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Enhancing the bioaccessibility of lycopene from tomato processing byproducts via supercritical carbon dioxide extraction

Ali Ubeyitogullari [a], Ozan N. Ciftci [a,b,*]

[a] Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE, 68588-6205, USA
[b] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE, 68588-6205, United States

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ABSTRACT

Tomato peel and seed from tomato processing industry are treated as waste; however, they contain lycopene, a high-value bioactive compound. In this study, lycopene was extracted from tomato peel and seed using supercritical carbon dioxide (SC–CO_2) and hexane, and the bioaccessibilities of lycopene in the SC-CO_2- and hexane-extracted oleoresins were investigated for the first time. The (Z)-lycopene content of the SC-CO_2-extracted oleoresin (69%) was higher than that of hexane-extracted oleoresin (45%). Separation of the insoluble fraction from the oleoresins increased the (Z)-lycopene contents of the SC-CO_2- and hexane-extracted oil fractions to 80% and 49%, respectively. The bioaccessibility of total-lycopene in the oleoresins was increased by 3.3-fold via SC-CO_2 extraction, which was attributed to higher (Z)-lycopene content, and small-sized uniform distribution of lycopene in the oleoresin. SC-CO_2 extraction is not only a green method for extraction of bioactive compounds, but also has the potential to improve health benefits of bioactive compounds.

1. Introduction

In recent years, food industry has prioritized the incorporation of health-promoting bioactives into foods as the consumers' demand for functional foods is increasing. To meet the growing demand, there has been considerable efforts to extract bioactive compounds from various sources. In particular, lycopene received much attention due to its protective action against cardiovascular diseases and a number of cancers such as prostate, lung and stomach cancers (Costa-Rodrigues et al., 2018; Giovannucci, 1999; Story et al., 2010). Lycopene is a non-provitamin-A, acyclic, symmetrical carotenoid with 13 double bonds (11 conjugated and 2 non-conjugated double bonds) (Colle et al., 2010; Longo et al., 2012). It is the most abundant carotenoid in red-ripe tomatoes (over 90% of total carotenoids). Lycopene is only synthesized by microorganisms and plants but not human body, therefore it must be consumed in the diet. When the poor bioavailability of lycopene is considered (Colle et al., 2010), extracts with high lycopene concentrations are needed for food industry to produce lycopene-enriched food products.

Tomato and tomato-based products are the main sources of lycopene in a typical western diet (Colle et al., 2010). Industrial waste of tomato-based products (e.g. ketchup, juice, and soup) ranges between 3 and 5% of the tomato weight which mainly consists of tomato peel and seed (Del Valle, Cámara and Torija, 2006). According to World Processing Tomato Council (WPTC), approximately 1,400,000 tons of tomato processing byproducts (tomato peel and seed) are produced annually worldwide (The World Processing Tomato Council WPTC, 2018). The disposal of those byproducts possesses a major problem for the tomato industry. The current practice is either to dispose them as waste or use them for animal feed production. However, real value of tomato byproducts is underestimated as these byproducts (especially tomato peel) have 72–92% of the total lycopene in tomatoes (Kaur et al., 2008).

Lycopene is predominantly present in all-E form in the nature. Specifically, (all-E)-lycopene accounts for 80–97% of total lycopene in tomatoes and tomato-based products (Longo et al., 2012; Murakami et al., 2018). Nevertheless, (Z)-lycopene isomers represents 50–90% of total lycopene in human serum and tissue suggesting higher bioavailability of (Z)-lycopene isomers than (all-E)-lycopene form in humans (Unlu et al., 2007). The higher absorption of (Z)-lycopene isomers in small intestine is hypothesized to be due to greater solubilization in mixed micelles and lower aggregation tendency of (Z)-lycopene isomers compared to

* Corresponding author. Departments of Food Science and Technology and Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE, 68588-6205, USA.
E-mail address: ciftci@unl.edu (O.N. Ciftci).

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(all-E)-lycopene (Boileau et al., 2002; Cooperstone et al., 2015; Unlu et al., 2007). (Z)-lycopene isomers are also considered to be more preferably taken up by the epithelial cells in the small intestine (Cooperstone et al., 2015).

Considering the demand and health benefits, there has been great efforts to obtain tomato extracts (oleoresins) with high (Z)-lycopene content (Colle et al., 2010; Honda et al., 2017; Longo et al., 2012; Urbonaviciene and Viskelis, 2017). There have been some efforts on Z-isomerization of lycopene using different methods such as heat treatment, microwave treatment, light irradiation, electrolysis treatment, and catalytic treatment (Honda et al., 2015a, 2015b, 2018; Murakami et al., 2018; Wei et al., 1997). However, above-mentioned methods have some drawbacks. For example, heat and microwave treatments may cause thermal degradation because of the high temperatures. Light irradiation may cause light degradation; therefore, photosensitizers need to be used to prevent light degradation. If toxic photosensitizers are used, they need to be removed after the treatment. Similarly, electrolytes and catalysts must be removed after electrolysis treatment if toxic ones are used. In some carotenoids, such as lycopene and astaxanthin, by combining chemical approaches such as Z-isomerization treatment and the above physical approaches, it may be possible to further improve the bioavailability. Moreover, in the above-mentioned methods, the tomato oleoresin first may need to be extracted using organic solvents before the treatment for Z isomerization or they may need to be dissolved in a solvent during the treatment, but finding a food grade solvent is a challenge.

Lycopene is conventionally extracted using organic solvents like hexane, chloroform and acetone (Poojary and Passamonti, 2015). However, the toxicity of those solvents is a major problem for food applications. Therefore, there is a critical need for a clean method for the extraction of lycopene. Use of SC-CO_2 received much attention as a green method to extract lycopene. Although several studies reported extraction of lycopene from tomato or tomato byproducts using SC-CO_2, there is no information on the function and efficacy of the lycopene in the oleoresins. Recently, tomato oleoresins were extracted from tomato peel and seed using SC-CO_2 by our research group (Vallecilla-Yepez and Ciftci, 2018). In that study, it has been found that the oleoresins extracted with SC-CO_2 contains high amount of (Z)-lycopene compared to the oleoresin extracted with hexanes suggesting improved bioaccessibility of the SC-CO_2-extracted lycopene. Although there are studies on SC-CO_2 extraction of lycopene, there is no information on the microstructure of the lycopene in the SC-CO_2-extracted oleoresin in literature. To the best of our knowledge, the use of SC-CO_2 to enhance the bioaccessibility of lycopene has not been reported before. Creating changes in the physicochemical properties of lycopene via extraction that results in improved function and efficacy is a breakthrough. With this approach, energy-intensive and/or complicated methods to improve lycopene's bioaccessibility such as microencapsulation (Szabo et al., 2021) and emulsion formation (Nemli et al., 2021) can be eliminated. In this study, a new phenomenon on SC-CO_2 extraction and microstructure of the lycopene extracted using SC-CO_2 is reported. This study will be the first report on increasing bioaccessibility of lycopene via an extraction process.

Therefore, the main objective of this study was to investigate the effect of SC-CO_2 extraction on the bioaccessibility of lycopene in the tomato oleoresin. The specific objectives were to: (a) extract lycopene from tomato peel and seed blend using SC-CO_2 and hexane extractions, (b) characterize the SC-CO_2- and hexane-extracted oleoresins for their lipid composition, namely, lycopene content and isomers, phytosterol and tocol contents, and fatty acid composition, and (c) compare the bioaccessibility of the lycopene in the oleoresins extracted using SC-CO_2 and hexane using a simulated digestion model.

2. Materials and methods

2.1. Materials

Tomato peel and seed that were by-products of a ketchup plant in California were kindly provided by ConAgra Brands (NE, USA). Moisture content of the tomato peel and seed were 6.2 and 4.9% (w/w), respectively. Based on hexane extraction, lycopene content of the peel was 2.4 mg lycopene/g, whereas no lycopene was detected in the seed. The oil content of the seed was 8.2% (w/w), whereas no oil was detected in the peel. Tomato seeds were ground and passed through a sieve with an opening size of 1.0 mm, whereas the tomato peel powder was used as is. Blend of tomato peel and seed (70:30, w/w) was used for both SC-CO_2 and hexane (Soxhlet) extractions. Liquid CO_2 (99.99% purity) was supplied by Matheson Tri-Gas, Inc. (NE, USA). (all-E)-lycopene standard (≥90%) and β-carotene (99%) were purchased from Sigma-Aldrich (MO, USA) and Alfa Aesar (MA, USA), respectively. Sylon BFT [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA):trimethylchlorosilane (TMCS), 99:1] was purchased from Supelco Inc. (PA, USA) and 5α-cholestane (>98%) was obtained from Acros Organics (NJ, USA). Pyridine was purchased from EMD Chemicals, Inc. (NJ, USA). Tocopherol standards (α-tocopherol (95.5%), β-tocopherol (>90%), γ-tocopherol (≥96%), and δ-tocopherol (95%)) were purchased from Sigma-Aldrich (MO, USA), whereas tocotrienol standards (α-, γ-, and δ-tocotrienol (>98%)) were obtained from Santa Cruz Biotechnology (CA, USA). β-Tocotrienol (>98%) was from LifeSpan BioSciences (WA, USA). The fatty acid standards were purchased from Nu-Chek-Prep, Inc. (MN, USA) and dilaurin (≥98%) was obtained from Cayman Chemical Company (MI, USA).

α-Amylase from *Bacillus subtilis* was obtained from MP Biomedicals (OH, USA). Amano lipase A12 (from fungus *Aspergillus niger*, 132,000 U/g) was kindly supplied by Amano Enzyme Inc. (IL, USA). All other enzymes (pancreatin and lipase from porcine pancreas, and pepsin from porcine gastric mucosa) and bile extract porcine were purchased from Sigma-Aldrich (MO, USA).

