Rapid and Selective Concentration of Lycopene Z-isomers from Tomato Pulp by Supercritical CO$_2$ with Co-solvents

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(Received April 26, 2017; Accepted June 26, 2017)

This study aimed to efficiently separate and concentrate lycopene Z-isomers from tomato pulp using supercritical CO$_2$ (SC-CO$_2$). The separation relies on the different solubility of (all-\(E\))-lycopene and the Z-isomers. Total lycopene recovery using SC-CO$_2$ at 50 °C and 30 MPa for 1 h was extremely low (1.2%). Thus, before the separation test, an optimal co-solvent was selected from water, organic chemicals, and edible vegetable oils; hazelnut oil, which had the highest lycopene recovery (21.6%), was adopted. When using hazelnut oil as a co-solvent, the extraction of lycopene Z-isomers was completed in a short time compared to (all-\(E\))-lycopene. Furthermore, when the extraction was conducted at higher pressure (50 MPa) and temperature (80 °C), the Z-isomer content decreased due to the improvement of (all-\(E\))-lycopene solubility. Thus, to selectively and efficiently extract lycopene Z-isomers, addition of a co-solvent, and a relatively short contact time, low pressure, and low temperature extraction were important.

1. Introduction

Carotenoids are beneficial to human health due to having strong antioxidant properties and they significantly lower the risk of cancer and atherosclerosis [1–3]. Tomatoes contain abundant carotenoids such as phytoene, phytofluene, \(\beta\)-carotene, and lycopene; particularly lycopene with a content of more than 80–90% of total carotenoids [4–6]. Thus, tomatoes are good sources for carotenoid material, in particular lycopene. In plants, most lycopene is present as the all-\(E\)-configuration, which constitutes about 80–97% of total lycopene in tomatoes. In contrast, Z-isomers of lycopene are primarily found in the human body, e.g. more than 50% of total lycopene is present as Z-isomers in serum and tissues [7, 8]. Several studies have reported that Z-isomers of lycopene have a greater potential for bioavailability than the (all-\(E\))-isomer [9–11]. In addition, lycopene Z-isomers are also expected to show higher antioxidant capacity than the all-\(E\)-form [12, 13]. Thus, the intake of the Z-isomers-rich foods may offer benefits for human health, and it is therefore important to gain a better understanding of methods for concentration of the Z-isomers and for Z-isomerization of (all-\(E\))-lycopene.

Extraction and concentration of carotenoids from plant sources are generally carried out using organic
chemicals such as ethyl acetate and hexane [4, 7, 14]. However, these solvents have many disadvantages; e.g.,
toxicity, the presence of solvent traces in the final product, and danger in handling. On the other hand,
supercritical CO2 (SC-CO2) which is non-toxic and easily separated from the extract is a suitable alternative
to be applied in food processing including carotenoids extraction [15–18]. Thus, in this study, we attempted
to develop a method that can efficiently separate and concentrate Z-isomers of lycopene using SC-CO2.

There is only limited evidence but Z-isomers of carotenoids are more soluble in solvents than the all-
E-isomer, e.g. the solubility of (9Z)-β-carotene in SC-CO2 was reported to be approximately four times higher
than that of the (all-E)-isomer [19, 20]. In this study, we found that the Z-isomers are more soluble in SC-
CO2 than the all-E-isomer. Thus, by utilizing the higher solubility of Z-isomers of lycopene, selective
extraction of the Z-isomers was carried out from dried tomato pulp (15.3% lycopene Z-isomers of total
lycopene) using SC-CO2. Since the extraction efficiency of lycopene isomers from tomato pulp by SC-CO2
was extremely low, before the separation test, an optimal co-solvent capable of improving the efficiency was
selected. The addition of small amount of organic chemicals and edible vegetable oils to SC-CO2 could
improve total lycopene recovery from plant materials [21–23]. Subsequently, in the presence of the co-
 solvent (hazelnut oil), we investigated the effect of the SC-CO2 extraction time, pressure, and temperature
on the Z-isomer content of the extract, and selected the conditions capable of efficiently separating and
concentrating Z-isomers of lycopene.

