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Thermal isomerization of (all-*E*)-lycopene and separation of the *Z*-isomers by using a low boiling solvent: Dimethyl ether

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ABSTRACT

Thermal isomerization of (all-*E*)-lycopene and separation of generated *Z*-isomers were conducted using a low boiling solvent, dimethyl ether (DME). Because of the low boiling point (–24.8 °C), DME is easily separated from lipids and other residues and is extremely low residual. The efficiency of thermal *Z*-isomerization of (all-*E*)-lycopene in DME was almost equivalent to using hexane. The thermally generated lycopene *Z*-isomers were separated by utilizing the solubility differences among lycopene isomers and a characteristic of DME that allows the solubility of compounds to be controlled by changing temperature. Finally, a lycopene mixture containing 72.0% *Z*-isomers was obtained from (all-*E*)-lycopene.

Keywords: Carotenoid, Dimethyl ether, Thermal isomerization, Physical property, Separation

Introduction

Lycopene ($C_{40}H_{56}$) is an acyclic carotenoid possessing eleven conjugated and two non-conjugated double bonds, abundant in vegetables and fruits with a bright-red color, such as tomato, watermelon, and gac (*Momordica cochinchinensis*).[1,2] Lycopene has strong antioxidant properties and can significantly reduce the risk of cancer, arteriosclerosis, and atherogenesis.[3,4] Tomatoes contain carotenoids in abundance, with lycopene accounting for more than 85% of its total carotenoids. Lycopene in fresh tomatoes occurs predominantly in the all-*E*-configuration. In contrast, *Z*-isomers of lycopene are primarily found in the human body; for example, >50% of total lycopene in serum and tissues is present as *Z*-isomers.[5,6] Their abundance in the human body suggests that *Z*-isomers of lycopene are more bioavailable than the all-*E*-isomer, and the intake of foods containing large amounts of *Z*-isomers would offer health benefits. In fact, both in vitro tests, using cultured small intestinal cells, and in vivo tests, using ferrets, strongly support these suggestions.[7,8] Moreover, in humans, the ingestion of tomato sauce rich in lycopene *Z*-isomers resulted in a practical increase in plasma lycopene concentrations compared to results obtained with a sample abundant in the all-*E*-isomer.[9] Lycopene *Z*-isomers are also expected to show a higher antioxidant capacity than that of all-*E*-isomer.[10,11] Therefore, the intake of lycopene *Z*-isomers, rather than the all-*E*-isomer, is preferable for human health, and appropriate methods are required to enable efficient *Z*-isomerization of (all-*E*)-lycopene. A number of studies have reported that (all-*E*)-carotenoids, including lycopene, promoted thermal *Z*-isomerization in organic solvents such as hexane and acetone.[12–16] However, those organic solvents have many disadvantages such as toxicity, presence of solvent traces in final product, and high energy cost for the evaporation because of high boiling point.

Thus, low toxic and low boiling point solvents are paid attention to application for food field. As one of such solvents, the use of dimethyl ether (DME, boiling point; –24.8 °C) is expected in recent years.[17–19] Dimethyl ether has been approved as a safe solvent for the production of foodstuffs and food ingredients by the European Food Safety Authority (EFSA)[20] and Food Standards Australia New Zealand (FSANZ).[21] Europe has permitted the use of DME as an extraction solvent only to extract fat from animal proteins at a maximum residue limit of 9 μg/kg, and Australia and New Zealand have also approved the use of DME for food processing except for dairy ingredients and dairy products where a maximum residue limit of 2 mg/kg is defined.[21] Dimethyl ether has high oxidation stability[22] and could prevent intrusion of oxygen in the apparatus due to the high inner pressure. Thus, the use of DME would be suitable for processing of easily oxidizable substance such as lycopene.[23] Figure 1 describes predicated *Z*-isomerization process of (all-*E*)-lycopene by using general organic solvents and DME. When organic solvents are used, in addition to a risk of a health hazard, high energy cost is require to remove and condense them (Figure 1A). On the other hand, the use of DME as a solvent has many advantages compared with organic solvents as following: 1) easy to separate from lycopene without the use of a high-power heat source, 2) extremely low-residual in final product, 3) low risk of a health hazard, 4) inhibit to oxidative decomposition of lycopene (Figure 1B).

