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Lisbeth Vallecilla Yezpe

University of Nebraska-Lincoln, lvallecilla@huskers.unl.edu

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INCREASING *CIS*-LYCOPENE CONTENT OF THE OLEORESIN FROM TOMATO
PROCESSING BYPRODUCTS USING SUPERCRITICAL CARBON
DIOXIDE AND ASSESSMENT OF ITS BIOACCESSIBILITY

Lisbeth Vallecilla Yepez

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Food Science and Technology

Under the Supervision of Professor Ozan N. Ciftci

Lincoln, Nebraska

December 2017

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PROCESSING BYPRODUCTS USING SUPERCRITICAL CARBON DIOXIDE AND
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Lisbeth Vallecilla Yopez, M.S.

University of Nebraska, 2017

Advisor: Ozan N. Ciftci

In recent years, health- and wellness-promoting foods have been one of the major focus of consumers; therefore, the food industry is increasing its efforts in developing these food products. Tomatoes, tomato-food products and their byproducts have gained special attention in virtue of the carotenoids-rich source that they represent, especially lycopene (~90%). The role of lycopene in human diet has been highlighted as a result of its direct relationship with the decrease of chronic diseases. Nevertheless, lycopene is water-insoluble making its extraction simple with organic-toxic solvents; in addition, lycopene degrades easily with light, time, and storage. Lycopene exist in nature mostly in *trans*-lycopene (~95%); however, *cis*-lycopene isomers provide potentially better health benefits than the *trans*-lycopene isomeric form due to their higher bioavailability.

The main objective of this thesis was to increase the bioaccessibility of the lycopene in the tomato oleoresin by SC-CO₂ extraction. Specific objectives were to extract oleoresin from tomato processing byproducts, namely, tomato seed and peel, using SC-CO₂ and optimize the extraction conditions for the highest *cis*-lycopene content, and to test the bioaccessibility of the SC-CO₂-extracted lycopene in vitro.

The highest oleoresin yield (24.6%) was obtained from 100% seed blend with SC-CO₂ extraction at temperature of 40 °C and pressure of 50 MPa, whereas the highest *cis*-lycopene content was achieved at temperature of 80 °C and pressure of 30 MPa from 100% peel. SC-CO₂-extracted oleoresins contained 67% of *cis*-lycopene, while the hexane-extracted ones had 34%. When insoluble fraction was removed, the oil fraction contained up to 82% of *cis*-lycopene in SC-CO₂ extracted oleoresins. SC-CO₂ extraction increased the bioaccessibility of lycopene approximately 2-folds in the oleoresin and 2.4-folds in the oil fraction compared to hexane extraction ($p < 0.05$). The bioaccessibility of the hexane-extracted oleoresin was 2.7%, whereas it was 5.2% for the SC-CO₂-extracted oleoresin. The bioaccessibility of the lycopene in the oil fraction was 1.7 and 4.0% for the hexane and SC-CO₂-extracted oleoresins, respectively. Lycopene concentration in the digesta increased when the tomato oleoresin was used compared to oil fraction for both SC-CO₂ and hexane extractions. After digestion of tomato peel oleoresin, lycopene concentration in the final digesta was 4.6 and 13.4 µg/g for hexane- and SC-CO₂-extracted oleoresin, respectively. Oil fraction of the tomato peel oleoresin released concentrations of lycopene of 1.3 µg/g for hexane and 3.0 µg/g for SC-CO₂ extracted oleoresin.

SC-CO₂ can enhance the efficacy of lycopene during extraction stage in a simple and clean way. The use of *cis*-lycopene-rich extract from tomato processing byproducts in traditional foods can improve the functional properties of the product while adding value to the byproducts of the tomato industry processing industry.

FOR MY MOM, MY SISTER, MY UNCLE, AND MY “HERMOSO”

ACKNOWLEDGMENTS

Firstly, I would like to thank God and the universe for this opportunity. I would like to express my sincere gratitude to my advisor, Dr. Ozan N. Ciftci, for his support, patience and guidance during my M.S. program. His assistance helped me to complete my research project, and most importantly, to gain knowledge and critical thinking. Also, I would like to thank Dr. John Rupnow and Dr. Changmou Xu as my committee members for their helpful suggestions, ideas, and encouragement of my research. I really appreciate their support and knowledge through this path.

I would like to express my gratitude to Dr. Rupnow for bringing me here and changing my life. I would also like to thank my first advisor Dr. George Cavender, for accepting my application, his patience, and his unconditional support while I was under his supervision. I appreciate their efforts and treasure the experience working with them.

I would like to thank all my lab mates, Henok Belayneh, Ali Ubeyitogullari, Junsu Yang, and Tahmasb Hatami, for their all-the-time support, collaboration, and friendship. I appreciate my lab mates who helped me complete my projects, giving their valuable time and effort. Also, I would like to thank my friends, Ana Arciniega, Berena Herrera, Elena Echeverria, Erika Gonzalez, Hollman Motta, Jilmar Robledo, Leila Vega, Leydi Vidal, Lorena Montano, Luz Pulido, Patricia Murillo, Rafael Segura, and Sabrina Vasquez, for all the encouragement they gave me in this process. Also, I would like to thank Dr. Martha Lilliam Mayorga for all her advices, her support and encouragement.

I owe my deep gratitude to my mom (Fraccedes Yepez Amaris); thanks, mommy, for all your love, care and support. I do not have words for expressing it. I would also like

to express my sincere gratitude to my sister (Maria Fernanda Vallecilla Yepez) and my uncle (Juan Bautista Yepez Amaris). Without all of your support, even from afar, I would not be the person I am.

I am grateful for and appreciate the care, support and love from my husband (Diego David Riascos Hurtado). Thank you, honey, for giving me support, love, laughs and advice all these 53 “moons.” Love you my “Hermoso.”

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Organization

This Thesis is organized as follows: introduction and thesis objectives (Chapter 1), a literature review (Chapter 2) followed by two research projects (Chapter 3 and 4), and summary, conclusions, and recommendations (Chapter 5). All chapters have been formatted using guidelines for Food Research International.

References can be found at the end of each chapter.

Chapter 1 . Introduction and Thesis Objectives

1.1 Introduction

The reduction of chronic diseases associated with changes in the human diet along with the growing demand for “natural” products have led the scientific community and the food industry to develop health- and wellness-promoting foods (Kaur & Das, 2011). One major focus of the consumers has become to understand the relationship between diet and health, hence the increased demand for information about health- and wellness-promoting foods (Urbonaviciene & Viskelis, 2017). Health- and wellness-promoting foods refer to those foods that may help to promote health benefits and decrease the risk of chronic diseases. These types of foods are known as functional foods (Kaur & Das, 2011).

Scientists are studying and developing functional foods from a great variety of fruits and vegetables and their byproducts based on the various types and the larger amounts of phytochemicals that they contain (Urbonaviciene & Viskelis, 2017). Considering its impacts on human health, lycopene from tomatoes and its derived products and byproducts have gained special attention from the scientific community (Canene-Adams, Campbell, Zaripheh, Jeffery, & Erdman, 2005; Ellinger, 2010; Kopsell & Kopsell, 2010; Longo, Leo, & Leone, 2012).

The tomato is one of the top crops used in human nutrition globally (Rao, Waseem, & Agarwal, 1998). Today, the waste generated by the tomato processing industry represents up to 40% of byproducts including peels, seeds, pulp and vascular tissues. These materials have no commercial value and are disposed of as a solid waste,

generating a negative impact on the environment. (Chandrasekaran, 2012;Zuorro, Lavecchia, Medici, & Piga, 2014). Nevertheless, the real value of these byproducts is underestimated in light of the rich source of carotenoids, principally lycopene (>90 %), that they represent (Longo, Leo, & Leone, 2012). For instance, tomato peel is rich in phenolic compounds and contains five times more lycopene than tomato pulp (Sharma & Le Maguer, 1996).

An industrial scale, “green” lycopene extraction with a minimum loss of bioactivities is highly desirable for the cosmetic, food, and pharmaceutical industries. Also, it represents a low-cost source of lycopene, which is in great demand from different industries. Moreover, utilization of the tomato peel and seed provides a solution for the waste disposition problem (Topal, Sasaki, Goto, & Hayakawa, 2006; Urbonaviciene & Viskelis, 2017).

Lycopene is a high antioxidant compound present principally in ripe red tomatoes and tomato products; humans and animals cannot produce it (Machmudah et al., 2012; Rao & Agarwal, 2000). Lycopene is very important given its several properties that enhance human health; it is a powerful antioxidant, possessing anticancer and anti-inflammatory benefits (Giovannucci et al., 1995; Palozza et al., 2010; Rao & Agarwal, 2000; Ried & Fakler, 2011). The most abundant lycopene isomer in human tissue and blood is *cis*- configuration, with up to 73% of total lycopene (Clinton et al., 1996; Singh & Goyal, 2008). However, dietary lycopene represented in the plant sources is about 95% of all-*trans*-lycopene. Some research reported that *cis*-lycopene provides a better bioavailability and is more easily absorbed than *trans*-lycopene (Clinton et al., 1996).

Therefore, it is suggested that *cis*-lycopene may offer potentially higher health benefits than *trans*-lycopene.

Lycopene is water-insoluble; therefore it is extracted with organic solvents; these solvents are hazardous, and may remain in the final product (Topal et al., 2006). Lycopene also degrades easily in the presence of light and over time (Machmudah et al., 2012). Therefore, it is necessary to develop a technology that is sensitive to environmental impact and considers the safety of the solvent for the extraction of food grade bioactive compounds from agricultural products. Supercritical fluid extraction using carbon dioxide (SC-CO₂) has been used as a green extraction method for various lipid compounds.

This thesis reports increasing *cis*-lycopene content of the oleoresin obtained from tomato peel and seed, both byproducts of tomato processing, using SC-CO₂ at the extraction stage. The effects of SC-CO₂ extraction conditions such as pressure, temperature, and peel to seed mass ratio on oleoresin yield; total lycopene content; isomeric composition (*cis* vs. *trans*) of the extracted lycopene were determined. Also, the results were compared with hexane extraction. Moreover, the *cis*-lycopene content of the soluble and insoluble fractions of the extracted oleoresin was determined in an effort to obtain a *cis*-lycopene-rich fraction with high biological functions. Furthermore, an in vitro approach was applied in order to screen the lycopene absorption in the digestion process. An in vitro simulated digestion model was used to assess the lycopene release from: the tomato peel oleoresin as well as its respective soluble and insoluble fractions. The in vitro model was used to evaluate the bioaccessibility of the supercritical extracted oleoresin and compared it to the oleoresin obtained with hexane.

1.2 Hypothesis

It was hypothesized that the lycopene in the SC-CO₂-extracted tomato peel oleoresin has higher bioaccessibility compared to the one obtained with conventional hexane extraction.

1.3 Thesis objectives

The main objective was to increase the bioaccessibility of the lycopene in the tomato oleoresin by SC-CO₂ extraction. The specific objectives were to:

- 1) To extract oleoresin from tomato processing byproducts, namely, tomato seed and peel, using SC-CO₂ and optimize the extraction conditions for the highest *cis*-lycopene content.
- 2) To test the bioaccessibility of the SC-CO₂-extracted lycopene in vitro.

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Chapter 2 . Literature Review

2.1 Lycopene

The work presented in this thesis focuses on lycopene, which is part of the carotenoids group. Carotenoids are pigments synthesized by photosynthetic organisms (Olson & Krinsky, 2016). The carotenoids group includes over 750 compounds, that represent one of the major colorful, fat-soluble and phyto-nutritional groups (Nobre et al., 2012). Carotenoids are responsible for the red, orange and yellow colors of many fruits, vegetables, and organisms. Human blood and tissues principally contain *alpha*-carotene, *beta*-carotene, lycopene, lutein, zeaxanthin, xanthophylls and *beta*-cryptoxanthin (Maiani et al., 2009). Lycopene is a lipid soluble carotenoid which presents the geometrical isomers *cis*- and *trans*- as a result of the presence of methyl groups bonded to acyclic open-chain structure consisting of 13 double-bonds (Nobre et al., 2012; Holzapfel et al., 2013). It is responsible for the red color of tomatoes and tomato-based products, and is the dominant carotenoid in this fruit.

2.1.1 Chemical structure and properties

Lycopene is a polyene hydrocarbon with the molecular formula of $C_{40}H_{56}$, that consists of an acyclic open-chain structure with 11 linearly arranged conjugated double bonds and two non-conjugated, resulting in a chromophore of variable length (Fig. 2.1) (Clinton, 1998; Sharma & Le Maguer, 1996).

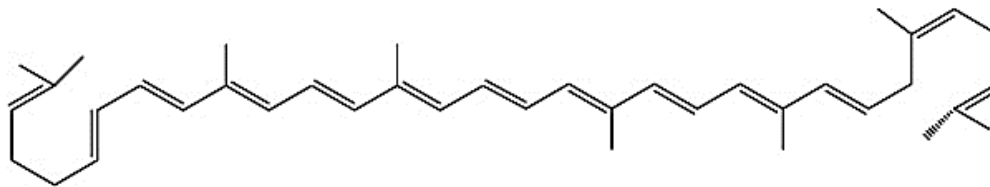


Figure 2.1 Structure of lycopene (O'Keefe, 1991).

Lycopene has a hydrophobic character, which makes it nearly insoluble in ethanol, methanol and water, but highly soluble in chloroform, hexane, petroleum ether, carbon disulfide, acetone, and benzene. It is also very sensitive to light, oxygen, heat, certain chemical reactions and acids, and its oxidation is catalyzed by certain metallic ions such as Fe^{3+} , Cu^{2+} (Shi, Dai, Kakuda, Mittal, & Xue, 2008; Sharma & Le Maguer, 1996).

Lycopene's large conjugated polyene structure is responsible for its ruby color and its antioxidant activity. Lycopene lacks a β -ionone ring structure, resulting in an absence of provitamin A activity and an increase in quenching activity (Clinton, 1998; Shi & Maguer, 2000). The quenching activity is influenced by the carotenoid end groups and the type of substituents in carotenoids containing cyclic end groups. It also has a direct relationship with the numbers of conjugated double bonds (Stahl, Sundquist, Hanusch, Schwarz, & Sies, 1993). The antioxidant activities of lycopene and other carotenoids are determined by their singlet oxygen quenching properties and their ability to inactivate peroxy radicals. Sections that possess affinity for lipid nature can be damaged by the production of peroxy radicals that comes from the lipid peroxidation process (Foote & Denny, 1968; Holzapfel et al., 2013). Lycopene has the ability to inhibit these reactions and protect membranes from lipid peroxidation (Chasse et al., 2001).

Additionally, lycopene has a considerable quenching-rate constant (Table 2.1) and is one of the most potent single oxygen quenchers when compared to other carotenoids (Conn, Schalch, & Truscott, 1991).

