.[29] The stability of these two isomers is also caused by their much lower relative energy compared to all other isomers.[30] Both effects thus lead to the observed accumulation in contrast to 7-*cis*-lycopene, which has one of the highest potential energies of all mono-*cis*-isomers combined with a low rotational barrier ($\Delta E_r^{\ddagger}$ = 22.1 kcal/mol).[29,30] This aspect results in a dominant degradation of this unstable isomer during energy-rich irradiation.

In contrast to the literature the influence of specific light wavelengths on isomerization of lycopene was investigated in detail herein. Figures 2B and 2C illustrate the isomerization process of lycopene using the same halogen lamp with optical filters. Cutoff at 420 nm (Figure 2B) omitted light of smaller wavelengths to interact with tomato extracts. As a result almost no degradation of *all-trans*-lycopene was observed. The initial content of 80.27% just decreased to 75.01% during the whole lighting period, i.e. a total loss of only 5.26% compared to a loss of 16.11% observed in model system A. As expected all other isomers showed smaller changes, too. 5-*cis*-Lycopene presented the highest concentration after irradiation of all *cis*-isomers by increasing from 4.55% to 8.53%. Its formation was much slower but seemed to be linear again. The highest absolute formation was observed for 9-*cis*-lycopene again, which rose from 0.55% to 3.21%. However, this concentration is just a fraction compared to irradiation with the full wavelength spectrum (6.88%). Simultaneously the degradation of 13-*cis* and 7-*cis* was suppressed, too. 13-*cis* decreased from 10.72% to 8.80%, while

7-*cis* stayed almost at the same level starting from 2.38% to 2.10% after irradiation.

When a cutoff filter at 550 nm was used (Figure 2C), changes were even much less pronounced. Small decreases were observed for *all-trans*- (−3.14%) and 7-*cis*-lycopene (−0.16%), while concentrations of 9-*cis*- (+0.96%) and 5-*cis*-lycopene (+1.91%) increased marginally. Interestingly, differences in pattern were observed for 13-*cis*- and 15-*cis*-isomers. Figure 2C shows a slight increase of 13-*cis* (from 11.55% to 14.23%) and 15-*cis* (from 0.96% to 1.12%) after 10 min of irradiation, while in contrast both isomers decreased continuously during irradiation in model systems A and B. Further irradiation led again to degradation of these isomers, but final concentrations just reached starting levels (13-*cis* 11.33%, 15-*cis* 1.02%).

In addition to the photoinduced isomerization, thermal treatment at 65 °C on tomato extracts was performed to identify possible mixed effects (Figure 2D). In contrast to photoinduced isomerization a significant formation of 15-*cis*- and 13-*cis*-lycopene was observed. The concentration of 13-*cis*-lycopene rose from 12.39% to 20.65% (increase of 8.26%), while *all-trans*-lycopene underwent an intense degradation process, illustrated by a total loss of 16.39% (from 80.00% to 63.61%). After 120 min both isomers already reached constant levels and seemed to form an equilibrium. 15-*cis*-Lycopene was constantly formed during heating (from 1.36% to 3.00%). Such a strong formation of 13-*cis*- and 15-*cis*-isomer was unique for the thermal treatment model system. The concentration of 9-*cis*-lycopene also reached a mentionable level of 3.13%, i.e. a total increase of 2.74%. Other isomers showed no significant changes, e.g. 5-*cis*-lycopene rose from 3.49% to 3.85%. In the literature thermal pasteurization (at 60 or 90 °C) or sterilization (at 117 °C) of tomato purée led to similar results.[31] *all-trans*-Lycopene underwent degradation while the concentration of *cis*-isomers, mainly 13-*cis* and 9-*cis*, almost doubled to tripled for the sterilization procedure. Only high pressure sterilization resulted in formation of 5-*cis*-lycopene.[31] In essence, model system D showed that the energy during thermal processing was not sufficient to form energetic hindered isomers like 5-*cis*-lycopene. Predominantly, isomers with lower activation energy levels (13-*cis*, 15-*cis* and 9-*cis*) were formed. The thermal model D therefore also explains the initial increase of the 13-*cis*-lycopene in model C. Obviously irradiation at lower energy led to mixed thermal effects.

A simplified overview on the observed results during isomerization processes of lycopene is given in Figure 3.

