Enriched (Z)-lycopene in Tomato Extract via Co-Extraction of Tomatoes and Foodstuffs Containing Z-isomerization-accelerating Compounds

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Abstract: The aim of this study was to promote the Z-isomerization of lycopene in its extraction process from tomato pulp by adding foodstuffs containing Z-isomerization-accelerating compounds. The addition of onion, broccoli, mustard, makonbu (Saccharina japonica), or shiitake mushroom (Lentinus edodes) to the ethyl acetate extraction process significantly accelerated the Z-isomerization of lycopene. For example, when lycopene was extracted from tomato pulp at 70 °C without foodstuffs, the total Z-isomer ratio of lycopene in the extract was 38.4 ± 0.5%, whereas when onion, broccoli, mustard, makonbu, and shiitake mushroom were added to the process and the extraction was performed using the same procedure, the total Z-isomer ratios significantly increased to 53.6 ± 0.4%, 47.9 ± 0.3, 48.2 ± 0.1, 41.5 ± 0.9, and 42.0 ± 1.2%, respectively. Since the above foodstuffs contain large amounts of carotenoid Z-isomerization-accelerating catalysts, i.e., polysulfides, isothiocyanates, or iodine, those components would promote Z-isomerization of lycopene in the extraction process. Since lycopene Z-isomers potentially have higher bioavailability and biological effects than the all-E-isomer, lycopene extraction with foodstuffs having a Z-isomerization-promoting effect in ethyl acetate should enhance the health benefits of tomato extracts.

Keywords: carotenoid; isomerization; tomato pulp; onion; broccoli; mustard; makonbu (Saccharina japonica); shiitake mushroom (Lentinus edodes)

1. Introduction

Lycopene is an acyclic carotenoid that imparts the characteristic bright-red color to ripe tomatoes and processed tomato products [1,2]. In recent years, lycopene has received increasing attention because of its potential anti-cancer and anti-obesity activities, as well as strong antioxidant capacity [3,4]. However, the oral bioavailability of carotenoids is generally very low, which is a serious problem in their industrial use [5]. Recently, as a means of increasing carotenoid bioavailability, their Z-isomerization has received much attention. In nature, lycopene predominantly occurs in its all-E-configuration, whereas in the living body, such as in the blood and in several tissues, more than 50% of the total lycopene exists as Z-isomers (Figure 1) [2,6,7]. This suggests that lycopene Z-isomers have greater bioavailability and tissue accumulation efficiency than the all-E-isomer. In fact, several in vitro and in vivo tests and human oral administration tests have strongly supported the higher bioavailability of the Z-isomers [7–9]. Moreover, recent studies have indicated the potential of some lycopene Z-isomers to show higher antioxidant and anti-obesity activities than the all-E-isomer [10,11]. Hence, the ingestion of lycopene Z-
isomer-rich diets would have beneficial health effects for humans, compared with all-E-isomer-rich ones.

Figure 1. Chemical structures of (a) (all-E)-lycopene and (b) lycopene Z-isomer (5Z,9Z-isomer).

Lycopene materials are generally produced by organic solvent extraction from tomatoes, and the all-E-isomer is the most predominant isomer in them. Thus, improving the Z-isomer ratio of lycopene in tomato extracts should lead to increased health benefits of lycopene products such as supplements. A few studies have shown that high-temperature carotenoid extraction and the addition of Z-isomerization-promoting catalysts, such as iron(III) chloride, enhance the total Z-isomer ratio of carotenoids in the extract [12,13]. Our most recent evidence indicates that several foodstuffs, such as onion, broccoli, and mustard, enhance the thermal Z-isomerization of lycopene in tomatoes [14,15]. Those foodstuffs contain compounds having a Z-isomerization-accelerating effect, i.e., polysulfides, isothiocyanates, and iodine. For example, onion contains large amounts of polysulfides such as diallyl disulfide and mustard contains allyl isothiocyanate (Figure 2) [16–18]. The possible mechanism of catalytic isomerization of lycopene by polysulfides is as described below [19–21]:

\[
\text{(RS-SR)} \rightleftharpoons 2\text{(RS•)} \quad (1) \\
\text{(Lycopene)}_E + \text{(RS•)} \rightleftharpoons \text{(RS-Lycopene•)_E} \quad (2) \\
\text{(RS-Lycopene•)_E} \rightleftharpoons \text{(RS-Lycopene•)_Z} \quad (3) \\
\text{(RS-Lycopene•)_Z} \rightleftharpoons \text{(Lycopene)_Z} + \text{(RS•)} \quad (4)
\]

Figure 2. Chemical structures of (a) diallyl disulfide and (b) allyl isothiocyanate.