Table 2.1 Antioxidant activities of carotenoids: singlet oxygen quenching

Carotenoid	Singlet oxygen quenching, $10^9 \times K_q \text{ (m}^{-1} \text{ s}^{-1})^*$
Lycopene	31
γ -carotene	25
α -carotene	19
β -carotene	14
Lutein	8
Astaxanthin	24
Canthaxanthin	21
Zeaxanthin	10

*Data adapted from Shi & Maguer (2000) and citations therein.

Lycopene presents a diversity of isomeric forms, from the *trans*-isomer to mono-*cis* and poly-*cis* forms. *Trans*-lycopene is the most prevalent isomer in fresh tomatoes (about 95% of total lycopene) and the most thermodynamically stable configuration (Longo, Leo, & Leone, 2012). Nevertheless, an isomerization from *trans*- to *cis*- forms or from *cis*- to *trans*- (retroisomerization) occurs during tomato processing and storage and takes place in both pure lycopene and tomato products (Lambelet, Richelle, Bortlik, Franceschi, & Giori, 2009). Moreover, the isomerization process not only promotes the formation of *cis*-isomers, but also competes with degradation, and the latter is faster at temperatures higher than 50 °C for a long heating time. The stability of lycopene isomers increases in the order: 11-*cis* < 7-*cis* < 15-*cis* < 13-*cis* < 9-*cis* < *all-trans* < 5-*cis*. (Lee &

Chen, 2002; Longo, Leo, & Leone, 2012). The 5-*cis*, 9-*cis*, 13-*cis* and 15-*cis* isomers are the most abundant isomers of lycopene in human serum and tissues and in some tomato-based products (Fig. 2.2) (Clinton et al., 1996; Schierle et al., 1997; Yeum et al., 1996).

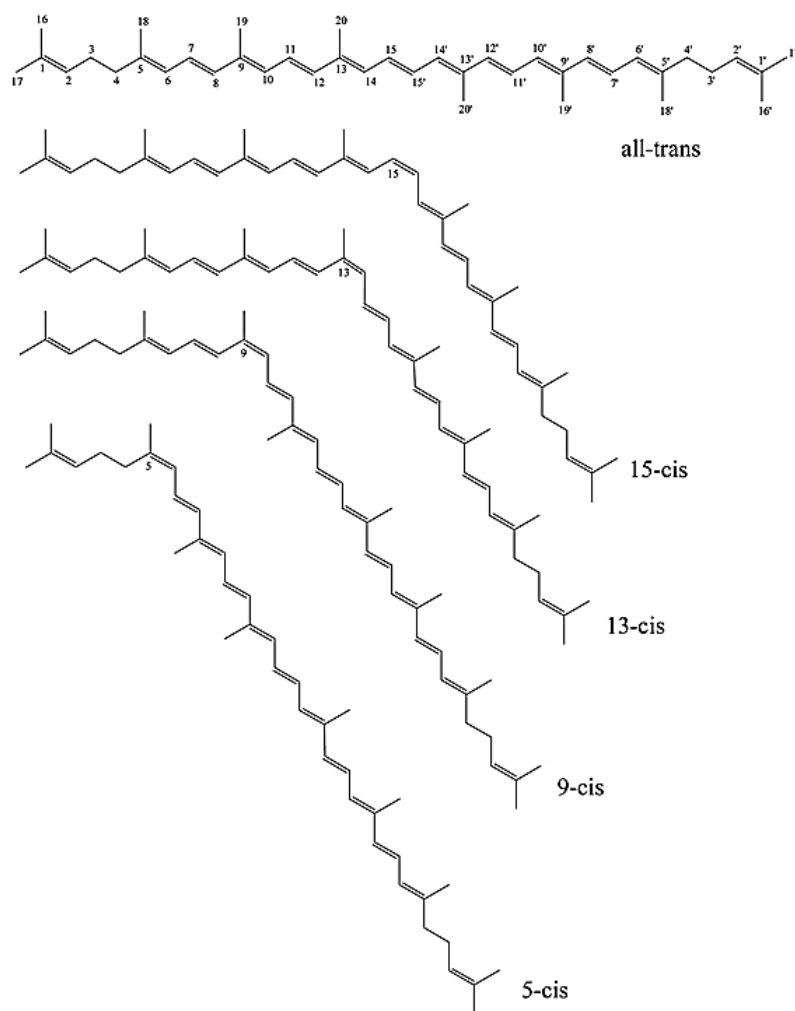


Figure 2.2 The most abundant isomers of lycopene in human serum and tissues and in some tomato-based products (Sun et al., 2016).

2.1.2 Lycopene content in tomato fruit.

In contrast to other carotenoids, lycopene is available in few fruits and vegetables. Tomatoes and tomato foods are the major sources of lycopene (Clinton, 1998). Other natural sources of lycopene are watermelon, papaya, pumpkins, pink guava, pink grapefruit, apricot, and rosehip fruit (Table 2.2) (Clinton, 1998; Machmudah, Kawahito, Sasaki, & Goto, 2007; Mangels, Holden, Beecher, Forman, & Lanza, 1993; Tonucci et al., 1995).

Table 2.2 Lycopene content in some fruits. Data based on (Mangels et al., 1993; Shi & Maguer, 2000).

Source	Lycopene content (mg/100 g wet basis)
Fresh tomato fruit	0.72–20
Cooked tomatoes	3.7
Watermelon	2.3–7.2
Guava (pink)	5.23–5.50
Pumpkin	0.38–0.46
Papaya	0.11–5.3
Rosehip puree	0.68–0.71
Apricot	0.01–0.05

Lycopene content varies in tomato fruits depending on the type, maturity, geographic location and the environmental conditions influencing the ripeness of tomatoes. Generally, tomato fruit contains 3-5 mg lycopene per 100 grams of raw material. The yellow variety is less rich in lycopene and contains around 0.5 mg per 100 g, while some red types consist of more than 15 mg per 100 grams (Holzapfel et al., 2013; Shi & Maguer, 2000). Some studies report high lycopene content in tomatoes that

are more mature, produced outdoors, or grown in the summer time. On the other hand, a lower amount of lycopene was found in tomatoes grown in winter, in greenhouses, and at low temperatures (Shi & Maguer, 2000).

Furthermore, some researchers have investigated the lycopene content in tomatoes. In general, they found that the tomato peel is very rich in lycopene, accounting for up to five times more lycopene (53.9 mg per 100 grams) than the whole tomato pulp (11 mg per 100 grams) (Sharma & Le Maguer, 1996). Also, higher concentrations of lycopene and other carotenoids are found in the stem than in the blossom end of the fruit (Shi & Maguer, 2000).

In addition, other authors have studied the lycopene content in tomato processed products. Tonucci et al. (1995) determined the lycopene content of some tomato-based products and found that processed tomato products have more lycopene than raw fruit (Table 2.3).

Table 2.3 Lycopene content of some tomato products. According to (Rao & Agarwal, 1999; Tonucci et al., 1995).

Tomato-based food	Lycopene content (mg/100 g)
Ketchup	17.23
Spaghetti sauce	15.99
Tomato paste	55.45
Tomato puree	16.67
Tomato sauce	17.98
Tomato soup (condensed)	79.9
Pizza sauce	127.1

Those studies indicated that processing enhances the bioavailability of lycopene. This may happen as a result of several factors: the breaking down of cell walls and further release of lycopene from the tomato tissue matrix, the presence of dietary lipids, and heat-induced isomerization from all *trans* to *cis* configuration (Clinton, 1998; Rao & Agarwal, 1999; Shi & Maguer, 2000).

2.1.3 Lycopene and human health

Around 60 carotenoids out of 750 have been identified in the human diet, and six of these constitute up to 95% of total blood carotenoids (Maiani et al., 2009). Dietary intakes or plasma levels of lycopene have been associated with a decreased risk of cardiovascular diseases and chronic diseases such as prostate, lung and stomach cancer (Giovannucci et al., 1995; Rao & Agarwal, 2000; Ried & Fakler, 2011). Also, some studies have found that lycopene protects against the rise of cholesterol levels and has an anti-inflammatory effect (Boileau, Boileau, & Erdman Jr, 2002; Palozza et al., 2010).

The conjugated double bond system present in lycopene possesses the characteristics of being a potent antioxidant (Rao & Agarwal, 2000), having a higher singlet oxygen- quenching ability compared to other carotenoids (Rao & Agarwal, 1999), and being a free radical scavenger. (Miller, Sampson, Candeias, Bramley, & Rice-Evans, 1996). It has also been suggested that the formation of atheroma and cancer development can be prevented by lycopene, because of its protection in the oxidation process of some critical biomolecules including low-density lipoprotein (LDL) lipids, DNA, and proteins (Rao & Agarwal, 1999).

Tissue culture systems have shown evidence for the biochemical and molecular effects of lycopene in malignant and normal cells, for example, lycopene inhibits about 40% of cell growth in human leukemia cell lines (Rao & Agarwal, 1999). Animal models have shown that dietary lycopene increased the survival rate of mice exposed to x-irradiation, decreased the development of ascites tumors and protected them from bacterial infections (Lingen, Ernster, & Lindberg, 1959). Epidemiological studies have mostly focused their attention on the relationship between lycopene and cancers; they have found that a high ingestion of lycopene from tomatoes was associated with a low mortality rate from cancer (Franceschi et al., 1994; Giovannucci et al., 1995; Rao & Agarwal, 1999). In addition, other authors have shown the positive relationship between lycopene and other chronic diseases such as cardiovascular risk, myocardial infarction risk and chronic heart disease (Rao & Agarwal, 1999). However, further research is required to better understand the role of lycopene in human health.

2.1.4 Bioavailability and bioaccessibility of lycopene

It is crucial to know the amount of lycopene in different food sources, but it is also important to know the grade of absorption of lycopene in the human body based on its bioavailability. Shi & Maguer (2000) define bioavailability as “the fraction of an ingested nutrient that is available to the body through absorption for utilization in normal physiological functions and for metabolic processes.”

Lycopene bioavailability is a complex process that can be affected by different means such as the food matrix and lipid medium of the sources, lifestyle factors, and age (Clinton et al., 1996; Holzapfel et al., 2013; Rao & Agarwal, 1999; Rao, Waseem, &

Agarwal, 1998). It has been suggested that some mechanisms, such as viscosity increase in the gastrointestinal tract, leads to the binding of phospholipids and bile acid micelles. These mechanisms can also result in impaired activity of enzymes such as lipase, decreasing carotenoid migration and consequently bioavailability (Svelander et al., 2010). Nevertheless, it is important to mention that lycopene uptake is never complete; its absorption from dietary sources is in the range of 10 to 30% in humans (Holzapfel et al., 2013).

Lycopene bioavailability from tomatoes also depends on presentation. In a raw tomato, lycopene is encapsulated within the chromoplasts in a crystalline form; therefore, its release and successive absorption is not very significant (Svelander et al., 2010). Moreover, the pectin that composed the tomato cell walls has shown to reduce the bioavailability of various carotenoids, including lycopene (Svelander et al., 2010). Although the influence of the processing techniques on lycopene bioavailability is not well established, studies have shown that absorption from processed tomato products is better than that from tomato fruit (Gartner, Stahl, & Sies, 1997; Svelander et al., 2010). The thermal and mechanical steps associated with processing can break the structures that contain lycopene, increasing its bioavailability (Svelander et al., 2010).

The naturally occurring configuration of lycopene in plant foods is usually the all-*trans*- isomer. Nevertheless, the most abundant isomeric form in human tissue and blood is *cis*-lycopene (58–73% of total lycopene in human serum, and up to 88% in prostate tissues) (Clinton et al., 1996; Singh & Goyal, 2008). Therefore, it is suggested that *cis*-configuration of lycopene has a higher bioavailability compared to *trans*-lycopene form (Clinton et al., 1996). Compared to the *trans*- configuration, *cis*- isomeric forms of

lycopene possess less susceptibility to aggregate and higher solubility in bile acid micelles (Boileau et al., 2002). In addition, *cis* isomers are more difficult to crystallize, and more soluble in organic solvents and oily phases (Castenmiller & West, 1998) resulting in a upper availability for intestinal cell uptake (Failla, Chitchumroonchokchai, & Ishida, 2008). Moreover, the changes in structural shapes modify the potency of the bioactivity of *cis*- configuration (Shi & Maguer, 2000). In addition, other investigations suggest that *trans*- forms of lycopene build crystals in the intestine, reducing the lycopene's uptake through the micelles (Holzapfel et al., 2013).

Other research studying the bioavailability of lycopene found that the isomerization phenomenon can occur in the stomach, which may explain the presence of *cis*-lycopene in human tissues when compared to *trans*-lycopene (Boileau et al., 2002). Furthermore, Re, Fraser, Long, Bramley, & Rice-Evans (2001) found that lycopene isomerization was constant when the pH was adjusted to 7, whereas it was improved when the pH was lowered to 2. These findings support the theory that *cis*-isomer formation may take place in the acidic conditions of the stomach.

In general, carotenoid bioavailability in humans has been determined by measuring the carotenoid levels in plasma following the ingestion of carotenoid-rich products (Garrett, Failla, & Sarama, 1999). Because of the constraints related to the use of clinical trials in humans, and specialized needs in animal models to assess *in vivo* lycopene uptake, intestinal metabolism and absorption of lycopene in *in vivo* models is limited (Garrett et al., 1999). *In vitro* models offer a cost-effective solution and have been reported to predict *in vivo* levels of lycopene bioavailability (Dehghan-Shoar, Mandimika, Hardacre, Reynolds, & Brennan, 2011; Eboul, Ichelle, & Loi, 2006; Garrett

et al., 1999). The mechanism of lycopene absorption requires that the amount that is released from the food matrix must be dispersed into the micellar fraction of the digesta in the intestinal lumen and combined with bile salts (Dehghan-Shoar et al., 2011). Hence, lycopene bioaccessibility is calculated as the fraction of lycopene in the meal that is released into the micellar phase throughout the process of simulated digestion. This release of lycopene from the tomato matrix through the digestive process, namely bioaccessibility, is the first step to measure the lycopene bioavailability (Goñi, Serrano, & Saura-Calixto, 2006). For this reason, in vitro digestion models may provide a better understanding of the bioavailability of lycopene from the tomato matrix and the factors that determine its availability (Goñi et al., 2006). Few studies report on the in vitro bioaccessibility of lycopene from tomatoes and tomato-based products, but studies have shown bioaccessibility values for the micellar fraction in the range of 2–8% (Garrett et al., 1999). These values only show the amount of lycopene release after in vitro digestion, but not the total lycopene amount that is incorporated into micelles.