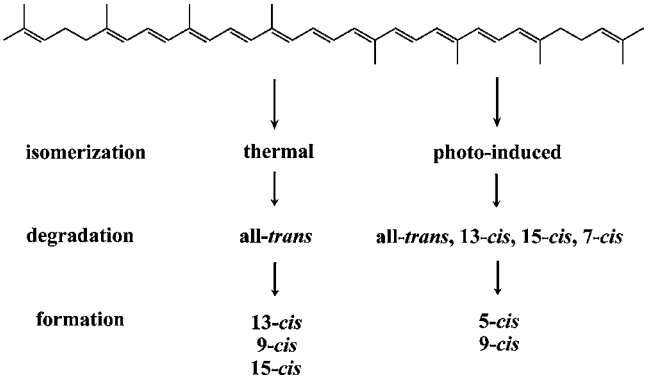

Figure 3. Thermal and photoinduced isomerization leads to degradation and formation of lycopene isomers.

Thermal isomerization is characterized by a strong degradation of *all-trans*-lycopene to form mainly 13-*cis*-, 9-*cis*- and 15-*cis*-isomers. Energy-rich photoinduced degradation is more complex, i.e. a decrease of *all-trans*-, 13-*cis*-, 15-*cis*- and 7-*cis*-lycopene was observed paralleled by formation of 5-*cis* and 9-*cis*. Wavelength cutoff during irradiation causes characteristics of thermal treatment verified by an increase of 13-*cis*- and 15-*cis*-lycopene combined with constant concentrations of 5-*cis* and 7-*cis*. Figure 3 illustrates the exceptional position of the 5-*cis*- and 7-*cis*-isomer during isomerization, because their formation and degradation is just triggered by photoinduced processes. This makes both isomers potent parameters for the differentiation of growing conditions. Thus we chose them for follow-up studies on authentic tomato samples. Concentrations of isomers 13-*cis* and 15-*cis* point to thermal treatments, showing considerable formation only during thermal treatment but decrease during irradiation. 9-*cis*-Lycopene is formed during both processes and, therefore, does not allow for any differentiation.

Lycopene Composition of Authentic Field- and Greenhouse-Grown Tomatoes. The results of the model systems were applied to real tomato samples. Altogether 55 samples of authentic field-grown tomatoes (*var. "Harzfeuer"*), exposed to sunlight during cultivation, and 49 samples of authentic greenhouse-grown tomatoes (*var. "Harzfeuer"*), planted under lamps with limited light spectra, were analyzed. Mean concentrations of all eight analyzed lycopene isomers are summarized in Table 1. Indeed significant differences between

Table 1. Mean Concentration (± Confidence Intervals), Expressed as Percentage of Total Lycopene, of All Analyzed Lycopene Isomers Observed in Different Sets of Tomato Samples

	mean concn (± confidence intervals) (%)		
	authentic field-grown tomatoes	authentic greenhouse-grown tomatoes	customer available tomatoes
15-*cis*	0.69 ± 0.14	0.77 ± 0.11	0.93 ± 0.12
13-*cis*	12.47 ± 0.51	12.41 ± 0.58	11.13 ± 0.61
isomer 11	0.06 ± 0.01[a]	0.12 ± 0.03	0.09 ± 0.01
9-*cis*	0.43 ± 0.03	0.51 ± 0.07	0.44 ± 0.05
isomer 13	0.48 ± 0.05	0.48 ± 0.07	0.69 ± 0.06
7-*cis*	1.67 ± 0.11[b]	2.65 ± 0.14	1.60 ± 0.11
all-trans	77.39 ± 0.79	78.33 ± 0.70	79.80 ± 0.94
5-*cis*	5.78 ± 0.26[b]	4.14 ± 0.08	4.71 ± 0.25
lycopene content (mg/100 g fresh wt)	2.0−3.9	1.4−2.4	1.8−5.1

[a]Significant difference compared to greenhouse-grown tomatoes calculated by *t* test (α = 0.01). [b]Significant difference compared to greenhouse-grown tomatoes calculated by WELCH test (α = 0.01).

field-grown and greenhouse-grown tomatoes were detected for three different lycopene isomers, as suggested in the model systems. A mean concentration of 5.78 ± 0.26% was assured for 5-*cis*-lycopene in field-grown tomatoes, which was significantly higher (α = 0.01) compared to greenhouse-grown tomatoes with 4.14 ± 0.08%. At the same time a significantly lower concentration of 7-*cis*-lycopene was established in field-grown tomatoes (α = 0.01) with 1.67 ± 0.11% compared to 2.65 ± 0.14% in greenhouse-grown tomatoes. The third significant difference was detected for the uncharacterized isomer **11**. Its mean concentration in field-grown tomatoes (0.06 ± 0.01%)

was only half as compared to greenhouse-grown tomatoes (0.12 ± 0.03%). All remaining isomers led to no significant differences (α = 0.05) at more or less the same concentration in both groups.

