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# Thermal isomerization pre-treatment to improve lycopene extraction from tomato pulp

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ABSTRACT

The effect of thermal *Z*-isomerization pre-treatment on lycopene extraction from dried tomato pulp by organic solvents and supercritical carbon dioxide (SC-$CO_2$) was evaluated. Although total *Z*-isomer content of lycopene in fresh tomato pulp was only 6.1%, it increased by thermal treatment at 120 and 150 °C for 1 h to 10.0% and 56.2%, respectively. Furthermore, by adding 1 g/100 g of olive oil to the pulp, the thermal *Z*-isomerization efficiencies of lycopene at 120 and 150 °C for 1 h improved significantly such that the total *Z*-isomer contents were 30.4% and 75.7%. After freeze-drying of the thermal treated tomato pulp, lycopene was extracted by ethanol, ethyl acetate, and SC-$CO_2$. When any solvents were used for the extraction, lycopene recovery increased according to the *Z*-isomer content of dried tomato pulp, e.g. in the case that ethanol extraction was conducted from the pulp containing 6.1%, 30.4%, and 75.7% *Z*-isomer content of lycopene, lycopene recoveries were 4.3%, 28.1%, and 75.9%, respectively. These results strongly indicated that *Z*-isomers of lycopene are more soluble in solvents than the all-*E*-isomer.


## 1. Introduction

Carotenoid-rich foods offer multiple health benefits such as a decreased risk of cancer and atherosclerosis (Dahan, Fennal, & Kumar, 2008; Palozza, Parrone, Simone, & Catalano, 2010; Tapiero, Townsend, & Tew, 2004). Lycopene, a carotenoid that imparts the characteristic deep-red color to ripe tomatoes and tomato products, plays a major role in these health benefits. Lycopene is often extracted from tomatoes or tomato byproducts with organic solvents and used as a supplement and dye in food products. Given its high hydrophobicity and crystallinity, lycopene is insoluble in water and sparingly soluble in oils and polar solvents. As a result, its extraction efficiency is low and thus many studies have been conducted to improve lycopene extraction efficiency from plant materials by optimizing pre-treatment and extraction condition (Borel et al., 1996; Cadoni, De Giorgi, Medda, & Poma, 2000; Calvo, Dado, & Santa-María, 2007; Kubola, Meeso, & Siriamornpun, 2013; Strati & Oreopoulou, 2016; Tuyen, Nguyen, Roach, & Stathopoulos, 2013). In this study, we first focused on "*Z*-isomerization of lycopene as pre-treatment" for improving the extraction efficiency.

In plants, lycopene predominantly occurs in its (all-*E*) configuration. In the human body and processed food, on the other hand, it exists mainly as *Z*-isomers. For instance, more than half of total lycopene in serum and tissues exists as the *Z*-isomers (Clinton et al., 1996; Schierle et al., 1997; Stahl, Schwarz, Sundquist, & Sies, 1992). *In vitro* and *in vivo* experiments using a Caco-2 human intestinal cell model and lymph cannulated ferrets, respectively, revealed higher bioavailability for lycopene *Z*-isomers than the all-*E*-form (Boileau, Merchen, Wasson, Atkinson, & Erdman, 1999; Failla, Chitchumroonchokchai, & Ishida, 2008). In addition, the oral administration of the tangerine tomato juice (containing 94% total *Z*-isomer content of lycopene) increased the human plasma lycopene concentration as compared with red tomato juice (containing 10% total *Z*-isomer content of lycopene) (Cooperstone et al., 2015). Moreover, studies have reported *Z*-isomers to exhibit higher antioxidant capacity than the (all-*E*)-isomer (Böhm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002; Müller et al., 2011). Thus, the dietary intake of *Z*-isomers of lycopene is preferred over that of the (all-*E*)-isomer for health reasons.

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Physical characteristics such as crystallinity and solubility differ between the (all-*E*)-carotenoids and *Z*-isomers (Cooperstone et al., 2015; Gamlieli-Bonshtein, Korin, & Cohen, 2002; Hempel, Schädle, Leptihn, Carle, & Schweiggert, 2016; Honda, Kudo et al., 2017). For instance, (all-*E*)-carotenoids such as β-carotene and lycopene display crystalline nature, but the *Z*-isomers exist in an oily aggregate state (Cooperstone et al., 2015; Hempel et al., 2016). The data available on the solubility of *Z*-isomers are limited but *Z*-isomers of carotenoids are more soluble than the all-*E*-isomers, e.g. the solubility of (9*Z*)-β-carotene in SC-$CO_2$ was reported to be approximately four times higher than that of the (all-*E*)-isomer (Gamlieli-Bonshtein et al., 2002). However, only a few studies have exploited these characteristics of *Z*-isomers for technological development. Therefore, the aim of this study was to improve solvent extraction efficiency of lycopene from plant source by utilizing the higher solubility of the *Z*-isomers than the all-*E*-isomer. Namely, we investigated whether lycopene extraction efficiency could be improved by increasing the *Z*-isomer content in extraction material (dried tomato pulp). The *Z*-isomerization of lycopene in the extraction material was conducted by thermal treatment (Honda, Murakami et al., 2017; Schierle et al., 1997) and ethanol, ethyl acetate, and supercritical $CO_2$ (SC-$CO_2$) were used as extraction solvents. Ethanol and ethyl acetate were organic solvents approved for using in food processing, and SC-$CO_2$ is an attractive alternative for the extraction of natural pigments, attributable to its nontoxicity and easy separation from the extract (Gomez-Prieto, Caja, & Santa-Maria, 2002; Topal, Sasaki, Goto, & Hayakawa, 2006; Zuknik, Norulaini, & Omar, 2012).