2.2. Hexane (Soxhlet) extraction

Tomato peel and seed blend (12 g) was extracted with hexane as control in a lab scale Soxhlet extraction system for 6 h. In order to prevent photodegradation, the extractions were carried out under dim light and the Soxhlet extractor was covered with aluminum foil. After extraction, hexane was removed from the extract using a rotary vacuum evaporator (R-200, Büchi Labortechnik AG, Flawil, Switzerland) at 22 °C. The residue was flushed with nitrogen and stored at -80 °C in an ultralow freezer (TLE Series, Thermo Scientific, NC, USA) until analyzed. Oleoresin yield was reported as (weight of oleoresin/weight of tomato peel and seed blend used for extraction) × 100. Tomato seed (100%) also underwent the same Soxhlet (hexane) extraction process to quantify the oil and lycopene content.

2.3. SC-CO_2 extraction

SC-CO_2 extractions were carried out in a custom-made laboratory scale SC-CO_2 extraction system. The details of the SC-CO_2 extraction system were given somewhere else (Ubeyitogullari and Ciftci, 2017). Firstly, the extraction vessel was loaded with 12 g of the tomato peel and seed blend (70:30, w/w) and glass wool was placed at both ends of the vessel. Then, the vessel was flushed with CO_2 at the tank pressure (ca. 6 MPa) to eliminate the air in the vessel and heated to 80 °C using the temperature controller. Then, the system was pressurized to 30 MPa with CO_2 using a double head high pressure syringe pump (Model 260D, Teledyne Isco Inc., NE, USA). The pressure was monitored by a pressure gauge and kept constant. After 20 min of static extraction, the needle valve was opened, and the flow rate was adjusted to 1 L/min (measured by the flow meter at ambient conditions) by the micrometering valve.

The extracted oleoresin was continuously collected in a sample vial that was kept in the cold trap at -10 °C for 4 h. The peel:seed blend ratio and the SC-CO_2 extraction conditions were based on the optimized extraction yield and lycopene content of the oleoresins in our previous study (Vallecilla-Yepez and Ciftci, 2018). The oleoresin yield was expressed as (weight of oleoresin/weight of tomato peel and seed blend used for extraction) × 100. The oleoresin samples were flushed with nitrogen and stored at -80 °C until analyzed.

2.4. Separation of the insoluble fraction of the extracted oleoresins

Oleoresins extracted by hexane and SC-CO_2 were centrifuged at 9000 *g* using a refrigerated centrifuge (Sorvall™ Legend™ X1R, Thermo Scientific, Osterode am Harz, Germany) for 30 min at 20 °C (Longo et al., 2012). The obtained supernatants (hexane- and SC-CO_2-extracted oil fractions) were analyzed for their composition, and bioaccessibility of lycopene.

2.5. Fatty acid composition analysis

Fatty acid compositions of the hexane- and SC-CO_2-extracted oleoresin and oil fractions were determined following the method of Belayneh et al. (2015) using a GC (Hewlett-Packard (HP) 6890 GC, Agilent Technologies, DE, USA) equipped with a flame ionization detector (GC-FID). Briefly, 3 μL of sample was placed into a screw-cap test tube, and then 1.5 mL of 2.5% (v/v) sulfuric acid in methanol (containing 0.01% w/v butylated hydroxyl toluene, BHT), 200 μL of triheptadecanoin solution (10 mg/mL in toluene) and 400 μL of toluene were included. Subsequently, the tubes were flushed with nitrogen, capped and kept at 90 °C for 1.5 h for the formation of fatty acid methyl esters (FAMEs). After cooling, 1 mL of water and 1.5 mL of heptane were added into the tubes and then vortexed for 10 s. Finally, FAMEs in the heptane layer were injected onto a GC column (HP-INNOWAX, 30 m × 0.25 mm x 0.25 μm; Agilent Technologies, CA, USA) at splitless mode. The oven temperature was programmed with an initial hold at 90 °C for 1 min, then followed by an increase to 235 °C at 30 °C/min, and lastly kept at 235 °C for 5 min. The injector and detector temperatures were 200 and 240 °C, respectively. Helium was used as the carrier gas with a constant inlet pressure of 35 psi. Fatty acids were identified by comparing the retention times of the authentic fatty acid standards and reported as percentages of the total fatty acids.

2.6. Analysis of free fatty acids, mono-, di-, and triacylglycerols

The analyses of free fatty acids (FFAs), monoacylglycerols (MAGs), diacylglycerols (DAGs), and triacylglycerols (TAGs) were carried out using a GC-FID (Hewlett-Packard (HP) 6890 GC). The samples were silylated according to the method of Ciftci and Temelli (2013). Briefly, 10 mg of sample was added into a screw-cap test tube along with 50 μL of dilaurin solution (20 mg/mL in chloroform) as an internal standard. Then, the samples were dissolved in 0.5 mL of pyridine and subjected to silylation with 0.1 mL of Sylon BFT at 70 °C for 20 min. After diluting with 5 mL of chloroform, an aliquot (1 μL) of each sample was injected onto a GC column (MXT-Biodiesel TG, 15 m × 0.32 mm x 0.10 μm; Restek, PA, USA) with a retention gap (2 m × 0.53 mm). The oven conditions were adapted from the method of Pacheco et al. (2014). First, the oven temperature was set to 40 °C and maintained at 40 °C for 4 min. Then, the temperature was raised to 350 °C at a rate of 25 °C/min and finally increased to 354 °C at a rate of 0.2 °C/min. The injector and detector temperatures were 320 and 380 °C, respectively. Helium with a flow rate of 2.3 mL/min was used as the carrier gas. Authentic standards of FFA, MAG, DAG, and TAG were used for identification of the peaks. Relative response factors (RRFs) were used for the quantification of the analytes, where RRFs were calculated by comparing the area and concentration of each analyte to the area and concentration of the internal standard dilaurin.

2.7. Phytosterols analysis

Phytosterols in the hexane- and SC-CO_2-extracted oleoresin and oil fractions were quantified by a GC-FID (Hewlett-Packard (HP) 6890 GC) according to the method of Ubeyitogullari et al. (2018). Briefly, 50 μL of 5α-cholestane (2.25 mg/mL) was added onto 0.1 g of sample and then the mixture was saponified with 4 mL of 1 N KOH in methanol at 40 °C for 1 h. After keeping the mixture at room temperature for 18 h, 2 mL of deionized water was added. Then, the unsaponifiable fraction was extracted with methyl tert-butyl ether/hexane (50:50, v/v) solution and the extract was brought to dryness under nitrogen at room temperature (21 °C). Finally, the dry residues were dissolved in 0.3 mL of pyridine and silylated with 1 mL of Sylon BFT at 50 °C for 30 min. The silylated sterols (1 μL) were injected onto a GC column (DB-35MS, 25 m × 0.20 mm x 0.33 μm; J&W, Agilent Technologies, CA, USA) at splitless mode. The oven temperature was programmed with an initial hold at 100 °C for 5 min, then increased to 250 °C at a rate of 25 °C/min and held at that temperature for 1 min, afterwards raised to 290 °C at a heating rate of 3 °C/min and kept at 290 °C for 40 min. Helium was used as the carrier gas at a flow rate of 0.5 mL/min. The injector and detector temperature were set to 270 and 300 °C, respectively.

2.8. Tocols analysis

Tocols contents of the hexane- and SC-CO_2-extracted oleoresin and oil fractions were determined according to the method of Ciftci et al. (2011) with minor modifications. Briefly, the samples (50 mg) were dissolved in hexane (5 mL) and separated using an HPLC system (Agilent 1100 Series, Agilent Technologies, Germany) equipped with an Agilent 1200 Series fluorescence detector set for an excitation at 295 nm and an emission at 325 nm. An aliquot of 10 μL was injected onto a normal-phase Luna Silica column (250 × 4.6 mm, 5 μm; Phenomenex, CA, USA). The column temperature was 25 °C, while the flow rate of the mobile phase (7% *tert*-butyl mehyl ether in hexane) was 1.5 mL/min. Authentic standards of α-, β-, γ-, and δ-tocopherols; and α-, β-, γ-, and δ-tocotrienols were used for identification and quantification of the tocol isomers. External calibration curves were prepared for quantification of each tocol isomer separately. The coefficient of determination (R^2) of the calibration curves ranged between 0.997 and 0.999.

2.9. Simulated digestion

Tomato oleoresins and their oil fractions were subjected to a sequential oral, gastric and intestinal digestion according to the method of Ubeyitogullari et al. (2018). Simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) stock solutions were prepared as previously reported by Minekus et al. (2014). All the enzyme unit calculations were conducted based on the activity of the enzymes declared by the manufacturers. Simulated digestion experiments were conducted in triplicate under dim light. The sample headspace was flushed with nitrogen before each digestion step.

2.9.1. Simulated oral digestion

First, 3.5 mL of SSF stock solution (pH 7.0) was added into a 50 mL-Erlenmeyer flask following the method of Ubeyitogullari et al. (2018). The sample (0.5 g) and α-amylase solution (0.5 mL, 750 U/mL) were then added into the flask where an α-amylase concentration of 75 U/mL was achieved in final mixture. Next, 25 μL of 0.3 M $CaCl_2$ and 0.475 mL of deionized water were included. Finally, the pH was adjusted to pH 7.0 and the mixture was incubated for 30 s at 37 °C and 150 rpm in a shaking water bath (Boekel Scientific, PA, USA).

2.9.2. Simulated gastric digestion

After the oral digestion, 3.25 mL of SGF stock solution (pH 3.0) was added onto 5 mL of oral bolus (Ubeyitogullari et al., 2018). Following the pH adjustment to pH 3.0 using 1 M HCl solution (75 μL), 0.5 mL of

porcine pepsin (40,000 U/mL) and 0.25 mL of fungal lipase (1000 U/mL) solutions were added. Next, 2.5 μL of 0.3 M $CaCl_2$ solution and 0.923 mL deionized water were included into the mixture which resulted in a final ratio of oral bolus to SGF of 50:50 (v/v). Finally, the samples were incubated at 37 °C and 100 rpm for 2 h in the shaking water bath. The pH of the mixture was monitored and re-adjusted to pH 3.0 using 1 M HCl solution throughout the gastric digestion.