2. Experimental

2.1. Materials

Dried tomato pulp (average particle size, about 0.25 mm; moisture content, about 8%) used in this
study was kindly provided by Kagome Co., Ltd. (Tokyo, Japan). Carbon dioxide was obtained from Sogo
Kariya Sanso, Inc. (Nagoya, Japan). High-performance liquid chromatography (HPLC)-grade ethanol, ethyl
acetate, and 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).
Eight commercially available edible vegetable oils were used as co-solvents: linseed (Nippon Flour Mills,
Tokyo), soybean (Ajinomoto, Tokyo), corn (Ajinomoto, Tokyo), sesame (Takemoto Oil & Fat, Aichi, Japan),
rapeseed (Ajinomoto, Tokyo), hazelnut (Huilerie Jean Leblanc, Iguerande, France), safflower seed
(Ajinomoto, Tokyo), and olive oil (Nisshin OilliO, Tokyo).

2.2. Supercritical CO2 extraction

The SC-CO2 extraction apparatus includes a chiller (CCA-1111, Eyela, Tokyo), a high-pressure pump
(NU 2086 Plus, Jasco, Tokyo) for CO2, a co-solvent pump (LC-6AD, Shimadzu, Kyoto, Japan), a heating
chamber (ST-110B1, Tabai Espec, Osaka), a 10mL vessel (Thar Tech, Pittsburgh, USA), a back pressure
regulator (Akico, Tokyo), and a wet gas meter (Sinagawa Seiki, Tokyo) as previously described [17]. Liquid
CO2 flowing from the CO2 cylinder was compressed and controlled with the high-pressure pump which was
cooled with a chiller to keep CO2 in a liquid state. In the heating chamber, CO2 was transformed into a
supercritical state by heating.

2.2.1. Effect of co-solvents

To select an optimum co-solvent which can extract and concentrate Z-isomers of lycopene efficiently,
SC-CO2 extraction tests with various co-solvents were conducted. A four gram sample was loaded into the
vessel and lycopene was extracted by SC-CO$_2$ with or without co-solvents. The flow rate of CO$_2$ with co-
solvent was 3 mL/min, the co-solvents concentrations were approximately 5% (w/w) in SC-CO$_2$, and the
extraction was performed for 1 h. As described below (section 3.3.2), in order to avoid Z-isomerization of
(all-E)-lycopene during the extraction process, the extraction temperature and pressure were kept at 50 °C
and 30 MPa, respectively. As co-solvents, water, 3 organic chemicals (ethanol, ethyl acetate, and hexane) and
8 edible vegetable oils (linseed, soybean, corn, sesame, rapeseed, hazelnut, sunflower seed, and olive oils)
were used. The extracts were collected in brown sample vials to inhibit light exposure.

2.2.2. Effect of extraction period

To investigate the effect of the SC-CO$_2$ extraction period on the lycopene recovery and total Z-isomer
content, time course extraction tests were conducted under the same conditions as above (section 2.2.1) with
and without hazelnut oil, which is the most effective co-solvent for lycopene isomers extraction. These tests
were carried out for 8 h.

2.2.3. Effect of extraction temperature and pressure

A number of studies has shown that the extraction efficiency of lycopene isomers was affected by the
extraction temperature and pressure [5, 15, 18]. Thus, in order to selectively and efficiently concentrate the
Z-isomers of lycopene, a good understanding of the effect of extraction temperature and pressure on the Z-
isomers content in the extract is important. We therefore conducted extraction tests at 50 °C and 50 MPa, and
80 °C and 30 MPa for 5 h with hazelnut oil as a co-solvent, and compared with the result of the test at 50 °C
and 30 MPa.