Overall, bioaccessibility is the fraction of lycopene released from the tomato matrix to micelles and is determined by an in vitro digestion model (Dehghan-Shoar et al., 2011; Svelander et al., 2010). Lycopene uptake by intestinal cells is estimated using human intestinal cell lines such as Caco-2 cells, which estimate the absorption of the micellized lycopene into the cells (Dehghan-Shoar et al., 2011; Eboul et al., 2006; Garrett et al., 1999). Bioavailability is the lycopene fraction recovered in plasma and is estimated using animal models, human studies, or in vitro digestion models (Eboul et al., 2006; Garrett et al., 1999; Svelander et al., 2010). Each case presents advantages and disadvantages; however, studies about lycopene absorption, bioavailability, and

bioaccessibility are not clear, so there is a need for more research to further understand these phenomena.

2.2 Supercritical fluid (SCF) technology

2.2.1. Introduction

Supercritical fluid technology uses a solvent that is under supercritical conditions. Supercritical fluids (SFs) are compounds whose temperatures and pressures cross their critical point. In the supercritical state, the solvating power of the solvent is enhanced and the fluid possesses unique physical properties in which it is neither liquid nor gas, but releases properties of both (Leitner, 2000). Moreover, at these conditions, the density of the fluids behaves as in the liquid phase, while the compressibility, diffusivity and viscosity perform as in the gas phase (Bozan & Temelli, 2002). For example, diffusivity and viscosity of the SF play an important role in the extraction process. The viscosity of SF is around 10^{-4} g/cm.s, a very low value compared to the liquid viscosities. Likewise, the liquids exhibit diffusion coefficients of the order of 10^{-6} cm²/s while supercritical fluids solvents have diffusivities of approximately 10^3 cm²/sec. This “gas-like” behavior of SFs allows them to have superior penetration properties into the target material, compared to liquid solvents (Sapkale, Patil, Surwase, & Bhatbhage, 2010).

Supercritical fluids act as solvents that facilitate heat and mass transfer dictated by the difference from the equilibrium state (Brunner, 2005). The critical pressures and temperatures can be modified efficiently to obtain a solvent with a high capacity and selectivity for the desired process. Changing the temperature and pressure can adjust

some properties of the fluid, such as density, diffusivity, and viscosity. This leads to acquiring the proper solvent not only for the target compound, but also for obtaining a product without solvent residues that can be recycled (Cansell et al., 1999; Morin, C. Loppinet-Serani, A. Cansell, F. Aymonie, 2012; Sapkale et al., 2010). Some of the processes that can use supercritical fluids are lipid extraction, bioactive compounds impregnation, polymer modification, polymer blending, nanomaterials synthesis, food processing, and particle formation (Belayneh, Wehling, Cahoon, & Ciftci, 2015; Brunner, 2005; Nalawade, Picchioni, & Janssen, 2006; Philippot, Elissalde, Maglione, & Aymonie, 2014; Ubeyitogullari & Ciftci, 2017; Yang & Ciftci, 2016). The selection of the fluid to be used as supercritical fluid depends on the purpose, cost, properties of the fluid, economic returns, and applicability (Sapkale et al., 2010).

2.2.2 Supercritical carbon dioxide (SC-CO₂) technology

SC-CO₂ is one of the most common fluids used in the food industry (Brunner, 2005). SC-CO₂ extraction is a promising method for isolation of bioactive compounds from plant materials under mild conditions (Sovilj, 2010). CO₂'s low cost, safety, non-toxicity, non-flammability, and environmental compatibility make it the most popular solvent in supercritical fluid extraction (King, 2002). Its high diffusivity and tunable solvent strength make it very attractive for food processing. When it is recycled, it does not support the environmental CO₂ problem, which is why it is called a green solvent. The supercritical conditions for CO₂ are defined in the upper right-hand sector of the diagram of phases (Fig. 2.3). Critical temperature and pressure (T_c and P_c) are 31.1 °C and 73.8 bar, respectively (Brunner, 1994). CO₂'s ability to be operated at a low

temperature is very important since this allows a suitable process of extraction for thermally labile or easily oxidized compounds (Machmudah et al., 2012). SC-CO₂ also has the advantage that it is gaseous at room temperature and ordinary pressure, which ensures an innocuous separation process both for humans and for the environment, and provides a simple extraction process (de Melo, Silvestre, & Silva, 2014).

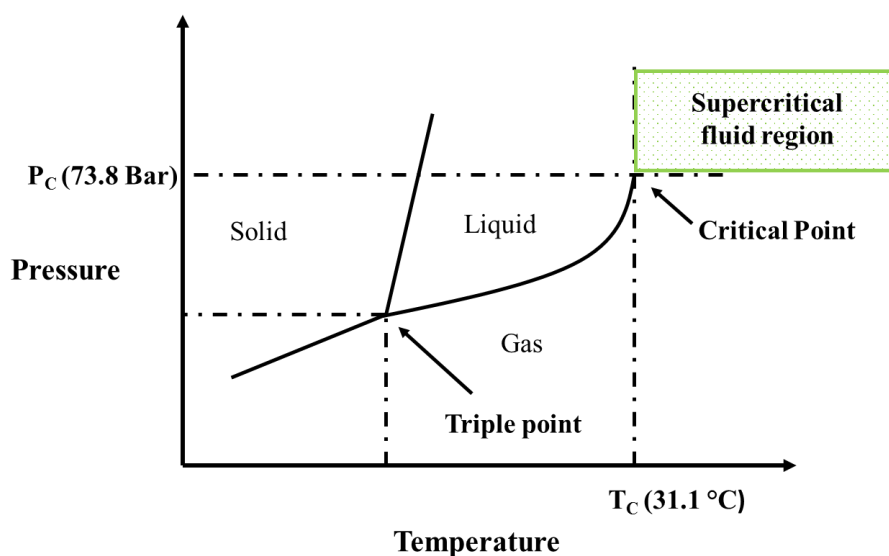


Figure 2.3 A Phase diagram for carbon dioxide. Adapted from Brunner (1994).

Solubility is one of the most important properties in supercritical fluid technology. CO₂ is a nonpolar solvent and is best suited for dissolving nonpolar and slightly polar compounds. It has the ability to form specific solvent-solute interaction with low molecular weight, oxygenated organic compounds, and non-polar lipophilic substances (Catchpole, et al., 2012). Compounds such as free fatty acids, pigments, and water have a very low solubility in SC-CO₂, while mineral salts, sugars, proteins, and polysaccharides

are non-soluble (Brunner, 2005). Nevertheless, its polarity can be modified with the addition of polar solvents (modifiers), such as ethanol, that can enhance its solvent power and maintain the status of a “green” solvent (Catchpole et al., 2012; Shi et al., 2009).

SC-CO₂ has been used for the extraction of high value compounds, essential oils, flavorings, the removal of pesticides from natural sources, the extraction of edible oils, and the extraction of oils rich in omega 3 and minor lipid compounds (phytosterols, carotenoids, etc.) (Belayneh et al., 2015; Bozan & Temelli, 2002; Machmudah et al., 2012; G. Brunner, 2005). Moreover, lipid extraction by SC-CO₂ has focused on specialty seed oils such as oil from grape seeds (Molero-Gómez, Pereyra-López, & Martinez de la Ossa, 1996), tomato seeds (Lenucci et al., 2010; Roy, Goto, & Hirose, 1996), and rosehip seeds (Machmudah et al., 2007). Additionally, some other special products obtained by SC-CO₂ technology may be found in daily life, such as vitamin additives, de-alcoholized beverages, decaffeinated coffee and tea, defatted potato chips, and spice extracts (Brunner, 2005).

2.2.3 SC- CO₂ extraction of lycopene from tomato matrices

SC-CO₂ extraction of lycopene has shown less decomposition when compared to other methods of extraction (Shi et al., 2009). There are some studies reporting lycopene extraction by SC-CO₂ from different tomato matrices, such as tomato- based products, seeds, pulp, peel, and the whole tomato (Egydio, Moraes, & Rosa, 2010; Lenucci et al., 2010; Longo, Leo, & Leone, 2012; Machmudah et al., 2012; Roy et al., 1996; Gómez-Prieto, Caja, Herraiz, & Santa-María, 2003; Rozzi, Singh, Vierling, & Watkins, 2002).

Moreover, there are some studies reported the SC-CO₂ extraction of lycopene using co-solvents or modifiers, principally ethanol (Egydio et al., 2010; Nobre et al., 2012; Shi et al., 2009; Vasapollo, Longo, Rescio, & Ciurlia, 2004).

In general, lycopene content can be increased significantly with the pre-treatment of the raw material (e.g., operations like grinding, drying, and sieving). For instance, drying the starting tomato materials can cause a decrease in lycopene content. Samples with low moisture content allow for obtaining higher yields of lycopene compared to raw material with a higher moisture content.

Temperature and pressure are one of the principal parameters studied in lycopene extraction using SC-CO₂. The most frequently reported optimum extraction temperature is 80 °C (Cadoni, De Giorgi, Medda, & Poma, 1999; Egydio et al., 2010). Moreover, some researchers stated that an increase in temperature cause an increase in lycopene yield; however, these studies consider only lycopene final amount, but not the thermal degradation of lycopene associated with the process (Cadoni et al., 1999; Ollanketo, Hartonen, Riekkola, Holm, & Hiltunen, 2001; Rozzi et al., 2002; Topal, Sasaki, Goto, & Hayakawa, 2006).

For pressure parameter, it has been found in the literature that lycopene yield increased with the increase of pressure; the best pressure condition in lycopene extraction reported is 400 bar with different temperatures range from 40 °C to 100 °C; however, these investigations did not study the effect of higher extraction pressures (> 450 bar) (Lenucci et al., 2010; Rozzi et al., 2002; Topal et al., 2006).

Moreover, studies also showed a shorter time of extraction using SC-CO₂ compared to conventional solvent extraction (Longo et al., 2012; Machmudah et al., 2012), and the interaction of time and pressure has a positive effect on the antioxidant activity of lycopene (Egydio et al., 2010). Furthermore, the effect of CO₂ flow rate showed no significant effect (Machmudah et al., 2012, 2007; Salgın & Salgın, 2013), although other researchers stated that CO₂ flow rate increases efficiency along with other extraction factors (Rozzi et al., 2002; Vasapollo et al., 2004).

There is also extensive research showing an increase on lycopene yield with the use of modifiers (Shi et al., 2009; Vasapollo et al., 2004), while other studies report a high yield of lycopene with the optimization of processing parameters (Gómez-Prieto et al., 2003; Longo et al., 2012; Machmudah et al., 2012; Topal et al., 2006).

However, those SC-CO₂ extraction studies mainly focused on lycopene solubility in CO₂, antioxidant activity, total lycopene yield and the adjustment of some technical parameters for an optimum lycopene extraction. Few studies have reported on the isomerization of lycopene from tomato and tomato byproduct fractions extracted by SC-CO₂.

2.2.3.1 SC-CO₂ isolation of *cis*-lycopene from tomato processing byproducts

Few papers have reported on the use of SC-CO₂ to isolate *cis*-lycopene from tomato byproducts. Urbonaviciene & Viskelis (2017) studied the optimization of SC-CO₂ extraction parameters for the isolation of the tomato byproduct oleoresin and *cis*-lycopene isomers using a central composite rotatable design model with response surface

methodology. They predicted the best results for oleoresin byproduct yield at a temperature of 73.9 °C, a pressure of 53.7 MPa, and high content of *cis*-lycopene isomers (62%) at 52 °C temperature, 180 min extraction time and 55 MPa pressure.

Gómez-Prieto, Caja, Herraiz, & Santa-María (2003) investigated the possibility of using SC-CO₂ to obtain an all-*trans* form of lycopene from tomatoes. They observed that the amount of the *trans* form extracted rises whereas the *cis* form content decreases with an increase of the extraction pressure.

Longo, Leo, & Leone (2012) reported the isomeric profile in SC-CO₂-extracted oleoresins from tomato and tomato/hazelnut matrices. They compared their composition with the one obtained by solvent extraction performed with tetrahydrofuran (THF). They indicated that the solvent extracted oleoresin contained mainly all-*trans*-lycopene (*cis*-lycopene was 9.92% of the total carotenoids) in the tomato powder, and the amount of *cis*-lycopene was higher in the SC-CO₂ extracted oleoresins accounting for 47.7% and 40.7% in the tomato and tomato/hazelnut matrices.

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Chapter 3 . Increasing *cis*-lycopene content of the oleoresin from tomato processing byproducts using supercritical carbon dioxide

3.1 Abstract

Cis-lycopene is more bioavailable than *trans*-lycopene; however, 95% of the lycopene in natural sources is in *trans* form. Tomato processing byproducts represent a low-cost source of high-value lycopene. The objective of this study was to obtain a *cis*-lycopene-rich oleoresin from tomato peels and seeds using supercritical carbon dioxide (SC-CO₂) and to compare it with the conventional hexane extraction. The highest oleoresin yield (24.6%) was obtained with 0:100 peel:seed (w:w) blend with SC-CO₂ extraction at lower temperature (40 °C) and higher pressure (50 MPa), whereas the highest *cis*-lycopene content was obtained at higher temperature (80 °C) and lower pressure (30 MPa) from 100% peel. SC-CO₂-extracted oleoresins contained 67% of *cis*-lycopene, whereas it was only 34% in the hexane-extracted ones. When insoluble fraction was removed, the oil fraction contained up to 82% of *cis*-lycopene. SC-CO₂ can enhance the efficacy of lycopene during extraction stage in a simple and clean way.

Keywords: Supercritical carbon dioxide; extraction; lycopene; tomato byproducts.

3.2 Introduction

Lycopene ($C_{40}H_{56}$) is a polyene hydrocarbon with 11 conjugated double bonds that represents up to 90% of the total carotenoid content in tomatoes (Longo, Leo, & Leone, 2012). Lycopene is a powerful natural antioxidant without provitamin A activity responsible for the red pigment of ripe tomatoes and tomato products. It is naturally produced by plants and microorganisms, but animals and humans cannot synthesize it (Machmudah et al., 2012; Rao & Agarwal, 2000; Topal, Sasaki, Goto, & Hayakawa, 2006). Recent studies have shown that serum and tissue lycopene have an inverse relationship with chronic disease risk (Rao & Agarwal, 2000). Moreover, the consumption of tomatoes and tomato-based products containing lycopene has promising health benefits; for instance, it protects against the rise of cholesterol levels and has an anti-inflammatory effect. In addition, clinical evidence associates the dietary intake of lycopene with a decreased risk of cardiovascular diseases and chronic diseases such as prostate, lung and stomach cancer (Giovannucci et al., 1995; Palozza et al., 2010; Rao & Agarwal, 2000; Ried & Fakler, 2011). *Trans*-lycopene is the predominant configuration in raw red tomato fruits at approximately 95%. However, the most abundant isomeric form in human tissue and blood is *cis*- configuration accounting for 58–73% of total lycopene in human serum, and up to 88% in benign or malignant prostate tissue (Clinton et al., 1996; Singh & Goyal, 2008). This suggested that *cis*-isomers of lycopene are preferably absorbed and have a higher bioavailability than the *all-trans*-lycopene form (Clinton, et al., 1996). Compared to the *trans*-lycopene configuration, *cis*-lycopene forms have a greater solubility in bile acid micelles, resulting in a lower tendency to aggregate (Boileau, Boileau, & Erdman Jr, 2002); are less feasible to crystallize, more polar, more

soluble in oil (Castenmiller & West, 1998); and are more available for the intestinal cell uptake (Failla, Chitchumroonchokchai, & Ishida, 2008).