2.9.3. Simulated intestinal digestion

Following the gastric digestion, 10 mL of the gastric chyme was mixed with 6.125 mL of SIF stock solution (pH 7.0) (Ubeyitogullari et al., 2018). The amount of pancreatin was determined based on the trypsin activity aiming to achieve a final trypsin activity of 100 U/mL. Extra porcine pancreatic lipase was included to achieve a lipase activity of 2000 U/mL in the final mixture. Thus, 1.25 mL of pancreatin (2000 U trypsin) and porcine pancreatic lipase (39,630 U) solution was added into the mixture. Then, 0.625 mL of 320 mM fresh bile solution (prepared in SIF), 0.66 mL of 0.3 M $CaCl_2$, and 1.29 mL of deionized water were included into the flask. Subsequently, the pH was adjusted to pH 7.0 using 50 μL of 1 M HCl solution. Therefore, the final ratio of gastric chyme to SIF was 50:50 (v/v). Lastly, the samples were incubated at 37 °C and 100 rpm for 2 h in the shaking water bath. The pH of the mixture was monitored and re-adjusted to pH 7.0 using 1 M HCl solution throughout the intestinal digestion.

2.9.4. Bioaccessible fraction

The bioaccessible fraction was separated according to the method of Ubeyitogullari et al. (2018). The digested samples were centrifuged at 4000 rpm and 4 °C for 90 min using a refrigerated centrifuge (Sorvall™ Legend™ X1R, Thermo Scientific, Osterode am Harz, Germany) to obtain the bioaccessible fraction (supernatant). The bioaccessibility of the lycopene in the extracts was calculated using the following formula:

$$Bioaccessibility\ (\%) = \frac{Weight\ of\ the\ lycopene\ in\ the\ bioaccessible\ fraction}{Weight\ of\ the\ total\ lycopene\ included} * 100 \quad (1)$$

2.10. Carotenoids analysis

Lycopene content and composition of the oleoresins and bioaccessible fractions were determined by an HPLC (Agilent 1100 Series, Agilent Technologies, Germany) equipped with a variable wavelength detector (VWD) following the method of Urbonaviciene and Viskelis (2017) with minor modifications. Briefly, 0.1 g of extract or 4 mL of bioaccessible fraction was mixed with 10 mL of hexane/ethanol/acetone (2:1:1, v/v/v) solution. Then, 10 mL of deionized water was added onto the mixture and vortexed for 5 min. After the phase separation, an aliquot of the hexane layer (20 μL) was injected onto a reversed-phase C30 column (250 × 4.6 mm, 5 μm; YMC, PA, USA) that was maintained at 25 °C. The elution was monitored at 473 nm. The mobile phase consisted of methanol (solvent A) and methyl *tert*-butyl ether (solvent B). The gradient was 40% B for 5 min, then changed to 83% B in 50 min. Finally, gradient altered back to 40% B in 5 min. The flow rate of the mobile phase was kept constant at 0.65 mL/min.

An external calibration curve was prepared using the authentic (all-E)-lycopene standard (1–30 μg/mL in hexane) for quantification of total lycopene, (all-E)- and (Z)-lycopene in the samples. The coefficient of determination (R^2) of the calibration curve was 0.995. Since commercial standard was available for only (all-E)-lycopene, the peaks of (Z)-lycopene isomers (5-Z-lycopene, 7-Z-lycopene, 9-Z-lycopene, 13-Z-lycopene, and 15-Z-lycopene) and all-E-lycopene were identified based on the authentic standard and literature (Lee and Chen, 2001; Urbonaviciene and Viskelis, 2017; Honda et al., 2017; Watanabe et al., 2018) (Fig. 1). In literature, the Z isomers were mainly identified based on the retention times, visible spectral data, and relative intensities of the Z peaks. Quantification was done using an external calibration curve prepared by plotting standard concentrations of the standards (1.9–31 μg/mL) versus their peak areas. Total lycopene was determined by summing up the contents of all (Z) and (E) isomers, whereas (Z) and (E) isomers were determined by summing up the contents of each isomer group.

Likewise, β-carotene content was quantified using the same HPLC method and an external calibration curve (1–20 μg/mL) that was prepared using the authentic β-carotene standard. The coefficient of determination (R^2) of the β-carotene calibration curve was 0.999.

2.11. Microstructural analysis

The microstructures of the extracts, and bioaccessible fractions obtained after simulated digestion were characterized using a confocal scanning fluorescence microscope (Nikon A1R–Ti2, Nikon, Tokyo, Japan). Carotenoids naturally exhibit fluorescence, therefore no staining procedure was applied before confocal analysis. A small aliquot (5 μL) of each sample was placed on the microscope slide and covered with a cover slip. The fluorescence signal was excited using laser source with wavelength of 488 nm and the images were recorded at wavelength of 525 nm. All images were captured using a 60× oil immersion objective

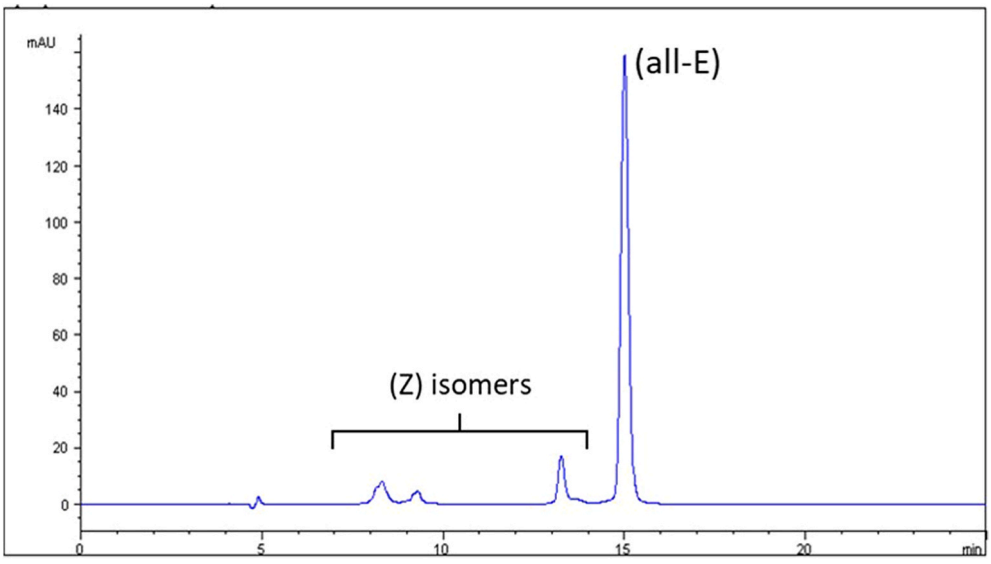

Fig. 1. HPLC chromatogram of the lycopene standard.

lens. The hexane- and SC-CO_2-extracted oleoresins and their oil fractions were analyzed at a lower laser power compared to their bioaccessible fractions due to the high concentration of lycopene in the extracts. (all-E)-lycopene standard and tomato seed oil were analyzed under confocal microscopy at the same settings as positive and negative controls, respectively.

2.12. Statistical analysis

Statistical evaluation of the data was carried out using Minitab® 16.1.1 software (Minitab Inc., PA, USA). All the experiments were performed in triplicate and the results were expressed as the mean ± standard deviation. The multiple comparison of the means was conducted by Tukey's test at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Oleoresin yield

The oleoresin yield of SC-CO_2 extraction at 30 MPa and 80 °C in 4 h with a CO_2 flow rate of 1 L/min was 11.5 ± 0.9% (w/w). The oleoresin yield was vastly dependent on the tomato peel:seed ratio because tomato seed contained high amount of oil (25.9 ± 0.2%, w/w). Slightly different tomato seed oil yields were obtained in the literature depending on the extraction method; 20% with accelerated solvent extraction using hexane (Eller et al., 2010) and 34% with Soxhlet (hexane) extraction for 15 h (Machmudah et al., 2012). Even though almost all of the lycopene are available in the peel, tomato peel and seed blend (70:30, w/w) was used to improve the extraction of lycopene from the tomato peel, because lycopene is lipophilic and tomato seed oil acts as a co-solvent for lycopene during SC-CO_2 extraction, which in turn improves recovery of lycopene (Vallecilla-Yepez and Ciftci, 2018). Previously, Machmudah et al. (2012) also observed an increase in the recovery of lycopene (from 18 to 56%) in the presence of tomato seed oil during SC-CO_2 extraction.

Hexane extraction was carried out as the conventional extraction technique. The oleoresin yield by hexane extraction (11.1 ± 0.3%, w/w) was comparable to that attained by SC-CO_2 extraction. However, SC-CO_2 extraction was more time-efficient than hexane extraction when the extraction times are considered (4 h versus 6 h).

3.2. Fatty acid and lipid composition

The oil fractions of the oleoresins were obtained by separating the insoluble matter from the oleoresins. The oil fractions consisted of 84 ± 2 and 96 ± 1% (w/w) of the oleoresins extracted by SC-CO_2 and hexane, respectively. After centrifugation, the oil fractions (Fig. 2 c1,d1) had a clearer appearance compared to the whole oleoresins (Fig. 2 a1,b1). Hexane-extracted oleoresin (Fig. 2a1) had a dark red color, whereas SC-CO_2-extracted oleoresin (Fig. 2b1) had an orange red color. This color difference between the hexane-and SC-CO_2-extracted oleoresins was due to their lycopene content (Table 1).