2.3. HPLC analysis

Normal-phase HPLC analysis with a photodiode array detector (L-2455, Hitachi., Japan) was
performed according to the method described previously with some modification [24, 25]. This analytical
method can clearly separate each lycopene isomer. The detection wavelength of lycopene isomers was set at
460 nm, at which the differences in molar extinction coefficients among lycopene isomers are relatively small
[26, 27]. The mobile phase consisted of hexane containing 0.075% DIEPA and the stationary phase consisted
of four Nucleosil 300-5 columns connected in tandem (4 × 250 mm in length, 4.6-mm inner diameter, 5-μm
particle size; GL Sciences Inc., Japan). The flow rate and column temperature were set at 1.0 mL/min and
40 °C, respectively. The peaks of lycopene Z-isomers were identified according to the retention times in
HPLC, visible spectral data, and the relative intensities of the Z-peak as % $D_0/D_II$ described in the previous
researches [26–29]. Before the analysis, the extracted samples were dissolved in hexane and filtered through
a 0.2-μm polytetrafluoroethylene membrane filter (Advantec, Tokyo, Japan). The lycopene recovery (%) was
expressed as a percentage of the amount of lycopene extracted by SC-CO$_2$ to the amount of lycopene
extracted by acetone. Acetone extraction was conducted according to the procedure as previously described
[7], which can extract all lycopene from tomato materials.

3. Results and Discussion

3.1. Effect of co-solvents

3.1.1. Lycopene recovery

Without co-solvent, total lycopene recovery was only 1.2%, while it was significantly improved by the
addition of co-solvents (Figure 1). In particular, when using edible vegetable oils as co-solvents, lycopene
recovery extremely improved compared to the use of water and organic chemicals. The differences in lycopene extraction efficiency by adding co-solvents is mainly due to polarity and impact on the tomato matrix such as swelling and penetration of the co-solvent [30, 31].

Figure 1. Effect of co-solvents on lycopene recoveries by SC-CO₂ extraction at 50 °C and 30 MPa for 1 h.

With water as a co-solvent, lycopene recovery slightly improved (2.3%), and further improvement in lycopene recovery was observed using organic chemicals (5.2–8.7%). The improvement in lycopene recovery by SC-CO₂ extraction using 5–15% water as a co-solvent has also been confirmed by Shi et al. [30]. Ethyl acetate had the highest extraction efficiency (8.7%) of the organic chemicals, and ethanol and hexane have approximately equivalent lycopene recovery. It is considered that, by using ethyl acetate as a co-solvent, SC-CO₂ become more suitable polarity to dissolve lycopene. These extraction trends using organic chemicals as co-solvents were similar to previous studies [21, 22], and therefore the validity of this study was confirmed.

Ample studies have demonstrated that the addition of a small amount of vegetable oils such as olive oil [31] and canola oil [30] to the SC-CO₂ could improve lycopene recovery. However, since these studies have conducted SC-CO₂ extractions under different extraction condition and raw material, it was difficult to discuss efficiency among those oils. In the present study, by performing the SC-CO₂ extraction under the same condition (temperature, 50°C; pressure, 30MPa; extraction time, 1 h) and raw material (dried tomato pulp), evaluation of lycopene extraction efficiency among edible vegetable oils was possible. When edible vegetable oils were used as co-solvents, hazelnut oil had the highest extraction efficiency (21.6%). The improvement of SC-CO₂ extraction efficiency of lycopene has also been confirmed by the addition of hazelnut oil to the raw material (dried tomato powder) [23, 32]. The highest lycopene recovery of hazelnut oil among edible vegetable oils is considered to be independent of the iodine value, saponification value, and fatty acid composition, because these values are similar to those of olive oil [33, 34], wherein the lowest lycopene recovery was obtained among vegetable oils. Thus, the differences in the recovery tendencies among oils is probably caused by the presence of specific minor components contained in the oil such as tocopherols and sterols [33] and/or the effect on the tomato cell such as swelling and penetration [30, 31].

