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Apparent solubility of lycopene and β-carotene in supercritical CO_2, CO_2 + ethanol and CO_2 + canola oil using dynamic extraction of tomatoes

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ABSTRACT

Lycopene and β-carotene were extracted from freeze-dried tomatoes (skin + pulp) with pure SC CO_2 and SC CO_2 + 5% w/w co-solvent at 40 °C, 400 bar and flow rates of 0.5 and 1.2 L/min. The apparent solubility of lycopene and β-carotene in the multicomponent complex system was determined from dynamic extraction experiments using a laboratory-scale supercritical extraction system. Solubility of pure lycopene and β-carotene in SC CO_2 (binary system) was reported in the literature to be of the order of 10^{-6} mole fraction. The apparent solubility of lycopene extracted from tomatoes with SC CO_2 (multicomponent complex system) under the same conditions was almost one order of magnitude smaller. The apparent solubility obtained using oil as a co-solvent was higher than that obtained with ethanol as a co-solvent or pure SC CO_2. The differences in solubility are mainly due to the polarity of the co-solvent and the impact of the tomato matrix in the multicomponent complex system.

1. Introduction

The demand for healthy food products and natural food colorants such as carotenoids is growing due to consumer concerns for food safety and quality. Of all the carotenoids, β-carotene has the highest provitamin A activity, while lycopene has potent antioxidant activity. These carotenoids have been the subject of numerous studies to demonstrate their health benefits. It was concluded that long-term supplementation with β-carotene provided neither benefit nor harm in terms of cancer, cardiovascular disease, stroke or overall mortality (Hennekens et al., 1996). On the other hand, there is growing evidence (Stacewicz-Sapuntzakis and Bowen, 2005) that lycopene reduces the risk of prostate cancer and the US Food and Drug Administration has allowed a qualified health claim for lycopene (FDA, 2005).

Tomatoes are a rich source of carotenoids, with lycopene being the major constituent (70–80% or 0.72–20 mg/100 g wet basis) (Cadoni et al., 2000). Lycopene and other carotenoids are found mostly in the outer pericarp (McCollum, 1955) with tomato skin containing 12 mg lycopene/100 g skin (wet basis) while whole mature tomato contains only 3.4 mg lycopene/100 g (wet basis) (Al-Wandawi et al., 1985).

An environmentally friendly lycopene extraction process with minimal loss of bioactivity is highly desirable for the food, feed, cosmetic, and pharmaceutical industries. To date, traditional solvent extraction (TSE) has been used to extract carotenoids from tomatoes (Schmitz et al., 1989; Jay et al., 1991). However, it requires long extraction times, consumes large amounts of organic solvents, requires heat treatment of the extract for solvent removal and may result in solvent residues being left in the final extracted products. On the other hand, supercritical fluid extraction (SFE) uses supercritical carbon dioxide (SC CO_2), which reduces the potential for oxidation of carotenoids and eliminates any harmful solvent residue in the products. In addition, the low critical temperature of CO_2 (31 °C) is beneficial for thermally labile carotenoids, resulting in minimal carotenoid degradation during extraction.

Table 1 shows the lycopene and β-carotene removal from different tomato matrices reported in the literature. Of the fourteen studies identified, seven used skin + seed while the others used skin + pulp, skin, paste, saponified tomato pomace or a mixture of tomato skin + pulp and hazelnut oil as the feed material for extraction. In addition, all studies focused on lycopene extraction while only a few also evaluated simultaneous β-carotene extraction. Furthermore, most of the previous research was conducted using only pure SC CO_2 at pressures of 77–530 bar, temperatures of 32–100 °C and flow rates of 0.4–185 L/min (measured at ambi-

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Nomenclature

SC CO_2	supercritical CO_2	w/w	weight/weight
EtOH	ethanol	v/v/v	volume/volume/volume

ent conditions). The extraction recoveries reported in Table 1 varied over a wide range (10–100% and 40–98% for lycopene and β-carotene, respectively), depending on the different tomato varieties and the part of tomato used, solvent/feed ratio, flow rate, time, extraction conditions and specifics of the HPLC analysis protocols and instrumentation used for the quantification of carotenoids. For the studies summarized in Table 1, the effects of temperature and pressure on the extraction of lycopene from tomato by-products were evaluated. The extraction yield increased with temperature and pressure using pure SC CO_2 to extract lycopene from skins (Topal et al., 2006) and from skin + pulp (Gomez-Prieto et al., 2003). However, Vagi et al. (2007) indicated that only temperature had a significant effect ($p < 0.05$) on the yield. Different lycopene recoveries were reported, such as 61% at 86 °C and 345 bar (Rozzi et al., 2002), 80% at 80 °C and 300 bar (Sabio et al., 2003) and 94% at 100 °C and 400 bar (Topal et al., 2006). Furthermore, Gomez-Prieto et al. (2003) observed that the amount of the *trans* form of lycopene extracted increased and the *cis* form decreased at higher extraction pressures. In a study on the stability of lycopene at very high hydrostatic pressures of 1000–6000 bar, Qui et al. (2006) highlighted that lycopene was stable up to 4000 bar. In addition, lycopene extraction from other sources such as watermelon was also reported using SC CO_2 with ethanol as a co-solvent, reaching a maximum yield at 70 °C, 21 MPa and ethanol at 15% by volume (Vaughn et al., 2008). Improving the extraction efficiency of carotenoids with SC CO_2 is essential for developing processes at an industrial scale. To date, some attempts to use co-solvents like chloroform or hexane (Cadoni et al., 2000), ethanol (Baysal et al., 2000), hazelnut oil (mixed with feed material) (Vasapollo et al., 2004), water, ethanol and olive oil (mixed with feed material) (Shi et al., 2009b) were also reported for tomato extraction. However, the literature has only few studies on the solubility of lycopene in SC CO_2. De la Fuente et al. (2006) reported solubility for lycopene ranging from 0.3 to 1.5×10^{-6}, using purified lycopene as the feed material. Gomez-Prieto et al. (2002) reported lower lycopene solubility (ranging from 4.1 to 11×10^{-8}) using tomato skin waste as their starting material and only 30 min of dynamic extraction at a flow rate of 4 mL/min. Shi et al. (2009a) also performed a dynamic extraction for 90 min using tomato skin to measure the solubility of lycopene ($0.7–1.9 \times 10^{-6}$). Both Gomez-Prieto et al. (2002) and Shi et al. (2009a) used tomato matrix rather than pure lycopene in their solubility measurements. In addition, both studies use a dynamic solubility measurement method but based on a single point measurement with relatively high flow rates and do not include a discussion to demonstrate that equilibrium was achieved. More studies are therefore needed to determine the solubility of lycopene and β-carotene in SC CO_2 and to understand the effects of the matrix and the presence of co-solvents on the solubility.