Fatty acid and lipid composition of the hexane- and SC-CO_2-extracted oleoresins and their oil fractions are shown in Table 2. The extraction method or the separation of the insoluble fraction did not have a significant effect on the fatty acid composition ($p > 0.05$). The majority of

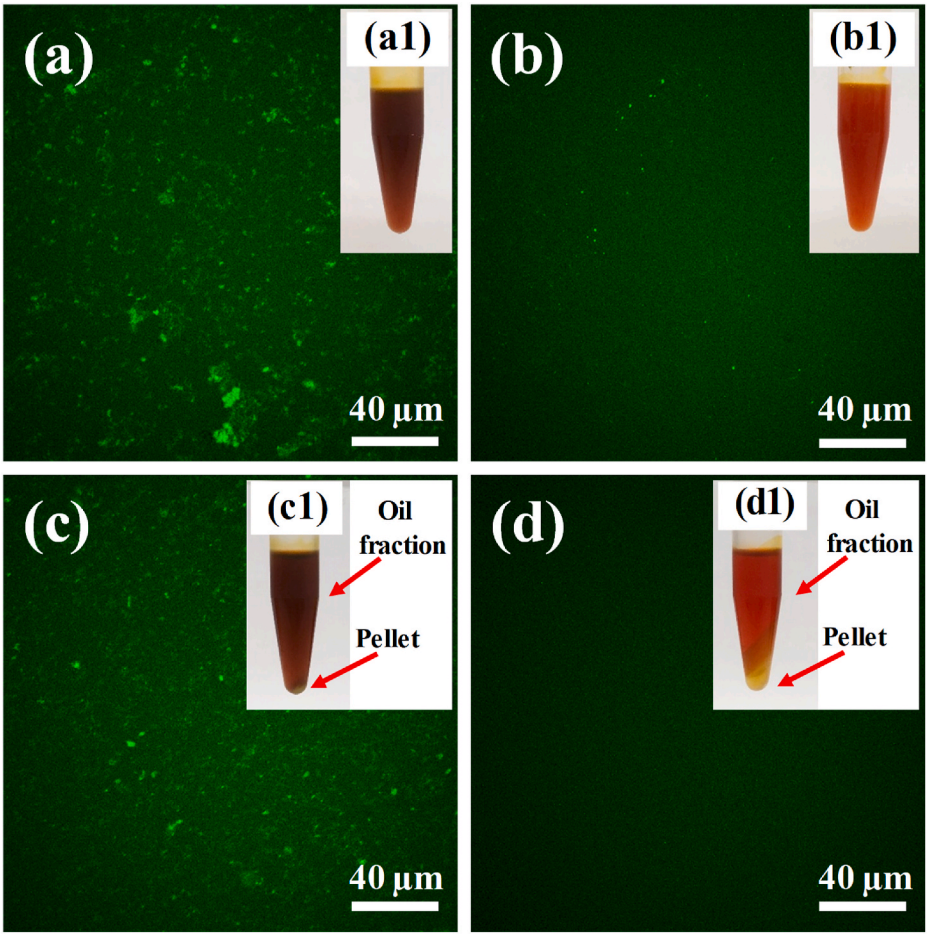

Fig. 2. Confocal images of the (a) hexane-extracted oleoresin, (b) SC-CO_2-extracted oleoresin, (c) hexane-extracted oil fraction, and (d) SC-CO_2-extracted oil fraction. Insets show pictures of the oleoresins extracted by (a and c) hexane, and (b and d) SC-CO_2 before and after centrifugation, respectively.

Table 1
Lycopene, β-Carotene, phytosterol and tocopherol content of the hexane and SC-CO_2-extracted oleoresins and their oil fractions.

Compound (mg/100 g sample)	Hexane-extracted oleoresin	Hexane-extracted oil fraction	SC-CO_2-extracted oleoresin	SC-CO_2-extracted oil fraction
Total lycopene	135±9[a]	137±8[a]	99±7[b]	106±3[b]
β-Carotene	19±1[b]	19±0[b]	22±0[a]	20±0[b]
Phytosterols	767 ± 12[c]	755 ± 15[c]	918±9[a]	846±4[b]
Total tocopherols	188±1[b]	178±2[b]	182±7[b]	213±4[a]
α-tocopherol	71±1[b]	68±1[b]	68±2[b]	86±2[a]
β-tocopherol	5±0[a]	4±0[a]	4±0[a]	4±0[a]
γ-tocopherol	111±0[b]	105±1[b]	109±4[b]	122±2[a]
δ-tocopherol	2±0[a]	2±0[a]	2±0[a]	2±0[a]

*Data are expressed as mean ± standard deviation. Means in the same row with different superscript letters are significantly different ($p < 0.05$).

Table 2
Fatty acid and lipid (FFA, MAG, DAG, and TAG) composition of the hexane and SC-CO_2-extracted oleoresins and their oil fractions.

Fatty acids	Hexane-extracted oleoresin	Hexane-extracted oil fraction	SC-CO_2-extracted oleoresin	SC-CO_2-extracted oil fraction
C14:0	0.2 ± 0.0[a]	0.15 ± 0.0[a]	0.2 ± 0.0[a]	0.2 ± 0.0[a]
C16:0	14.1 ± 0.1[b]	14.1 ± 0.1[b]	14.5 ± 0.0[a]	14.4 ± 0.0[a]
C16:1	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.4 ± 0.0[a]
C18:0	5.6 ± 0.0[a]	5.5 ± 0.0[a]	5.3 ± 0.1[b]	5.3 ± 0.0[b]
C18:1	22.4 ± 0.0[a]	22.4 ± 0.0[a]	22.2 ± 0.1[b]	22.1 ± 0.0[b]
C18:2	54.8 ± 0.2[a]	55.1 ± 0.2[a]	55.1 ± 0.2[a]	55.2 ± 0.1[a]
C18:3 n-6	0.4 ± 0.0[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.2 ± 0.1[a]
C18:3 n-3	2.2 ± 0.0[a]	2.3 ± 0.0[a]	2.2 ± 0.1[a]	2.3 ± 0.0[a]
Groups				
SFA	19.9 ± 0.1[a]	19.8 ± 0.1[a]	19.9 ± 0.1[a]	19.9 ± 0.1[a]
MUFA	22.8 ± 0.0[a]	22.7 ± 0.0[a]	22.6 ± 0.1[b]	22.5 ± 0.0[b]
PUFA	57.3 ± 0.1[b]	57.5 ± 0.1[a]	57.5 ± 0.1[a]	57.6 ± 0.1[a]
Lipids				
FFA	1.0 ± 0.2[a]	1.1 ± 0.2[a]	1.3 ± 0.1[a]	1.2 ± 0.1[a]
MAG	0.7 ± 0.0[b]	0.7 ± .0.1[b]	1.3 ± 0.1[a]	0.7 ± 0.1[b]
DAG	3.9 ± 0.1[b]	3.9 ± 0.2[b]	4.7 ± 0.1[a]	3.9 ± 0.0[b]
TAG	94.4 ± 0.0[a]	94.4 ± 0.5[a]	92.7 ± 0.1[b]	94.2 ± 0.1[a]

*Data are expressed as mean ± standard deviation. Means in the same row with different superscript letters are significantly different ($p < 0.05$).

the fatty acids were polyunsaturated fatty acids (PUFA) (57%), followed by monounsaturated fatty acids (MUFA) (23%) and saturated fatty acids (SFA) (20%). In terms of the individual fatty acids, linoleic acid (55%) was the most predominant fatty acid in all the samples. Linoleic acid was followed by oleic (22%) and palmitic (14%) acids. Other minor fatty acids present in the samples were stearic (5%), α-linolenic (2%), palmitoleic (0.4%), γ-linolenic (0.3%), and myristic (0.2%) acids. The fatty acid composition of the tomato oleoresins is in a good agreement with the previously reported studies (da Silva and Jorge, 2017; Yang et al., 2018; Zuorro et al., 2014).

Furthermore, hexane-extracted oleoresin and its oil fraction exhibited similar lipid composition with the SC-CO_2-extracted oil fraction (Table 2). The majority of the samples were composed of TAG (94.4%) and followed by DAG (3.9%), FFA (1.1%) and MAG (0.7%). However, the SC-CO_2-extracted oleoresin had lower amount of TAG (92.7%) than the other samples. In the same manner, SC-CO_2-extracted oleoresin showed slightly higher FFA (1.3%), MAG (1.3%) and DAG (4.7%) contents than the SC-CO_2-extracted oil fraction, suggesting the precipitation of some FFA, MAG, and DAG during centrifugation.

The FFA contents of hexane-and SC-CO_2-extracted oleoresins and oil fractions are within the range of the previously reported data, where the FFA content of the tomato seed oil extracted by petroleum ether was 1.0% (Lazos et al., 1998), while cold pressed tomato seed oil contained higher FFA content of 1.5% (Zuorro et al., 2014). Higher TAG contents and low FFA values show that the oil fractions of the tomato by-product oleoresins are suitable for food applications.

3.3. Microstructure

The microstructures of the oleoresins and their oil fractions were studied using a confocal scanning fluorescence microscope (Fig. 2). Since carotenoids naturally fluoresce, it was possible to observe the distribution of carotenoids (mainly lycopene) in the samples as green color (Fig. 2). Hexane-extracted oleoresin (Fig. 2a) exhibited noticeably different microstructure than the SC-CO_2-extracted oleoresin (Fig. 2b) where the appearance of hexane-extracted oleoresin was brighter and had much higher number of green spots compared to that of SC-CO_2-extracted oleoresin. The bright green spots in Fig. 2 represent the lycopene concentrated areas (lycopene aggregates). Hence, the oleoresin extracted by SC-CO_2 can be speculated to have a better dispersion/dissolution of lycopene in the tomato seed oil (Fig. 2b). To the best of our knowledge, this is the first reported data on the microstructure of the oils extracted by SC-CO_2 and hexane. The extraction method may have played a significant role in forming lycopene-dense spots. During hexane extraction, hexane was removed from the lycopene-oil-hexane mixture by a rotary evaporator and consequently lycopene was concentrated in tomato seed oil, which may cause the formation of those lycopene-dense spots upon recrystallization. However, lycopene was continuously accumulated in tomato oleoresin from the tomato seed oil-lycopene–SC–CO_2 solvato complex during SC-CO_2 extraction. Moreover, the solubility of lycopene in SC-CO_2 at 30 MPa and 80 °C is only about 0.01–0.02 g/L, which was calculated using the density data by NIST Chemistry WebBook (Lemmon et al., 2018) and the Chrastil models proposed by Machmudah et al. (2012) and Topal et al. (2006). Tomato seed oil significantly improved the lycopene content in the solvato complex during extraction as the solubility of lycopene is much higher in vegetable oil (0.2 g/L at room temperature) than SC-CO_2 (Machmudah et al., 2012), which in turn improved the extraction of lycopene as discussed above.

