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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/100g 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.

Keywords:

Carotenoid

E/*Z* Isomerization

Separation

Supercritical CO_2

Solubility

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

71 the human plasma lycopene concentration as compared with red tomato juice
72 (containing 10% total *Z*-isomer content of lycopene) (Cooperstone et al., 2015).
73 Moreover, studies have reported *Z*-isomers to exhibit higher antioxidant capacity than
74 the (all-*E*)-isomer (Böhm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002; Müller et
75 al., 2011). Thus, the dietary intake of *Z*-isomers of lycopene is preferred over that of the
76 (all-*E*)-isomer for health reasons.
77 Physical characteristics such as crystallinity and solubility differ between the
78 (all-*E*)-carotenoids and *Z*-isomers (Cooperstone et al., 2015; Gamlieli-Bonshtein, Korin,
79 & Cohen, 2002; Hempel, Schädle, Leptihn, Carle, & Schweiggert, 2016; Honda et al.,
80 2017a). For instance, (all-*E*)-carotenoids such as β-carotene and lycopene display
81 crystalline nature, but the *Z*-isomers exist in an oily aggregate state (Cooperstone et al.,
82 2015; Hempel, Schädle, Leptihn, Carle, & Schweiggert, 2016). The data available on
83 the solubility of *Z*-isomers are limited but *Z*-isomers of carotenoids are more soluble
84 than the all-*E*-isomers, e.g. the solubility of (9*Z*)-β-carotene in SC-CO_2 was reported to
85 be approximately four times higher than that of the (all-*E*)-isomer (Gamlieli-Bonshtein,
86 Korin, & Cohen, 2002). However, only a few studies have exploited these
87 characteristics of *Z*-isomers for technological development. Therefore, the aim of this
88 study was to improve solvent extraction efficiency of lycopene from plant source by
89 utilizing the higher solubility of the *Z*-isomers than the all-*E*-isomer. Namely, we
90 investigated whether lycopene extraction efficiency could be improved by increasing
91 the *Z*-isomer content in extraction material (dried tomato pulp). The *Z*-isomerization of
92 lycopene in the extraction material was conducted by thermal treatment (Honda et al.,
93 2017b; Schierle et al., 1997) and ethanol, ethyl acetate, and supercritical CO_2 (SC-CO_2)
94 were used as extraction solvents. Ethanol and ethyl acetate were organic solvents

95 approved for using in food processing, and SC-CO_2 is an attractive alternative for the
96 extraction of natural pigments, attributable to its nontoxicity and easy separation from
97 the extract (Gomez-Prieto, Caja, & Santa-Maria, 2002; Topal, Sasaki, Goto, &
98 Hayakawa, 2006; Zuknik, Norulaini, & Omar, 2012).
99
100 **2. Materials and methods**
101 *2.1. Materials*
102 Fresh tomato pulp (moisture content, 91.6 g/100g) used in this study was kindly
103 provided by Kagome Co., Ltd. (Tokyo, Japan). The fresh tomato pulp was a precipitate
104 obtained by centrifuging tomato juice. (all-*E*)-Lycopene (HPLC, ≥98% purity) for
105 preparing the calibration curve was isolated from tomato oleoresin (Lyc-O-Mato® 15%,
106 LycoRed Ltd., Beer-Sheva, Israel) according to the previous descriptions (Honda et al.,
107 2017b; Takehara et al., 2014). Analytical-grade acetone, ethanol, and ethyl acetate as
108 well as high performance liquid chromatography (HPLC)-grade hexane were purchased
109 from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *N*,*N*-Diisopropylethylamine
110 (DIPEA) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Olive
111 oil was purchased from the Nisshin OilliO Group Ltd. (Tokyo, Japan). Carbon dioxide
112 was obtained from Sogo Kariya Sanso, Inc. (Nagoya, Japan).
113
114 *2.2. Preparation of extraction material*
115 Olive oil was added to fresh tomato pulp at a final concentration of 1 g/100g and
116 the mixture was homogenized in a food processor. The sample (approximately 60 g)
117 was transferred into a 100 mL glass bottle and heated in an oil bath at 120 or 150 °C for
118 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 et al., 2017b; Schierle et al., 1997). The samples were freeze dried to achieve the final moisture content of less than 10 g/100g 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 et al. (2017b). 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 to 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 to 100 μg/ml and it also applied to the *Z*-isomers.