The main objective of this study was to investigate the extraction of lycopene from tomatoes using pure SC CO_2 and continuous addition of ethanol and canola oil as co-solvents in SC CO_2. First, the extraction of lycopene from tomato skin + pulp in pure SC CO_2 was performed to determine the apparent solubility of lycopene in SC CO_2 at different temperature and pressure conditions. The apparent solubility determined using this dynamic method is representative of the solubility of lycopene in a multicomponent complex system (lycopene + tomato + SC CO_2). Then, dynamic extractions of lycopene from tomatoes using SC CO_2 + ethanol and SC CO_2 + canola oil were carried out to demonstrate the impact of continuous co-solvent addition on the apparent solubility of lycopene.

2. Materials and methods

2.1. Materials

Lycopene (90–95% purity) and β-carotene (99.9% purity) standards were purchased from Fluka–Sigma Aldrich Co. (St. Louis, MO). CO_2 bone dry (99.8% purity) and nitrogen (99.95% purity) were purchased from Praxair Canada Inc. (Mississauga, ON, Canada). Solvents such as HPLC grade methanol, ethanol, dichloromethane, and tetrahydrofuran (THF) were obtained from Fisher Scientific (Fair Lawn, NJ).

Tomatoes were bought at a local market (Edmonton, AB, Canada). After washing, the seeds were removed and tomatoes were chopped into cubes at room temperature. Then, tomato cubes were freeze-dried for 5 days until a moisture content of ca. 0.8% was reached and then ground by a Quadro Comil grinder (Model 197S, Emerson Industrial Controls, Grand Island, NY), mixed and sieved (W.S. Tyler Company of Canada Ltd., St. Catharines, ON) until a desired particle size distribution (0.5–1 mm) was achieved. All samples were vacuum packed in several moisture and O_2-barrier bags and stored at −18 °C in the dark until use. Samples were used within two months of preparation. Canola oil (No Name®, 100% pure) was bought from a local supermarket.

2.2. Supercritical fluid extraction

A laboratory-scale SFE system equipped with a 300 mL extraction cell (Newport Scientific Inc., Jessup, MD) was used as described previously (Saldaña et al., 2006). The extraction temperature was monitored by a thermocouple immersed in the centre of the extractor. A stainless steel basket (24.6 cm × 26 mm ID) was fitted into the extraction vessel for easy loading and unloading of the freeze-dried tomato sample (10 g). CO_2 was compressed to the desired pressure using a diaphragm compressor and the extraction pressure was controlled by a back pressure regulator. The CO_2 flow rate (0.5 and 1.2 L/min, measured at ambient conditions) was controlled by a micrometering valve and measured using a gas meter. Each extraction run was carried out over 6 h to establish the full extraction curve. Extract fractions were collected every 60 min in side-armed glass tubes attached to the depressurization valve, which were held in a refrigerated circulating bath at −20 °C and weighed. The extracted material was transferred into a pre-weighed glass vial by washing side-armed glass tubes with ethanol, which was then evaporated under gentle nitrogen flow. The extracts were weighed and stored at −18 °C in the dark until HPLC analysis. At least two replicates were carried out for each experiment. Experiments were conducted at temperatures of 40 and 70 °C and at a pressure of 400 bar.

For the extractions with co-solvent addition, the flow rate of CO_2 was maintained at 0.5 L/min into which 5% (w/w) of ethanol or canola oil was introduced continuously using a piston pump (Gilson 305, Gilson Inc., Middleton, WI). Experiments with co-sol-

Table 1
Extraction of lycopene and β-carotene from tomato matrix with pure SC CO_2 and SC CO_2 + co-solvent.