The heat treatment induces the S–S bond cleavage of polysulfides (1), and the resulting thiyl radicals (RS•) act as the catalyst for carotenoid isomerization (2–4). The possible isomerization mechanism by isothiocyanates is as described below [13,19,22]:

\[
\text{(Lycopene)}_E + \text{(Isothiocyanate)} \rightleftharpoons \text{(Lycopene••)}_E + \text{(Isothiocyanate••)} \quad (5) \\
\text{(Lycopene••)_E} \rightleftharpoons \text{(Lycopene••)_Z} \quad (6) \\
\text{(Lycopene••)_Z} + \text{(Isothiocyanate••)} \rightleftharpoons \text{(Lycopene)_Z} + \text{(Isothiocyanate)} \quad (7)
\]

Isothiocyanates show powerful electrophilicity, and their properties accelerate the thermal isomerization of carotenoids (5–7).

Hence, we considered that when lycopene extraction from tomatoes is performed in the presence of foodstuffs having a Z-isomerization-promoting effect, a tomato extract rich in lycopene Z-isomers might be obtained. Namely, in the extraction process,
Z-isomerization-accelerating compounds are extracted from the foodstuffs and the compounds would enhance the Z-isomerization of (all-E)-lycopene extracted from tomatoes. However, there are no trials that have attempted to isomerize tomato lycopene using the foodstuffs at the same time as the extraction. Moreover, the impact of the foodstuffs on the lycopene isomerization in organic solvents is unknown, i.e., previous studies performed lycopene isomerization using the foodstuffs in vegetable oils [14,15]. The aim of this study was to investigate the effect of co-extraction of tomatoes and foodstuffs having a Z-isomerization-promoting effect on the Z-isomer ratio of lycopene in the resulting extract, and to clarify the extraction conditions capable of producing lycopene Z-isomer-rich tomato extract. As foodstuffs, onion; broccoli; mustard; an edible brown seaweed, makonbu (Saccharina japonica); and shiitake mushroom (Lentinus edodes) were used [14,15]. The lycopene extraction was carried out using organic solvents such as ethanol, 2-propanol, acetone, and ethyl acetate, which are commonly used in food processing.

2. Results and Discussion
2.1. Typical Profile of Lycopene Isomers in Tomato Pulp and Extracts
Lycopene isomers in an extracted raw material (tomato pulp) and the extracts obtained by the ethyl acetate extraction at 70 °C for 1 h were analyzed by normal-phase HPLC according to previous studies [2,14,15,22–24]. Lycopene isomers eluted within 15–30 min in this HPLC system, and good separation between them was obtained (Figure 3). Several peaks of lycopene Z-isomers, such as the 5Z-, 9Z-, and 13Z-isomers, were tentatively identified from the retention time and visible spectral data [2,14,15,22–24]. The HPLC chromatograms clearly showed that most lycopene in tomato pulp was present as the all-E-isomer, whereas various peaks related to the Z-isomers, especially the 13Z-isomer, were observed in the extracts. Moreover, the number and area of (Z)-lycopene peaks in the extracts were markedly increased by adding foodstuffs, such as onion and mustard, in the extraction step. Several studies have indicated that thermal treatment of (all-E)-lycopene in organic solvents at 50–60 °C induces the Z-isomerization, and mainly increases the levels of the 13Z-isomer [19,22]. Furthermore, previous studies have shown that cooking tomatoes with onion and mustard enhances the thermal Z-isomerization of lycopene [14,15]. From the above results and the information from previous studies, it is considered that (all-E)-lycopene is considered to be thermally isomerized in the high-temperature extraction step, and the efficiency is enhanced by adding onion and mustard, which have a Z-isomerization-promoting effect.

2.2. Effects of Temperature and Added Foodstuffs on Lycopene Extraction
The effects of extraction temperature (50, 60, and 70 °C) and the addition of foodstuffs (onion, broccoli, mustard, makonbu, and shiitake mushroom) on the total Z-isomer ratio in the resulting extract and recovery ratio of lycopene are shown in Figure 4 and Table 1, respectively. The foodstuffs (0.5 or 1 g) were added to the extraction process, and ethyl acetate was used as the extraction solvent. Commercially available tomato extracts (tomato oleoresin) are generally produced via ethyl acetate extraction [6]. Lycopene recovery was not markedly affected by the addition of foodstuffs; however, the total Z-isomer ratio and the isomer profile in the extract were greatly affected.
Figure 3. Normal-phase HPLC chromatograms of (a) extraction raw material (tomato pulp) and extracts obtained (b) without foodstuffs, and with (c) onion and (d) mustard using ethyl acetate at 70 °C for 1 h. (all-E)-, (5Z)-, (9Z)-, (13Z)-, (5Z,9Z)-, (5Z,9′Z)-, and (9Z,13′Z)-Lycopene designated in the chromatograms were identified according to the previous studies [2,14,15,22–24]. The peaks (1–9) were unknown lycopene Z-isomers [2,22].