143

144 *2.4. Organic solvent extraction*

145 The extraction of lycopene from dried tomato pulp was carried out using ethanol
146 and ethyl acetate by referring to the method described previously (Calvo, Dado, &
147 Santa-María, 2007; Strati & Oreopoulou, 2016). Briefly, 3 g of extraction material was
148 treated with 30 mL of ethanol or ethyl acetate in a 100 mL screw-capped tube and then
149 the tube was tightly capped to prevent solvent evaporation during extraction. The
150 mixture was agitated using a magnetic stirring bar at 1,000 rpm for 60 min at 20 °C. The
151 residue was removed by suction filtration on a Kiriyama funnel (number 5B filter paper)
152 and the solution collected was evaporated to dryness under reduced pressure. The
153 extract was weighed and then dissolved in 5 to 10 mL of hexane and filtered through a
154 0.2 μm PTFE membrane filter for HPLC analysis. The experiment was independently
155 performed twice and values are presented as mean ± standard error.

156

157 *2.5. Supercritical fluid extraction*

158 Supercritical CO_2 extraction was performed with the apparatus shown in Fig 1. The
159 apparatus includes a chiller (CCA-1111, Eyela, Tokyo, Japan), high-pressure pump (PU
160 2086 Plus, Jasco, Tokyo, Japan) for CO_2, heating chamber (ST-110B1, Tabai Espec,
161 Osaka, Japan), 10 mL vessel (Thar Tech, Pittsburgh, USA), back-pressure regulator
162 (Akico, Tokyo, Japan), and wet gas meter (Sinagawa Seiki, Tokyo, Japan). Although the
163 chiller maintains CO_2 in the liquid state between the CO_2 cylinder and heat chamber,
164 CO_2 exists in its supercritical state in the heating chamber. The extraction was
165 performed dynamically from 3 g of sample loaded into the vessel at SC-CO_2 flow rate
166 of 3 mL/min for 8 h. The extraction temperature and pressure were maintained at 50 °C

and 50 MPa, respectively, to avoid decomposition and isomerization of lycopene (Zuknik, Norulaini, & Omar, 2012). The obtained extract was analyzed by HPLC in the same procedure as the organic solvent extracts.

2.6. HPLC analysis

Normal-phase HPLC analysis was performed with 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) according to the method described previously (Honda et al., 2017b). Briefly, the mobile phase hexane containing 0.75 mL/L DIPEA was used at a flow rate of 1 mL/min, with the column temperature of 40 °C. The detection wavelength was set at 460 nm such that the difference in molar extinction coefficients of the lycopene isomers is relatively small (Hengartner, Bernhard, Meyer, Englert, & Glinz, 1992; Honda, Kawana, Takehara, & Inoue, 2015; Honda et al., 2017a). Lycopene isomer peaks were identified according to HPLC retention times, visible spectral data, and relative intensities of the *Z*-peak (% D_B/D_{II}), as described in previous reports (Fröhlich, Conrad, Schmid, Breithaupt, & Böhm, 2007; Hengartner, Bernhard, Meyer, Englert, & Glinz, 1992; Honda et al., 2017a). The lycopene recovery (%) with organic solvents and SC-CO_2 was expressed as the amount of the extracted lycopene to the amount of lycopene contained in extraction material. The *Z*-isomer content (%) was estimated from the amount of total *Z*-isomers to the amount of total lycopene isomers, including the (all-*E*)-isomer.