Tomato matrix			*P* (bar)	*T* (°C)	Flow rate (L/min at STP)	Time (h)	Co-solvent	Yield[a]/Recovery[b]		Reference
Feed (g)	Part used	Particle size (mm)						Lycopene	β-carotene	
3	Skin + seed	n.i.	138–483	32–86	0.0025[*]	0.3	No	61[b]		Rozzi et al. (2002)
40–50	Skin + seed	0.08–0.35	250, 300	60, 80	7–13	n.i.	No	80[b*]	88[b*]	Sabio et al. (2003)
0.5	Skin + pulp	n.i.	77–281	40	0.004[n.i.]	0.5	No	42[b]	98[b]	Gomez-Prieto et al. (2003)
n.i.	Skin	n.i.	200–500	40–100	0.0015–0.0045[*]	5.5	No	94[b]		Topal et al. (2006)
1,000	Skin + seed	0.3–0.6	460	80	n.i.	n.i.	No	31.4[a]		Vagi et al. (2007)
2.5	Skin + seed	n.i.	172–276	40–80	0.5	0.5	Chloroform, hexane (1 mL)[c]	64[a]	35[a]	Cadoni et al. (2000)
100	Saponified tomato powder	n.i.	370–530	47–63	0.7	1.8	EtOH (16%)[d]	27.4[b]		Huang et al. (2008)
0.5	Skin + seed	1	250–350	45–75	3.5	1.5	SC CO_2 with 5 or 10% w/w EtOH, water, or olive oil mixed with feed material	3.2–6.1[a]		Shi et al. (2009b)
1.2	Skin + seed	1	250–450	40–70	3.5	0.5	SC CO_2 with 5 or 10%[d] EtOH	33[b]		Kassama et al. (2008)
n.i.	Skin + seed	1	200–400	40–100	0.001–0.002	1.5	No	3.1[a]		Yi et al. (2009)
0.3	Skin	n.i.	400	60–110	1.5	1.1	SC CO_2, SC CO_2 + 500 μL acetone, MeOH, EtOH, hexane, dichloromethane	100[b]		Ollanketo et al. (2001)
53	Paste	3	200–300	35–65	19–74	3	No	20[b]	40[b]	Baysal et al. (2000)
							SC CO_2 + 5, 10, 15% w/w EtOH	50[b]	50[b]	
3,000	Skin + pulp and roasted hazelnut	n.i.	400	60	1.5	8	No	72.5[b]		Ciurlia et al. (2009)
3,000	n.i.	1	335, 450	45, 66	46–185	7	No	10[b]		Vasapollo et al. (2004)
			450	66	93		Hazelnut oil (10% w/w mixed with feed material)	30[b]		

STP: standard temperature and pressure
[*] Measured at extraction temperature and pressure.
[a] Yield reported as mg lycopene/100 g feed
[b] Recovery (%) = 100 × (g lycopene in extract/g lycopene in feed); b[*] what seems like recovery data is reported by Sabio et al. (2003) as yield (%)
[c] Addition method not specified.
[d] Co-solvent concentration not specified in terms of w/w, v/v or mole %
[n.i.] Not indicated.

vent addition were conducted at 40 and 70 °C and at a pressure of 400 bar.

2.3. Soxhlet extraction

Soxhlet extraction of freeze-dried tomatoes was carried out to determine the initial amount of carotenoids present in the tomatoes. Two grams of freeze-dried tomatoes and 40 mL of dichloromethane were placed in a Soxhlet apparatus and refluxed for 6 h. The solvent was removed and the extract was dried under a gentle flow of nitrogen. Then, the dried extract sample was dissolved in THF prior to HPLC analysis. Three replicates of the Soxhlet extraction followed by HPLC analysis were performed with an average coefficient of variation (standard deviation/mean × 100%) of less than 13%.

2.4. High performance liquid chromatography (HPLC) analysis

Lycopene and β-carotene identification and quantification in the extracted samples were performed according to the method of Vasapollo et al. (2004) using an HPLC system (Shimadzu Scientific Instruments Inc., Columbia, MD) equipped with an XDB C18 column (15 cm × 4.6 mm × 5 μm) (Agilent Technologies Canada Inc., Mississauga, ON). Samples dissolved in THF were injected onto the column. The mobile phase consisted of methanol/THF/water (67/27/6 v/v/v) at a flow rate of 1.5 mL/min at isocratic conditions. The separated lycopene and β-carotene peaks were monitored by a Pro Star 325 UV–vis detector Model 410, Shimadzu at 475 nm. Lycopene and β-carotene standards were used for identification and quantification based on their respective calibration curves.

3. Results and discussion

3.1. Extraction of lycopene and β-carotene from tomatoes (skin + pulp) using pure SC CO_2 and SC CO_2 with co-solvents

Fig. 1 shows typical HPLC chromatograms of carotenoid standards (Fig. 1A) and tomato extracts obtained by supercritical fluid extraction (Fig. 1B). The carotenoids in the extract were comprised mainly of lycopene and β-carotene and there were some additional unidentified small peaks. The error on the HPLC analysis was less than 10% also accounting for the differences between replicate samples. The literature (Baloch et al., 1997; Zanoni et al., 1999; Anguelova and Warthesen, 2000; Goula et al., 2006) demonstrates some lycopene degradation after spray drying and oven drying in the presence or absence of additives. However, there is no such data for freeze-dried tomato samples.