The number of bright green spots in the samples decreased after centrifugation (Fig. 2c and d) due to the precipitation of large lycopene crystals (mostly (all-E)-lycopene). Forming homogenous lycopene distribution (smaller particle size) with SC-CO_2 extraction (Fig. 2b,d) may improve the solubilization of lycopene in the mixed micelles and therefore enhance its bioaccessibility.

3.4. β-Carotene, phytosterol and tocol content

β-Carotene contents of the oleoresins and their oil fractions are presented in Table 1. SC-CO_2 extraction resulted in significantly higher β-carotene content (22 mg/100 g) in the oleoresin compared to hexane extraction (19 mg/100 g) ($p < 0.05$). Likewise, SC-CO_2 was more effective than hexane in extracting β-carotene in a previously reported study (Longo et al., 2012). However, some studies reported similar β-carotene content in the oleoresins extracted from tomato seed and peel blend by SC-CO_2 at 27.6 MPa and 80 °C (35 mg/100 g), and hexane (38 mg/100 g) (Cadoni, Rita De Giorgi, Medda, & Poma, 1999). Furthermore, the concentration of β-carotene was slightly decreased after separating the insoluble fraction, indicating the precipitation of some β-carotene during centrifugation.

The phytosterol content of the samples followed a similar trend to that of β-carotene (Table 1), where SC-CO_2 was significantly more effective than hexane in extracting the phytosterols ($p < 0.05$). The phytosterol contents of the SC-CO_2- and hexane-extracted oleoresins were 918 and 767 mg/100 g, respectively. Vági et al. (2007) also attained higher phytosterol content in the extracts from industrial tomato byproducts (tomato skin and seed) with SC-CO_2 at 38 MPa and 80 °C compared to hexane extraction. In another study, SC-CO_2 extraction of phytosterols from tomato seed yielded 1230 mg

phytosterols/100 g oil, whereas accelerated solvent extraction using hexane resulted in significantly lower phytosterol content as 1081 mg/100 g (Eller et al., 2010). Those results suggest that most of the phytosterols are sourced from tomato seeds rather than the tomato peel since higher phytosterol contents were obtained with only tomato seeds (100%).

In addition to the β-carotene and phytosterol contents, tocopherol contents (α-, β-, γ-, and δ-tocopherol) of the samples are also reported in Table 1. No tocotrienols were detected in the samples. The extraction method did not have a significant effect on the total tocopherol content of the oleoresins (Table 1) ($p > 0.05$). Nevertheless, after the separation of the insoluble fraction from the SC-CO_2-extracted oleoresin, the total tocopherol content significantly increased from 182 to 213 mg/100 g sample since tocopherols were concentrated in the oil fraction due to their high solubility in oil (Grilo et al., 2014). Previously, Belayneh et al. (2015) reported that the extraction method (hexane versus SC-CO_2) did not significantly affect the tocopherol content of the camelina seed oil. γ-Tocopherol was the leading tocopherol (ca. 60%) in the extracts, which was followed by α-tocopherol (37%), β-tocopherol (2%) and δ-tocopherol (1%). Vági et al. (2007) reported that tocopherols were mainly sourced from the tomato seed, and tocopherol content of the extracts greatly differed from 34 to 596 mg/100 g extract according to the source of the tomato pomace. Moreover, tomato seed oil extracted by accelerated solvent extraction using hexane or SC-CO_2 had similar total tocopherol contents of 108 and 111 mg/100 g extract, respectively (Eller et al., 2010). However, in both studies, γ-tocopherol was the dominant tocopherol, which is in accordance with the tocopherol composition stated in this study (Eller et al., 2010; Vági et al., 2007).

3.5. Lycopene content

SC-CO_2-extracted oleoresin had a total lycopene (Z- and all-E-lycopene) content of 99 mg/100 g oleoresin, whereas the oil fraction contained slightly higher total lycopenes (106 mg/100 g) owing to the separation of insoluble fraction that had lower lycopene concentration (Table 1). On the other hand, total lycopene content of hexane-extracted oleoresin was 135 mg/100 g oleoresin, which was not significantly affected by the centrifugation ($p > 0.05$) (Table 1).

Tomato seed oil extracted by hexane contained only 1 mg total lycopene/100 g, indicating tomato peel was the major source of lycopene. Therefore, the total lycopene content is significantly affected by the ratio of tomato seed:peel, which makes it difficult to compare the lycopene content in the oleoresins with other studies. For example, the total lycopene content of tomato seed oil extracted by accelerated solvent extraction using hexane was 3 mg/100 g (Eller et al., 2010). Topal et al. (2006) obtained a total lycopene content of 80 mg/100 g oleoresin by SC-CO_2 extraction at 80 °C, 30 MPa for 5.5 h from tomato peel. Although higher total lycopene concentration (1%, w/w) was achieved by Kehili et al. (2017) from tomato peel by SC-CO_2 extraction, the oleoresin yield was lower (~6%, w/w). Recently, Vallecilla-Yepez and Ciftci (2018) reported similar total lycopene contents as 90 and 180 mg/100 g in tomato oleoresins extracted from tomato peel and seed blend (70:30, w/w) by SC-CO_2 and hexane, respectively.

The lycopene isomers in the oleoresins and oil fractions were also identified and quantified in this study (Fig. 3). The major lycopene isomers in the samples were 15-Z-, 13-Z-, 9-Z-, 7-Z-, 5-Z- and all-E-lycopene. Among the Z-lycopene isomers, the 5-Z-lycopene isomer was the predominant form in the oleoresins. (Z)-lycopene content (total of 15-Z-, 13-Z-, 9-Z-, 7-Z-, and 5-Z-lycopene) of hexane-extracted oleoresin was 61 mg/100 g. Although total lycopene content of the SC-CO_2-extracted oleoresin and its oil fraction was lower than that of hexane-extracted ones, SC-CO_2-extracted oleoresin and its oil fraction showed higher (Z)-lycopene contents of 69 and 84 mg/100 g, respectively (Fig. 3). SC-CO_2 extraction (69%) gave significantly higher (Z)-lycopene ratio in the oleoresins compared to hexane extraction (45%). The (Z)-lycopene content was increased in both SC-CO_2 (80%) and hexane

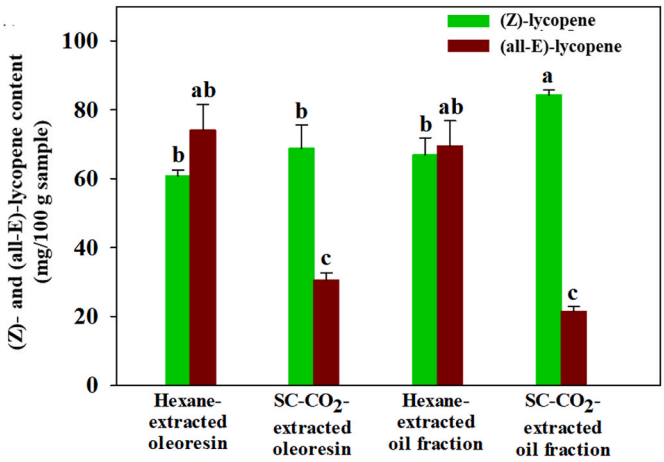

Fig. 3. (Z)- and (all-E)-lycopene content of the hexane and SC-CO_2-extracted oleoresins and their oil fractions.

(49%) extracted oil fractions after centrifugation which was mainly due to eliminating the large (all-E)-lycopene crystals in the oleoresins. Similar findings were also attained by Longo et al. (2012), where lycopene was extracted by SC-CO_2 at 60–70 °C and 40–45 MPa for 7 h from tomato and tomato/hazelnut matrixes. The (Z)-lycopene content increased from ~61 to 76% after separation of the insoluble fraction (Longo et al., 2012). In another study, lycopene was extracted from tomato byproducts (peels, vascular tissues, seeds and small amounts of pulp) by SC-CO_2 at 52 °C and 55 MPa for 3 h, however the highest (Z)-lycopene ratio was 62% (Urbonaviciene and Viskelis, 2017).

Although SC-CO_2 extraction was able to generate oleoresins with high (Z)-lycopene content (Longo et al., 2012; Urbonaviciene and Viskelis, 2017; Vallecilla-Yepez and Ciftci, 2018), the mechanism behind is not well understood. Some speculate that SC-CO_2 favorably extracted the (Z)-lycopene isomers compared to their (all-E) form (Leone et al., 2010; Longo et al., 2012). Also, the isomerization of the (all-E)- to (Z)-lycopene in SC-CO_2 environment is proposed for the increase in the (Z)-lycopene content of the SC-CO_2-extracted oleoresins (Vallecilla-Yepez and Ciftci, 2018), where the isomerization was associated with the supercritical phase making the rotation of the conjugated double bonds on the (all-E)-lycopene easier (Spanos et al., 1993). Nevertheless, some researchers argue in favor of the increased solubility explanation rather than the all-E to Z isomerization in the SC-CO_2 environment (Leone et al., 2010).

Furthermore, (all-E)-lycopene has higher crystallinity compared to (Z)-lycopene isomers, which therefore makes (all-E)-lycopene less soluble than (Z)-lycopene isomers (Murakami et al., 2017). This is one possible explanation for the lycopene dense spots observed under confocal microscopy in the hexane-extracted oleoresin and oil fractions (Fig. 2a,c), where the (all-E)-lycopene content of the hexane-extracted samples (51–55%) was significantly higher than that of SC-CO_2-extracted ones (20–31%) (Fig. 3).