3. Results and discussion

3.1. Profile of extraction material

191 Table 2 summarizes the lycopene and total *Z*-isomer content of each extraction
192 material (dried tomato pulp). Although almost no decomposition of lycopene in tomato
193 pulp was observed by heating at 120 °C for 1 h, approximately 20% was decomposed
194 by heating at 150 °C for 1 h. The total *Z*-isomer content of lycopene increased with an
195 increase in the heating temperature. In the absence of olive oil, heating at 120 °C for 1 h
196 yielded *Z*-isomer content of 10.0%; however, the *Z*-isomer content reached 56.2% at
197 150 °C for 1 h. In the presence of olive oil, heating at 120 and 150 °C resulted in
198 significant conversion of the (all-*E*)-lycopene to the *Z*-isomers as content of 30.4% and
199 75.7%, respectively. Typical chromatograms of thermally-treated and non-treated
200 tomato pulp are shown in Fig. 2. Heat treatment increased the (5*Z*)-, (9*Z*)- and
201 (13*Z*)-lycopene content, which is consistent with previous reports (Honda et al., 2017b;
202 Schierle et al., 1997). In addition, heating samples at 150 °C resulted in the formation of
203 additional *Z*-isomers such as (multi-*Z*)-lycopene. Lycopene was extracted from the
204 above dried tomato pulp with different content of the *Z*-isomers, and lycopene content
205 and lycopene recovery of the extracts were compared.
206
207 *3.2. Profile of extracts obtained with organic solvent extraction*
208 The choice of solvent used for the extraction determines the extraction efficiency of the
209 target compound from the extraction material. In general, nonpolar solvents are shown
210 to be more suitable for nonpolar solutes as well as more polar solvents for more polar
211 solutes. As lycopene is made up of hydrogen and carbon atoms ($C_{40}H_{56}$), it exhibits
212 extremely low polarity and is difficult to dissolve in highly polar solvents such as
213 ethanol (Aizawa et al., 2011; Calvo, Dado, & Santa-María, 2007; Strati & Oreopoulou,
214 2016). On the other hand, it is easily soluble in nonpolar solvents such as benzene and

chloroform ($CHCl_3$) (Zuknik, Norulaini, & Omar, 2012). However, these nonpolar solvents display high toxicity and are deemed unsuitable for food processing. Commercially available tomato extracts with high lycopene content are generally extracted with ethyl acetate (Honda, Higashiura, & Fukaya, 2017), however, the extraction efficiency is low (Calvo, Dado, & Santa-María, 2007; Strati & Oreopoulou, 2016). Therefore, there is still considerable room for the development of a method that improves lycopene extraction efficiency using solvents widely used for food processing, such as ethanol and ethyl acetate.

The weight, lycopene content, and total *Z*-isomer content of the extract as well as the lycopene recovery rate with ethanol and ethyl acetate extraction are summarized in Table 3, and typical chromatograms of the extracts are shown in Supplementary material Fig. S1 and S2, respectively. With ethanol as the extraction solvent, an increase in the weight and lycopene and *Z*-isomer contents was observed with an increase in the processing temperature of samples (Table 3 and Fig. S1). The extract weight increase may be attributed to the change in the structural integrity of the cellular matrix and/or disassembling of cell walls by the heating process, thereby improving the extraction efficiency of membrane-bound lipids such as glycolipid and phospholipid (Murador, da Cunha, & de Rosso, 2014; Sugawara & Miyazawa, 1999). An extremely strong correlation was observed between *Z*-isomer content in extraction materials and lycopene recovery rate. Amount of extracted (all-*E*)-lycopene with ethanol was almost constant regardless of the thermal pre-treatment and addition of olive oil (Fig. S1). Furthermore, the extraction residue from the heat-treated tomato pulp showed small *Z*-isomer content but high (all-*E*)-lycopene content (Fig. 3). These results indicate that the improvement in lycopene content and recovery was associated with the higher solubility of *Z*-isomers

than that of the all-*E*-isomer in ethanol, but not the heat-induced change in the cell wall structure. Since the solubility of (all-*E*)-lycopene in ethanol is extremely low, the total *Z*-isomer content was high in ethanol extracts, e.g. tomato pulp heated at 150 °C with or without 1 g/100g of olive oil before extraction showed the *Z*-isomer content of 92.3 ± 0.1% and 93.5 ± 0.1%, respectively. Thus, the ethanol extraction is effective for the isolation and concentration of *Z*-isomers of lycopene that display higher bioavailability (Boileau, Merchen, Wasson, Atkinson, & Erdman, 1999; Failla, Chitchumroonchokchai, & Ishida, 2008) and antioxidant capacity (Böhm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002; Müller et al., 2011) than the (all-*E*)-lycopene.