In this study, in addition to the thermal *Z*-isomerization of (all-*E*)-lycopene in DME, the differences of physical properties between (all-*E*)-lycopene and the *Z*-isomers were investigated by scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) analyses. Moreover, we examined the separation of the thermally generated *Z*-isomers by utilizing the differences in physical properties between

(all-*E*)-lycopene and the *Z*-isomers and a characteristic of DME that allows the solubility of compounds to be controlled by changing the temperature.[7,24–27] As shown in Figure 1, (all-*E*)-lycopene would be trapped by a filter because it is low solubility and has high crystallinity compared to the *Z*-isomers (refer to the "Results and discussion" section). Thus, it is considered that by repeatedly *Z*-isomerizing the filtered (all-*E*)-lycopene, almost all of the initially-loaded (all-*E*)-lycopene can be isomerized to the *Z*-isomers. We herein report the first instance of using DME as a solvent to promote *Z*-isomerization of (all-*E*)-lycopene and the separation of thermally generated *Z*-isomers in a model system. This new isomerization procedure could lead to improved product quality, lowered manufacturing costs, and a reduction in environmental loading.

Experimental

Reagents

Analytical grade acetone, ethanol, and benzene, and high-performance liquid chromatography (HPLC)-grade hexane were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *N*,*N*-Diisopropylethylamine (DIPEA) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Dimethyl ether was obtained from Tamiya, Inc. (Shizuoka, Japan).

Preparation of (all-E)-lycopene

(All-*E*)-lycopene was obtained from tomato oleoresin (Lyc-O-Mato® 15%, LycoRed Ltd., Beer-Sheva, Israel) according to a previous description with some modifications.[15,28] Briefly, oleoresin (3.2 g) was dissolved in benzene (100 mL), and insoluble substances were collected by suction filtration on a Kiriyama funnel (number

5B filter paper). The residue was rinsed with benzene (50 mL), acetone (50 mL), and then ethanol (50 mL), and dried in vacuo to afford 354 mg of fine red crystalline (all-*E*)-lycopene (normal-phase HPLC, ≥96% purity).

Thermal isomerization of lycopene in DME

Purified (all-*E*)-lycopene was dissolved in liquefied DME at a concentration of approximately 0.05 mg/mL in a reaction vessel (TVS-1; Taiatsu Techno Corp., Saitama, Japan), and the solution-filled vessel was heated in water bath. The reaction temperature and absolute pressure were set as 40, 60, and 80 °C, and 0.9, 1.4, and 2.2 MPa, respectively. The reaction time was set at 1, 3, and 6 h for 60 °C, and 3 h for 40 and 80 °C. According to previous studies,[12,29] the thermal isomerization reaction of (all-*E*)-lycopene in organic solvents at the above temperature have reached equilibrium within approximately 6 h. Thus, the time course of *Z*-isomerization at 60 °C was set at maximally 6 h. In all isomerization tests, DME is not a supercritical state but a liquid state; critical temperature and pressure of DME are 126.85 °C and 5.37 MPa, respectively.[18] After isomerization of (all-*E*)-lycopene, DME was removed by returning to room temperature and atmospheric pressure, and the resulting red substance was dissolved in hexane at a concentration of approximately 0.05 mg/mL, filtered through a 0.2-μm polytetrafluoroethylene (PTFE) membrane filter (Advantec Co., Ltd., Tokyo), and then applied to normal-phase HPLC. To compare the *Z*-isomerization tendencies of liquefied DME and general organic solvents, the thermal isomerization of (all-*E*)-lycopene in hexane was also conducted under the same temperature and lycopene concentration conditions as for DME described above.