Nowadays, new efforts to improve consumers' health and quality of life have led to the introduction and development of health-promoting types of food, such as functional foods. Scientists are studying and developing functional foods from a broad range of fruits and vegetables and their byproducts due to the various types of phytochemicals contained therein. Considering its impacts on human health, lycopene from tomatoes and its derived products and byproducts have gained special attention from the scientific community (Canene-Adams, Campbell, Zaripheh, Jeffery, & Erdman, 2005; Ellinger, 2010; Kopsell & Kopsell, 2010; Longo, Leo, & Leone, 2012).

The tomato is one of the top crops of the human diet globally (Rao, Waseem, & Agarwal, 1998). Currently, millions of tons of tomato are processed per year to produce tomato-based products such as tomato juice, ketchup, tomato paste, canned tomatoes and many other products. The industrial waste generated by this process accounts up to 40% of tomato byproducts consisting mainly of peel and seed (Chandrasekaran, 2012; Zuorro, Lavecchia, Medici, & Piga, 2014). The disposal and handling of these materials represent a major problem for the tomato industry; currently, these byproducts are wasted or used for animal food production. Nevertheless, their real value is underestimated. According to some studies, tomato seed contains a high nutritional quality oil (Eller, Moser, Kenar, & Taylor, 2010) and tomato peel is a very rich source of flavonoids and carotenoids containing five times more lycopene than tomato pulp (Sharma & Le Maguer, 1996). Therefore, the possibility of transforming these byproducts into a high value product could represent a low-cost source of lycopene, which is in great demand for supplements

to enhance the nutritional value of food, and for products in the pharmaceutical and cosmetic industries. This possibility also offers a solution for the disposal problem.

Stability and solubility are known as the principal problems in the lycopene extraction process (Shi, Yi, Xue, Jiang, Ma, & Li, 2009). Lycopene is extracted with organic solvents; it is insoluble in water but highly soluble in benzene, hexane, chloroform, and methylene chloride. These solvents are hazardous, toxic and may remain in the final product (Topal, Sasaki, Goto, & Hayakawa, 2006). Lycopene also degrades easily in the presence of light and over time (Machmudah et al., 2012). Hence, there is a critical need for an alternative green solvent for the extraction of oleoresins rich in lycopene. These oleoresins contain bioavailable lycopene free of toxic residues of conventional solvents that can be consumable for humans.

Supercritical carbon dioxide (SC-CO₂) extraction has been used as a green extraction method for various lipid bearing materials. Several studies have reported on lycopene extraction from tomatoes, tomato-based products and tomato byproducts using SC-CO₂. Those SC-CO₂ extraction studies mainly focused on lycopene solubility in carbon dioxide, antioxidant activity, total lycopene yield and the adjustment of some technical parameters for an optimum lycopene extraction (Gómez-Prieto, Caja, Herraiz, & Santa-María, 2003; Machmudah et al., 2012; Rozzi, Singh, Vierling, & Watkins, 2002; Singh & Goyal, 2008; Topal et al., 2006). However, studies have rarely reported on the isomerization of lycopene from tomato and tomato byproduct fractions extracted by SC-CO₂. In this study, effect of SC-CO₂ extraction conditions along with the effect of the peel and seed fractions on the content of *cis*-lycopene in the oleoresins were investigated to obtain a *cis*-lycopene-rich oleoresin.

Therefore, the main objective of this study was to increase the *cis*-lycopene content of the oleoresin obtained from tomato peel and seed, byproducts of tomato processing, using SC-CO₂ at the extraction stage. Specific objectives were to determine the effect of SC-CO₂ extraction conditions, namely, pressure, temperature, and peel to seed mass ratio, on the (i) oleoresin yield; (ii) total lycopene content; (iii) isomeric form (*cis* vs. *trans*) of the extracted lycopene, and to compare with hexane extraction. Moreover, *cis*-lycopene content of the soluble and insoluble fractions of the extracted oleoresin was determined in an effort to obtain a *cis*-lycopene-rich fraction with the possibility of having a high value product for its biological functions.

3.3 Materials and Methods

3.3.1. Materials

Tomato peel and seed, by-products of a ketchup plant in California, were provided by ConAgra Brands (Omaha, NE, USA). Seeds were separated from other materials, such as pieces of plants, then ground using a small analytical mill (IKA A11 basic, IKA Works, Inc., USA) and sieved to obtain fractions with particles smaller than 0.7, 1.0, and 1.4 mm. Tomato peel was in the powder form and used as is. Blends of tomato peel and tomato seed (peel:seed, w/w: 100:0, 70:30, 50:50, 30:70, 0:100, w:w) were prepared and stored at -20 °C in sealed bags.

CO₂ and nitrogen (both 99.99% purity) were acquired from Matheson (Lincoln, NE, USA). All lycopene standards (>90% purity) were purchased from Sigma Aldrich (St. Louis, MO, USA). Standard solutions were prepared by dissolving pure individual

lycopene in a mixture of hexane/acetone/ethanol. Hexane, acetone, ethanol, ethyl acetate, methanol, methyl butyl ether, and ethyl acetate were all HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other reagents and solvents were of analytical or chromatographic grade.

3.3.2. Hexane (Soxhlet) extraction

Tomato peel and seed blends (12 g) were loaded into cellulose thimbles and placed in a Soxhlet extractor. Extractions were carried out with hexane for 6 h under dim light and the Soxhlet extractor was covered with aluminum foil to prevent photodegradation. Hexane was separated from the oleoresin using a rotary vacuum evaporator (Buchi Labortechnik AG, model B-490, Flawil, Switzerland) at 37 °C after each extraction. The resulting oleoresin was weighed and the total oleoresin yield was reported as (weight of oleoresin/weight of sample used for extraction) x 100. The oleoresin extracts were flushed with nitrogen and stored at -20 °C until analyzed for lycopene content and composition. Extractions were carried out as triplicates and results were reported as mean \pm standard deviation.

3.3.3. SC-CO₂ extraction

SC-CO₂ extractions were carried out in a laboratory scale SC-CO₂ extractor (SFT 110, Supercritical Fluids, Inc., Newark, DE, USA). Details and operation of the system was reported previously (Belayneh, Wehling, Cahoon, & Ciftci, 2015). For each extraction, a blend of tomato peel and tomato seed was loaded into the extraction vessel. The system was flushed with CO₂ by opening the CO₂ cylinder to remove the air, and the

shut-off valve was then closed. Then, the temperature of the extraction vessel was set and maintained with the temperature controller. After the temperature reached the set point, the extractor was pressurized with CO₂ using the high-pressure CO₂ pump. The pressure was monitored and maintained constant using the pump. The shut-off valve was kept closed for 20 min to create a static extraction time. Then, the valve was opened and the CO₂ flow rate was maintained at 1 L/min (at ambient conditions), and measured by the gas flow meter. To avoid degradation of lycopene, extracted oleoresins were collected continuously in an amber glass vial placed in a cold bath at intervals of 30, 45, 60, 90, 120, 180, and 240 min. Sample vials were flushed with nitrogen and stored at -20 °C until analyzed. The extractions were carried out at varying temperatures (40 and 80 °C) and pressures (30 and 50 MPa), and peel:seed blends (100:0, 70:30, 50:50, 30:70, 0:100, w:w). A CO₂ flow rate of 1 L/min (at ambient conditions) and feed particle size of 1 mm were used after studying the effect of varying CO₂ flow rate (1 and 2 L/min) and particle size (0.7, 1.0 and 1.4 mm) on the oleoresin yield.

3.3.4. Analysis of lycopene isomers

Lycopene analysis was performed according to the method of Longo, Leo, & Leone (2012) with some modifications. Lycopene isomers were separated by mixing 0.1 g of each oleoresin sample in 10 mL of hexane/acetone/ethanol (2:1:1, v/v/v) mixture and an equal volume of deionized water. Then this mixture was vortexed for 2 minutes and stored at 4 °C for 2 hours or until the separation of the aqueous phase from the solvent mixture occurred. Next, 20 µL of the organic layer containing lycopene isomers were injected into a reverse phase high performance liquid chromatograph (RP-HPLC, Agilent

Technologies 1100 series, Waldbronn, Germany) equipped with a quaternary pump, and a variable wavelength detector. The analysis was carried out using a C30 column (5 μ m; 4.6 mm x 250 mm, YMC, Allentown, PA, USA). A methanol/methyl butyl ether/ethyl acetate (50:40:10) mixture was used as mobile phase at a flow rate of 1 mL/min. The column temperature was set at 30 °C by a column thermostat and detection was measured at 475 nm wavelength. Detector signals were recorded using Chem-Station Chromatography software. Peaks of *cis*- and *trans*-lycopene isomers were identified by comparing the retention time in the oleoresins with the ones obtained for the authentic standards. Quantification was done using an external calibration curve prepared by plotting standard concentrations of the standards (1.9-31 μ g/mL) versus their peak areas. For *cis*- and *trans*-lycopene, the correlation coefficients (R^2) of the calibration curves were 0.9930 and 0.9962, respectively. Total lycopene was determined by summing up the contents of all *cis*- and *trans*-lycopenes, whereas *cis* and *trans*-lycopenes were determined by summing up the contents of each isomer group.

3.3.5. Separation of the oil and insoluble fractions of the extracted oleoresins

Oleoresin samples (1 g) was centrifuged at 11,000 rpm for 30 min at room temperature (20 °C). The two fractions obtained, the oily fraction (supernatant) and the insoluble fraction (pellet), were analyzed by HPLC for their *trans*- and *cis*-lycopene content.

3.3.6. Statistical analysis

Extractions were carried out three times and the results were reported as a mean \pm standard deviation. Analyses of variance (ANOVA) was used to determine statistical differences between different groups at 95% confidence interval.

3.4 Results and Discussions

3.4.1. Selection of the CO₂ flow rate and particle size

CO₂ flow rate and the particle size of the tomato seed and peel blend that will be used in the study were determined by preliminary studies. Increasing the flow rate of CO₂ from 1 to 2 L/min did not have a significant effect on the oleoresin yield (Figure 3.1a). Many studies have shown that CO₂ flow rate does not have a great influence in SC-CO₂ extractions (Machmudah, Kawahito, Sasaki, & Goto, 2007; Machmudah, Winardi, Sasaki, Goto, Kusumoto, & Hayakawa, 2012; Salgın & Salgın, 2013). The higher the CO₂ flow rate, the lower the residence time and therefore the lower the contact between the CO₂ molecules and the matrix (Machmudah et al., 2012). If this time is limited, the solvent only passes around the sample and there is a minor possibility for the CO₂ to diffuse through the pores within the tomato blend; this phenomenon is known as the channeling effect (Machmudah et al., 2012). In addition, high flow rates of CO₂ can cause compression of the mixture, generating difficulties for the CO₂ to pass through the tomato blend and resulting in less contact with the sample. For all these reasons, a CO₂ flow rate of 1 L/min was chosen; since less CO₂ was used, this value would represent economy for the process on a larger scale.

Particle size of the material is an important parameter determining the extract yield. Decreasing the size of the material could decrease the mass transfer resistance and therefore increase the recovery of the target component (Machmudah et al., 2012). However, there was no significant difference in the oleoresin yield when the particle size was reduced from 1.0 mm to 0.7 mm (Figure 3.1b). The reduction of the particle size could cause the sample to compact or agglomerate, limiting the CO₂ that encounters the sample and decreasing the yield of the oleoresins. Thus, the 1.0 mm of particle size was used after considering the oleoresin yield obtained and the reduction of the energy consumption associated with the grinding process. Based on the results of these preliminary studies, CO₂ flow rate of 1 L/min and a particle size of 1.0 mm was used in all extractions.

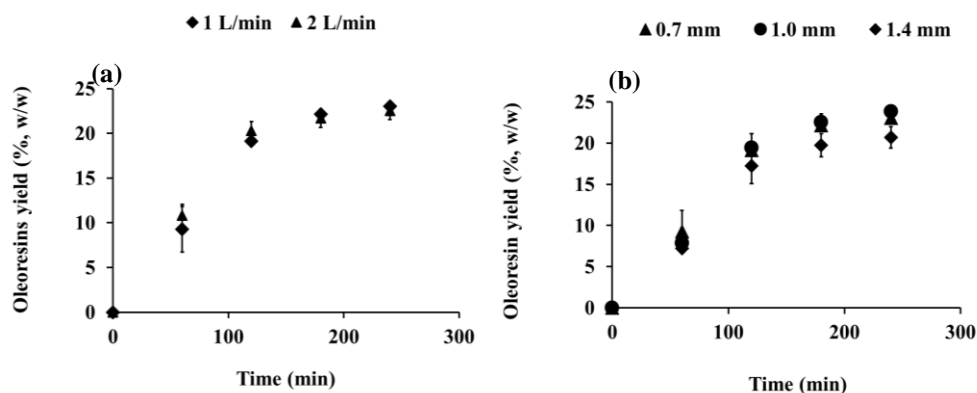


Figure 3.1 (a) Effect of CO₂ flow rate on the oleoresin yield. Conditions: T= 40 °C and P= 50 MPa; and (b) Effect of particle size on the oleoresin yield. Conditions: T= 40 °C, P= 50 MPa, and CO₂ Flow rate= 1 L/min.

3.4.2. Effect of the temperature and pressure on the oleoresin yield

Figure 3.2 presents the kinetic curves of the oleoresin extraction at varying peel:seed blends at two different pressures (30 and 50 MPa) and two different temperatures (40 and 80 °C). The curves of the peel:seed blend of 100:0 could not be plotted due to the very small amounts of oleoresins collected at each sampling point; instead, total amount of oleoresin collected at the end of the extraction was collected to determine the total yield. Unlike 100% peel, the mixtures containing tomato seed yielded considerable amount of extract because of the presence of oil in the seed. At 40 °C, oleoresin yield increased with increasing amount of seed in the blend (Figure 3.2a). The highest oleoresin yield of 18.2% was obtained with 30:70 peel:seed blend, whereas it was 16.9% for the 0:100 peel:seed blend. Even though there was no significant difference between the oleoresin yields of 30:70 and 0:100 blends ($p>0.05$), extraction rate, observed from the slope of the extraction curves, was higher for the 30:70. The highest yield of 18.2% for 30:70 blend was obtained in 180 min, time need to obtain the highest yield of 16.9% from the 0:100 blend was 240 min. In the first 100 min of the extraction, the lowest yields were obtained with the 0:100 blend.