With ethyl acetate as the extraction solvent, an increase in the extract weight, total *Z*-isomer content, and lycopene recovery was observed with an increase in the thermal pre-treatment temperature (Table 3 and Fig. S2). However, the lycopene content decreased in the extract treated at 150 °C compared to that treated at 120 °C. This may be attributed to the generation of compounds soluble in ethyl acetate and decomposition of lycopene at high temperature. In comparison to ethanol extraction, ethyl acetate extraction decreased the total *Z*-isomer content in the extracts and increased the lycopene recovery, indicative of the higher solubility of the (all-*E*)-lycopene in ethyl acetate than ethanol. In their study using tomato peel powder rich in the (all-*E*)-isomer, Calvo, Dado, & Santa-María (2007) reported higher extraction efficiency of lycopene with ethyl acetate than with ethanol. As lycopene recovery increased with an increase in the *Z*-isomer content of extraction materials, *Z*-isomers of lycopene are thought to be more soluble than the all-*E*-form in ethyl acetate. Thus, thermal pre-treatment is also effective for extraction with ethyl acetate. Most of the commercially available tomato oleoresins are obtained with ethyl acetate extraction and most lycopene contained is

present in the all-*E*-configuration (Honda, Higashiura, & Fukaya, 2017). Therefore, our finding may help improve not only lycopene productivity in the actual production process but also the bioavailability and antioxidant capacity of the product.

3.3. Profile of extracts obtained with SC-CO_2 extraction

Organic solvents such as benzene and $CHCl_3$ are widely used for the extraction of carotenoids from plant source (Kaur, Wani, Oberoi, & Sogi, 2008; Kubola, Meeso, & Siriamornpun, 2013). However, these solvents display several disadvantages such as toxicity, presence of solvent traces in the final product, and danger in handling. On the contrary, SC-CO_2 is non-toxic, easily separated from the extract, and therefore thought to be a suitable alternative in food processing (Sun, & Temelli, 2006; Zuknik, Norulaini, & Omar, 2012). Several studies have demonstrated SC-CO_2 for the extraction of lycopene from tomato materials (Gomez-Prieto, Caja, & Santa-Maria, 2002; Topal, Sasaki, Goto, & Hayakawa, 2006; Zuknik, Norulaini, & Omar, 2012). However, the relatively low solubility of lycopene in SC-CO_2 attributed to its large molecular weight and high crystallinity decreases the extraction efficiency (Reverchon & De Marco, 2006; Zuknik, Norulaini, & Omar, 2012). The extraction efficiency of lycopene from plant sources can be improved by the optimization of extraction conditions such as extraction temperature and pressure as well as the addition of co-solvents (Shi et al., 2009; Yi, Shi, Xue, Jiang, & Li, 2009; Zuknik, Norulaini, & Omar, 2012). Furthermore, addition of lipid co-matrix and digestion with cell-wall by hydrolytic enzymes have recently attracted attention as pre-treatment techniques (Lenucci et al., 2010 and 2015). However, no reports focused on the increase of the *Z*-isomer content of extraction material to improve lycopene extraction efficiency were found.

287 Figure 4A shows the time course of lycopene recovery from dried tomato pulp with
288 SC-CO_2 extraction at 50 °C and 50 MPa for 8 h. Despite of the *Z*-isomer content of
289 extraction material, lycopene recovery was approximately 1% in the absence of olive oil.
290 On the other hand, the addition of olive oil to the tomato pulp before the extraction
291 significantly improved the lycopene recovery based on the *Z*-isomer content of the
292 material. The recovery rates were 6.5%, 15.5%, and 27.6% for pre-treatment with no
293 heating, heating at 120 °C, and at 50 °C using 8h SC-CO_2 extraction, respectively.
294 Cadoni, De Giorgi, Medda, & Poma (2000) reported negligible lycopene extraction
295 from fresh and dried tomatoes at 40 °C and 27.6 MPa; however, the addition of a small
296 amount of organic solvents to extraction material significantly improved its recovery.
297 Vasapollo, Longo, Rescio, & Ciurlia (2004) found that lycopene extraction efficiency
298 from dried tomatoes was greatly enhanced with the addition of 10 g/100g hazelnut oil to
299 extraction material. Such organic solvents and vegetable oils directly added to
300 extraction material to improve the SC-CO_2 extraction efficiency of target compounds
301 are called modifiers (Rozzi, Singh, Vierling, & Watkins, 2002; Zuknik, Norulaini, &
302 Omar, 2012). In the SC-CO_2 extraction, the *Z*-isomerization pre-treatment is thought to
303 be extremely effective in the presence of modifiers. The improvement in lycopene
304 recovery is attributed to the increased solubility of *Z*-isomers as compared to the
305 all-*E*-isomer in SC-CO_2. This is supported by the result of time courses of total
306 *Z*-isomer content of lycopene in the extract (Fig. 4B). The ratio of lycopene *Z*-isomers
307 decreased with an increase in the extraction time for all samples. Thus, it is suggested
308 that the *Z*-isomers are preferentially extracted owing to their high solubility in SC-CO_2
309 as compared to the (all-*E*)-isomer. The recovery rate observed for the individual
310 mono-*Z*-isomer was as follows: (13*Z*)-lycopene > (9*Z*)-lycopene > (5*Z*)-lycopene (Fig.