Figure 4. Effects of extraction temperature [(a) 50, (b) 60, and (c) 70 °C] and foodstuffs on the total Z-isomer ratio of lycopene in the extract. The foodstuffs (0.5 or 1 g) were added to the extraction process and ethyl acetate was used as an extraction solvent. Error bars represent mean ± standard deviation (n = 3). Asterisks (*) indicate a statistically significant difference from the control group (* p < 0.05, ** p < 0.01, Dunnett’s t-test).
Effects of extraction temperature and foodstuffs on lycopene recovery and each lycopene isomer ratio in the extract.

| Foodstuff  | Temperature (°C) | Amount (g) | Recovery (%) | all-E | 5Z | 9Z | 13Z | Other Z 4 |
|------------|-----------------|------------|--------------|-------|----|----|-----|----------|
| Control    | 50              | –          | 65.5 ± 3.9   | 80.8 ± 0.9 | 3.2 ± 0.0 | 2.1 ± 0.2 | 9.6 ± 0.2 | 4.3 ± 0.7 |
|            | 60              | –          | 82.0 ± 0.8   | 68.2 ± 0.4 | 3.2 ± 0.1 | 2.4 ± 0.1 | 20.1 ± 0.2 | 6.1 ± 0.2 |
|            | 70              | –          | 93.4 ± 1.6   | 61.6 ± 0.5 | 2.7 ± 0.1 | 3.4 ± 0.1 | 25.2 ± 0.3 | 7.1 ± 0.3 |
| Onion      | 50              | 0.5        | 69.7 ± 2.7   | 72.2 ± 0.8 | 4.5 ± 0.3 | 3.9 ± 0.2 | 12.7 ± 0.1 | 6.7 ± 0.6 |
|            | 60              | 0.5        | 80.2 ± 2.5   | 54.5 ± 0.2 | 5.2 ± 0.1 | 6.5 ± 0.2 | 20.6 ± 0.2 | 13.2 ± 0.3 |
|            | 70              | 0.5        | 83.9 ± 2.2   | 49.6 ± 1.1 | 5.9 ± 0.3 | 7.8 ± 0.3 | 19.7 ± 0.2 | 17.0 ± 1.5 |
| Broccoli   | 50              | 0.5        | 62.7 ± 4.9   | 74.0 ± 0.2 | 3.9 ± 0.0 | 3.2 ± 0.1 | 12.4 ± 0.3 | 6.5 ± 0.2 |
|            | 60              | 0.5        | 74.8 ± 3.1   | 64.0 ± 0.4 | 4.2 ± 0.1 | 4.4 ± 0.1 | 20.9 ± 1.7 | 6.5 ± 2.1 |
|            | 70              | 0.5        | 83.2 ± 4.5   | 52.1 ± 0.3 | 4.0 ± 0.0 | 5.4 ± 0.2 | 25.2 ± 0.1 | 13.3 ± 0.3 |
| Mustard    | 50              | 0.5        | 67.0 ± 4.3   | 68.3 ± 1.4 | 5.8 ± 0.2 | 5.0 ± 0.1 | 12.6 ± 0.4 | 8.3 ± 0.8 |
|            | 60              | 0.5        | 73.6 ± 2.7   | 63.3 ± 0.2 | 6.6 ± 0.3 | 5.8 ± 0.2 | 13.8 ± 0.1 | 10.5 ± 0.4 |
|            | 70              | 0.5        | 81.7 ± 4.6   | 60.3 ± 0.8 | 5.1 ± 0.1 | 4.3 ± 0.1 | 20.4 ± 0.2 | 9.9 ± 0.8 |
| Makonbu    | 50              | 0.5        | 67.0 ± 4.3   | 68.3 ± 1.4 | 5.8 ± 0.2 | 5.0 ± 0.1 | 12.6 ± 0.4 | 8.3 ± 0.8 |
|            | 60              | 0.5        | 67.0 ± 4.3   | 68.3 ± 1.4 | 5.8 ± 0.2 | 5.0 ± 0.1 | 12.6 ± 0.4 | 8.3 ± 0.8 |
| Shitake    | 50              | 0.5        | 67.0 ± 4.3   | 68.3 ± 1.4 | 5.8 ± 0.2 | 5.0 ± 0.1 | 12.6 ± 0.4 | 8.3 ± 0.8 |
|            | 60              | 0.5        | 67.0 ± 4.3   | 68.3 ± 1.4 | 5.8 ± 0.2 | 5.0 ± 0.1 | 12.6 ± 0.4 | 8.3 ± 0.8 |
|            | 70              | 0.5        | 93.4 ± 5.9   | 38.9 ± 1.1 | 7.5 ± 0.2 | 11.5 ± 0.0 | 19.3 ± 0.0 | 22.8 ± 1.3 |