In this study, co-solvents were added to SC-CO₂, while interestingly, several studies have reported that co-extraction of oil-rich plant sources such as seeds and tomato material improved extraction efficiency of lycopene. For example, when lycopene was extracted by SC-CO₂ from tomato material mixed with tomato
seed [35] and hazelnut [32], the recovery significantly increased compared to the recovery in their absence.

3.1.2. Z-isomers content

Several studies have reported that SC-CO\textsubscript{2} extract contained Z-isomers of lycopene [5, 15, 32]. However, an accurate amount of the Z-isomers have not been reported in most of those studies due to problems of the analytical methods. Thus, in this study, with the HPLC method capable of clear separation of lycopene isomers, the Z-isomer content was accurately measured.

Although most lycopene in the dried tomato pulp was present in the all-E-configuration (84.7% of total lycopene; Figure 2a), a higher amount of Z-isomers of lycopene such as (5Z)- and (9Z)-lycopene existed in the extracts. For example, as shown in Figure 2b and 2c, 84.0% and 67.2% of lycopene Z-isomers were contained in the extract obtained by SC-CO\textsubscript{2} with or without hazelnut oil as the co-solvent, respectively. The effect of the co-solvents on E/Z-isomer of lycopene content in SC-CO\textsubscript{2} extract is summarized in Table 1. The total contents of the Z-isomers were higher in the order of no co-solvents (88.9%) > water (87.1%) > organic chemicals (80.9–85.3%) > edible vegetable oils (67.2–73.6%). The order of the Z-isomers content showed the opposite correlation with the order of the lycopene recovery (Figure 1). For instance, the test with no co-solvent resulted in the lowest lycopene recovery (1.2%) and the highest Z-isomer content (88.9%), while the test using hazelnut oil as the co-solvent resulted in the highest lycopene recovery (21.6%) and the lowest Z-isomer content (67.2%). In addition, before the extraction, the dried tomato pulp contained 15.3% of lycopene Z-isomers, while after the extraction, the tomato pulp (extraction residue) contained only 5.5% of lycopene Z-isomers. Furthermore, principal Z-isomers of lycopene contained in the extracts, which were (5Z)- and (9Z)-lycopene (Figure 2b and 2c), were the same as those included in the raw material (Figure 2a). These results indicate that Z-isomers of lycopene would be more soluble in SC-CO\textsubscript{2}, and be easily extracted from dried tomato pulp compared with the (all-E)-lycopene. This consideration supports the result that total Z-isomer content of the extract decreased by the use of edible vegetable oils as co-solvents, i.e. with edible vegetable oils as a co-solvent, since extraction capacity of (all-E)-lycopene which is extremely low solubility in SC-CO\textsubscript{2} improved, the Z-isomer content of extract decreased.

This result clearly shows the possibility of separation and concentration of Z-isomers of lycopene from plant material rich in the (all-E)-isomer using SC-CO\textsubscript{2}. However, since the extraction efficiency of lycopene

![Figure 2. Normal-phase HPLC chromatograms of lycopene isomers contained in (a) raw material (dried tomato pulp) and the extract obtained by SC-CO\textsubscript{2} extraction at 50 °C and 30 MPa for 1 h in the (b) absence and (c) presence of hazelnut oil as the co-solvent.](image-url)
isomers is extremely low, the addition of a small amount of co-solvent to SC-CO$_2$ is considered necessary. Thus, to optimize the separation and concentration condition of the $Z$-isomers using SC-CO$_2$, the effect of extraction time, pressure, and temperature on the total lycopene recovery and total $Z$-isomer content of the extract was investigated in the presence of hazelnut oil, which was the most effective co-solvent for lycopene isomers extraction, as a co-solvent.