311 4C), suggestive of the varying solubility among the *Z*-isomers in SC-CO_2. The relatively
312 high (5*Z*)-lycopene content in the ethanol extraction residue (Fig. 3) may be associated
313 with its low solubility in organic solvents as compared to (13*Z*)- and (9*Z*)-lycopene.
314 The *Z*-isomerization–induced change in the solubility of the (all-*E*)-lycopene may
315 be attributed to the change in the physical properties of lycopene. Although carotenoid
316 aggregation can be stabilized via π–π-stacking interactions of conjugated polyene chains,
317 *Z*-isomerization would result in enormous steric hindrance, thereby diminishing the
318 potential attractive π–π forces (Hempel, Schädle, Leptihn, Carle, & Schweiggert, 2016).
319 As a result, it would become impossible to arrange regularly between compounds, and
320 physical properties change. Lycopene exists in an oily aggregated state in the globular
321 chromoplast of tangerine tomato, which is rich in *Z*-isomers (Cooperstone et al., 2015).
322 The solubility order of lycopene isomers in SC-CO_2 (Fig. 4C) strongly supported this
323 suggestion: as the steric hindrance of the *Z*-isomers was large, i.e. the *Z*-part was near
324 the central part of the conjugated chain of lycopene, the solubility became higher.
325 Differences in the physical properties of the all-*E*- and *Z*-form of carotenoids were
326 revealed in recent years, which should be exploited for the development of new
327 technologies in the future.
328
329 **4. Conclusions**
330 Our study clearly showed that lycopene extraction efficiency using organic
331 solvents and SC-CO_2 from dried tomato pulp improved by the increase in the *Z*-isomer
332 content. It indicated that the *Z*-isomers are more soluble in organic solvents and SC-CO_2
333 than the all-*E*-isomer. Although the thermal *Z*-isomerization of lycopene and drying of
334 tomato pulp were separately performed in this study, simultaneous implementation of

these steps is preferable in practical application. While drying at high temperature is preferred to induce isomerization of the (all-*E*)-lycopene to *Z*-isomers, care must be taken to minimise lycopene decomposition. In addition, the thermal *Z*-isomerization pre-treatment increased the content of *Z*-isomers, which exhibit higher bioavailability and antioxidant capacity than the all-*E*-isomer, in the extract. These findings will improve lycopene productivity as well as functionality of the obtained extract.

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485 **Figure Captions**

486

487 **Fig. 1.** Scheme diagram of the SC-CO_2 extraction apparatus.

488

489 **Fig. 2.** Normal-phase HPLC chromatograms of extraction materials (dried tomato pulp):
490 (A) non-heated; (B) heated at 120 °C; (C) heated at 150 °C; (D) non-heated with olive
491 oil; (E) heated at 120 °C with olive oil; (F) heated at 150 °C with olive oil. Lycopene
492 isomers were eluted with the retention time ranging from 24 to 47 min. (5*Z*)-, (9*Z*)-, and
493 (13*Z*)-lycopene were identified according to previous studies (Fröhlich, Conrad, Schmid,
494 Breithaupt, & Böhm, 2007; Hengartner, Bernhard, Meyer, Englert, & Glinz, 1992;
495 Honda et al., 2017b).

496

497 **Fig. 3.** Normal-phase HPLC chromatograms of ethanol extraction residue. Lycopene
498 isomers were eluted with the retention time ranging from 24 to 47 min. Samples
499 subjected to thermal treatment at 150 °C in the (A) absence or (B) presence of olive oil
500 (1 g/100g fresh weight) before the extraction.