1 Results are expressed as the mean ± standard deviation (n = 3). The extraction was carried out using ethyl acetate for 1 h. 2 Addition amounts of foodstuffs to the extraction process. 3 The amount of the extracted lycopene to the amount of lycopene contained in the raw material (tomato pulp). 4 Sum of lycopene Z-isomers other than the 5Z-, 9Z-, and 13Z-isomers. 5 Adding no foodstuffs to the extraction process.

With or without foodstuffs, depending on the temperature rise in the extraction process, the total Z-isomer ratio and the recovery ratio of lycopene increased. Moreover, when foodstuffs were added to the process, the total Z-isomer ratios in the extracts were significantly enhanced compared to those without the addition of foodstuffs in most cases. For example, when lycopene was extracted from the tomato pulp (10 g, wet weight) without foodstuffs at 70 °C, the total Z-isomer ratio of lycopene in the extract was 38.4 ± 0.5%. On the other hand, when 0.5 g of onion, broccoli, mustard, makonbu, or shiitake mushroom was added to the process, the total Z-isomer ratios significantly increased to 53.6 ± 0.4, 47.9 ± 0.3, 48.2 ± 0.1, 41.5 ± 0.9, and 42.0 ± 1.2%, respectively. The total Z-isomer ratio increased with the addition amounts of foodstuffs, except for the extraction at 70 °C in the presence of makonbu. The increase in the lycopene Z-isomer ratio varied greatly depending on the type of foodstuff, and onion had a particularly high Z-isomerization efficiency. Several studies have reported that onion, broccoli, mustard, makonbu, and shiitake mushroom enhance the thermal Z-isomerization of tomato lycopene and the causative components are polysulfides, isothiocyanates, and iodine [14,15,19,25,26]. Hence, the Z-isomerization-promoting catalysts were considered to have been extracted from the foodstuffs in the extraction process,
which enhanced the thermal $Z$-isomerization of (all-$E$)-lycopene extracted from tomato pulp. One of the factors of the differences in isomerization efficiency among the foodstuffs is thought to be differences in the content of the catalytic components [16–18]. Moreover, the isomerization efficiency among the catalytic components would also affect the thermal isomerization of lycopene, i.e., dipropyl trisulfide, which is a major polysulfide contained in onion, shows greater lycopene $Z$-isomerization efficiency than that of allyl isothiocyanate, which is a major isothiocyanate contained in mustard [16–19]. In addition, onion showed extremely high $Z$-isomerization efficiency in the high temperature extraction. This was probably due to the efficient S–S bond cleavage at the high temperature, which produced a large amount of thyl radicals ($RS\cdot$), and thus enhanced thermal $Z$-isomerization of the extracted lycopene [21]. In fact, we recently reported that diallyl disulfide shows greater $Z$-isomerization efficiency for lycopene than that of allyl isothiocyanate at high temperature heating in ethyl acetate [19]. Moreover, our previous study showed that makonbu had greater $Z$-isomerization efficiency of lycopene than the other foodstuffs [14], but the isomerization promotion effect of makonbu was not so high in this test. This is probably due to the difference in the amount of $Z$-isomerization-accelerating compound (iodine) in the makonbu used and/or the difference in the medium used, i.e., the previous study used olive oil [14].

The addition of onion, broccoli, mustard, and makonbu improved the (5$Z$)-lycopene ratio in the extract (Table 1). Several studies have demonstrated that the 5$Z$-isomer potentially has higher antioxidant and anti-obesity activities than the other lycopene isomers [10,11,27]. These results indicate that co-extraction of tomatoes and foodstuffs having a $Z$-isomerization-promoting effect, such as onion, broccoli, and mustard, under high temperature should enhance not only the bioavailability, but also the biological effects of lycopene in the resulting extract.

2.3. Effects of Solvents and Added Foodstuffs on Lycopene Extraction

The effects of extraction solvents and the addition of foodstuffs on the total $Z$-isomer ratio in the extract and recovery ratio of lycopene are shown in Figure 5 and Table 2, respectively. After adding 10 and 1 g of tomato pulp and foodstuffs to the extraction process, respectively, the extraction was carried out at 60 °C. This process was performed with four organic solvents commonly used in food processing, that is, ethanol, 2-propanol, acetone, and ethyl acetate. These solvents have different polarities, and the solubility of lycopene in them is markedly different [28,29].