A feed amount of 10 g was selected so that the full extraction curve (including the diffusion-controlled plateau region) could be generated within a reasonable experimental time frame. The amount of sample used in this study should not impact the solubil-

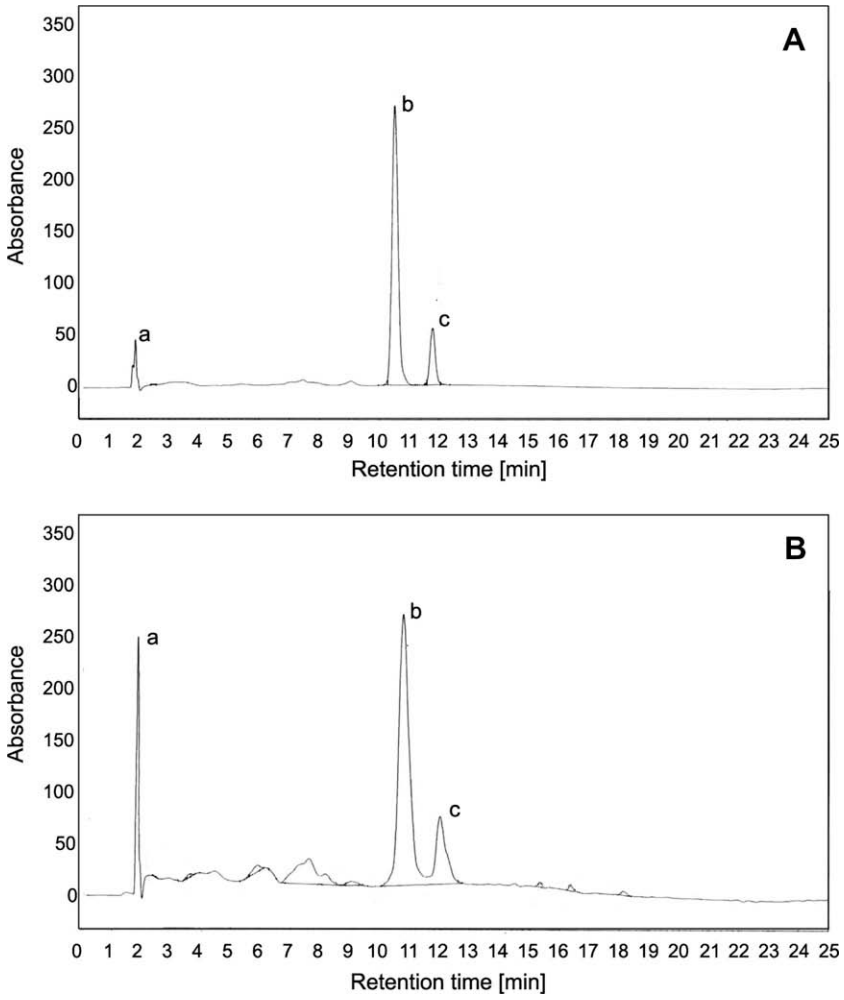

Fig. 1. Typical HPLC chromatogram of: (A) carotenoid standards and (B) tomato extract obtained by extraction with pure SC CO_2 (a = solvent peak, b = lycopene peak and c = β-carotene peak).

ity results since the solubility data were generated based on the initial part of the extraction curve using a low CO_2 flow rate and ensuring that there was sufficient material to saturate the CO_2.

Previous studies (Cadoni et al., 2000; Baysal et al., 2000; Ollanketo et al., 2001; Vasapollo et al., 2004; Huang et al., 2008; Kassama et al., 2008; Shi et al., 2009b; Ciurlia et al., 2009) were performed using SC CO_2 + co-solvent. However, Cadoni et al. (2000) did not specify how the 1 mL of co-solvent (chloroform or hexane) was added. Baysal et al. (2000) and Huang et al. (2008) added ethanol continuously to the SC CO_2 flow at 5–15% (w/w). Vasapollo et al. (2004), Ciurlia et al. (2009), Kassama et al. (2008) and Shi et al. (2009b) blended their co-solvents with the dried starting material prior to extraction rather than continuously mixing it into SC CO_2, thus it is difficult to assess the role of a co-solvent since the initial concentration of co-solvent changes over time as it is also extracted with SC CO_2. In the present study, ethanol and canola oil were used as co-solvents at 5% (w/w) level and were pumped into SC CO_2 in a continuous mode, ensuring that the co-solvent is present at a fixed concentration throughout the entire extraction period. Fig. 2 shows the extraction curves for lycopene obtained using pure SC CO_2, SC CO_2 + ethanol and SC CO_2 + canola oil at 40 °C, 400 bar and a CO_2 flow rate of 0.5 L/min. The amount of lycopene extracted at the end of 6 h increased substantially with the use of a co-solvent, and canola oil was a better co-solvent than ethanol. The use of a polar co-solvent such as ethanol enhances the extraction power of SC CO_2. Baysal et al. (2000) found that the addition of 5% ethanol was optimum to obtain the highest lycopene and β-carotene yield from tomato paste waste. When additional ethanol was added, the extraction was hindered due to the decreased homogeneity of the extraction mixture. As ethanol is a polar co-solvent, it may not be the best choice for improving the solubility of the non-polar lycopene and β-carotene in SC CO_2. Sun and Temelli (2006) used canola oil as a continuous co-solvent for the extraction of carotenoids from carrots and found that canola oil significantly ($p \leqslant 0.05$) increased the extraction yield of carotenoids from carrots. As carotenoids are fat soluble compounds, the use of canola oil increased carotenoid solubility and yield. Canola oil addition at 5% by weight corresponded to the highest solubility conditions reported by Sun and Temelli (2006). At the lower temperature and pressure conditions of this study, the solubility of canola oil in SC CO_2 is lower and may lead to a two phase system. But, instead of introducing another variable, oil addition was maintained at a constant level in this study. Additional interactions between the liquid oil and the solid matrix probably contributed to enhanced solubility.