501

502 **Fig. 4.** Time course of (A) lycopene recovery and (B) total *Z*-isomer content of
503 lycopene in extract from dried tomato pulp by SC-CO_2 extraction at 50 °C and 50 MPa:
504 (○) non-heated; (●) heated at 120 °C; (Δ) heated at 150 °C; (▲) non-heated with olive
505 oil; (□) heated at 120 °C with olive oil; (■) heated at 150 °C with olive oil, and (C)
506 recovery of each lycopene isomer from dried tomato pulp heated at 150 °C with olive
507 oil: (–) (all-*E*)-lycopene; (×) (5*Z*)-lycopene; (*) (9*Z*)-lycopene; (+) (13*Z*)-lycopene.

Tables

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.

Table 2
Profiles of extraction materials.[a]

Sample number[b]	Profiles	
	Lycopene content (mg/100 g)	Total *Z*-isomer content (%)
1	814.4	6.1
2	797.4	10.0
3	646.9	56.2
4	710.7	8.8
5	710.1	30.4
6	595.5	75.7

[a]Extraction materials mean freeze-dried tomato pulp which are pre-treated by the conditions described in Table 1.
[b]Sample numbers are corresponding to Table 1.

Table 3
Extracts profile with ethanol and ethyl acetate.[a]

Extraction solvent	Sample number[b]	Extract weight (mg)	Lycopene content (mg/100 g)	Total *Z*-isomer content (%)	Lycopene recovery (%)[c]
Ethanol	1	98 ± 4 A	1114.5 ± 86.4 B	77.4 ± 0.4 A	4.3 ± 0.4 A
	2	105 ± 2 A	2170.9 ± 16.7 C	84.9 ± 0.2 B	9.5 ± 0.3 A
	3	139 ± 0 B	9571.3 ± 142.5 E	92.3 ± 0.1 C	59.7 ± 0.9 C
	4	437 ± 4 C	307.6 ± 16.9 A	76.3 ± 2.1 A	6.3 ± 0.4 A
	5	427 ± 4 C	1398.8 ± 11.2 B	91.7 ± 0.1 C	28.1 ± 0.0 B
	6	487 ± 11 D	2783.8 ± 15.7 D	93.5 ± 0.1 C	75.9 ± 2.1 D
Ethyl acetate	1	62 ± 3 A	15529.2 ± 103.3 D	10.9 ± 0.3 A	39.1 ± 1.8 A
	2	68 ± 1 A	18013.8 ± 420.9 E	14.5 ± 0.6 A	51.2 ± 2.1 A
	3	129 ± 3 B	12831.7 ± 124.6 F	60.2 ± 0.2 C	85.0 ± 2.5 B
	4	422 ± 9 C	2648.2 ± 407.5 A	12.2 ± 2.0 A	48.5 ± 8.1 A
	5	402 ± 9 C	4235.3 ± 54.2 B	39.0 ± 0.1 B	79.5 ± 1.0 B
	6	488 ± 3 D	3524.8 ± 89.8 C	76.3 ± 0.2 D	96.3 ± 1.5 B

[a]Values are presented as mean ± standard error (n = 2). For each sample number and for each column, values followed by different letters are significantly different at $P<0.05$ (ANOVA, Tukey's test).
[b]Sample numbers are corresponding to Table 1.
[c]The recovery expressed as a percentage of the amount of ethanol- or ethyl acetate-extracted lycopene to the amount of acetone-extracted lycopene as previous work (Honda et al., 2017b; Schierle et al., 1997).