The clear differences for 5-*cis*- and 7-*cis*-lycopene in authentic field- and greenhouse-grown tomatoes correspond to the data from the model systems on irradiation of tomato extracts and are plotted in two dimensions in Figure 4. The energy-rich UV-

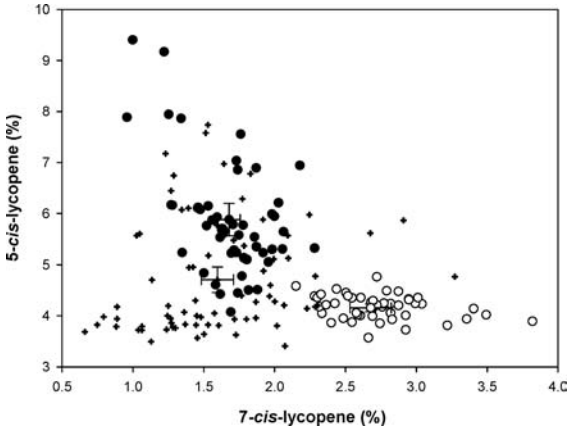

Figure 4. 5-*cis*- and 7-*cis*-lycopene concentrations of authentic field-grown (●), authentic greenhouse-grown (○) and customer-available tomato samples (+), expressed as percentage of total lycopene concentration (%).

light during sunlight exposure led to formation of the energetic hindered 5-*cis*-isomer while 7-*cis*-lycopene was reduced. These differences were paralleled by a slightly lower concentration of *all-trans*-lycopene in field-grown tomatoes. In round tomato types (e.g., *var. "Harzfeuer"*) Kuti et al. also observed a lower content of *all-trans*-lycopene compared to greenhouse-grown tomatoes, however *cis*-isomers were not analyzed particularly.[32] Interestingly our data provided no difference in 9-*cis*-lycopene concentration. Based on our model systems this parameter is influenced by both light and temperature (Figure 3). Obviously the formation of 9-*cis* was more influenced by thermal effects in this case. Cultivation of tomatoes in greenhouses is coupled to a higher average temperature, which is also caused by a light period of about 16 h.

A total of 74 samples from local markets and food stores were then analyzed to test for the hypothesis that the cultivation can also be differentiated in consumer available vegetables based on the two specific lycopene isomers. The results are summarized in Table 1 and Figure 4. 5-*cis*-Lycopene was analyzed at a mean concentration of 4.71 ± 0.25% which lay between the two authentically grown sets with a strong tendency toward authentic greenhouse-grown tomatoes. This notion reflects the higher market share of greenhouse cultivation, typical for the European market. In contrast, the mean concentration of 7-*cis*-lycopene (1.60 ± 0.11%) corresponds predominantly to the reference group of authentic field-grown tomatoes. Confidence intervals of customer available tomatoes were almost identical to authentically grown sets. In detail Figure 4 shows some crossovers between authentic field-grown tomatoes and commercial samples, while only a few crossovers to authentic greenhouse-grown tomatoes were observed. The major part of the commercial samples lay between the two authentic groups, which makes a clear assignment impossible. On the one hand, studies have shown that the concentration of *cis*-/*trans*-lycopene also depends on the particular tomato variety.[32] Additional analyses in the present study proved this observation. Nine authentic field-grown cherry tomato samples showed a slightly higher concentration of 5-*cis* (6.15 ± 0.83%) and a significantly lower level of 7-*cis* (0.91 ± 0.10%) compared to authentic field-grown tomatoes of the variety *"Harzfeuer"*. Otherwise analyses of 8 authentic field-grown beefsteak tomatoes resulted in a significantly lower concentration of 5-*cis*-isomer (4.56 ± 0.51%), while 7-*cis*-lycopene showed a significantly higher level (2.40 ± 0.44%). These results suggest that the caliber of tomatoes has a strong impact on isomerization. The relative depth of penetration by daylight or other sources of light seems to be much deeper in smaller varieties to cause a higher grade of isomerization resulting in formation of 5-*cis*- with a simultaneous degradation of *all-trans*- and 7-*cis*-lycopene. On the other hand authentically grown tomatoes were harvested only at full red ripe state and were frozen immediately after sampling. The isomeric composition of customer available tomatoes could have been influenced by storage conditions and times, e.g. during transportation. Thus, postharvest processing could explain the wide variability between customer available samples, too.

In summary, 5-*cis*- and 7-*cis*-lycopene were identified in different model systems as potent markers for photoinduced isomerization. This resulted in a clear differentiation between authentic field- and greenhouse-grown tomato samples within the same variety "*Harzfeuer*". In a first attempt classification of customer-available tomatoes was not possible. However, based on the present study, follow-up studies expanding the data on lycopene isomeric composition growing conditions of specific tomato varieties will also allow defined statements.

■ ASSOCIATED CONTENT

Supporting Information

NMR data, characteristics of halogen lamp used for irradiation experiments and UV/vis data and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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