When temperature was increased to 80 °C, similar extraction curves were obtained for all blends except for 0:100 blend (Figure 3.2b). Similar to lower temperature (40 °C), extraction rate of the 0:100 blend was lower; however, same yields were obtained at the end of 240 min. Even though a similar extraction behavior was observed at higher temperature, the highest oleoresin yield (11.0%) was significantly lower at 80 °C ($p<0.05$).

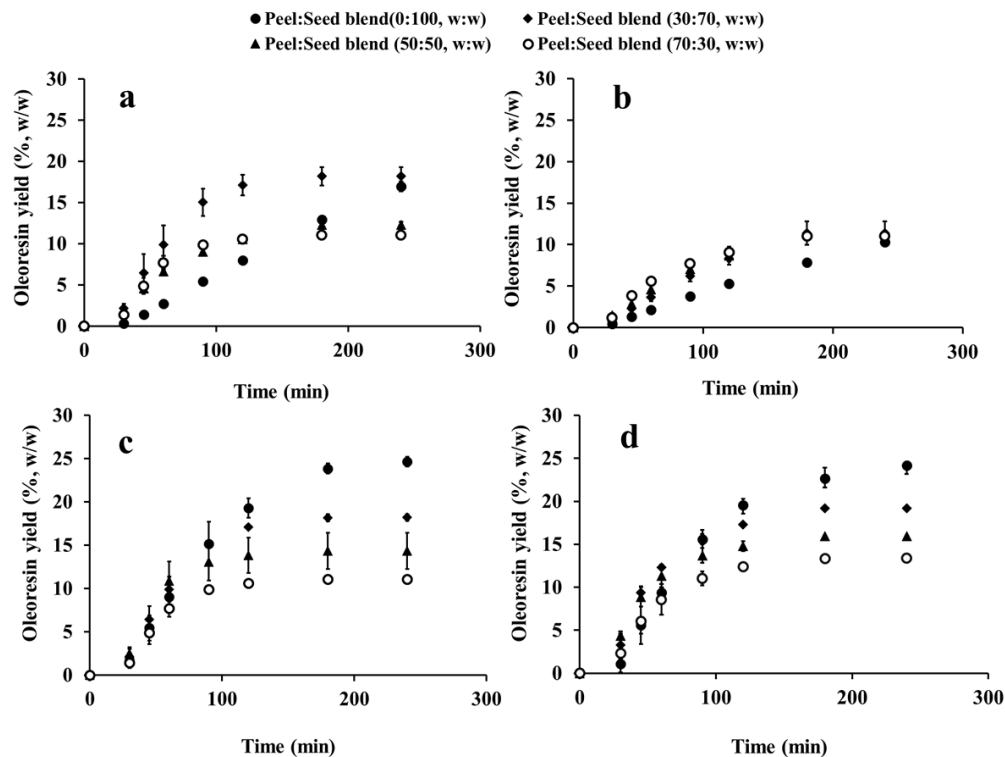


Figure 3.2. Effect of the SC-CO₂ temperature on the oleoresin yield at 30 MPa. (a) 40 °C, (b) 80 °C.

Moreover, extraction rate was lower at 80 °C compared to 40 °C. At constant pressure, increasing the temperature decreased the density of the SC-CO₂ and in turn the solubility of the tomato oleoresins in the SC-CO₂, causing a decline in the extraction rate of the oleoresins (Roy, Goto, & Hirose, 1996). Lycopene is mainly present in the peel, and the seed does not contain lycopene but it is rich in oil. The main objective of studying varying peel:seed blends was to use the oil in the seed as a co-solvent to increase the solubilization of lycopene in the oil and to improve its mobility in the matrix, and in turn to increase lycopene yields.

On the other hand, increasing pressure from 30 to 50 MPa increased the oleoresin yield significantly ($p < 0.05$) (Figure 3.2 c and 3.2 d). Increasing pressure increases the SC-CO₂ density and thus the solubility of the oleoresin in the SC-CO₂. In general, the highest oleoresin yields were obtained at low temperature (40 °C) and high pressure (50 MPa). Similar trends were observed in other studies (Roy, Goto, & Hirose, 1996; Topal, Sasaki, Goto, & Hayakawa, 2006). However, different from 30 MPa, the highest oleoresin yields were obtained from 0:100 blends. The highest yield of 24.6% was obtained at 50 MPa and 40 °C with 0:100 blend in 240 min. Under those conditions, the rate of extraction was similar for all blends in the first 60 min of the extraction and stayed almost constant after 120 min for all blends other than 0:100, and the yields were higher for the blends with higher seed content. The same trend was observed for the extractions at the same pressure but higher temperature (80 °C); the highest yield of 24.2% was obtained from 0:100 blend. These results indicated that seed oil was not completely extracted at 30 MPa at both temperatures, whereas increased pressures extracted more seed oil due to increased solvent power of the SC-CO₂ resulted from increased SC-CO₂ density. At 30 MPa, the seed oil acted as a co-solvent for the extraction of non-oil fraction from the peel, whereas at 50 MPa, increased seed oil extraction overweighed the amount of non-oil fraction, shown as increased yields at 0:100 blend (Fig. 3.2).

At higher pressures, SC-CO₂ was found be more efficient to extract the oleoresin compared to hexane. For hexane extraction, the highest oleoresin yield (19.3%) were obtained from the tomato seed (0:100 blend), whereas the lowest (6.5%) from peel (100:0). Oleoresin yield increased with increasing seed content of the blend (Figure 3.3).

SC-CO₂ was also more efficient in terms of extraction time needed for the highest yields was 4 h for SC-CO₂ extraction whereas it was 6 h for hexane extraction.

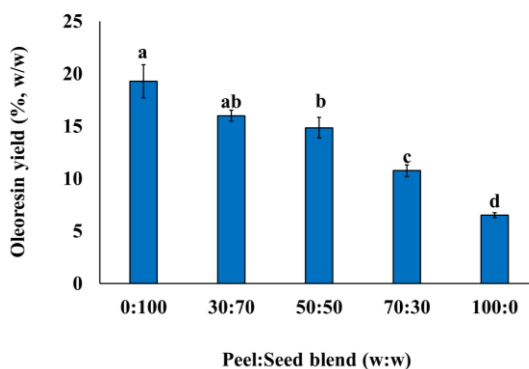


Figure 3.3. Oleoresin yield by hexane (Soxhlet) extraction.

3.4.3. Effect of the temperature and pressure on the content and isomers of lycopene in the oleoresins

Figure 3.4 shows the total lycopene content of the oleoresins extracted from the five different blends with SC-CO₂ and hexane. Although higher oleoresin yields were obtained with SC-CO₂ extraction compared to hexane, lycopene content of the hexane-extracted oleoresin was comparable to that of SC-CO₂-extracted ones. The highest quantities of lycopene were found in the 100% peel oleoresins (100:0 blend), and there was not any detectable lycopene in the 100% seed oleoresin (0:100% blend). Furthermore, these results showed that for tomato peel oleoresins, the total amount of lycopene extracted was 238.7 mg per 100 grams of oleoresin for the hexane extraction, whereas the total lycopene detected with the SC-CO₂ extraction method at 30 MPa/80 °C and 50 MPa/80 °C was 193.4 mg and 258.9 mg per 100 grams of oleoresin, respectively.

However, there was no significant difference in the total lycopene amount among these three conditions ($p>0.05$). The results showed that the lycopene amount was enhanced by increasing both temperature and pressure. As was mentioned, at high pressures the density of SC-CO₂ increases, which increases its solvating power and therefore the solubility of the lycopene in the solvent (Topal, Sasaki, Goto, & Hayakawa, 2006). These results were also explained by Brunner, who reported that at high pressures the SC-CO₂ polarity changes and it behaves as chloroform, while at low pressures, the polarity performs similarly to hexane (Brunner, 1994). Carotenoids, including lycopene, have more affinity and higher solubility in chloroform in contrast to hexane; consequently, higher percentages of lycopene were extracted with SC-CO₂ at 50 MPa (Cadoni, De Giorgi, Medda, & Poma, 1999).

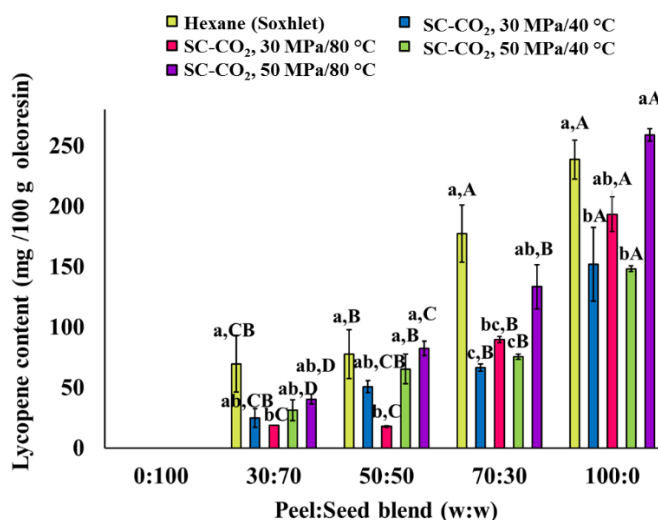


Figure 3.4. Total lycopene content of the extracted oleoresins. Data are mean \pm SD of two measures from three independent samples. Different capital letters represent significant differences among the peel:seed blend groups at the same extraction conditions ($p<0.05$),

and different lower-case letters represent significant differences within the same peel:seed blend group ($p < 0.05$).

Moreover, the results showed a positive effect with the increase of temperature. This effect is more significant than that of pressure because lycopene content increased dramatically by increasing temperature when compared with the increase of pressure. This effect was more pronounced for the blends containing more than 50% peel. Usually, an isobaric increase in temperature decreases the density of the supercritical solvent and hence its solubility, resulting in lower yields of the solute (Topal, Sasaki, Goto, & Hayakawa, 2006). Nevertheless, the temperature increase creates an increase in vapor pressure and volatility of lycopene, producing an increment of lycopene solubility and higher lycopene yield (Machmudah et al., 2012; Topal, Sasaki, Goto, & Hayakawa, 2006). Consequently, it seems that the magnitude of the vapor pressure and volatility is greater than the density change. Additionally, increasing temperatures led to the breaking of vegetable structures that contain lycopene, improving the mass transfer of the tomato byproducts matrix and from this matrix to the SC-CO₂, and causing higher recoveries of lycopene (Vasapollo, Longo, Rescio, & Ciurlia, 2004). As a result, 100% peel (100:0 blend) extracted with both hexane and SC-CO₂ at 50 MPa/80 °C contained highest amount of lycopene even though its oleoresin yield was only 5.3%.

The lycopene isomers detected in all oleoresin samples were principally 15-*cis*-, 13-*cis*-, 9-*cis*-, 5-*cis*-, others *cis*-isomers and all-*trans*-lycopene. The hexane-extracted oleoresins contained principally all-*trans*-lycopene (Figure 3.5). On the other hand, the *trans*- and *cis*-lycopene composition of the SC-CO₂-extracted oleoresins depended on the extraction conditions (Figures 3.6 and 3.7). The hexane extraction yielded oleoresins with

a high content of *trans*-lycopene for all types of blends, whereas the SC-CO₂ extractions produced different types of oleoresins in terms of *trans*- and *cis*-lycopene composition. The *cis*-lycopene content represented up to 34% of total lycopene (78.3 mg per 100 grams of oleoresin) in the hexane-extracted oleoresins (Fig. 3). The percentage of *cis*-lycopene detected in the oleoresins extracted by SC-CO₂ was 34% (51.2 mg and 49.4 mg per 100 grams of oleoresin) of the total lycopene when the extraction was performed at 40 °C and both pressures (30 and 50 MPa) respectively. The *cis*-lycopene content varied when the temperature was increased to 80 °C; 67% and 56% of *cis*-lycopene (128.7 mg and 143.6 mg per 100 grams of oleoresin) at 30 and 50 MPa, respectively.

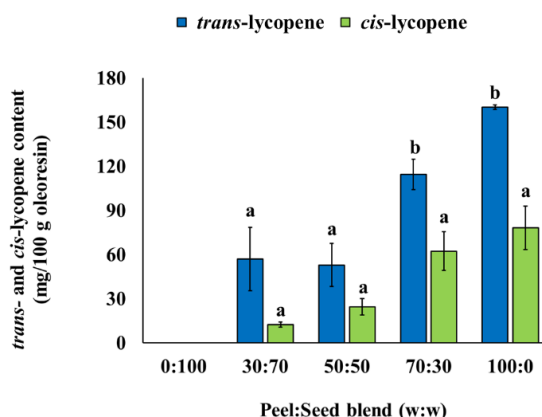


Figure 3.5. *Trans*- and *cis*-lycopene content of the oleoresins extracted by hexane (Soxhlet). Data are mean \pm SD of two measures from three independent samples. Different lower-case letters represent significant differences in *trans*- and *cis*-lycopene within the same the peel:seed blend group ($p < 0.05$).

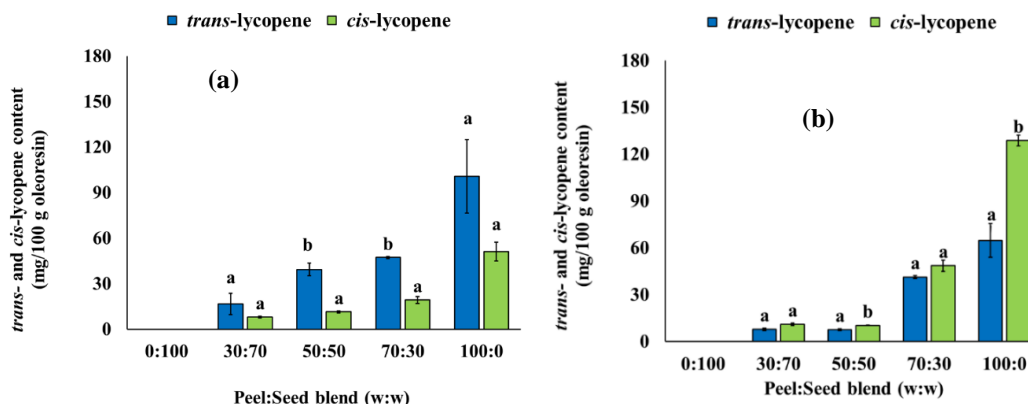


Figure 3.6. *Trans*- and *cis*-lycopene content of the oleoresins extracted by SC-CO₂ at 30 MPa. (a) 40 °C, (b) 80 °C. Data are mean \pm SD of two measures from three independent samples. Different lower-case letters represent significant differences in *trans*- and *cis*-lycopene within the same the peel:seed blend group ($p < 0.05$).