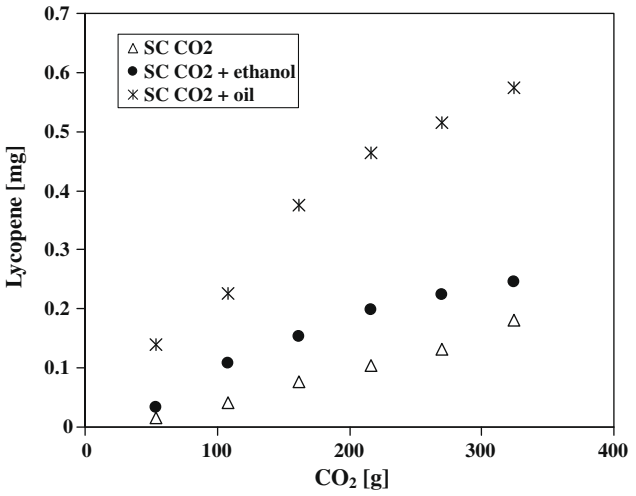

Fig. 2. Comparison of lycopene extraction with pure SC CO_2, SC CO_2 + 5% ethanol and SC CO_2 + 5% canola oil at 40 °C, 400 bar, and 0.5 L/min.

Earlier, Temelli (1992) reported the solubility of canola oil in SC CO_2 at 345–620 bar and 40–70 °C. At 400 bar and temperatures of 40 and 70 °C, oil solubility was reported to be 10 and 15 mg oil/g CO_2, corresponding to 1.36×10^{-3} and 2.04×10^{-3} mole fraction, respectively. In the present study, similar solubility values of canola oil in SC CO_2 (11.14–13.16 mg oil/g CO_2, corresponding to 1.52×10^{-3} and 1.79×10^{-3} mole fraction, respectively) were obtained at the same pressure and temperature using a flow rate of 0.5 L/min. The high level of carotenoids extracted in SC CO_2 + oil could be attributed to the molecular interactions between the carotenoids and the triglycerides of canola oil that increased the driving force, which is also manifested by the reduced chemical potential. Bamberger et al. (1988) also observed that the solubility of a less volatile lipid component was significantly enhanced by the presence of a more volatile triglyceride compound in the system. The overall finding of Shi et al. (2009b) in terms of the effectiveness of various modifiers at increasing the yield of lycopene extraction using SC CO_2 (olive oil > ethanol > water) is in agreement with the results found herein. However, the lycopene yields of 37.7 and 35.2 μg/g reported by Shi et al. (2009b) at 35 MPa and 45 °C for SC CO_2 + 5% ethanol and SC CO_2 + 5% olive oil, respectively, are not in agreement with expectations. Since lycopene is more soluble in oil than in ethanol, one would expect a higher yield when extracting with SC CO_2 + olive oil than with SC CO_2 + ethanol. It is possible that the unexpected results may be due to the fact that the co-solvent was mixed in with the feed material and not fed in continuously. Therefore, these results are a reflection of the different rates of removal of ethanol and olive oil by SC CO_2 under the tested conditions.

One important effect that the co-solvent may have on the matrix is swelling, which may affect the cell structure, improve diffusion of carotenoids from the matrix and enhance dissolution into the solvent. When the co-solvent is pumped continuously into SC CO_2 the interaction of the co-solvent with the matrix is anticipated to be greater at the initial stages of extraction but then it should stabilize so that the co-solvent concentration in the system is constant for the rest of the extraction period. The complex interactions among the tomato matrix, lycopene, canola oil and SC CO_2 lead to the higher amounts of lycopene being extracted. The average coefficient of variation (standard deviation/mean × 100%) in the supercritical fluid extraction and HPLC analysis data over different time points, extraction conditions and replicates obtained in this study was less than 13%.

Fig. 3 shows the effect of CO_2 flow rate (0.5 vs 1.2 L/min) on the amount of lycopene extracted using SC CO_2 + 5% canola oil at 70 °C and 400 bar as a function of the mass of CO_2 used. Under these conditions, the amount of lycopene obtained at the end of the 6 h extraction was similar with both flow rates. Topal et al. (2006) observed a similar behavior using pure SC CO_2 when the CO_2 flow rate was increased from 2.5 to 4.5 mL/min (measured at extraction conditions) at 400 bar and 90 °C, with decreasing amounts of lycopene extracted from tomato skin at the later stages of extraction. This trend could be attributed to the reduced residence or contact time of the supercritical fluid with the tomato matrix. At the higher flow rate, the solvent passes quickly around the solid matrix and does not have sufficient residence time to diffuse through the pores within the sample matrix. At a constant flow rate of 0.5 L/min (319 min of residence time), the initial portion of the plateau of the extraction curve can be observed. However, the plateau could not be established at the high flow rate (1.2 L/min, corresponding to a residence time of 133 min). Furthermore, the highest amount of extracted lycopene was reached after 324 g CO_2 at flow rate of 0.5 L CO_2/min and after 778 g of CO_2 at 1.2 L CO_2/min. That is, the amount of the CO_2 required to extract the greatest amount of