Table 1. Effect of the co-solvents on $E/Z$-isomer ratio of lycopene content in SC-CO$_2$ extract.$^a$

<table>
<thead>
<tr>
<th>Co-solvent</th>
<th>Content (%)</th>
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|                  | (all-$E$)   | Total $Z^b$ | (5$Z$)   | (9$Z$)   | (13$Z$)  | Other $Z^c$
| $^d$             | 11.1 ± 0.3  | 88.9 ± 0.3 | 11.5 ± 0.2 | 23.3 ± 0.3 | 8.0 ± 0.0 | 46.2 ± 0.2 |
| Water            | 12.9 ± 0.5  | 87.1 ± 0.5 | 10.8 ± 0.1 | 22.6 ± 0.7 | 6.8 ± 1.3 | 47.0 ± 1.6 |
| Organic chemicals|             |          |          |          |          |          |
| Ethanol          | 16.0 ± 0.1  | 84.0 ± 0.1 | 10.5 ± 0.0 | 22.5 ± 0.1 | 5.6 ± 0.1 | 45.4 ± 0.2 |
| Ethyl acetate    | 16.9 ± 0.4  | 83.1 ± 0.4 | 12.6 ± 1.2 | 24.5 ± 0.3 | 6.5 ± 0.9 | 39.5 ± 1.0 |
| Hexane           | 14.7 ± 0.1  | 85.3 ± 0.1 | 12.8 ± 0.4 | 22.9 ± 0.4 | 4.4 ± 0.0 | 45.3 ± 0.2 |
| Vegetable oils   |             |          |          |          |          |          |
| Linseed          | 32.6 ± 0.0  | 67.4 ± 0.0 | 10.8 ± 1.0 | 18.7 ± 0.1 | 6.4 ± 1.5 | 31.5 ± 0.6 |
| Soybean          | 27.0 ± 0.8  | 73.0 ± 0.8 | 10.9 ± 0.0 | 19.0 ± 0.5 | 5.6 ± 0.6 | 37.5 ± 1.8 |
| Corn             | 31.0 ± 2.9  | 69.0 ± 2.9 | 12.0 ± 0.2 | 18.2 ± 1.2 | 7.0 ± 2.8 | 31.8 ± 1.3 |
| Sesame           | 30.0 ± 2.7  | 70.0 ± 2.7 | 10.9 ± 0.0 | 17.4 ± 0.6 | 4.5 ± 0.4 | 37.3 ± 1.7 |
| Rapeseed         | 27.8 ± 1.1  | 72.2 ± 1.1 | 12.7 ± 0.0 | 20.0 ± 0.4 | 4.6 ± 0.1 | 35.0 ± 1.5 |
| Hazelnut         | 32.8 ± 1.6  | 67.2 ± 1.6 | 12.5 ± 0.5 | 18.5 ± 0.7 | 4.7 ± 0.0 | 31.5 ± 0.4 |
| Safflower seed   | 27.9 ± 1.0  | 72.1 ± 1.0 | 12.2 ± 0.6 | 18.7 ± 1.0 | 5.0 ± 0.2 | 36.1 ± 0.3 |
| Olive            | 28.6 ± 1.2  | 71.4 ± 1.2 | 11.4 ± 0.2 | 18.0 ± 0.2 | 4.7 ± 0.0 | 37.4 ± 0.8 |

$^a$Values are presented as mean ± standard error ($n = 2$). SC-CO$_2$ extraction was carried out at 50 °C and 30 MPa for 1 h.

$^b$Total content of $Z$-isomers of lycopene.

$^c$Sum of $Z$-isomer of lycopene other than 5$Z$-, 9$Z$-, and 13$Z$-forms.

$^d$Used no co-solvent.

$^e$Oil with high oleic acid content.

3.2. Effect of extraction period

3.2.1. Lycopene recovery

In order to investigate the effect of SC-CO$_2$ extraction period on the total lycopene recovery, the extraction was carried out for 8 h, at 50 °C and 30 MPa with hazelnut oil (Figure 3a). As a comparison, the extraction test without hazelnut oil was also conducted at the same temperature and pressure condition. When the co-solvent was not used, total lycopene recovery hardly increased: 2.4% after 8 h SC-CO$_2$ extraction. On the other hand, the recovery dramatically improved with hazelnut oil: 66.4% after 8 h SC-CO$_2$ extraction. Thus, it was once again confirmed that the presence of co-solvents is very important to extract lycopene isomers efficiently using SC-CO$_2$. 