Figure
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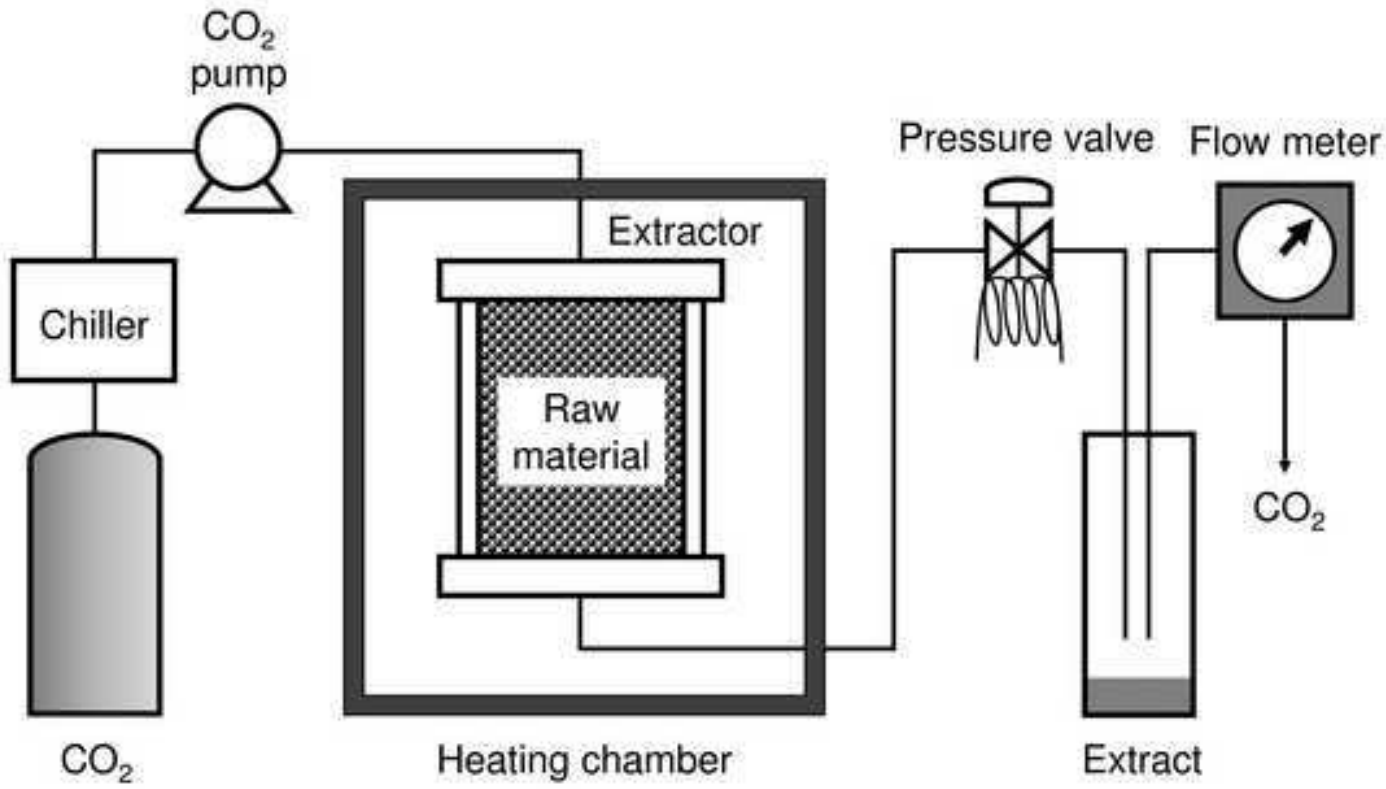