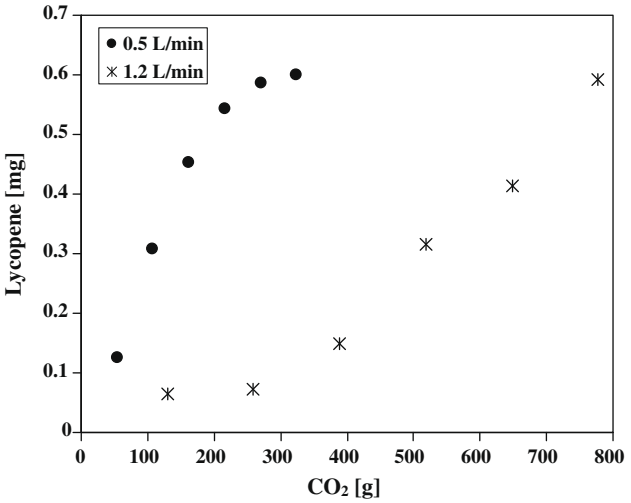

Fig. 3. Extraction of lycopene from skin + pulp of tomato at flow rates of 0.5 and 1.2 L/min, 70 °C, and 400 bar with SC CO_2 + 5% canola oil.

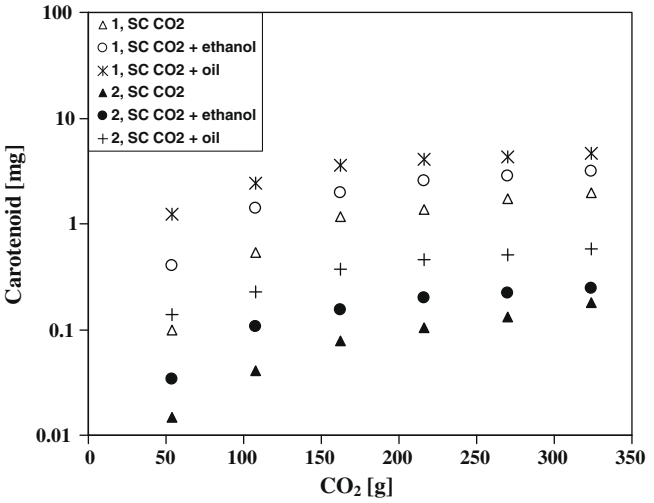

Fig. 5. Extraction of β-carotene (1) and lycopene (2) from skin + pulp of tomato with pure SC CO_2 and SC CO_2 + 5% co-solvent at 40 °C, 400 bar, and CO_2 flow rate of 0.5 L/min.

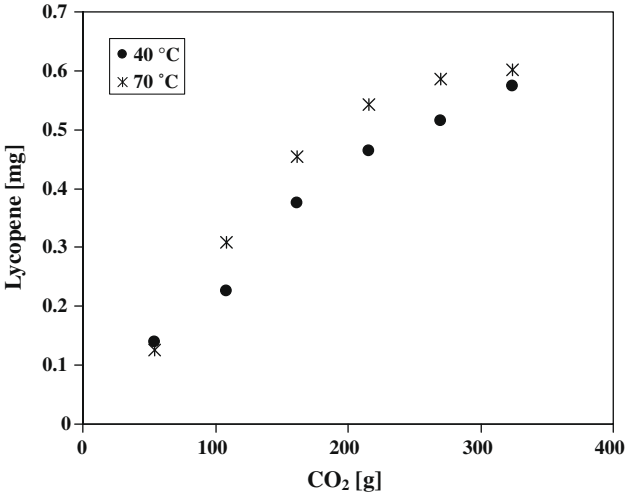

Fig. 4. Extraction of lycopene from skin + pulp of tomato at temperatures of 40 and 70 °C, 400 bar, and flow rate of 0.5 L/min with SC CO_2 + 5% canola oil.

lycopene can be reduced by using the low flow rate since CO_2 loading is below the solubility limit at high flow rates.

Fig. 4 shows the effect of temperature (40 vs 70 °C) on the extraction of lycopene from tomato skin + pulp at 400 bar and a flow rate of 0.5 L/min with SC CO_2 + canola oil as a function of the mass of CO_2 used. A temperature increase results in an increase in the solute vapor pressure as well as the solute diffusivity. The density of SC CO_2 at constant pressure decreases with temperature, but the magnitude of such a density drop becomes smaller at elevated pressures. Therefore, a temperature increase reduces the solvent density and consequently reduces the solubility of lycopene, but promotes the transport of solute in the matrix and/or from the matrix into the solvent. The amount of lycopene extracted after 6 h was similar (0.60 vs 0.57 mg) at both temperatures studied. A similar behavior was also reported when extracting lycopene from tomato skin + seed using pure SC CO_2 (Rozzi et al., 2002). An increase in temperature from 90 to 100 °C at 400 bar yielded almost the same amount of lycopene (1.17 and 1.18 mg of lycopene/g of sample at 90 and 100 °C, respectively) with pure SC CO_2 (Topal et al., 2006). Presumably, some degradation of lycopene at such elevated temperatures occurred. Although increased lycopene yield at high temperatures has been identified as an advantage (Cadoni et al., 2000; Rozzi et al., 2002; Vagi et al., 2007), others (Gomez-Prieto et al., 2003; Topal et al., 2006) reported the risk of undergoing *trans–cis* isomerization during high temperature extraction. To minimize isomerization, extraction temperatures of 40 and 70 °C were used in this study.