HPLC analysis

Normal-phase HPLC analysis with a photodiode array detector (L-2455; Hitachi Ltd., Tokyo, Japan) was conducted according to the method described previously with some modifications.[30,31] Briefly, analysis was performed on four Nucleosil 300-5 columns connected in tandem (4 × 250 mm length, 4.6 mm inner diameter, 5 μm particle size; GL Sciences Inc., Tokyo, Japan) with hexane containing 0.075% DIPEA, at a flow rate of 1.0 mL/min, and a column temperature at 40 °C. The quantification of lycopene isomers was performed by peak area integration at 460 nm. Lycopene isomer peaks were identified according to HPLC retention times, visible spectral data, and the relative intensities of the *Z*-peak (% D_B/D_{II}), as described in previous research.[30–34]

SEM analysis

The shape and surface characteristics before and after thermal *Z*-isomerization of lycopene in DME at 60 °C for 3 h were observed by scanning electron microscopy (SEM; JSM-6390LV JEOL, Tokyo, Japan). The samples were sputter-coated with gold in a high-vacuum evaporator and examined using SEM at 10 kV. The sample before isomerization, (all-*E*)-lycopene crystal, was dissolved once in DME, and then the solvent was removed by returning the solution to room temperature and atmospheric pressure.

DSC analysis

The melting point of purified (all-*E*)-lycopene and thermally isomerized lycopene in DME at 60 °C for 3 h were measured by differential scanning calorimetry (DSC) using a DSC-60 system (Shimadzu, Kyoto, Japan). DSC measurements were carried out with

aluminum sample pans and the mass of the sample was approximately 4 mg. Both samples were scanned at a heating rate of 10 K/min under a nitrogen atmosphere.

Separation of lycopene Z-isomers

Lycopene *Z*-isomers were separated by utilizing solubility characteristics, with the *Z*-forms having a putative higher solubility than the all-*E*-configuration,[7,24] and the phase behavior property of liquefied DME that allows the solubility of compounds to be controlled by changing the temperature.[25–27] The device configuration for separating lycopene *Z*-isomers is shown in Figure 2. A 0.5-μm stainless steel filter was placed between the reaction vessel and recovery vessel to remove insoluble lycopene isomers, putative mainly (all-*E*)-lycopene. (All-*E*)-lycopene was dissolved in DME at a concentration of 1 mg/mL, and *Z*-isomerization was performed at 60 °C for 3 h in the reaction vessel. After cooling the vessel to 4 °C in ice-cold water (absolute pressure: 0.3 MPa), the thermally isomerized lycopene solution was fed to the recovery vessel through the filter using a vacuum pump. Dimethyl ether was then removed by returning to room temperature and atmospheric pressure. Filter-trapped lycopene and recovered lycopene in the recovery vessel were dissolved in a defined volume of hexane, filtered through a 0.2-μm PTFE filter, and the *Z*-isomerization ratios were measured by normal-phase HPLC. Moreover, the mass balance of the filter-trapped lycopene and recovered lycopene was determined by comparing HPLC peak area of total lycopene isomers.

Results and discussion

Thermal isomerization of (all-E)-lycopene

Thermal isomerization of (all-*E*)-lycopene to the corresponding *Z*-isomers was investigated in liquefied DME and hexane. The total *Z*-isomers contents rapidly increased within a few hours in both solvents at 60 °C, reaching more than 50% in both solvents during the test periods (Figure 3A). Isomerization of (all-*E*)-lycopene was enhanced by increasing the temperature both solvents to almost the same level (Figure 3B); when the temperatures were 40, 60, and 80 °C, the total *Z*-isomers contents of lycopene were 27.8, 49.6, and 62.5%, respectively, in liquefied DME, and 27.3, 50.4, and 61.9%, respectively, in hexane, after heat treatment for 3 h. These results indicated that liquefied DME had an isomerization promoting effect on the total amount of lycopene *Z*-isomers, similar to that of general organic solvents. In addition, it was confirmed that lycopene was hardly decomposed in any reaction time and temperature in both solvents from the result of HPLC analysis.[31]