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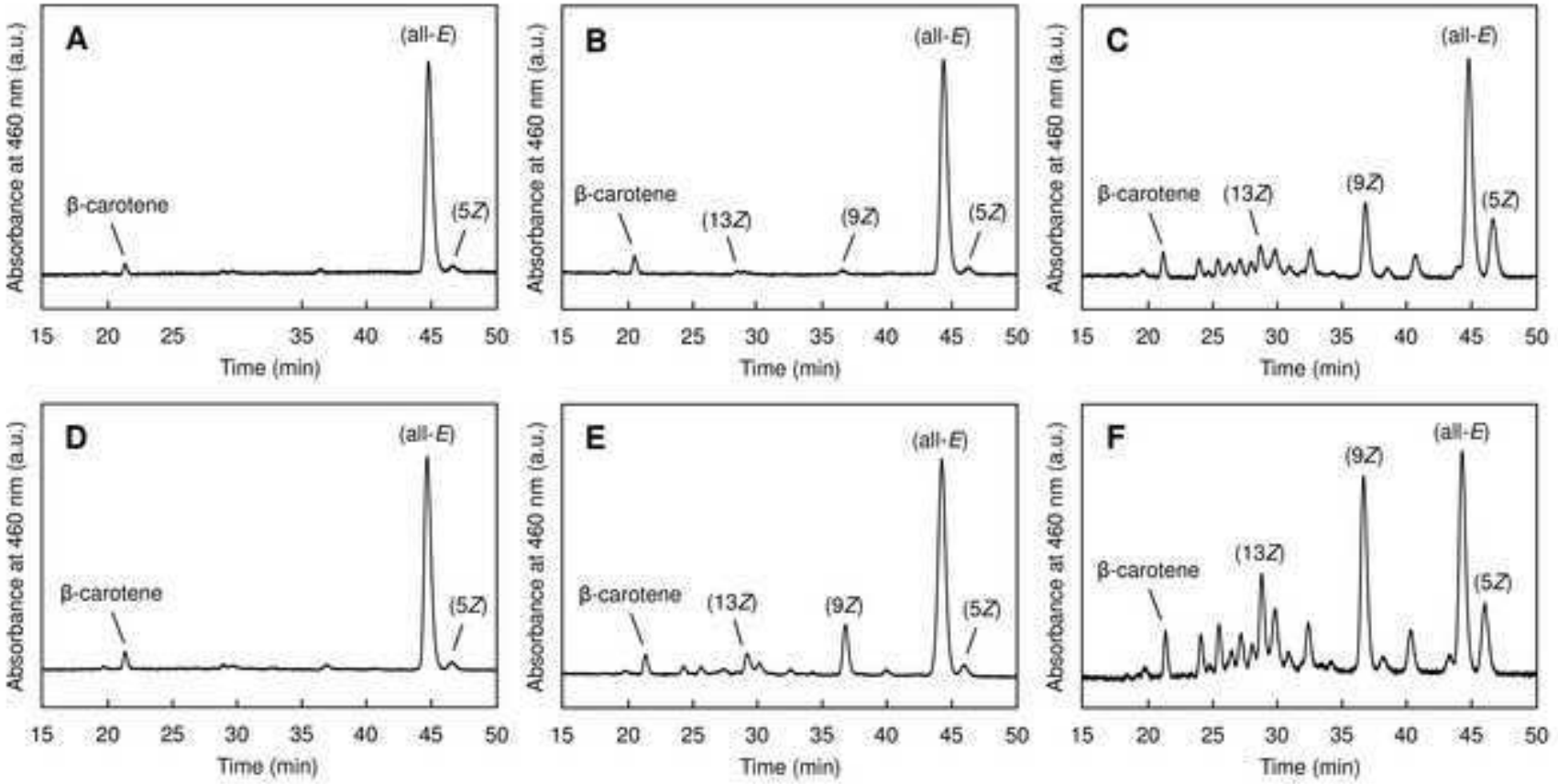

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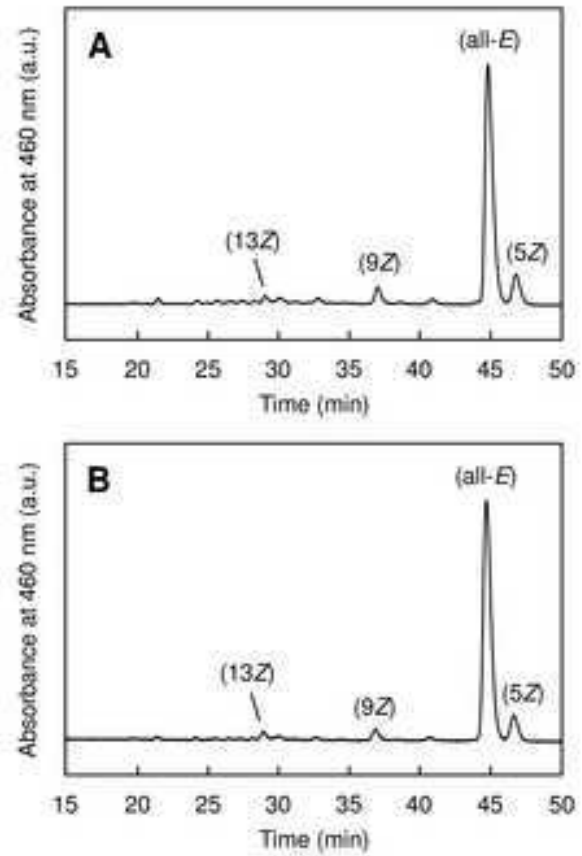

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