Fig. 5 shows the comparison of extraction curves of lycopene and β-carotene obtained from tomato skin + pulp using pure SC CO_2, SC CO_2 + ethanol and SC CO_2 + canola oil at 40 °C, 400 bar, and 0.5 L/min flow rate as a function of the mass of CO_2 used. The extraction of both β-carotene and lycopene using three different supercritical solvents followed the order: SC CO_2 + canola oil > SC CO_2 + ethanol > SC CO_2. The quantities of β-carotene extracted by SC CO_2 were almost one order of magnitude higher than those of lycopene. At 40 °C and 276 bar, Cadoni et al. (2000) observed that β-carotene was extracted almost exclusively from tomato skin + seed using SC CO_2.

3.2. Apparent solubility of lycopene and β-carotene in the multicomponent complex system by dynamic extraction of tomatoes

Dynamic extraction of tomato skin + pulp was used to determine the apparent solubility of lycopene and β-carotene in a multicomponent complex system (tomatoes + SC CO_2 and tomatoes + SC CO_2 + co-solvent). Dynamic extraction data (in particular, the mass of carotenoids extracted as a function of mass of CO_2) can be used to determine the apparent solubility provided that two conditions are met: (i) sufficient lycopene and β-carotene are present to saturate SC CO_2 and reach the solubility limit, and (ii) the SC CO_2 flow rate is sufficiently low to allow equilibrium to be reached. In order to ensure that the first condition was satisfied, the amount of lycopene and β-carotene present in the freeze-dried tomato sample was compared to an estimate of the amount of lycopene and β-carotene needed to satisfy the solubility limit. Based on the initial lycopene and β-carotene contents of the freeze-dried tomatoes (16.48 mg lycopene and 1.07 mg β-carotene/g dry tomato, as determined by Soxhlet extraction) and assuming a solubility of the order of 10^{-6} mole fraction for lycopene (De la Fuente et al., 2006) and β-carotene (Saldaña et al., 2006) based on the highest level of binary solubility reported in the literature, the calculation reveals that sufficient lycopene and

Table 2
Solubility of lycopene and β-carotene in SC CO_2 or SC CO_2 + co-solvent in binary and complex systems.

P (bar)	*T* (°C)	Method	Flow rate (L/min at STP)	Time (h)	Solubility (mole fraction)		Reference
					Lycopene	β-Carotene	
120–200	40, 50	Static		8		4.3 ± 0.4 to $9.4 \pm 0.9 \times 10^{-7}$[a]	Saldaña et al. (2006)
171–418	40, 50, 60	Static		12	0.3 ± 0.1 to $1.5 \pm 0.2 \times 10^{-6}$[b]		De la Fuente et al. (2006)
400	70	Dynamic[d]	0.0015–0.0045	5.5	1.9×10^{-6}[c]		Topal et al. (2006)
400	40	Dynamic[e]	0.5	6	$4.0 \pm 0.7 \times 10^{-8}$[c]	$6.2 \pm 0.9 \times 10^{-7}$[c]	This study
400	40	Dynamic[e] ($SC CO_2$ + 5% EtOH)	0.5	6	$7.0 \pm 0.7 \times 10^{-8}$[c]	$9.1 \pm 1.0 \times 10^{-7}$[c]	This study
400	40, 70	Dynamic[e] ($SC CO_2$ + 5% canola oil)	0.5	6	1.9 ± 0.3 to $2.2 \pm 0.3 \times 10^{-7}$[c]	0.9 ± 0.04 to $1.8 \pm 0.4 \times 10^{-6}$[c]	This study

STP: standard temperature and pressure
[a] Solubility of pure β-carotene in SC CO_2 by Quartz Crystal Microbalance (QCM) (mean ± standard deviation).
[b] Solubility of purified lycopene obtained from 0.2 g of tomato paste in SC CO_2 (mean ± standard deviation).
[c] Solubility determined by dynamic extraction of tomatoes with SC CO_2 or SC CO_2 + co-solvent (mean ± standard deviation).
[d] The particle size and the initial quantity of tomato skin sample used was not indicated
[e] Skin+pulp of tomoto (10 g) with a particle size of 0.5–1 mm.

β-carotene were present to saturate approximately 108 g of CO_2. Thus, the slope of the initial linear portion of the dynamic extraction curve in Fig. 5 was used for apparent solubility calculations. The second condition that must be satisfied to use dynamic extraction data to calculate the apparent solubility is the use of low flow rate. Sun and Temelli (2006) also evaluated the effect of flow rate (0.5 and 1.0 L/min) on the extraction of β-carotene from carrots with SC CO_2 + canola oil as a co-solvent. The solubility-controlled region of the extraction curves for the two flow rates tested overlapped in a single line, indicating that solubility limit was reached even at 1 L/min. Therefore, the flow rate of 0.5 L/min was chosen for this study and it was assumed that equilibrium was indeed reached.