![Figure 5](image-url)

*Figure 5.* Effects of extraction solvents and foodstuffs on the total $Z$-isomer ratio of lycopene in the extract. The foodstuffs were added to the extraction process and (a) ethanol, (b) 2-propanol, (c) acetone, and (d) ethyl acetate were used as extraction solvents. Error bars represent mean ± standard deviation ($n = 3$). Asterisks (*) indicate a statistically significant difference from the control group (*$p < 0.05$, **$p < 0.01$, Dunnett’s $t$-test).*
The total Z-isomer ratio and lycopene recovery varied depending on the type of extraction solvent used. Namely, with or without foodstuffs, lycopene recovery decreased in the following order: ethyl acetate > acetone > 2-propanol > ethanol, whereas the total Z-isomer ratio showed the opposite order (Figure 3 and Table 2). This was due to the differences in lycopene solubility in different solvents, as well as the solubility difference among lycopene isomers. In general, lycopene recovery depends on its solubility in the extraction solvent, and such a tendency was clearly observed in this test; that is, the recovery of lycopene was directly proportional to its solubility in the extraction solvent [29,30]. On the other hand, several studies have demonstrated that Z-isomers of carotenoids, including lycopene, have greater solubility than the all-E-isomers [28,31], and the use of solvents with low carotenoid solubility for lycopene extraction from tomatoes results in a high Z-isomer ratio in the extract [12,32]. Specifically, (all-E)-lycopene is practically insoluble in ethanol, while the Z-isomers show high solubility; Murakami et al. [28] reported that (all-E)-lycopene barely dissolved in ethanol (0.6 mg/L) at 20 ºC, whereas the solubility of Z-isomer-rich lycopene was 2401.7 mg/L. Thus, it was considered that the Z-isomers were preferentially extracted by ethanol extraction in comparison to the all-E-isomer, and this resulted in a high Z-isomer ratio in the extract. On the other hand, owing to the relatively high solubility of (all-E)-lycopene in acetone and ethyl acetate [28], the total Z-isomer ratio in the extract was lower than that obtained with ethanol and 2-propanol.

The degree of influence of the foodstuffs on the total Z-isomer ratio of lycopene in the extract depended on the type of extraction solvent used (Figure 5). When 2-propanol and acetone were used as the extraction solvents, no significant difference in the total Z-isomer ratio was observed compared to that without the addition of foodstuffs (control), except for broccoli in the 2-propanol extraction. On the other hand, in ethanol extraction with added

Table 2. Effects of extraction solvents and foodstuffs on lycopene recovery and each lycopene isomer ratio in the extract. 1.