Even though the crystalline (all-E)-lycopene isomer is more thermodynamically stable than the Z isomers of lycopene (Unlu et al., 2007), considerable efforts have been recently dedicated to increase the (Z)-lycopene content as the amorphous form of the bioactives is more bioaccessible than their crystalline counterparts (Ubeyitogullari et al., 2018). Thermal isomerization and photoisomerization are the most common approaches to increase the (Z)-lycopene content. To illustrate, Takehara et al. (2014) investigated the thermal isomerization of lycopene in hexane and benzene at 50 °C. All-tans-lycopene (100%) was converted to (Z)-lycopene isomers (80 and 45%) in hexane and benzene, respectively. Moreover, thermal isomerization of lycopene was studied in an olive oil/tomato emulsion at 80–140 °C. The (Z)-lycopene content was increased up to 55% after keeping the emulsion at 140 °C for 60

min, nonetheless about 30% of the total lycopene was degraded at those conditions (Colle et al., 2010). The other common method used to increase the (Z)-lycopene content is photoisomerization, where visible to middle-infrared light irradiation is applied in the presence of sensitizers. For example, Honda et al. (2017) examined photoisomerization of (all-E)-lycopene dissolved in hexane, acetone, and ethyl acetate with various sensitizers (chlorophyll *a*, erythrosine, rose bengal, and methylene blue). The highest (Z)-lycopene content (80%) was achieved with 60 min treatment of erythrosine in hexane under light irradiation of 480–600 nm. Nevertheless, the use of toxic organic solvents and degradation of lycopene during the process are still major drawbacks of those techniques. On the other hand, SC-CO_2 extraction not only overcomes those problems but also generates a valuable product with increased (Z)-lycopene content from tomato processing byproducts in a simple and clean way.

3.6. Bioaccessibility of lycopene

Fig. 4 depicts the bioaccessibility of lycopene in the hexane- and SC-CO_2-extracted oleoresins and their oil fractions after a sequential oral, gastric, and intestinal digestion. The bioaccessibility of total lycopene in the oleoresins was significantly increased from 3.5 to 11.5% by using SC-CO_2 instead of hexane as a solvent for extraction. Both the homogenous distribution of lycopene in the SC-CO_2-extracted samples (Fig. 2b,d) and increased (Z)-lycopene content (Fig. 3) played a critical role in the improvement of lycopene's bioaccessibility. Furthermore, the total lycopene bioaccessibility in the hexane-extracted oleoresin (3.5%) slightly increased when the oil fraction (4.0%) underwent the simulated digestion, which can be attributed to the increase in the (Z)-lycopene content of the oil fraction. Nevertheless, separation of the insoluble fraction from the SC-CO_2-extracted oleoresin decreased the bioaccessibility of total lycopene from 11.5 to 8.1% (Fig. 4). Even though the (Z)-lycopene content was higher in the oil fraction compared to the oleoresin extracted by SC-CO_2 (Fig. 3), MAG and DAG contents of the oil fraction were significantly lower than that of the oleoresin (Table 2), which may have negatively affected the bioaccessibility of lycopene. Especially, MAG is known to contribute to the formation of mixed micelles and therefore increase the bioaccessibility of lipophilic bioactives such as β-carotene (Salvia-Trujillo et al., 2019; Verkempinck et al., 2018). Thus, the elimination of those compounds from the SC-CO_2-extracted oleoresin affected the bioaccessibility of lycopene more than the change in the (Z)-lycopene content (from 69 to 80%). Nonetheless, (Z)-lycopene is expected to exhibit higher bioavailability compared to (all-E)-lycopene due to its reduced crystallinity, lower tendency to aggregate, shorter chain length that fits into micelles, and greater solubility in the micellar phase (Boileau et al., 2002; Honest et al., 2011).

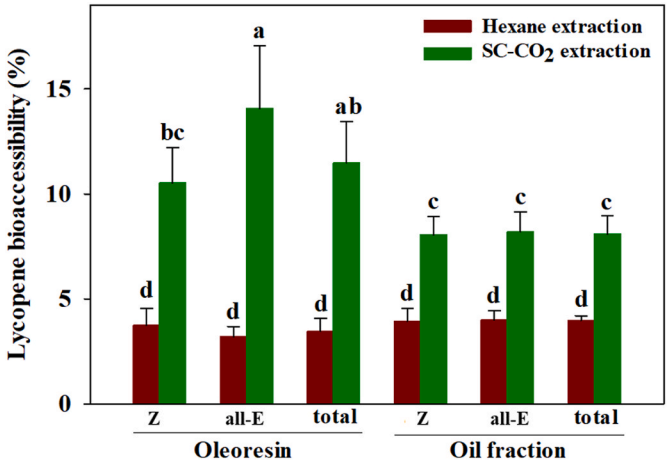

Fig. 4. Bioaccessibility of (Z)-, (all-E)- and total lycopene in the oleoresin and oil fractions obtained by hexane and SC-CO_2 extraction.

Although there was no clear difference between the bioaccessibility of (Z)- and (all-E)-lycopene isomers, the samples with higher (Z)-lycopene content resulted in improved total lycopene bioaccessibility (Fig. 4). Moreover, the (Z)-lycopene contents in the bioaccessible fractions of SC-CO_2-extracted oleoresin and oil fraction were still forming the majority of the total lycopene as 67 and 80%, respectively. On the other hand, the (Z)-lycopene content of the bioaccessible fractions of the hexane-extracted samples (~51%) was slightly higher compared to the (Z)-lycopene content of the hexane-extracted oleoresin (45%) and oil fraction (49%) (Fig. 4). Similar conclusions were also drawn by Colle et al. (2010), where the tomato pulp with higher (Z)-lycopene content resulted in higher bioaccessibility. Nonetheless, there was not a noticeable difference between the bioaccessibility of (Z)- and (all-E)--lycopene isomers (Colle et al., 2010). Furthermore, the bioaccessibility of (Z)-, (all-E)- and total-lycopene obtained in this study corroborates with the *in vivo* study conducted by Unlu et al. (2007), where two tomato sauces (sauce A: 95% (all-E)-lycopene and sauce B: 55% (all-E)-lycopene) were served to 12 healthy human subjects in a randomized cross-over design. The fractional absorptions of (Z)-, (all-E), and total-lycopene from the (all-E)-lycopene-rich sauce (A) were 1.29, 1.90, and 3.19%, respectively. The fractional absorption values increased to 2.57, 2.40, and 4.97% for (Z)-, (all-E), and total-lycopene, respectively when (Z)-lycopene-rich sauce (B) was consumed (Unlu et al., 2007). Even though the fractional absorption of total lycopene significantly increased from 3.19 to 4.97% by (Z)-lycopene-rich sauce, the fractional absorption of (Z)-lycopene (2.57%) was not noticeably different than that of (all-E)-lycopene (2.40%). However, *in vivo* studies have clearly demonstrated that the (Z)-lycopene content of the serum and tissues is much greater than (Z)-lycopene content of the foods consumed (Honest et al., 2011). For instance, mesenteric lymph secretions (77%) and serum (52%) of the ferrets fed with dose containing 9% (Z)-lycopene contained significantly higher (Z)-lycopene content compared to the stomach (6%) and small intestinal (18%) contents (Boileau et al., 2002). It has been suggested that the isomerization from (all-E)- to (Z)-lycopene might have taken place continuously in the body (Unlu et al., 2007).

Aggregated lycopene particles were observed in the bioaccessible fractions of hexane-extracted oleoresin (Fig. 5a) and oil fraction (Fig. 5c) under confocal microscopy. However, lycopene was homogenously dispersed in the bioaccessible fractions of SC-CO_2-extracted samples (Fig. 5b,d). The distribution of lycopene in the bioaccessible fractions (Fig. 5) agreed with that in the oleoresins and oil fractions (Fig. 2). Thus, it can be inferred that lycopene aggregates were kept intact during digestion, which negatively affected the solubilization of lycopene in the micellar phase and in turn resulted in lower bioaccessibility.

To the best of our knowledge, there is no study that investigates the bioaccessibility of lycopene in oleoresins extracted by hexane or SC-CO_2. However, many studies have focused on the bioaccessibility improvement of lycopene using different methods such as emulsions and thermal treatment. For example, Salvia-Trujillo and McClements (2016) intended to improve the bioaccessibility of lycopene in tomato juice by excipient emulsions. However, the bioaccessibility of lycopene was increased only from 7.5% (emulsion-free samples) to 12.5% (excipient emulsions with small droplets) (Salvia-Trujillo and McClements, 2016). In another study, the effect of heat treatment on isomerization and bioaccessibility of lycopene in tomato pulp was investigated at temperatures between 60 and 140 °C for a treatment time of 30 min (Colle et al., 2010). Only high temperature treatments (130 and 140 °C) significantly increased the bioaccessibility of lycopene by ~1.5 and 2-fold, respectively. The (Z)-lycopene content in the tomato pulp was increased from 11% to 12 and 16% by thermal treatments at 130 and 140 °C, respectively. However, about 26% of the total lycopene was degraded upon treatments at those temperatures (Colle et al., 2010).