Typical chromatograms of thermally isomerized lycopene solutions, as well as that of purified (all-*E*)-lycopene, are shown in Figure 4. (13*Z*)-Lycopene increased the most in both solvents, and (9*Z*)-lycopene also increased notably. These *Z*-isomerization tendencies of (all-*E*)-lycopene have also been observed in other organic solvents, such as acetone and benzene.[12,15] According to Takehara et al. (2015),[14] the magnitude of the activation energies of isomerization from (all-*E*)-lycopene to (mono-*Z*)-lycopene are in the order (5*Z*)-lycopene > (9*Z*)-lycopene > (13*Z*)-lycopene. This computational result was in good agreement with our experimental findings. Namely, the amount of *Z*-isomers generated was in the order (13*Z*)-lycopene > (9*Z*)-lycopene > (5*Z*)-lycopene in almost all experiments. (5*Z*)-Lycopene content was only increased in liquefied DME. (5*Z*)-Lycopene has been reported as having higher bioavailability[35] and antioxidant capacity[11] than (all-*E*)-lycopene, as well as possibly (9*Z*)- and (13*Z*)-lycopene. Thus,

liquefied DME would be more effective in obtaining the highly functional lycopene isomer than general organic solvents. In addition, when using liquefied DME, many types of lycopene *Z*-isomer, putative multi-*Z*-isomers of lycopene such as 9*Z*,13*Z*- and 5*Z*,9*Z*-isomers,[12] were observed, compared to results obtained using hexane. Reasons for this difference between generated lycopene *Z*-isomers using liquefied DME and hexane would be the differences of their polarizability and polarity.[12,31]

Physical properties of thermally isomerized lycopene

Thermally *Z*-isomerized lycopene had different appearance and character than non-isomerized lycopene. Namely, (all-*E*)-lycopene was present in a solid state, while lycopene after isomerization treatment in hexane and DME was present in a high-viscosity oil state. Thus, to investigate the effect of the thermal *Z*-isomerization treatment on physical properties of lycopene, SEM and DSC analysis were conducted. Figure 5 shows SEM images of lycopene before and after thermal *Z*-isomerization in DME. (All-*E*)-lycopene formed plate-like crystals, while lycopene after thermal *Z*-isomerization treatment did not form a crystal state, but an oily aggregate state. Recently, Sun et al. (2016)[36] reported that catalytically *Z*-isomerized lycopene could be less prone to crystallize. Similarly, an oily aggregate state has been proposed for lycopene *Z*-isomers found in globular chromoplasts of tangerine tomato.[37] However, this is the first instance that *Z*-isomers of lycopene generated by heat treatment, which is a commonly-used method to obtain those isomers, form an oily aggregate state. Furthermore, differences of the properties between (all-*E*)- and *Z*-form were also observed in β-carotene.[24] Namely, (all-*E*)-β-carotene aggregates showed a flake-like morphology, while (9*Z*)-β-carotene, obtained from microalga *Dunaliella bardawil*, did

not show any sign of aggregation in chloroform. Figure 6 shows the DSC charts for the purified (all-*E*)-lycopene and thermally isomerized lycopene. The DSC chart of the purified (all-*E*)-lycopene showed a large peak of 175.32 °C, which was consistent with the value obtained by Takehara et al. (2014).[15] On the other hand, there was no peak around 175.32 °C in the DSC curve of thermally isomerized lycopene, and two possible melting points 42.97 and 142.92 °C were observed, which indicated that melting point depression occurred. Tests and measurements were done three times independently, but all showed the same trend. This result strongly suggests that physical properties of lycopene aggregate changed drastically by the thermal *Z*-isomerization. Carotenoid aggregates can be stabilized via π–π-stacking interactions of conjugated polyene chains. When the *Z*-isomer content is increased, enormous steric hindrance would occur, diminishing the potential attractive π–π forces. [24] Therefore, it is considered that the regular arrangement of lycopene molecules was prevented by the coexistence of the *Z*-isomers, resulting in the change the physical properties.

The difference of the physical properties between all-*E*- and *Z*-form of carotenoids is a very interesting field that became apparent in recent years. Thus, development of processing technology of carotenoids utilizing the difference is expected in the future. In the next section, by utilizing the property of lycopene *Z*-isomers that would not easily form crystals and the nature of liquefied DME that can easily control compounds solubility by changing the temperature,[25–27] we attempted to separate the lycopene *Z*-isomers by DME.