| Foodstuff | Solvent  | Recovery 2 (%) | all-E | 5Z | 9Z | 13Z | Other Z 3 |
|-----------|----------|----------------|-------|----|----|-----|----------|
| Control   | Ethanol  | 5.0 ± 0.3      | 11.1 ± 0.2 | 10.6 ± 0.2 | 20.0 ± 0.7 | 13.8 ± 0.1 | 44.5 ± 1.3 |
|           | 2-Propanol | 8.9 ± 0.3      | 32.4 ± 1.2 | 10.6 ± 0.3 | 13.7 ± 0.6 | 16.0 ± 0.8 | 27.3 ± 0.4 |
|           | Acetone  | 51.8 ± 0.8     | 52.5 ± 1.4 | 7.3 ± 0.3  | 6.8 ± 0.1  | 18.3 ± 0.3 | 15.1 ± 1.2 |
|           | Ethyl acetate | 82.0 ± 0.8   | 68.2 ± 0.4 | 3.2 ± 0.1  | 2.4 ± 0.1  | 20.1 ± 0.2 | 6.1 ± 0.2  |
| Onion     | Ethanol  | 5.4 ± 0.6      | 12.4 ± 0.3 | 13.4 ± 0.2 | 13.9 ± 0.3 | 14.3 ± 0.1 | 46.0 ± 0.5 |
|           | 2-Propanol | 9.6 ± 0.2      | 31.5 ± 1.1 | 11.8 ± 0.2 | 12.1 ± 0.3 | 14.8 ± 0.2 | 29.8 ± 0.7 |
|           | Acetone  | 70.6 ± 1.1     | 47.2 ± 0.4 | 8.0 ± 0.1  | 9.3 ± 0.1  | 16.2 ± 0.2 | 19.3 ± 0.6 |
|           | Ethyl acetate | 83.9 ± 2.2    | 49.6 ± 1.1 | 5.9 ± 0.3  | 7.8 ± 0.3  | 19.7 ± 0.2 | 17.0 ± 1.5 |
| Broccoli  | Ethanol  | 2.6 ± 0.1      | 40.1 ± 0.4 | 15.5 ± 0.3 | 11.3 ± 0.3 | 12.0 ± 0.3 | 21.1 ± 0.5 |
|           | 2-Propanol | 6.2 ± 0.1      | 36.9 ± 1.0 | 12.1 ± 0.1 | 12.4 ± 0.1 | 13.3 ± 0.1 | 25.3 ± 1.0 |
|           | Acetone  | 55.7 ± 1.6     | 53.2 ± 1.3 | 7.5 ± 0.2  | 8.3 ± 0.2  | 15.6 ± 0.3 | 15.4 ± 0.9 |
|           | Ethyl acetate | 68.2 ± 1.5    | 57.4 ± 0.3 | 5.1 ± 0.1  | 6.5 ± 0.4  | 18.8 ± 0.5 | 12.2 ± 0.4 |
| Mustard   | Ethanol  | 3.4 ± 0.1      | 22.9 ± 0.8 | 16.0 ± 0.2 | 13.1 ± 0.3 | 14.7 ± 0.4 | 33.3 ± 0.7 |
|           | 2-Propanol | 9.0 ± 0.0      | 32.1 ± 0.5 | 10.3 ± 0.1 | 12.3 ± 0.2 | 17.2 ± 0.2 | 28.1 ± 0.4 |
|           | Acetone  | 52.1 ± 1.8     | 55.0 ± 1.8 | 6.5 ± 0.2  | 5.9 ± 0.1  | 18.8 ± 0.4 | 13.8 ± 0.5 |
|           | Ethyl acetate | 75.8 ± 3.2    | 55.2 ± 0.6 | 6.8 ± 0.2  | 6.1 ± 0.1  | 19.4 ± 0.1 | 12.5 ± 0.6 |
| Makonbu   | Ethanol  | 5.0 ± 0.1      | 11.7 ± 0.5 | 15.1 ± 0.6 | 19.9 ± 0.2 | 13.3 ± 0.1 | 40.0 ± 1.1 |
|           | 2-Propanol | 8.8 ± 0.1      | 29.2 ± 3.0 | 14.2 ± 0.7 | 13.8 ± 0.5 | 12.6 ± 0.1 | 30.2 ± 2.7 |
|           | Acetone  | 58.3 ± 4.0     | 57.5 ± 0.6 | 8.5 ± 0.2  | 7.0 ± 0.3  | 13.4 ± 0.5 | 13.6 ± 0.7 |
|           | Ethyl acetate | 72.7 ± 1.8    | 61.1 ± 1.2 | 4.8 ± 0.1  | 4.0 ± 0.2  | 19.4 ± 0.5 | 10.7 ± 1.0 |
| Shiitake  | Ethanol  | 5.0 ± 0.0      | 10.7 ± 0.3 | 12.9 ± 0.1 | 18.8 ± 0.1 | 13.4 ± 0.2 | 44.2 ± 0.0 |
|           | 2-Propanol | 8.6 ± 0.2      | 31.6 ± 0.2 | 9.9 ± 0.1  | 12.8 ± 0.3 | 17.5 ± 0.0 | 28.2 ± 0.3 |
|           | Acetone  | 53.6 ± 6.2     | 50.9 ± 2.3 | 7.1 ± 0.3  | 7.0 ± 0.5  | 19.2 ± 0.8 | 15.8 ± 0.8 |
|           | Ethyl acetate | 79.0 ± 4.0    | 66.3 ± 0.3 | 3.4 ± 0.1  | 2.8 ± 0.2  | 20.7 ± 0.1 | 6.8 ± 0.2  |

1 Results are expressed as the mean ± standard deviation (n = 3). The extraction was carried out at 60 ºC for 1 h. 2 The amount of the extracted lycopene to the amount of lycopene contained in raw material (tomato pulp). 3 Sum of lycopene Z-isomers other than the 5Z-, 9Z-, and 13Z-isomers. 4 Adding no foodstuffs to the extraction process.
broccoli and mustard, the total Z-isomer ratios were significantly decreased compared to the control. Our group recently reported that the addition of a certain amount of allyl isothiocyanate in ethanol lycopene solution inhibited its thermal Z-isomerization [19]. Thus, as broccoli and mustard contain isothiocyanates in common [33], the total Z-isomer ratios might be lowered by their action. When ethyl acetate was used as the extraction solvent, the total Z-isomer ratio was significantly increased, except for shiitake mushroom. It is considered that ethyl acetate could efficiently extract Z-isomerization-promoting components (i.e., polysulfides, isothiocyanates, and iodine) from the foodstuffs and thus, the thermal Z-isomerization reaction of extracted lycopene was promoted in the solution. Therefore, the use of ethyl acetate as the extraction solvent for lycopene is effective not only for obtaining high extraction efficiency but also for improving (Z)-lycopene content in the extract during extraction with Z-isomerization-promoting foodstuffs.