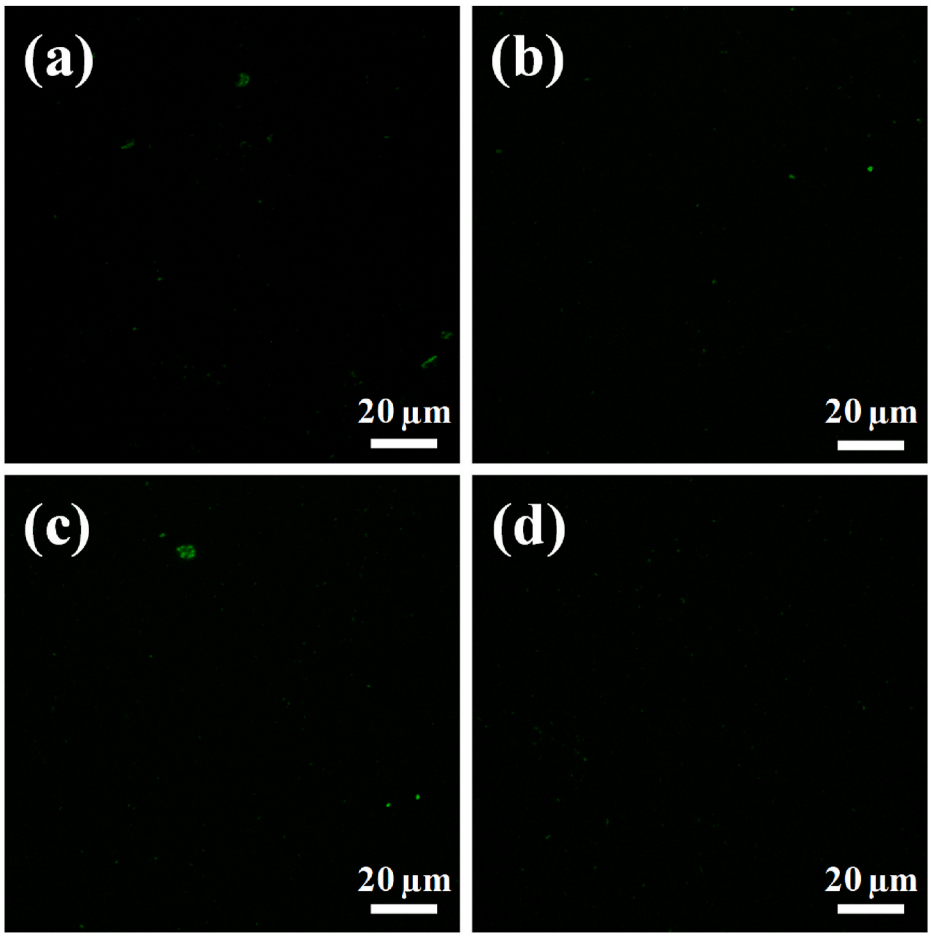

Fig. 5. Confocal images of the bioaccessible fractions of the (a) hexane-extracted oleoresin, (b) SC-CO_2-extracted oleoresin, (c) hexane-extracted oil fraction, and (d) SC-CO_2-extracted oil fraction.

4. Conclusions

This study increased the (Z)-lycopene content of the oleoresins obtained from tomato processing byproducts by simply using SC-CO_2 extraction. Similar oleoresin yields were attained by SC-CO_2 (11.5%) and hexane (11.1%) extractions. Although hexane extraction (135 mg/100 g oleoresin) resulted in higher total lycopene content than SC-CO_2 extraction (99 mg/100 g oleoresin), the hexane-extracted oleoresins showed lycopene aggregates which were not observed in the SC-CO_2-extracted samples. The (Z)-lycopene content of the oleoresin extracted by SC-CO_2 (69%) was significantly higher than that by hexane (45%). Furthermore, separation of the insoluble fraction from the oleoresins increased the (Z)-lycopene contents of the SC-CO_2 (80%) and hexane (49%) extracted oil fractions. The bioaccessibility of lycopene in the oleoresins was significantly enhanced by SC-CO_2 extraction (11.5%) compared to hexane extraction (3.5%) owing to the higher (Z)-lycopene content of the SC-CO_2-extracted oleoresin. Likewise, the lycopene in the SC-CO_2-extracted oil fraction (8.1%) was more bioaccessible than that in the hexane-extracted sample (4.0%). The lycopene in the bioaccessible fractions of the hexane-extracted samples was still intact, whereas a uniform lycopene distribution was observed in the bioaccessible fractions of the SC-CO_2-extracted samples. This study produces high (Z)-lycopene content oleoresins using SC-CO_2, which (i) eliminates the use of toxic chemicals, (ii) protects the lycopene during processing, and (iii) utilizes only food-grade materials which allows the incorporation of oleoresins into foods to prepare functional foods. The enhanced bioaccessibility improves the efficacy of lycopene and maximizes its health benefits upon consumption. However, the mechanism of increased (Z)-lycopene contents in the SC-CO_2-extracted oleoresins is not well understood; therefore, there is a need for more research on the fundamentals associated with increased (Z)-lycopene contents by SC-CO_2 extraction. Moreover, more research is needed in *in vivo* studies to demonstrate the superior bioavailability of lycopene in the SC-CO_2-extracted tomato oleoresin.

With this study, it has been shown that SC-CO_2 is not only a green extraction method, but also has the potential to improve health and wellness promoting properties of lycopene via improving its bioavailability. This approach is a clean and simple alternative to the conventional (Z) isomerization approaches. With this approach, a lycopene extract rich in (Z) isomers can be obtained by a single extraction step without any further chemical treatment.

CRediT authorship contribution statement

Ali Ubeyitogullari: Investigation, Methodology, Data curation, Writing – original draft. **Ozan N. Ciftci:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Belayneh, H.D., Wehling, R.L., Cahoon, E., Ciftci, O.N., 2015. Extraction of omega-3-rich oil from Camelina sativa seed using supercritical carbon dioxide. J. Supercrit. Fluids 104, 153–159. https://doi.org/10.1016/j.supflu.2015.06.002.

Boileau, T.W.M., Boileau, A.C., Erdman, J.W., 2002. Bioavailability of all-trans and cis–isomers of lycopene. Exp. Biol. Med. 227 (10), 914–919. https://doi.org/10.1177/153537020222701012.

Cadoni, E., Rita De Giorgi, M., Medda, E., Poma, G., 1999. Supercritical CO2 extraction of lycopene and β-carotene from ripe tomatoes. Dyes Pigments 44 (1), 27–32. https://doi.org/10.1016/S0143-7208(99)00065-0.

Ciftci, O.N., Temelli, F., 2013. Enzymatic conversion of corn oil into biodiesel in a batch supercritical carbon dioxide reactor and kinetic modeling. J. Supercrit. Fluids 75, 172–180. https://doi.org/10.1016/j.supflu.2012.12.029.

Ciftci, O.N., Przybylski, R., Rudzinska, M., Acharya, S., 2011. Characterization of fenugreek (Trigonella foenum-graecum) seed lipids. J. Am. Oil Chem. Soc. 88 (10), 1603–1610. https://doi.org/10.1007/s11746-011-1823-y.

Colle, I.J.P., Lemmens, L., Tolesa, G.N., Van Buggenhout, S., De Vleeschouwer, K., Van Loey, A.M., Hendrickx, M.E., 2010a. Lycopene degradation and isomerization kinetics during thermal processing of an olive oil/tomato emulsion. J. Agric. Food Chem. 58 (24), 12784–12789. https://doi.org/10.1021/jf102934u.

Colle, I.J.P., Lemmens, L., Van Buggenhout, S., Van Loey, A., Hendrickx, M., 2010b. Effect of thermal processing on the degradation, isomerization, and bioaccessibility of lycopene in tomato pulp. J. Food Sci. 75 (9), C753–C759. https://doi.org/10.1111/j.1750-3841.2010.01862.x.

Cooperstone, J.L., Ralston, R.A., Riedl, K.M., Haufe, T.C., Schweiggert, R.M., King, S.A., Schwartz, S.J., 2015. Enhanced bioavailability of lycopene when consumed as cis-isomers from tangerine compared to red tomato juice, a randomized, cross-over clinical trial. Mol. Nutr. Food Res. 59 (4), 658–669. https://doi.org/10.1002/mnfr.201400658.

Costa-Rodrigues, J., Pinho, O., Monteiro, P.R.R., 2018. Can lycopene be considered an effective protection against cardiovascular disease? Food Chem. 245, 1148–1153. https://doi.org/10.1016/j.foodchem.2017.11.055.

da Silva, A.C., Jorge, N., 2017. Bioactive compounds of oils extracted from fruits seeds obtained from agroindustrial waste. Eur. J. Lipid Sci. Technol. 119 (4), 1600024 https://doi.org/10.1002/ejlt.201600024.

Del Valle, M., Cámara, M., Torija, M.-E., 2006. Chemical characterization of tomato pomace. J. Sci. Food Agric. 86 (8), 1232–1236. https://doi.org/10.1002/jsfa.2474.

Eller, F.J., Moser, J.K., Kenar, J.A., Taylor, S.L., 2010. Extraction and analysis of tomato seed oil. J. Am. Oil Chem. Soc. 87 (7), 755–762. https://doi.org/10.1007/s11746-010-1563-4.

Giovannucci, E., 1999. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J. Natl. Cancer Inst.: J. Natl. Cancer Inst. 91 (4), 317–331. https://doi.org/10.1093/jnci/91.4.317.

Grilo, E.C., Costa, P.N., Gurgel, C.S.S., Beserra, A.F.d.L., Almeida, F.N.d.S., Dimenstein, R., 2014. Alpha-tocopherol and gamma-tocopherol concentration in vegetable oils. Food Sci. Technol. 34 (2), 379–385. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-20612014000200024&nrm=iso.

Honda, M., Takahashi, N., Kuwa, T., Takehara, M., Inoue, Y., Kumagai, T., 2015a. Spectral characterisation of Z-isomers of lycopene formed during heat treatment and solvent effects on the E/Z isomerisation process. Food Chem. 17, 323–329. https://doi.org/10.1016/j.foodchem.2014.09.004.

Honda, M., Kawana, T., Takehara, M., Inoue, Y., 2015b. Enhanced E/Z isomerization of (all-E)-lycopene by employing iron(III) chloride as a catalyst. J. Food Sci. 80, C1453–C1459. https://doi.org/10.1111/1750-3841.12916.

Honda, M., Watanabe, Y., Murakami, K., Takemura, R., Fukaya, T., Wahyudiono, ., Goto, M., 2017. Thermal isomerization pre-treatment to improve lycopene extraction from tomato pulp. Lebensm. Wiss. Technol. 86, 69–75. https://doi.org/10.1016/j.lwt.2017.07.046.

Honda, M., Sato, H., Takehara, M., Inoue, Y., Kitamura, C., Takemura, R., Fukaya, T., Wahyudiono Kanda, H., Goto, M., 2018. Microwave-accelerated Z-isomerization of (all-E)-lycopene in tomato oleoresin and enhancement of the conversion by vegetable oils containing disulfide compounds. Eur. J. Lipid Sci. Technol. 120, 180060 https://doi.org/10.1002/ejlt.201800060.

Honest, K.N., Zhang, H.W., Zhang, L., 2011. Lycopene: isomerization effects on bioavailability and bioactivity properties. Food Rev. Int. 27 (3), 248–258. https://doi.org/10.1080/87559129.2011.563392.

Kaur, D., Wani, A.A., Oberoi, D.P.S., Sogi, D.S., 2008. Effect of extraction conditions on lycopene extractions from tomato processing waste skin using response surface methodology. Food Chem. 108 (2), 711–718. https://doi.org/10.1016/j.foodchem.2007.11.002.