- 52 -
3.2.2. Z-isomers content

Figure 3b shows the changes in the total Z-isomer content in SC-CO2 extracts with or without hazelnut oil as a co-solvent. Although the initial stage of the extracts contained high amounts of lycopene Z-isomers, the content of the Z-isomers decreased with extraction time in both extraction conditions. It indicated that Z-isomers of lycopene were preferentially extracted by SC-CO2 compared to (all-E)-lycopene, i.e. Z-isomers of lycopene are more soluble in SC-CO2 than (all-E)-lycopene.

When hazelnut oil was used as the co-solvent, the decrease of total Z-isomers content in the extract almost stopped by 2-h extraction. It indicated that the extraction of the Z-isomers was almost completed in a short time. On the other hand, when without a co-solvent, a large amount of the Z-isomers (more than 40%) was contained in the extract after 8-h extraction: the extraction of lycopene Z-isomers was not completed. Thus, to obtain a lycopene extract rich in the Z-isomers efficiently, a short extraction time and the presence of a co-solvent were important.

![Graph](image)

Figure 3. Change in (a) total lycopene recovery and (b) total Z-isomer content in the SC-CO2 extract at 50 °C and 30 MPa from tomato pulp in the (●) absence and (○) presence of hazelnut oil as the co-solvent.

3.3. Effect of extraction temperature and pressure

3.3.1. Lycopene recovery

Many studies have demonstrated that lycopene recovery improved by increasing the extraction pressure and temperature [5, 15, 36]. Also in this study, the same results were obtained (Figure 4a). Although total lycopene recovery after 5 h extraction at 50 °C and 30 MPa with hazelnut oil as the co-solvent was 60.1%, it was improved significantly by extraction at 50 °C and 50 MPa (78.9%) and at 80 °C and 30 MPa (97.0%).

Ciurlia et al. have conducted lycopene extraction by SC-CO2 with hazelnut oil added as the co-solvent to dried tomato powder and with dried hazelnut powder added as a co-matrix to the tomato powder [32]. When the SC-CO2 extraction was carried out at 60–70 °C and 40–45 MPa for 5 h, lycopene recovery was approximately 25% for the former and approximately 70% for the later. Thus, it is highly possible that the extraction efficiency of lycopene could be improved by adding the co-solvent to SC-CO2 rather than adding the co-solvent and co-matrix to the raw material.

3.3.2. Z-isomers content

Figure 4b shows the effect of extraction pressure and temperature on the total Z-isomer content in the
extract during 5 h. When the extraction was conducted at high pressure condition (50 °C and 50 MPa), the
trend of change in the Z-isomer content in the extract was almost similar to the low pressure extraction
condition (50 °C and 30 MPa). However, the Z-isomer content of the initial stage (after 1 h extraction) was
lower compared to the low pressure extraction condition. It indicated that the solubility of (all-\(E\))-lycopene
improved by increasing extraction pressure. Gómez-Prieto et al. also reported that the Z-isomer content in
extract obtained by SC-CO\(_2\) extraction at the same temperature decreased with increasing pressure [5]. Thus,
to separate Z-isomers of lycopene from tomato pulp efficiently, a short extraction time and relatively low
pressure extraction were preferable.

When the SC-CO\(_2\) extraction was conducted at high temperature condition (80 °C and 30 MPa), the Z-
isomer content of the extract was around 60% in any extraction period (Figure 4b). It indicated that (all-\(E\))-
lycopene isomerized to the Z-isomers during the extraction process by heating. Several studies have also
reported that the total Z-isomers content in the SC-CO\(_2\) extract increased as the extraction temperature rose
[5, 15, 36].