2.4. Effects of Extraction Time and Added Foodstuffs on Lycopene Extraction

The time course of lycopene extraction with or without Z-isomerization-promoting foodstuffs was investigated. The extraction was conducted at 60 °C using ethyl acetate, and 10 and 1 g of tomato pulp and foodstuffs, respectively, were added to the extraction process. The total Z-isomer ratio of lycopene in the resulting extract and the recovery ratio of lycopene are shown in Figure 6. The total Z-isomer ratio and lycopene recovery increased with extraction time. The Z-isomerization efficiency of foodstuffs during this extraction period decreased in the following order: onion > mustard > broccoli > makonbu ≈ shiitake mushroom ≈ control (Figure 6a). A higher Z-isomerization efficiency was obtained with the use of onion, mustard, and broccoli in all extraction periods compared to that without the addition of foodstuffs (control). When makonbu was added, the total Z-isomer ratio was higher than that of the control at 1 h of extraction, whereas, at all other time points, the ratios were almost the same as the control. The total Z-isomer ratio obtained with the addition of shiitake mushroom was almost the same as that of the control under these extraction conditions. When shiitake mushroom was used, the total Z-isomer ratio significantly increased during high-temperature extraction (Figure 3). Thus, the Z-isomerization-promoting components present in shiitake mushroom, that is, lenthionine [14], could efficiently act at high temperatures. In fact, several studies have reported that shiitake mushroom enhances the Z-isomerization of tomato lycopene at 80 °C [14,15]. When broccoli and mustard were added, a slight decrease in lycopene recovery was observed. Broccoli and mustard contain isothiocyanates, which promote thermal Z-isomerization and the thermal degradation of carotenoids [19]. Thus, it is possible that lycopene was degraded during the extraction process due to the presence of isothiocyanates extracted from broccoli and mustard, resulting in a lower recovery rate.

The time courses of each lycopene isomer are shown in Figure 7. With or without foodstuffs, (13Z)-lycopene was mainly contained in the resulting extracts. Although the ratio of the 13Z-isomer increased during 0.5–2 h of the extraction period, the ratio gradually decreased concomitantly with the increase in the other Z-isomers. These observations have been described in previous studies that investigated the thermal isomerization of pure (all-E)-lycopene in organic solvents [22,34], and they indicated that this isomerization tendency is caused by the differences in the potential energy and activation energy among lycopene isomers. Several computational studies have shown that the potential energies of (all-E)- and (mono-Z)-lycopene are in the order of 13Z > 9Z > 5Z ≈ all-E-isomers, and the activation energies of the isomerization from the all-E-isomer to the mono-Z-isomers are in the order of 5Z > 9Z > 13Z-isomers [24,35]. Thus, the kinetically favored 13Z-isomer was initially generated by thermal treatment. However, as the 13Z-isomer is thermodynamically unpreferred, its ratio gradually decreased over time and, along with this, the other thermodynamically preferred isomers increased. When onion and makonbu were added to the extraction process, the content of (5Z)-lycopene, which exhibits higher antioxidant and anti-obesity activities than the other lycopene isomers [10,11,27], increased with the extraction time. The addition of onion to the process not only did not affect the
lycopene recovery but also increased the total Z-isomer ratio of lycopene as well as the valuable (5Z)-lycopene ratio in the extract. Therefore, the addition of onion to the extraction process of tomato lycopene results in value addition to the extract.

**Figure 6.** Effects of extraction time and foodstuffs on (a) the total Z-isomer ratio of lycopene in the extract and (b) the recovery ratio of lycopene. The foodstuffs were added to the extraction process and the extraction was performed using ethyl acetate at 60°C. Error bars represent mean ± standard deviation (n = 3).

**Figure 7.** Effects of extraction time and foodstuffs on each lycopene ratio in the extract. The extraction was conducted (a) without foodstuffs and with (b) onion, (c) broccoli, (d) mustard, (e) makonbu, or (f) shiitake mushroom. The extraction was performed using ethyl acetate at 60°C. Error bars represent mean ± standard deviation (n = 3).
3. Materials and Methods

3.1. Materials

High-performance liquid chromatography-grade ethanol, 2-propanol, acetone, ethyl acetate, and hexane were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). N,N-Diisopropylethylamine (DIPEA) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Heat-sterilized tomato pulp (lycopene content, 45 mg/100 g; total Z-isomer ratio of lycopene, 7.3%; moisture content, 90.2%) was gifted by Kagome Co., Ltd. (Tokyo, Japan). Dried powders of onion, broccoli, mustard, makonbu, and shiitake mushroom were purchased from S&B Foods Inc. (Tokyo, Japan), Namisato Corporation (Tochigi, Japan), Aarti Japan Co., Ltd. (Hyogo, Japan), Kobayashi Shouten (Hokkaido, Japan), and Harashima Shiitake Production Union (Nagasaki, Japan), respectively.