Separation of lycopene Z-isomers

Lycopene *Z*-isomers are thought to be more bioavailable than (all-*E*)-lycopene

because they are more soluble in bile acid micelles and preferentially incorporated into chylomicrons than the (all-*E*)-isomer.[7] Lycopene *Z*-isomers are also considered to be more soluble in organic solvents than the (all-*E*)-isomer. This difference in solubility is thought to be related to lycopene *Z*-isomers having difficulty in forming crystals, as shown in Figure 5. Liquefied DME can control compound solubility by changing the temperature.[25–27] We therefore experimented with *Z*-isomer separations in lycopene by utilizing the solubility difference among lycopene isomers and the characteristic of liquefied DME by an apparatus as shown in Figure 2. The chromatograms of filter-trapped lycopene isomers and recovered lycopene isomers in the recovery vessel are shown in Figure 7, with the total amount of lycopene *Z*-isomers being 6.3 and 72.0%, respectively. When (all-*E*)-lycopene was heated under the same conditions as this test (60 °C for 3 h), the *Z*-isomer content became 49.6% (Figure 3A and 4C). Thus, the *Z*-isomer content could increase over 20% using DME system (Figure 2). This result indicated that lycopene *Z*-isomers could be dissolved in liquefied DME at 4 °C (absolute pressure: 0.3 MPa), and pass through the filter. However, (all-*E*)-lycopene would crystallize in liquefied DME due to low solubility and be trapped by the filter. These data strongly support the fact that lycopene *Z*-isomers are more soluble than the all-*E*-isomer. In addition, the mass balance of the filter-trapped lycopene isomers to the recovered lycopene isomers was 39.6:60.4. Considering the change in total *Z*-isomer content before and after the separation, the above mass balance was appropriate.

One of the efficient ways to deliver lycopene into body is the ingestion of supplements.[38,39] As the raw material of the supplement, tomato oleoresin is widely used. However, most lycopene in the oleoresin is present as the all-*E*-configuration.[40] The *Z*-isomerization and separation method using DME could be applied to tomato

oleoresin. Namely, by utilizing the processing method as shown in Figure 1B, tomato oleoresin having high functionality and safety would be obtained.

Conclusions

This study has demonstrated thermal *Z*-isomerization of (all-*E*)-lycopene and separation of generated *Z*-isomers by using a low boiling solvent, DME, which is easily separated from lipids and other residues, extremely low residual, and low toxicity, in a model system. The efficiency of thermal *Z*-isomerization of (all-*E*)-lycopene in DME was almost the same level as in hexane. Although (all-*E*)-lycopene was present as crystal state, lycopene suffered thermal *Z*-isomerization treatment in DME and hexane became an oily aggregate state. By utilizing such the difference of physical properties between all-*E*- and *Z*-form, and a characteristic of liquefied DME which was used to adjust compound solubility by changing the temperature, the thermally generated lycopene *Z*-isomers have been separated. Finally, a lycopene mixture containing 72.0% *Z*-isomers was obtained from (all-*E*)-lycopene.

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Figure Captions

Figure. 1. Schematic diagram for procedure comparison of (A) general organic solvent method with (B) DME method to obtain lycopene composition rich in *Z*-isomers of lycopene.

Figure. 2. Scheme of laboratory-scale apparatus for dimethyl ether separation.

Figure. 3. Changes in the total amount of lycopene *Z*-isomers contents when thermally isomerized at (A) 60 °C for 1, 3, and 6 h, and (B) 40, 60, and 80 °C for 3 h in hexane (open bars) and dimethyl ether (shaded bars). Isomerization (%) is expressed as a percentage of the amount of *Z*-isomers in the total amount of lycopene isomers, including (all-*E*)-lycopene.