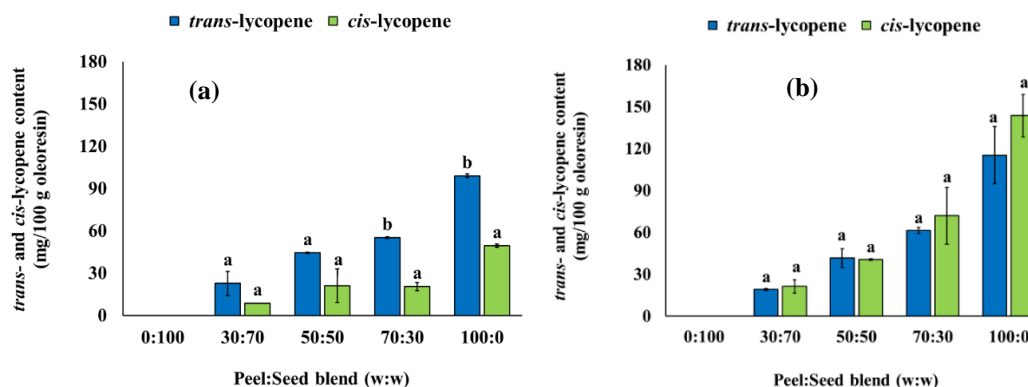


Figure 3.7. *Trans*- and *cis*-lycopene content of the oleoresins extracted by SC-CO₂ at 50 MPa. (a) 40 °C, (b) 80 °C. Data are mean \pm SD of two measures from three independent samples. Different lower-case letters represent significant differences in *trans*- and *cis*-lycopene within the same the peel:seed blend group ($p < 0.05$).

Consequently, the *cis*-lycopene concentration in the oleoresins extracted at both SC-CO₂ pressures and high temperature (80 °C) was higher compared to the SC-CO₂

oleoresins at low temperature (40 °C) as well as those that were hexane-extracted. At lower temperature (40 °C) at both pressures, lycopenes contained higher amount of *trans* isomer. When temperature was increased to 80 °C, oleoresins contained higher amount of *cis*-lycopene. However, this effect was more pronounced at 30 MPa/80 °C for the 100:0 blend, which the *cis* isomer content of the lycopene was 67% (128.7 mg per 100 grams oleoresin). Even though the total lycopene was higher in the oleoresin extracted at 50 MPa/80 °C (Figure 3.7b), the *cis*-lycopene content of the one extracted at 30 MPa/80 °C was higher ($p < 0.05$) (Figure 3.6b).

High *cis*-isomer profile of the SC-CO₂-extracted oleoresin could be explained by considering a favorable solubility of *cis*-isomers in SC-CO₂ at certain conditions or through a specific isomerization phenomenon that occurs in SC-CO₂. The differences in the solubility of *cis*- and *trans*-lycopene in SC-CO₂ depends on the pressure and temperature. As explained earlier, density of the CO₂ has a direct relationship with pressure; increasing the pressure will cause an increase in the density of the SC-CO₂. On the other hand, the rising of the temperature will produce a decrease in the SC-CO₂ density, and increase in the solute's vapor pressure. Total lycopene content showed that at a high pressure and high temperature, the temperature had a more considerable effect; consequently, solubility in the SC-CO₂ has a substantial effect over density. Moreover, lycopene composition of the oleoresins showed a high content of *cis*-lycopene at high temperatures, while large amounts of *trans*-lycopene were determined at low temperatures. Hence, it seems that the *cis*-lycopene content of the oleoresin could be increased with high temperatures and low pressures. (Gómez-Prieto, Caja, Herraiz, & Santa-María, 2003) stated that differences in the solubility of *cis*- and *trans*- forms in the

SC-CO₂ rather than their trend to undergo isomerization may explain the different isomer profile in the tomatoes oleoresins. They found that *trans*-lycopene extraction is favored at high SC-CO₂ densities while *cis* content decreased.

Some other studies proposed that the lycopene isomer profile is linked to an isomerization process that occurs during the extraction. How lycopene undergoes from *trans*- to *cis*- isomers is not totally understood; however, there are some theories that attempt to explain this mechanism. To explore this, it should be mentioned that the isomerization process not only promotes the formation of *cis*-isomers, but also competes with degradation, and the latter is faster in high temperature environments (>50 °C) and with a long heating time (Lee & Chen, 2002). Isomerization and stability of lycopene can be achieved under the use of different aids such as heat, active surfaces, light, the presence of oxygen, and specific chemical reactions (Shi, Dai, Kakuda, Mittal, & Xue, 2008; Spanos, Chen, & Schwartz, 1993). Among all these different means, heating and light have been amply studied because these are the major factors to affect food quality in processing (Shi, Dai, Kakuda, Mittal, & Xue, 2008). Some studies investigated the influence of heating and light on the isomerization process of lycopene as a pure compound (standard), and in some tomato matrices such as raw tomato, tomato cooking preparations, tomato-based products, powder mixture of tomato and tomato seed, and non-isomerized tomato raw extracts (Colle et al., 2010; de Alvarenga et al., 2017; Lambelet, Richelle, Bortlik, Franceschi, & Giori, 2009; Lee & Chen, 2002; Longo et al., 2012; Schierle et al., 1997). In fact, catalytic thermal isomerization and photoisomerization are the principal methods used to increase the content of *cis*-lycopene in natural lycopene. Chasse et al. (2001) suggested that in lycopene, all *trans*- and some

cis-isomers are very close on the free energy scale isomerization, and the isomers interconversion requires a significant amount of energy of activation. This energy can be reached with the presence of a catalyst until obtaining the isomerization equilibrium. (Bortlik, Richelle, Lambelet, & Saucy, 2005) studied this equilibrium in tomato oleoresins rich in lycopene, and they found that this energy is achieved by photoisomerization in CH₂Cl₂ and using iodine as a catalyst. Additionally, (Sun, Yang, Li, Aboshora, Raza, & Zhang, 2016) reported that *trans-cis* isomerization of lycopene catalyzed by iodine-doped titanium dioxide (I-TiO₂) can be performed with results up to 80% of *trans-cis* conversion. Nevertheless, these methods are not suitable at the industrial scale due to some drawbacks such as complexity on the separation of the catalyst from the final product (non-edible I₂ is used in photoisomerization), and because non-convenient and specialized reactors are required in larger scale (Sun, Yang, Li, Aboshora, Raza, & Zhang, 2016).

Because SC-CO₂ extractions are carried out in closed pressurized vessels, it is challenging to investigate the transformations occurring during the extraction. It can be speculated that SC-CO₂ facilitates the rotation of conjugated double bonds on the *trans*-lycopene to *cis* isomers. A similar explanation for the *cis* isomerization of *beta*-carotene was done by Spanos, Chen, & Schwartz (1993). Moreover, the isomerization process may be also promoted by the contact between the lycopene molecules solubilized in SC-CO₂ and the metal surface of the extraction vessel.

The analysis of the *trans-cis*-lycopene was also expanded to the oily and insoluble fractions of the oleoresins extracted with hexane, and compared with the fractions of the oleoresins extracted with SC-CO₂ at 30 MPa/80 °C, and 50 MPa/80 °C (Figure 3.8). The

profiles of the lycopene isomers in both fractions of the three oleoresins were similar, but the quantities were different. *Cis*-lycopene was mostly present in the oily fractions while *trans*-lycopene was more predominant in the insoluble fraction. This outcome is significant because the lipids present in the oleoresins affect the lycopene stability in two opposite manners; the oil may protect the lycopene from degradation, but also can increase its deterioration due to the influence of some lipids present in the oil that oxidize efficiently (Colle et al., 2010). Very recently, it was reported that the *trans* to *cis* isomerization of lycopene was significantly increased in the presence of oil during heat treatment (Honda et al., 2017). However, we have not observed such a behavior in this study.

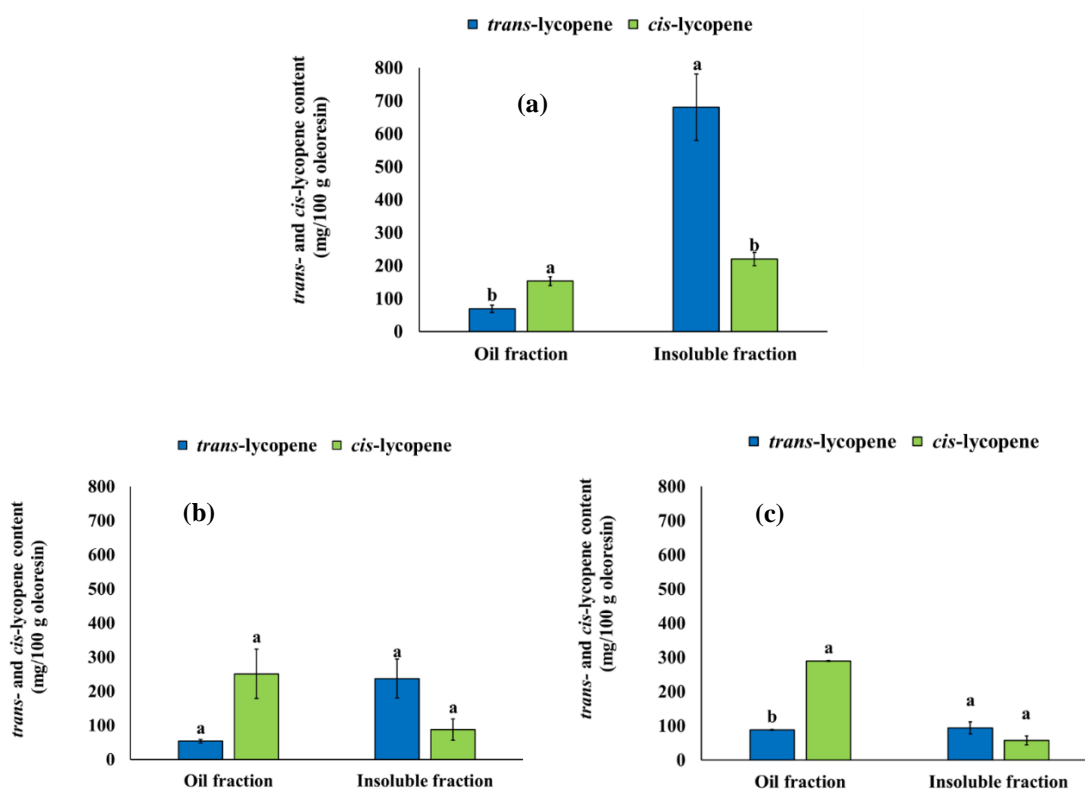


Figure 3.8. *Trans*- and *cis*-lycopene composition of the oil and insoluble fractions of the tomato peel oleoresin obtained by (a) hexane (Soxhlet) extraction, (b) SC-CO₂ extraction

at 30 MPa/80 °C, and (c) SC-CO₂ extraction at 50 MPa/80 °C. Data are mean \pm SD of two measures from three independent samples. Different lower-case letters represent significant differences of *trans*- and *cis*-lycopene within the same fraction group ($p < 0.05$).

As shown in Figures 3.5, 3.6, and 3.7, the *cis*-lycopene content was not higher in the blends containing higher amount of seed, which contains oil, extracted with both hexane and SC-CO₂, rather, it was higher in the 100% peel that does not contain oil. The *cis*-lycopene content of the oleoresins extracted by hexane were 68 and 24% (152.9 and 220.5 mg per 100 grams oleoresin) in the oil and insoluble fractions, respectively. In the SC-CO₂ oleoresins obtained at 30 MPa/80 °C the *cis*-isomers content was 82% (251.2 mg per 100 grams oleoresin) in the oil fraction and 26% (87.9 mg per 100 grams oleoresin) in the insoluble fraction, and in the oleoresins obtained at higher pressure, 50 MPa/80°C, this composition consisted of 76 and 38% of *cis*-lycopene (289.7 and 57.9 mg per 100 grams oleoresin) in the oil and insoluble fractions, respectively (Figure 3.8). Therefore, the insoluble fractions were found to be mostly *trans*-lycopene, while the oil fractions were enriched in *cis*-lycopene.

3.5 Conclusions

SC-CO₂ was found to be an environmentally friendly method to obtain *cis*-lycopene-rich oleoresins from tomato processing byproducts. The oleoresins with the highest content of *cis*-isomers of lycopene were obtained at lower SC-CO₂ pressure. The oily fraction of the oleoresin contained higher *cis*-lycopene than the insoluble fraction. After a simple centrifugation, an oil fraction containing up to 82% *cis*-lycopene can be

obtained after SC-CO₂ extraction. Since *cis* form of the lycopene is more bioavailable than the *trans* form, SC-CO₂ has the potential to create highly bioavailable lycopene formulations during extraction stage. Because of its biological properties, the *cis*-lycopene-rich oleoresin can be used as a food ingredient to develop health and wellness promoting foods and reduce the food waste generated by the tomato processing industry.

Acknowledgements

We would like to thank ConAgra Brands for providing tomato seed and peel samples. We also thank Ric Gonzalez and Indarpal Singh for the analytical support.

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Chapter 4 . In vitro bioaccessibility of the lycopene in the tomato peel oleoresin obtained by supercritical carbon dioxide extraction

4.1 Abstract

The objective of this study was to determine the bioaccessibility of the lycopene in the tomato peel oleoresin obtained by supercritical carbon dioxide (SC-CO₂) and hexane using a simulated digestion method. Tomato peel oleoresins were obtained by SC-CO₂ at 30 MPa and 80 °C. SC-CO₂ extraction increased the bioaccessibility of lycopene approximately 2-folds in the oleoresin and 2.4-folds in the oil fraction compared to hexane extraction ($p < 0.05$). The bioaccessibility of the hexane-extracted oleoresin was 2.7%, whereas it was 5.2% for the SC-CO₂-extracted oleoresin. The bioaccessibility of the lycopene in the oil fraction was 1.7 and 4.0% for the hexane and SC-CO₂-extracted oleoresins, respectively. Lycopene concentration in the digesta increased when the tomato oleoresin was used compared to oil fraction for both SC-CO₂ and hexane extractions. After digestion of tomato peel oleoresin, lycopene concentration in the final digesta was 4.6 and 13.4 µg/g for hexane- and SC-CO₂-extracted oleoresin, respectively. Oil fraction of the tomato peel oleoresin released concentrations of lycopene of 1.3 µg/g for hexane and 3.0 µg/g for SC-CO₂ extracted oleoresin. Furthermore, fatty acid composition analysis showed oleoresins rich in linoleic and oleic acids, and oxidative test showed good stability of tomato peel oleoresins.

Keywords: Bioaccessibility, Tomato peel oleoresin, Supercritical carbon dioxide, Fatty acid composition, Oxidative stability.

4.2 Introduction

Clinical studies have shown that the dietary intake of lycopene decreased the risk of chronic diseases such as cardiovascular risk, atherosclerosis, and some types of cancer such as prostate, stomach, and lung cancer (Giovannucci et al., 1995; Palozza et al., 2010; Rao & Agarwal, 2000; Ried & Fakler, 2011; Svelander et al., 2010). In order to attain the health properties of lycopene, it must be absorbed into the blood stream, distributed, and metabolized by the organism; this means that lycopene needs to be bioavailable. The concept of bioavailability is related to the amount of nutrient release from the food matrix that is available to the body for its absorption and subsequent utilization (Shi & Maguer, 2000; Svelander et al., 2010).