Table 2 shows the available data for the solubility of lycopene and β-carotene in pure SC CO_2 at pressures ranging from 87 to 418 bar and temperatures of 40–70 °C. Only two other studies reported the solubility of lycopene, where either a static method with 12 h of equilibration and a starting material of purified lycopene from 0.2 g of tomato paste (De la Fuente et al., 2006) or a dynamic method at flow rates of 1.5–4.5 mL/min (measured at extraction conditions) with a starting material of tomato skin (Topal et al., 2006) were used. The reports of Gomez-Prieto et al. (2002) and Shi et al. (2009a) were not included in this analysis because the data claimed to be solubility are in fact extraction yields since they are based on a single time point of 30 min (from 0.5 g tomato skin waste at 4 mL/min) and 90 min (from 5 g tomato skin + seed at 1.5 mL/min), respectively, without any of the equilibrium considerations discussed above. Without establishing the full extraction curve, it is not possible to ascertain whether this point lies in the solubility-controlled or in the diffusion-controlled region of the extraction curve.

In this study, the dynamic extraction of lycopene and β-carotene from tomato skin + pulp was performed using pure SC CO_2 at 400 bar, 40 °C and a flow rate of 0.5 L/min resulting in an apparent solubility of $4.0 \pm 0.7 \times 10^{-8}$ and $6.2 \pm 0.9 \times 10^{-7}$ mole fraction, respectively. De la Fuente et al. (2006) reported solubility of purified lycopene in pure SC CO_2 using the static method of the order of $0.3 \pm 0.1 \times 10^{-6}$ to $1.5 \pm 0.2 \times 10^{-6}$ mole fraction for pressures ranging from 171 to 418 bar and temperatures of 40, 50 and 60 °C. The results from these two studies highlight the differences between a multicomponent and a binary system, which is essential for a better understanding of the effects of the solid matrix of natural materials. In the multicomponent system presented here, the apparent solubility in SC CO_2 was lower than the true solubility in the binary system (as reported by De la Fuente et al. (2006)). Similar findings were previously reported for β-carotene in the binary and multicomponent (dynamic extraction from carrot) systems (Saldaña et al., 2006). The fact that the apparent solubility in a multicomponent system is lower is most likely due to the effects of the location of carotenoids within the matrix, the effects of interactions of carotenoids with other matrix components and possible changes in these interactions over time throughout the extraction period. On the other hand, it is interesting to note that the apparent solubility reported by Topal et al. (2006) based on dynamic extraction of tomato skin was somewhat similar to the solubility of purified lycopene reported by De la Fuente et al. (2006).

The apparent solubility of lycopene in the complex system using canola oil as a co-solvent ($2.2 \pm 0.3 \times 10^{-7}$ mole fraction) was higher than that obtained using ethanol as a co-solvent ($0.7 \pm 0.07 \times 10^{-7}$ mole fraction). According to Turner et al. (2001), small polar modifier molecules accelerate the desorption process by competing with the solutes for the active binding sites as well as by disrupting matrix structures. A similar behavior was reported by Sanal et al. (2005) on the extraction of β-carotene from apricot with SC CO_2 + ethanol. The ethanol molecules absorb at the active site of apricot matrix, resulting in a "loosening" of the matrix structure and consequently promoting desorption of β-carotene from the matrix. Furthermore, lycopene had a lower apparent solubility than β-carotene in SC CO_2 (Table 2 and Fig. 5) for all systems investigated in this study. This lower apparent solubility of lycopene could be explained in part by the fact that β-carotene has a slightly lower melting point (173 °C) than lycopene (183 °C). Similar trends of an overall increase in apparent solubility with the use of a co-solvent were observed in Fig. 5 for both lycopene and β-carotene. There was up to five times difference between the apparent solubilities obtained for the three solvent systems. Comparing the binary and the complex systems, one order of magnitude difference was obtained for lycopene. This difference might be attributed to the size of the tomato particles and the extent of the rupture of the cellular structure in which the lycopene and β-carotene is present freely or complexed with other components. The presence of other compounds may affect the solubility behavior of the lycopene and β-carotene, which are not freely available for solubilization in SC CO_2.

4. Conclusions

The extraction of lycopene and β-carotene from skin + pulp of tomato at 40 °C, 400 bar, and 0.5 L/min of CO_2 flow rate using three different supercritical solvents was as follows: SC CO_2 + 5% canola oil > SC CO_2 + 5% ethanol > SC CO_2. Lycopene apparent solubility values for the multicomponent complex system (lycopene extracted from tomatoes with SC CO_2 and SC CO_2 + co-solvent) under

the same processing conditions were in the range of $0.4 \pm 0.07 \times 10^{-7}$ to $2.2 \pm 0.3 \times 10^{-7}$ mole fraction, whereas those for β-carotene were in the range of $6.2 \pm 0.9 \times 10^{-7}$ to $18 \pm 4 \times 10^{-7}$ mole fraction. The findings show the difference between the true solubility based on binary system measurements reported in the literature and the apparent solubility based on multicomponent system measurements of this study. This difference can be attributed to the impact of tomato cell matrix on the free availability of lycopene and β-carotene for solubilization into the supercritical fluid as well as the interactions between different components, which can change over time throughout the dynamic extraction period. Based on this study, the use of oil as a continuous co-solvent is promising for industrial recovery of carotenoids from tomato matrix. The carotenoid-saturated vegetable oil product can be used as is in a variety of nutraceutical and functional food applications.

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