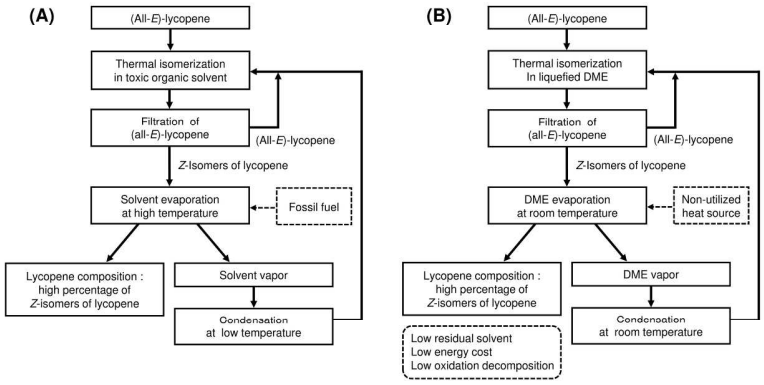
Figure. 4. Normal-phase HPLC chromatograms of (A) (all-*E*)-lycopene and thermally treated lycopene at 60 °C for 3 h in (B) hexane and (C) dimethyl ether. (5*Z*)-, (9*Z*)-, and (13*Z*)-lycopene designated in the charts were identified according to the previous studies.[29–33] Asterisk symbols show the *Z*-isomer of lycopene peaks which were observed only in DME.

Figure. 5. SEM images of lycopene (A) before and (B) after *Z*-isomerization in dimethyl ether at 60 °C for 3 h.

Figure. 6. DSC curves of lycopene (A) before and (B) after *Z*-isomerization in dimethyl

ether at 60 °C for 3 h.

Figure. 7. Normal-phase HPLC chromatograms of lycopene isomers after the separation treatment at 4 °C and 0.3 MPa by dimethyl ether, as shown in Figure 2: (A) trapped lycopene in 0.5-μm stainless steel filter, and (B) recovered lycopene in recovery vessel. Before the separation, (all-*E*)-lycopene was isomerized to the *Z*-isomers in dimethyl ether at 60 °C for 3 h.



Schematic diagram for procedure comparison of (A) general organic solvent method with (B) DME method to obtain lycopene composition rich in *Z*-isomers of lycopene.

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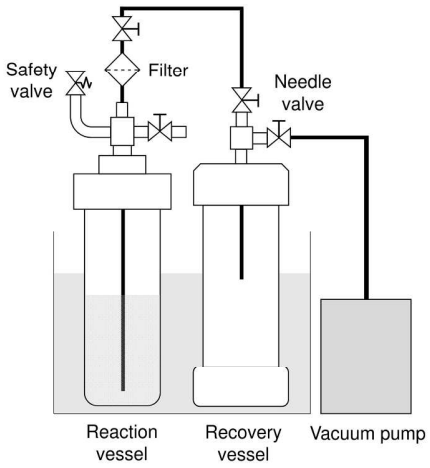
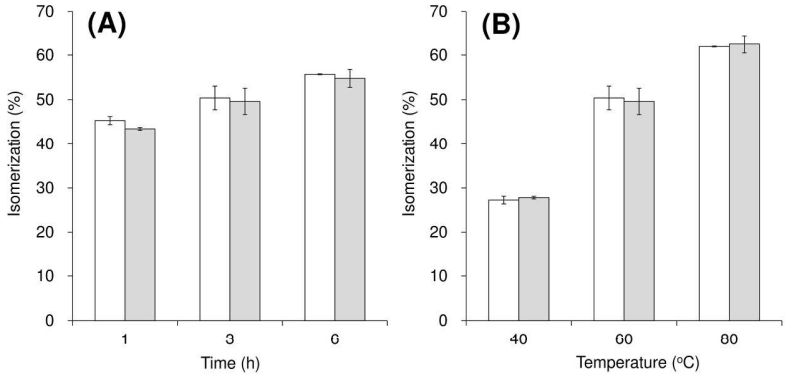


Scheme of laboratory-scale apparatus for dimethyl ether separation.