## 2. Materials and methods

### 2.1. Materials

Fresh tomato pulp (moisture content, 91.6 g/100 g) used in this study was kindly provided by Kagome Co., Ltd. (Tokyo, Japan). The fresh tomato pulp was a precipitate obtained by centrifuging tomato juice. (all-*E*)-Lycopene (HPLC, ≥98% purity) for preparing the calibration curve was isolated from tomato oleoresin (Lyc-*O*-Mato® 15%, LycoRed Ltd., Beer-Sheva, Israel) according to the previous descriptions (Honda, Murakami et al., 2017; Takehara et al., 2014). Analytical-grade acetone, ethanol, and ethyl acetate as well as high performance liquid chromatography (HPLC)-grade hexane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *N*,*N*-Diisopropylethylamine (DIPEA) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Olive oil was purchased from the Nisshin OilliO Group Ltd. (Tokyo, Japan). Carbon dioxide was obtained from Sogo Kariya Sanso, Inc. (Nagoya, Japan).

### 2.2. Preparation of extraction material

Olive oil was added to fresh tomato pulp at a final concentration of 1 g/100 g and the mixture was homogenized in a food processor. The sample (approximately 60 g) was transferred into a 100 mL glass bottle and heated in an oil bath at 120 or 150 °C for 1 h to isomerize the (all-*E*)-lycopene into the *Z*-isomers (Honda et al., 2016). The addition of olive oil to fresh tomato pulp promotes thermal *Z*-isomerization of lycopene (Honda, Murakami et al., 2017; Schierle et al., 1997). The samples were freeze dried to achieve the final moisture content of less than 10 g/100 g and were ground using a laboratory mill to obtain an average particle size of about 1 mm (corresponding to 18 mesh). Dried tomato pulp without olive oil and the thermal *Z*-isomerization pre-treatment was also prepared for comparison. Treatment conditions for each tomato pulp before freeze-drying are summarized in Table 1.

### 2.3. Determination of total lycopene in extraction material

Lycopene content in extraction material was determined as described by Honda, Murakami et al. (2017). Briefly, approximately 50 mg of the material was weighed into a 50 mL volumetric flask and treated with 30 mL acetone. The mixture was subjected to ultrasonic treatment for 5 min in ice water (approximately 4 °C) to prevent thermal *Z*-isomerization of lycopene (Schierle et al., 1997). The mixture was diluted with 50 mL acetone and the ultrasonic treatment repeated for 10 min in ice water. The solution was filtered using suction filtration on a Kiriyama funnel (number 5B filter paper). The residual color was rinsed with acetone until the filtrate was colorless. The collected lycopene solution was evaporated to dryness under reduced pressure at 35 °C and dissolved in 5–10 mL of hexane. The solution was filtered through a 0.2 μm polytetrafluoroethylene (PTFE) membrane filter (Advantec Co., Ltd., Tokyo, Japan) and analyzed using HPLC. Lycopene concentration was determined based on the calibration curve prepared by HPLC analysis as the sum of all lycopene isomers. A calibration curve was prepared with purified (all-*E*)-lycopene in the range of 25–100 μg/mL and it also applied to the *Z*-isomers.

### 2.4. Organic solvent extraction

The extraction of lycopene from dried tomato pulp was carried out using ethanol and ethyl acetate by referring to the method described previously (Calvo et al., 2007; Strati & Oreopoulou, 2016). Briefly, 3 g of extraction material was treated with 30 mL of ethanol or ethyl acetate in a 100 mL screw-capped tube and then the tube was tightly capped to prevent solvent evaporation during extraction. The mixture was agitated using a magnetic stirring bar at 1000 rpm for 60 min at 20 °C. The residue was removed by suction filtration on a Kiriyama funnel (number 5B filter paper) and the solution collected was evaporated to dryness under reduced pressure. The extract was weighed and then dissolved in 5–10 mL of hexane and filtered through a 0.2 μm PTFE membrane filter for HPLC analysis. The experiment was independently performed twice and values are presented as mean ± standard error.

### 2.5. Supercritical fluid extraction

Supercritical $CO_2$ extraction was performed with the apparatus shown in Fig. 1. The apparatus includes a chiller (CCA-1111, Eyela, Tokyo, Japan), high-pressure pump (PU 2086 Plus, Jasco, Tokyo, Japan) for $CO_2$, heating chamber (ST-110B1, Tabai Espec, Osaka, Japan), 10 mL vessel (Thar Tech, Pittsburgh, USA), back-pressure regulator (Akico, Tokyo, Japan), and wet gas meter (Sinagawa Seiki, Tokyo, Japan). Although the chiller maintains $CO_2$ in the liquid state between the $CO_2$ cylinder and heat chamber, $CO_2$ exists in its supercritical state in the heating chamber. The extraction was performed dynamically from 3 g of sample loaded into the vessel at SC-$CO_2$ flow rate of 3 mL/min for 8 h. The extraction temperature and pressure were maintained at 50 °C and 50 MPa, respectively, to

**Table 1**
Summary of treatments of tomato pulp before drying.

| Sample number | Amount of oil added (wt%) | Heating temperature (°C) |
|---|---|---|
| 1 | – | – |
| 2 | – | 120 |
| 3 | – | 150 |
| 4 | 1 | – |
| 5 | 1 | 120 |
| 6 | 1 | 150 |

–, No treatment.