It is noteworthy to mention that humans' dietary lycopene uptake occurs between 10 and 30%, so its absorption is never complete (Holzapfel et al., 2013). Many factors such as food matrix, fat content, dietary fiber, and interactions with other food components have been found to influence carotenoids absorption, including lycopene, after their ingestion.

Lycopene absorption from raw tomatoes is very low (Svelander et al., 2010) compared to the absorption from processed tomato foods (Gartner, Stahl, & Sies, 1997; Svelander et al., 2010). The thermal and mechanical steps associated with processing can disrupt the cellular structures that contain lycopene, increasing its bioavailability (Svelander et al., 2010). Carotenoids, including lycopene, exist within the chromoplast cells. Chromoplast organelles and cell walls in tomatoes constitute the first physical barriers for lycopene release from tomato matrix (Palmero, Lemmens, Hendrickx, & Van Loey, 2014). After lycopene is released from the food, it is dissolved into lipid droplets

within the stomach and duodenum, then transported into the small intestine micelles (Castenmiller & West, 1998; Clinton, 1998); afterwards, through the lymph system micelles are transported to the liver, then to the plasma by lipoproteins to be finally distributed to designated organs (Parker, 1996). Carotenoid composition of the ingested food may influence the amount of lycopene during intestinal absorption and metabolism. One study even suggests that *beta*-carotene can increase lycopene concentration in men (Wahlqvist et al., 1994). Nevertheless, a reduction in the lycopene content in serum and low-density lipoproteins was observed after *beta*-carotene supplementation in other research (Castenmiller & West, 1998; Gaziano et al., 1995).

In addition to absorption, lycopene bioavailability in humans has been estimated by measuring the lycopene levels in plasma following the ingestion of lycopene-rich product (Garrett, Failla, & Sarama, 1999). Such bioavailability can be studied through animal and human models. The limitations of using these models, such as cost and specialized needs, have resulted in the selection of in vitro studies. These methods are becoming more popular because they are rapid, less labour intensive, and also based on their cost-effective results in predicting in vivo levels of bioavailability (Dehghan-Shoar, Mandimika, Hardacre, Reynolds, & Brennan, 2011; Eboul, Ichelle, & Loi, 2006; Garrett, Failla, & Sarama, 1999).

Lycopene bioaccessibility is calculated as the fraction of lycopene in the meal that is released into the digesta phase throughout the process of simulated digestion. This release of lycopene, namely bioaccessibility, is the first step to measure lycopene bioavailability (Goñi, Serrano, & Saura-Calixto, 2006). For this reason, in vitro digestion

models may provide a better understanding of the bioavailability of lycopene from the tomato matrix and the factors that determine its availability (Goñi et al., 2006).

Previously (in Chapter 3), we have shown that SC-CO₂ extraction yields tomato peel oleoresin rich in *cis*-lycopene, whereas the traditional hexane extraction yields *trans*-lycopene-rich oleoresin. As explained in Chapter 3, the most abundant isomeric form of lycopene in human tissue and blood is *cis*- configuration accounting for 58–73% of total lycopene in human serum, and up to 88% in benign or malignant prostate tissue (Clinton et al., 1996; Singh & Goyal, 2008); hence, it has been suggested that *cis*-isomers of lycopene are preferably absorbed and have a greater bioavailability than the all-*trans*-lycopene form (Clinton, et al., 1996). Therefore, we hypothesized that the lycopene in the SC-CO₂-extracted tomato peel oleoresin is more bioaccessible than the lycopene in the hexane-extracted oleoresin.

The objective of this work was to assess the lycopene bioaccessibility from tomato peel byproduct oleoresin obtained by SC-CO₂ extraction using a simulated digestion model, and to compare with the bioaccessibility of the hexane-extracted oleoresin. Moreover, the bioaccessibility of the oil fraction of the oleoresins was determined. Furthermore, oxidative stability and fatty acid composition of tomato peel oleoresins were also investigated.

4.3 Materials and methods

4.3.1 Materials

Tomato peel oleoresins extracted by SC-CO₂ at 30 MPa and 80 °C (SC-CO₂-30/80) and hexane extraction using Soxhlet method, as well as their respective oil fraction (4 total samples) were used for simulated digestion. SC-CO₂- and hexane-extracted tomato peel oleoresins were obtained as described in Sections 3.3.2 and 3.3.3 in the Chapter 3, and their oil and insoluble fractions were obtained by the method described in the Section 3.3.5 in the Chapter 3. Oleoresin extracted with SC-CO₂ at 30 MPa/80°C was used because it contained the highest amount of *cis*-lycopene.

For digestion, the alpha-Amylase enzyme was acquired from MP Biomedicals, USA, and Lipase A “Amano” 12 enzyme was purchased from Amano Enzyme Inc. Japan. All other enzymes were obtained from Sigma Aldrich, USA. These enzymes were pepsin (from porcine gastric, 3,200-4,500 units/mg solids), pancreatin (from porcine pancreas, 8 USP), and lipase (from porcine pancreas, type II, 100-500 units/mg protein). Bile extract porcine (Sigma product) was also used. Fatty acid and lycopene (>90% purity) standards were also purchased from Sigma–Aldrich, USA. HPLC-grade solvents, including hexane, acetone, ethanol, ethyl acetate, methanol, methyl butyl ether, and ethyl acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other reagents and chemicals were of analytical or chromatographic grade.

4.3.1.1. Simulated digestion fluids

Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) electrolyte stock solutions were prepared according to Minekus et al. (2014). Simulated digestion electrolyte stock solutions were composed of various salts (potassium chloride, monopotassium phosphate, sodium bicarbonate, sodium chloride, magnesium chloride hexahydrate, and ammonium carbonate) (Table 4.1).

Table 4.1 Concentrations of electrolytes in Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF).

Constituent	Stock conc.		SSF (pH 7)		SGF (pH 3)		SIF (pH 7)	
			Volume of stock	Conc. in SSF	Volume of stock	Conc. in SGF	Volume of stock	Conc. in SIF
	g/L	mol/L	mL	mmol/L	mL	mmol/L	mL	mmol/L
KCl	37.3	0.5	7.55	15.1	3.45	6.9	3.4	6.8
KH ₂ PO ₄	68	0.5	1.85	3.7	0.45	0.9	0.4	0.8
NaHCO ₃	84	1	3.4	13.6	6.25	25	21.25	85
NaCl	117	2	—	—	5.9	47.2	4.8	38.4
MgCl ₂ (H ₂ O) ₆	30.5	0.15	0.25	0.15	0.2	0.1	0.55	0.33
(NH ₄) ₂ CO ₃	48	0.5	0.03	0.06	0.25	0.5	—	—

*The final volume of each simulated digestion fluid stock solution was made up to 200 mL with deionized water.

4.3.2 Fatty acid composition

Fatty acid composition of the oil fraction of tomato peel oleoresins was analyzed using a gas chromatograph-mass spectrometer (GC-MS; Agilent 7890A-Agilent 5975C, Santa Clara, CA, USA). The tomato peel oleoresin (50 µL) was mixed with

trimethylsulfonium hydroxide solution (100 μL) containing butylated hydroxyl toluene (0.1% v/v) and then incubating at room temperature for 2 hours and shaking at 60 rpm to form fatty acid methyl esters (FAME). After the reaction, 300 μL of hexane was added to each tube. Then, the FAMEs were separated onto an Agilent HP-Innowax capillary column (30m \times 0.25mm \times 0.25m) at 275 $^{\circ}\text{C}$. H_2 was used as carrier gas (0.75 mL/min). Oven temperature was hold at 185 $^{\circ}\text{C}$ for one minute, then increased to 240 $^{\circ}\text{C}$ at 7 $^{\circ}\text{C}/\text{min}$, and kept at 240 $^{\circ}\text{C}$ for 8 minutes. Analytes eluting from the column were detected using the mass spectrometer (scanning m/z 40 – 600, 1.35 scans/s), and identified by comparing mass spectra and retention times against those of entries in the NIST08 MS library and commercial authentic standards. The experiments were performed under light excluding conditions. Having identified the compounds in each sample, the relative abundance of each fatty acid in each sample was determined using a gas chromatography-flame ionization detection system that was identical to the GC-MS system except that it used a flame ionization detector (Agilent) held at 275 $^{\circ}\text{C}$ that burned H_2 (45 mL/min) in compressed air (375 mL/min). The traces from the GC-FID system were integrated using the Agilent ChemStation software, and the total area of all peaks was calculated. The relative contribution of each compound's peak to the total area was used to determine the relative abundance of each compound.

4.3.3 Oxidative stability

Oxidative stability of the tomato peel oleoresin samples was determined. Samples were analyzed for peroxide value to follow the formation of primary oxidation products, and anisidine value for the secondary oxidation products, as described below.

4.3.3.1 Peroxide value

Peroxide value (PV) was determined according to Bae & Lee (2008) with some modifications. An aliquot (1-30 mg) of tomato peel oleoresin was diluted with chloroform/methanol (7:3, v/v) and vortexed well, so that the final absorbance of the reacted sample remained below 1.0 cm^{-1} . The mixture (50 μL) was then mixed with 8.0 mL chloroform/methanol (7:3, v/v) solution in a glass tube and vortexed for 10 seconds, followed by the addition of 50 μL ammonium thiocyanate solution and vortexed for 10 seconds, and then 50 μL ferrous chloride solution was added and vortexed for 10 seconds. The final mixtures were then incubated for 20 min in a dark cabinet at room temperature. After incubation, the absorbance of the samples was measured with a UV/Vis spectrophotometer at 505 nm. PV of the samples were determined from an external calibration curve PV was expressed as milliequivalents (meq) hydroperoxide per kg of oleoresin (1 meq = 0.5 mmol).

4.3.3.2 Anisidine value

Anisidine value (AV) was determined according to AOCS Official Method Cd 18-90 with some modifications (Society & Firestone, 1994). The iso-octane is used as the reference (blank) solution. Absorbance of the mixture (A1) is measured at 350 nm using a UV/Vis spectrophotometer. An aliquot of sample (2.5 mL) is then transferred to test tubes, and 0.5 mL of p-anisidine solution (0.25%, w/v, solution in glacial acetic acid) is added to each test tube. The test tubes were then capped, vortexed, and allowed to react for 10 min in a dark cabinet at ambient temperature. The absorbance (A2) was measured at 350 nm against the iso-octane blank containing p-anisidine.

AV was calculated by Eq. (4.1):

$$AV = \frac{25 \times (1.2A2 - A1)}{\text{sample weight (g)}} \quad (4.1)$$

4.3.4 Simulated digestion

To assess the lycopene biaccessibility of tomato peel oleoresin, a sequential simulated digestion model was developed based on the method described by Minekus et al. (2014) with some modifications. Samples of 1 g were weighed into Erlenmeyer flasks to undergo oral, gastric and intestinal digestions, successively. The flasks were covered with aluminum foil to avoid light-degradation of the lycopene. To simulate the oral phase, 3.5 mL of SSF, 12.5 μ L of CaCl₂ (0.3 M), 0.987 mL of water and 0.5 mL of alpha-amylase (11.75 mg in 2.5 mL SSF) were added to the flasks. The mixtures were incubated for 30 seconds at 37 °C in a water bath shaking at 150 rpm. Then, to mimic the gastric phase, 3.25 mL of SGF was added into the flask that contained the mixture from

oral step and the pH of the solution was adjusted to pH 3.0 with 1 M HCl. Afterwards, 0.5 mL of porcine pepsin (15.6 mg in 1.25 mL SGF), 0.5 mL of fungal lipase (18.9 mg in 2.5 mL SGF), 2.5 μ L of CaCl_2 (0.3 M), 0.9 mL water were added. The mixtures were incubated at 37 °C and agitated at 100 rpm for 2 h. The pH was readjusted to 3.0 with 1 M HCl throughout the gastric digestion. To simulate the intestinal phase, after the gastric digestion, 6.125 mL SIF was added to the mixture and the pH was adjusted to 7.0 with 1 M NaOH. Then, 1.25 mL of porcine pancreatin and porcine lipase (36 mg and 188 mg respectively in 2.5 mL SIF), 0.625 mL fresh porcine bile (0.195 g in 1.5 mL SIF), 20 μ L CaCl_2 , and 1.95 mL deionized water were added. The intestinal phase of the samples was performed at 37 °C in a water bath shaking at 100 rpm for 2 h. The pH was readjusted to 7.0 with 1 M NaOH throughout the intestinal digestion. To stop digestion after the completion of the intestinal step, the flasks that contain the digested samples were immersed in an ice bath. These digested samples will be called digesta from now on.

4.3.4.1 Determination of the lycopene bioaccessibility

The digesta was centrifuged at 4 °C at 4000 rpm for 90min (Allegra X-15R, Beckman Coulter IN, USA). After centrifugation, three fractions were obtained from each digested sample (oily, bioaccessible, and pellet fractions). The bioaccessible fractions were separated from each digested sample and stored at -80 °C for future analysis. All the samples were protected from light throughout the digestion process and analysis. Total lycopene were quantified from the bioaccessible fraction of the digesta by HPLC using the procedure described in the Section 3.3.4 in the Chapter 3 and the bioaccessible lycopene was accounted using the method proposed by Alemany et al. (2013). The bioaccessibility

(%) of lycopene was calculated using Eq. 4.2. Triplicate digestions of each tomato peel oleoresin and oil fraction were made.

$$\text{Bioaccessibility (\%)} = \frac{\text{Lycopene in the bioaccessible fraction}}{\text{Total lycopene included}} * 100 \quad (4.2)$$

4.3.5 Statistical analysis

Data are presented as mean \pm standard deviation based on triplicate experiments and analyses. The data were analyzed by ANOVA to determine statistical differences at 95% confidence interval

4.4 Results and discussions

4.4.1 Oxidative stability of tomato peel oleoresins

Oxidative stability of the tomato peel oleoresins (SC-CO₂-30/80 and Hexane) was evaluated by peroxide and anisidine value to determine the effect of extraction conditions on the oxidation of the samples. Peroxide value is an indicator of the formation of primary oxidation products (hydroperoxides), whereas anisidine value indicates the formation of secondary oxidation products such as aldehydes, ketones, and alcohols that are formed by degradation of the primary oxidation products. No primary and secondary oxidation products formation was detected in both samples, indicating that both extraction method does not cause any oxidation in the oleoresins.

4.4.2 Fatty acid composition in tomato peel oleoresins

Lipid matrix composition is one of the key factors that affects the bioaccessibility of the carotenoids (including lycopene) from natural sources such as fruits and vegetables. It is well-known that for increasing carotenoids absorption, a lipid environment is preferable, since lipids play a fundamental role in the lycopene release, transfer to the fat phase of the gastrointestinal content and its solubilization in micelles (Colle, Van Buggenhout, Lemmens, Van Loey, & Hendrickx, 2012). In most cases, formulations of lycopene in an oily matrix may offer a better bioaccessibility (Yeum & Russell, 2002). Tomato peel oleoresin is a tomato matrix rich in oil. Additionally, the variety and amount of fat present in the compound can affect its accessibility (Fernández-García, Rincón, & Pérez-Gálvez, 2008).