Kehili, M., Kammlott, M., Choura, S., Zammel, A., Zetzl, C., Smirnova, I., Sayadi, S., 2017. Supercritical CO2 extraction and antioxidant activity of lycopene and β-carotene-enriched oleoresin from tomato (Lycopersicum esculentum L.) peels by-product of a Tunisian industry. Food Bioprod. Process. 102, 340–349. https://doi.org/10.1016/j.fbp.2017.02.002.

Lazos, E.S., Tsaknis, J., Lalas, S., 1998. Characteristics and composition of tomato seed oil. Grasas Aceites 49 (5–6), 6. https://doi.org/10.3989/gya.1998.v49.i5-6.755.

Lee, M.T., Chen, B.H., 2001. Separation of lycopene and its cis isomers by liquid chromatography. Chromatographia 54 (9–10), 613–617. https://doi.org/10.1007/BF02492187.

Lemmon, E.W., McLinden, M.O., Friend, D.G., 2018. Thermophysical properties of fluid systems. In: Linstrom, P.J., Mallard, W.G. (Eds.), NIST Chemistry WebBook, NIST Standard Reference Database Number 69. National Institute of Standards and Technology, Gaithersburg, MD.

Leone, A., Zefferino, R., Longo, C., Leo, L., Zacheo, G., 2010. Supercritical CO2-extracted tomato oleoresins enhance gap junction intercellular communications and recover from mercury chloride inhibition in keratinocytes. J. Agric. Food Chem. 58 (8), 4769–4778. https://doi.org/10.1021/jf1001765.

Longo, C., Leo, L., Leone, A., 2012. Carotenoids, fatty acid composition and heat stability of supercritical carbon dioxide-extracted-oleoresins. Int. J. Mol. Sci. 13 (4) https://doi.org/10.3390/ijms13044233.

Machmudah, S., Zakaria Winardi, S., Sasaki, M., Goto, M., Kusumoto, N., Hayakawa, K., 2012. Lycopene extraction from tomato peel by-product containing tomato seed using supercritical carbon dioxide. J. Food Eng. 108 (2), 290–296. https://doi.org/10.1016/j.jfoodeng.2011.08.012.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Brodkorb, A., 2014. A standardised static in vitro digestion method suitable for food - an international consensus. Food Funct. 5 (6), 1113–1124. https://doi.org/10.1039/C3FO60702J.

Murakami, K., Honda, M., Takemura, R., Fukaya, T., Kubota, M., Wahyudiono, ., Goto, M., 2017. The thermal Z-isomerization-induced change in solubility and physical properties of (all-E)-lycopene. Biochem. Biophys. Res. Commun. 491 (2), 317–322. https://doi.org/10.1016/j.bbrc.2017.07.103.

Murakami, K., Honda, M., Takemura, R., Fukaya, T., Wahyudiono Kanda, H., Goto, M., 2018. Effect of thermal treatment and light irradiation on the stability of lycopene with high Z-isomers content. Food Chem. 250, 253–258. https://doi.org/10.1016/j.foodchem.2018.01.062.

Nemli, E., Ozakdogan, S., Tomas, M., McClements, D.J., Capanoglu, E., 2021. Increasing the bioaccessibility of antioxidants in tomato pomace using excipient emulsions. Food Biophys. 16, 355–364. https://doi.org/10.1007/s11483-021-09674-y.

Pacheco, C., Palla, C., Crapiste, G.H., Carrín, M.E., 2014. Simultaneous quantitation of FFA, MAG, DAG, and TAG in enzymatically modified vegetable oils and fats. Food Anal. Meth. 7 (10), 2013–2022. https://doi.org/10.1007/s12161-014-9830-x.

Poojary, M.M., Passamonti, P., 2015. Optimization of extraction of high purity all-trans-lycopene from tomato pulp waste. Food Chem. 188, 84–91. https://doi.org/10.1016/j.foodchem.2015.04.133.

Salvia-Trujillo, L., McClements, D.J., 2016. Enhancement of lycopene bioaccessibility from tomato juice using excipient emulsions: influence of lipid droplet size. Food Chem. 210, 295–304. https://doi.org/10.1016/j.foodchem.2016.04.125.

Salvia-Trujillo, L., Verkempinck, S., Rijal, S.K., Van Loey, A., Grauwet, T., Hendrickx, M., 2019. Lipid nanoparticles with fats or oils containing β-carotene: storage stability and in vitro digestibility kinetics. Food Chem. 278, 396–405. https://doi.org/10.1016/j.foodchem.2018.11.039.

Spanos, G.A., Chen, H.A.O., Schwartz, S.J., 1993. Supercritical CO2 extraction of β-Carotene from sweet potatoes. J. Food Sci. 58 (4), 817–820. https://doi.org/10.1111/j.1365-2621.1993.tb09366.x.

Story, E.N., Kopec, R.E., Schwartz, S.J., Harris, G.K., 2010. An update on the health effects of tomato lycopene. Ann. Rev. Food Sci. Technol. 1 (1), 189–210. https://doi.org/10.1146/annurev.food.102308.124120.

Szabo, K., Teleky, B.E., Floricuta, R., Simon, E., Pop, O.L., Vanda, B.-F., Kapsalis, N., Vodnar, D.C., 2021. Bioaccessibility of microencapsulated carotenoids, recovered from tomato processing industrial by-products, using in vitro digestion model. Lebensm. Wiss. Technol. 152, 112285 https://doi.org/10.1016/j.lwt.2021.112285.

Takehara, M., Nishimura, M., Kuwa, T., Inoue, Y., Kitamura, C., Kumagai, T., Honda, M., 2014. Characterization and thermal isomerization of (all-E)-lycopene. J. Agric. Food Chem. 62 (1), 264–269. https://doi.org/10.1021/jf404497k.

The World Processing Tomato Council (WPTC), 2018. World production estimate of tomatoes for processing. https://www.wptc.to/releases-wptc.php. (Accessed 20 March 2019).

Topal, U., Sasaki, M., Goto, M., Hayakawa, K., 2006. Extraction of lycopene from tomato skin with supercritical carbon dioxide: effect of operating conditions and solubility analysis. J. Agric. Food Chem. 54 (15), 5604–5610. https://doi.org/10.1021/jf0606407.

Ubeyitogullari, A., Ciftci, O.N., 2017. Generating phytosterol nanoparticles in nanoporous bioaerogels via supercritical carbon dioxide impregnation: effect of impregnation conditions. J. Food Eng. 207, 99–107. https://doi.org/10.1016/j.jfoodeng.2017.03.022.

Ubeyitogullari, A., Moreau, R., Rose, D.J., Zhang, J., Ciftci, O.N., 2018. Enhancing the bioaccessibility of phytosterols using nanoporous corn and wheat starch bioaerogels. Eur. J. Lipid Sci. Technol. https://doi.org/10.1002/ejlt.201700229, 1700229-n/a.

Unlu, N.Z., Bohn, T., Francis, D., Clinton, S.K., Schwartz, S.J., 2007a. Carotenoid absorption in humans consuming tomato sauces obtained from tangerine or high-β-Carotene varieties of tomatoes. J. Agric. Food Chem. 55 (4), 1597–1603. https://doi.org/10.1021/jf062337b.

Unlu, N.Z., Bohn, T., Francis, D.M., Nagaraja, H.N., Clinton, S.K., Schwartz, S.J., 2007b. Lycopene from heat-induced cis-isomer-rich tomato sauce is more bioavailable than from all-trans-rich tomato sauce in human subjects. Br. J. Nutr. 98 (1), 140–146. https://doi.org/10.1017/S0007114507685201.

Urbonaviciene, D., Viskelis, P., 2017. The cis-lycopene isomers composition in supercritical CO2 extracted tomato by-products. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 85, 517–523. https://doi.org/10.1016/j.lwt.2017.03.034.

Vági, E., Simándi, B., Vásárhelyiné, K.P., Daood, H., Kéry, Á., Doleschall, F., Nagy, B., 2007. Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato by-products. J. Supercrit. Fluids 40 (2), 218–226. https://doi.org/10.1016/j.supflu.2006.05.009.

Vallecilla-Yepez, L., Ciftci, O.N., 2018. Increasing cis-lycopene content of the oleoresin from tomato processing byproducts using supercritical carbon dioxide. Lebensm. Wiss. Technol. 95, 354–360. https://doi.org/10.1016/j.lwt.2018.04.065.

Verkempinck, S.H.E., Salvia-Trujillo, L., Moens, L.G., Charleer, L., Van Loey, A.M., Hendrickx, M.E., Grauwet, T., 2018. Emulsion stability during gastrointestinal conditions effects lipid digestion kinetics. Food Chem. 246, 179–191. https://doi.org/10.1016/j.foodchem.2017.11.001.

Watanabe, Y., Honda, M., Higashiura, T., Fukaya, T., Machmudah, S., Diono, W., Kanda, H., Goto, M., 2018. Rapid and selective concentration of lycopene Z-isomers from tomato pulp by supercritical CO_2 with co-solvents. Solvent Extract. Res. Devel. Japan 25 (1), 47–57. https://doi.org/10.15261/serdj.25.47.

Wei, C.C., Gao, G., Kispert, L.D., 1997. Selected cis/trans isomers of carotenoids formed by bulk electrolysis and iron(III) chloride oxidation. J. Chem. Society, Perkin Transact. 2, 783–786. https://doi.org/10.1039/A605027A.

Yang, R., Zhang, L., Li, P., Yu, L., Mao, J., Wang, X., Zhang, Q., 2018. A review of chemical composition and nutritional properties of minor vegetable oils in China. Trends Food Sci. Technol. 74, 26–32. https://doi.org/10.1016/j.tifs.2018.01.013.

Zuorro, A., Lavecchia, R., Medici, F., Piga, L., 2014. Use of cell wall degrading enzymes for the production of high-quality functional products from tomato processing waste. Chem. Eng. Transact. 38, 355–360. https://doi.org/10.3303/CET1438060.