209x148mm (300 x 300 DPI)



Changes in the total amount of lycopene *Z*-isomers contents when thermally isomerized at (A) 60 °C for 1, 3, and 6 h, and (B) 40, 60, and 80 °C for 3 h in hexane (open bars) and dimethyl ether (shaded bars). Isomerization (%) is expressed as a percentage of the amount of *Z*-isomers in the total amount of lycopene isomers, including (all-*E*)-lycopene.

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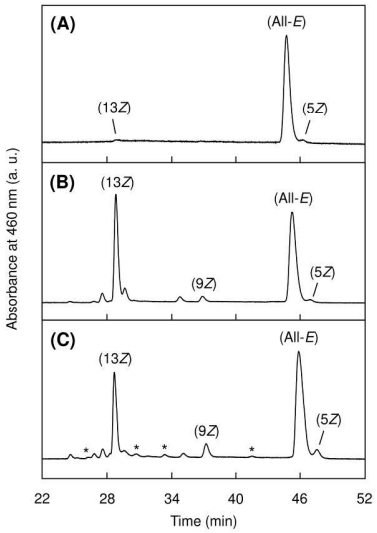

Normal-phase HPLC chromatograms of (A) (all-*E*)-lycopene and thermally treated lycopene at 60 °C for 3 h in (B) hexane and (C) dimethyl ether. (5*Z*)-, (9*Z*)-, and (13*Z*)-lycopene designated in the charts were identified according to the previous studies.[29–33] Asterisk symbols show the *Z*-isomer of lycopene peaks which were observed only in DME.

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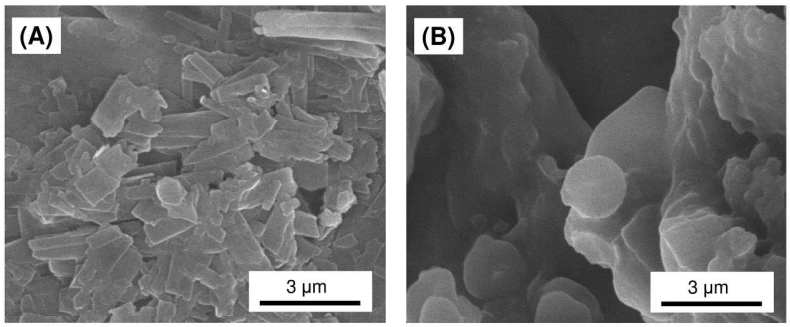

SEM images of lycopene (A) before and (B) after *Z*-isomerization in dimethyl ether at 60 °C for 3 h.

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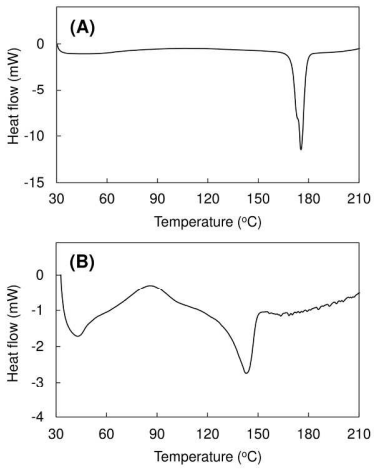
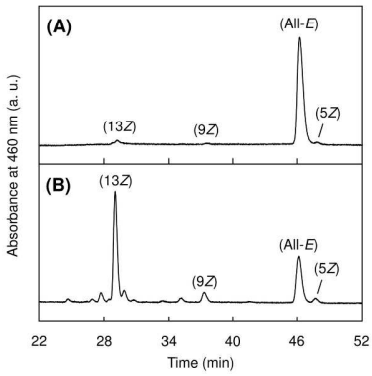


DSC curves of lycopene (A) before and (B) after *Z*-isomerization in dimethyl ether at 60 °C for 3 h.

209x148mm (300 x 300 DPI)



Normal-phase HPLC chromatograms of lycopene isomers after the separation treatment at 4 °C and 0.3 MPa by dimethyl ether, as shown in Figure 2: (A) trapped lycopene in 0.5-μm stainless steel filter, and (B) recovered lycopene in recovery vessel. Before the separation, (all-*E*)-lycopene was isomerized to the *Z*-isomers in dimethyl ether at 60 °C for 3 h.

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