Table 4.2 shows the fatty acid composition of the tomato peel oleoresins obtained by SC-CO₂ and hexane extractions. Both oleoresins were composed principally by saturated fatty acids such as palmitic and stearic acids, monounsaturated fatty acid such as oleic acid, and polyunsaturated fatty acids (PUFA) as linoleic and linolenic acids. Tomato peel oleoresins were rich in linoleic acid, which represented up to 59% of the total lipids, followed by oleic (20.4-21.2%), palmitic (12.7-12.8%), stearic (4.6%) and linolenic (2.6-2.7%) acids (Table 4.2). The lipid nature is very important due to its influence on the lycopene stability and isomer composition (Colle et al., 2010). Although, the amount of linoleic and linolenic detected in these samples could represent a likely susceptibility to oxidation of these PUFAs during extraction, no oxidation was detected during extraction. Colle, Van Buggenhout, Lemmens, Van Loey, & Hendrickx (2012) found that matrices rich in oleic and/or linoleic fatty acids rather than matrices containing

lauric (12:0) and palmitic fatty acids resulted in higher lycopene bioaccessibility. Similar trends were found by Huo, Ferruzzi, Schwartz, & Failla (2007) who reported better micellarization of lycopene when was ingested with long chain triglycerides (TGs) instead of medium chain TGs. In addition, some studies have suggested that a diet rich in polyunsaturated fatty acids rather than monounsaturated fatty acids can increase the beta-carotene response in serum (Castenmiller & West, 1998). However, there are no investigations that have studied such phenomenon in lycopene.

Table 4.2 Percentage of fatty acids of tomato peel oleoresins obtained by SC-CO₂ and hexane extraction. Values are expressed as percentage of the total lipids. Data are mean \pm SD of three independent samples.

Fatty acids	Percentage (%)	
	Hexane	SC-CO ₂ (30/80) ^a
Palmitic acid (16:0)	12.8 \pm 0.8	12.7 \pm 0.4
Stearic acid (18:0)	4.6 \pm 0.4	4.6 \pm 0.3
Oleic acid (18:1)	21.2 \pm 0.5	20.4 \pm 0.4
Linoleic acid (18:2)	58.8 \pm 1.6	59.7 \pm 0.8
Linolenic acid (18:3)	2.6 \pm 0.1	2.7 \pm 0.1

^a Oleoresin obtain by SC-CO₂ extraction at 30 MPa and 80 °C.

4.4.3 Bioaccessibility of lycopene

The capacity of the digestive process in releasing lycopene from the food matrix, namely bioaccessibility, may be the first tool to screen the lycopene bioavailability.

Lycopene bioaccessibility of the oleoresin and the oil fraction of the oleoresin is presented in Figure 4.1. SC-CO₂ extraction increased the bioaccessibility of lycopene approximately 2-folds in the oleoresin and 2.4-folds in the oil fraction compared to hexane extraction ($p < 0.05$). The bioaccessibility of the hexane-extracted oleoresin was 2.7%, whereas it was 5.2% for the SC-CO₂-extracted oleoresin. The bioaccessibility of the lycopene in the oil fraction was 1.7 and 4.0% for the hexane and SC-CO₂-extracted oleoresins, respectively. However, there was no significant difference between the bioaccessibility of the lycopene from the oleoresin and oil fractions extracted by both hexane and SC-CO₂ (Fig. 4.1).

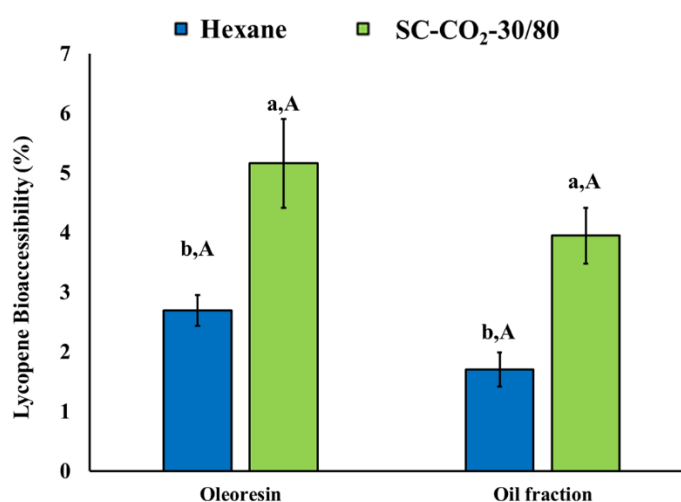


Figure 4.1. Bioaccessibility of tomato peel oleoresin and oil fraction of tomato peel oleoresins obtained with hexane (Soxhlet) and SC-CO₂ extraction at 30 MPa/80 °C. Data are mean \pm SD of triplicate experiments and analyses. Different capital letters represent significant differences among the extraction method groups ($p < 0.05$), and different lower-case letters represent significant differences within the same the tomato matrix group ($p < 0.05$).

As it was shown in Chapter 3, SC-CO₂-extracted oleoresins contain 67% of *cis*-lycopene in the total oleoresin, and 82% in its oil fraction (difference was not significant, $p>0.05$). Tomato peel oleoresin extracted with hexane as well as its oil fraction contained only 34% of *cis*-lycopene isomers. Results showed that even though there was no significant difference between the *cis*-lycopene percentage of the oleoresin and oil fractions of the SC-CO₂-extracted samples ($p>0.05$), bioaccessibility of the oleoresin was higher, suggesting the critical role of the matrix. It is plausible that during digestion and absorption, the tomato food matrix is broken to release lycopene; once this occurs, *trans*-lycopene isomerization also may occur. *Cis*-lycopene levels in plasma may suggest that *trans*- to *cis*- isomerization is a fundamental stage in lycopene absorption. In contrast, to the *trans*- configuration, *cis*- isomeric forms of lycopene possess less susceptibility to aggregate and higher solubility in bile acid micelles (Boileau, Boileau, & Erdman Jr, 2002). Moreover, *cis* isomers are more difficult to crystallize, and more soluble in organic solvents and oil phases (Castenmiller & West, 1998), resulting in a higher availability for intestinal cell uptake (Failla, Chitchumroonchokchai, & Ishida, 2008). In addition, other investigations suggest that *trans*- forms of lycopene build crystals in the intestine, reducing the lycopene's uptake through the micelles (Holzapfel et al., 2013).

Even though percent bioaccessibility is an indicator of its potential bioavailability, the amount of bioaccessible lycopene must be considered in addition to percent bioaccessibility. The content of lycopene measured in the digesta after simulated digestion are depicted in Figure 4.2. Lycopene concentration in the digesta increased when the tomato oleoresin was used compared to oil fraction for both SC-CO₂ and hexane extractions. After digestion of tomato peel oleoresin, lycopene concentration in

the final digesta was 4.6 and 13.4 $\mu\text{g/g}$ for hexane- and SC-CO₂-extracted oleoresin, respectively. Oil fraction of the tomato peel oleoresin released concentrations of lycopene of 1.3 $\mu\text{g/g}$ for hexane and 3.0 $\mu\text{g/g}$ for SC-CO₂ extracted oleoresin. Lycopene concentration in the digesta ranged between 0.2 and 11 $\mu\text{g/g}$ in the reported lycopene bioaccessibility studies in literature (Garrett et al., 1999; Goñi et al., 2006; Svelander et al., 2010). In the current study, lycopene concentration varied between 1.3 and 13.4 $\mu\text{g/g}$ of digesta. Values obtained suggest the approximate lycopene release from the tomato oleoresins, but do not necessarily indicate the amount of lycopene incorporated into micelles.

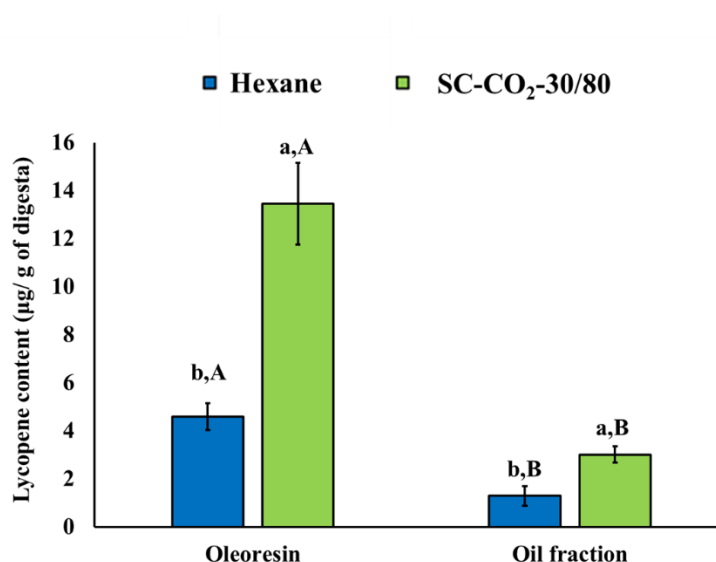


Figure 4.2. Concentration in the digesta of lycopene from tomato peel oleoresins and oil fractions obtained by hexane (Soxhlet) and SC-CO₂ extraction at 30 MPa/80 °C. Data are mean \pm SD of triplicate experiments and analyses. Different capital letters represent significant differences among the extraction method groups ($p < 0.05$), and different lower-case letters represent significant differences within the same the tomato matrix group ($p < 0.05$).

Little is known about lycopene bioaccessibility from tomato peel oleoresins. There is no study on the effect of extraction method on the bioaccessibility of lycopene. However, some studies reported carotenoid bioaccessibility, including lycopene from food matrices in presence of a lipid environment (Colle et al., 2013, 2012; Fernández-García, Mínguez-Mosquera, & Pérez-Gálvez, 2007; Lemmens et al., 2014). Moreover, some studies have shown lycopene bioaccessibility from raw tomato, tomato pulp, and processed tomato (Eboul, Ichelle, & Loi, 2006; Gartner, Stahl, & Sies, 1997; Goñi, Serrano, & Saura-Calixto, 2006; Svelander et al., 2010). In addition, some studies have reported lycopene bioaccessibility from tomato meals and tomato-based products using in vitro digestion models (Dehghan-Shoar et al., 2011; Garrett et al., 1999; Svelander et al., 2010). Lycopene concentration in the digesta, and lycopene bioaccessibility varied among all these studies. Although the assess of lycopene bioaccessibility in those in vitro methods are derived from earlier reported models, there are differences in the digestion parameters used that may affect the biocessibility lycopene values. Conditions such as amount of sample, pH, time of digestion, which influence on the enzyme activity; other factors such as the use of antioxidants and nitrogen, inclusion or not of the oral step, centrifugation type for the digesta are different. Therefore, it is challenging to compare lycopene bioaccessibility results among the reported studies.

4.5 Conclusions

Overall, SC-CO₂ oleoresin as well as its oil fraction offer a higher in vitro bioaccessibility of lycopene compared to hexane-extracted one. The lipid environment of the oleoresins may offer a positive effect on the digestion and further absorption of the

lycopene released from this type of matrix. Moreover, the in vitro digestion model provides a valuable tool to estimate the bioaccessibility of lycopene from tomato peel oleoresin. As expected, higher *cis*-lycopene content resulted in higher bioaccessibility; however, higher bioaccessibilities is possible for the similar *cis*-lycopene contents due to matrix effect. Tomato peel oleoresin was found to be a better source of lycopene due to both high bioaccessibility and higher lycopene content in the digesta. Moreover, using oleoresin as lycopene source does not require a separation step as required in obtaining the oil fraction.

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Chapter 5 . Summary, Conclusions and Recommendations

5.1 Summary and conclusions

This thesis has reported that SC-CO₂ is a green solvent that allows to obtain higher amounts of tomato byproducts oleoresins compared to hexane. Moreover, SC-CO₂ can increase the *cis*-lycopene content in such oleoresin increasing in the same way the bioaccessibility of lycopene.

In Chapter 3, it has been found that SC-CO₂ extraction at higher pressures (50 MPa) and lower temperatures (40 °C) yielded more oleoresin. The oleoresins obtained from tomato peel contained the highest amount of lycopene, and the oleoresins extracted with SC-CO₂ at 30 MPa/80°C contained the highest amount of *cis*-lycopene. In addition, after the insoluble fraction was removed, the oil fraction obtained contains the highest amount of *cis*-lycopene, representing up to 82% of *cis*-isomers.

In Chapter 4, the bioaccessibility of the tomato peel oleoresin, as well as its oil fraction was successfully tested through a simulated digestion method. The simulated digestion model provides a valuable tool to estimate the bioaccessibility of lycopene from tomato peel oleoresin. The highest percentage of lycopene bioaccessibility was found for the tomato peel oleoresin (5.2%) and its oil fraction (4.0%) obtained by SC-CO₂ at 30 MPa/80°C compared to hexane-extracted oleoresin (2.7%) and its oil fraction (1.7%). The concentration of lycopene in the bioaccessible phase was also higher in the SC-CO₂-extracted oleoresins accounting for 13.4 and 3.0 µg/g digesta in the oleoresin and its oil fraction, respectively. Hexane-extracted oleoresin released lycopene in a concentration of 4.6 µg/g, whereas its oil fraction delivered 1.3 µg/g of digesta. Moreover, the lipid

environment of the oleoresins may offer a positive effect on the digestion and further absorption of the lycopene released from this type of matrix.

The study suggested that the oleoresin obtained by this green technology can be bioaccessible and it is plausible to use as a food ingredient to develop health and wellness promoting foods.

5.2 Recommendations

Intake of lycopene-rich foods is associated with decreased risk for several chronic diseases. Recent studies have suggested that *cis*-lycopene is more bioavailable than *trans*-lycopene. For its biological functions, *cis*-lycopene could be used as a functional ingredient to develop health and wellness. In this thesis, we studied the bioaccessibility of the lycopene-rich oleoresin through an *in vitro* digestion model. In the future planning, cell cultures and *in vivo* animal studies should be investigated to determine the bioavailability of the lycopene in the oleoresin. We should be able to know if the bioaccessible lycopene can reach the blood system after its metabolism for action to expend health benefits. *In vivo* studies are also suggested to determine the bioactivity of the SC-CO₂-extracted oleoresin on selected cancer types.

Another future research that should be investigated to further complete this investigation is the incorporation of the tomato peel oleoresin in food products, its stability, and its *in vitro* and *in vivo* bioavailability. Incorporating the extracted tomato byproducts oleoresin into various delivery systems to develop dry powder formulations will make the

utilization of the oleoresin extracts as food ingredients convenient, and may enhance the function and efficacy, and storage stability.