![Figure 4](image)

Figure 4. Change in (a) total lycopene recovery and (b) total Z-isomer content in the extract by SC-CO\(_2\)
extraction with hazelnut oil as the co-solvent from tomato pulp at (○) 50 °C and 30 MPa, (△) 50 °C and 50
MPa, and (▲) 80 °C and 30 MPa.

Figure 5 shows normal-phase HPLC chromatograms (monitored at 460 nm) of lycopene isomers
contained in the extract obtained by SC-CO\(_2\) at 50 °C and 30 MPa and 80 °C and 30 MPa with hazelnut oil
as the co-solvent. When the extraction was conducted at 80 °C and 30 MPa, an extremely high amount of
(13Z)-lycopene was contained in the extract at all times during the extraction period compared to the
extraction condition of 50 °C and 30 MPa. (13Z)-Lycopene was easily isomerized from (all-\(E\))-lycopene in
solvents including SC-CO\(_2\) with heating [37, 38], and the content in the raw material was very low. Thus,
most (13Z)-lycopene in the extract was considered to be caused by thermal isomerization during extraction.
When the extraction was carried out at high temperature, the Z-isomer content of the initial stage (after 1 h
extraction) was lower than in the low temperature extraction conditions. Therefore, when selectively
extracting Z-isomers of lycopene from the raw material, high temperature extraction is not preferred.
However, since high temperature extraction can improve lycopene recovery and extract while isomerizing
(all-\(E\))-lycopene to the Z-isomers, it is suitable for obtaining an extract having a high lycopene concentration
and a relatively high Z-isomer content.
The result of this study suggests that if the content of lycopene Z-isomers in the raw material could be increased, lycopene recovery and the Z-isomers content in the extract by SC-CO2 extraction would improve. A number of studies has shown that heat and microwave processing could promote Z-isomerization of (all-E)-lycopene in tomato materials [25, 38, 39]. Therefore, by performing the above processing before SC-CO2 extraction, extraction efficiency of lycopene and functionality of the extract could improve. In addition, Ciurlia *et al.* have reported that by centrifuging the SC-CO2 extract obtained from a mixture of dried tomato and dried hazelnut, (all-E)-lycopene and the Z-isomers were separated: the all-E-isomer was collected as the precipitate, and the Z-isomers remained in solution in the oil phase [32]. That result indicated that further concentration of lycopene Z-isomers would become possible by adding a centrifugation-based procedure. Furthermore, although Z-isomers of lycopene were concentrated in this study, when concentration of the all-E-isomer is desired, it would be effective to extract it by relatively low temperature and high pressure SC-CO2 extraction conditions with a co-solvent from materials rich in (all-E)-lycopene. The above condition can efficiently extract (all-E)-lycopene while suppressing the thermal Z-isomerization during extraction process. In addition, as a material rich in the (all-E)-lycopene, the use of the residue from which the Z-isomers are extracted is effective.

![Figure 5](image.png)

Figure 5. Normal-phase HPLC chromatograms (monitored at 460 nm) of lycopene isomers extracted by SC-CO2 with hazelnut oil as a co-solvent from tomato pulp at (a) 50 °C and 30 MPa and (c) 80 °C and 30 MPa.
4. Conclusion

It becomes clear that Z-isomers of lycopene were preferentially extracted by SC-CO$_2$ compared to the all-E-isomer, probably because the Z-isomers are more soluble. Utilizing this characteristic, selective extraction of the Z-isomers from tomato pulp was successfully performed. When SC-CO$_2$ extraction was conducted at relatively low temperature and low pressure, e.g. 50 °C and 30 MPa, with hazelnut oil as the co-solvent, Z-isomers of lycopene could be efficiently concentrated in a short time. The result of this study suggests that by increasing the Z-isomer content of the raw material, lycopene recovery and the Z-isomers content in extract by SC-CO$_2$ extraction would improve.

Acknowledgement

The authors are grateful to Dr. Chitoshi Kitamura, Dr. Yoshinori Inoue, and Dr. Munenori Takehara (Department of Materials Science, The University of Shiga Prefecture) for their kind help and provision of analysis device.

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