3.2. Lycopene Extraction

Lycopene isomers were extracted from wet tomato pulp with dried powder of foodstuffs, that is, onion, broccoli, mustard, makonbu, and shiitake mushroom. The extraction conditions such as temperature, solvents, and the amount of extraction raw materials were determined based on previous studies \[29,30,32\]. Briefly, 10 g of tomato pulp and 0.5 or 1 g of foodstuff were treated with 40 mL of organic solvents (ethanol, 2-propanol, acetone, and ethyl acetate) in a 100-mL screw-cap glass bottle. The headspace of the bottle was purged with nitrogen gas, and the bottle was tightly capped to minimize oxygen exposure and prevent solvent evaporation during extraction. The extraction was conducted using a magnetic stirrer (SW-M60; Nissin Rika Co., Ltd., Tokyo, Japan) in a water bath. After the extraction, the residue was eliminated using a 0.22-µm PTFE membrane filter, and the resulting filtrate was evaporated to dryness under reduced pressure at 35 °C. The extract was dissolved in hexane for HPLC analysis.

3.3. HPLC Analysis

Lycopene isomer analysis in the extract was performed by normal-phase HPLC with a photodiode array detector (SPD-M20A; Shimadzu Co., Ltd., Kyoto, Japan) and three Nucleosil 300-5 columns connected in tandem (3 × 250 mm length, 4.6 mm inner diameter, 5 µm particle size; GL Sciences Inc., Tokyo, Japan) \[2,14,15\]. The analytical conditions were as follows: mobile phase, hexane containing 0.075% of DIPEA; mobile phase flow rate, 1.0 mL/min; column temperature, 40 °C; detection wavelength, 460 nm. The peaks of lycopene isomers were identified by HPLC retention time, visible spectral data, and the height ratio of the Z-peak at approximately 360 nm to the main absorption peak (Q-ratio) \[2,14,15,22–24\].

3.4. Data Expression and Statistical Analysis

All data were recorded as the mean ± standard deviation of triplicate experiments. Statistical significance was tested using one-way analysis of variance followed by Dunnett’s t-test using the EZR software program (version 1.54; Saitama Medical Center, Jichi Medical University, Saitama, Japan) and \(p < 0.05\) or \(p < 0.01\) was regarded as significant.

4. Conclusions

The effect of the presence of foodstuffs (onion, broccoli, mustard, makonbu, and shiitake mushroom) in the extraction process on the isomer profile of lycopene extracted from tomato pulp was investigated. The addition of the foodstuffs promoted thermal Z-isomerization of tomato-derived (all-E)-lycopene in the process. In particular, when onion was added to the ethyl acetate extraction process, the total Z-isomer ratio in the extract was markedly increased. Lycopene Z-isomers have higher bioavailability and potentially exhibit higher biological effects than the all-E-isomer. Therefore, the use of this extraction procedure (i.e., co-extraction of tomatoes and foodstuffs having a Z-isomerization-promoting effect) is expected to lead to the practical application of highly bioavailable and functional lycopene products.
Author Contributions: Conceptualization, M.H., K.I. and W.T.; methodology, M.H. and K.I.; validation, M.H., K.I. and W.T.; formal analysis, M.H., K.M. and K.I.; investigation, M.H., K.M. and K.I.; resources, K.I. and W.T.; data curation, M.H. and K.M.; writing—original draft preparation, M.H.; writing—review and editing, K.M., K.I., W.T. and M.G.; supervision, M.G.; project administration, W.T.; funding acquisition, M.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was partially supported by the Adaptable and Seamless Technology transfer Program through Target-driven R&D (A-STEP) from the Japan Science and Technology Agency (JST) (grant number: JPMJTR20U7) and the KAKENHI from the Japan Society for the Promotion of Science (JSPS) (grant number: 19K15779 and 20H02515).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to Takahiro Inakuma (Shinshu University) and Hiroyuki Ueda, Takahiro Kawana, Tetsuya Fukaya, Satoshi Otsuki, Takuma Higashiura, and Ryota Takemura (Kagome Co., Ltd.) for their kind help and constructive suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

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