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Supercritical Fluid Extraction of Lycopene from Tomato Processing Byproducts

N. L. ROZZI,† R. K. SINGH,‡ R. A. VIERLING,§ AND B. A. WATKINS*,†

Center for Enhancing Foods to Protect Health, Department of Food Science, and Department of Agronomy, Purdue University, West Lafayette, Indiana 47907

Tomato seeds and skins acquired from the byproduct of a local tomato processing facility were studied for supercritical fluid extraction (SFE) of phytochemicals. The extracts were analyzed for lycopene, β-carotene, α-carotene, α-tocopherol, γ-tocopherol, and δ-tocopherol content using high-performance liquid chromatography−electrochemical detection and compared to a chemically extracted control. SFEs were carried out using CO_2 at seven temperatures (32−86 °C) and six pressures (13.78−48.26 MPa). The effect of CO_2 flow rate and volume also was investigated. The results indicated that the percentage of lycopene extracted increased with elevated temperature and pressure until a maximum recovery of 38.8% was reached at 86 °C and 34.47 MPa, after which the amount of lycopene extracted decreased. Conditions for the optimum extraction of lycopene from 3 g of raw material were determined to be 86 °C, 34.47 MPa, and 500 mL of CO_2 at a flow rate of 2.5 mL/min. These conditions resulted in the extraction of 61.0% of the lycopene (7.19 μg lycopene/g).

KEYWORDS: Lycopene; supercritical fluid extraction; carotenoids; tocopherols; chromatography

INTRODUCTION

Lycopene is an acyclic carotenoid containing 13 double bonds and is chiefly found in tomatoes, where it provides their rich, red color. Other sources of lycopene include apricots, pink grapefruit, guava, and watermelon (*1*). Although there are other products that contain lycopene, tomatoes are the main source. Sharma and Le Maguer (*2*) evaluated the nutrient content of tomato juice fractions and determined that 72−92% of the lycopene in a tomato is found in the water insoluble fraction and the skin of the tomato. About two-thirds of the lycopene is located in the heat-denatured cytoplasmic material of the water insoluble fraction, while the remaining lycopene is found in the intracellular granules. Lindner et al. (*3*) determined that this distribution could be due to the location of coagulated cytoplasmic material between the two different fractions of insoluble solids.

Recent studies suggest that carotenoids have anticarcinogenic properties; some of these investigations have linked increased consumption of lycopene-containing foods, or increased levels of serum lycopene, with a decreased occurrence of cancer. Researchers found that individuals who reported a higher intake of tomatoes had a lower incidence of digestive tract cancer (*4*). It was observed that an increased intake of lycopene, but not other carotenoids, was associated with a decreased risk of prostate cancer (*5*−*7*). In a review of multiple studies, Giovannucci and Clinton (*8*) reported that epidemiological data supported these claims. In addition, Rao and Agarwal (*9*), Weisburger (*10*), Clinton (*11*), Astorg (*12*), and Gerster (*13*), examined the health benefits of lycopene and correlated the consumption of food containing lycopene to the prevention of several cancers.

Supercritical fluid CO_2 has been used to extract α- and β-carotene and lycopene from several plant sources (*14*−*17*). Marsili and Callahan (*14*) compared supercritical fluid extraction (SFE) of carotenoids to chemical solvent extraction from several vegetables. Their study on the supercritical CO_2 extraction of carotenoids is limited, however, because only a single extraction temperature and pressure was evaluated (*14*). Rather, numerous chemical modifiers (water, ethanol, methylene chloride, and hexane) were tested to enhance the extraction process. Modifiers are similar to cosolvents in that they aid in the extraction, but modifiers are added directly to the sample prior to extraction, instead of with the solvent (like cosolvents). In the aforementioned study, SFEs were equal to or greater than the chemical extractions for the percentage of carotenoids removed (*14*). In another experiment, Barth et al. (*15*) examined the solubility of carotenoids from carrot tissue in supercritical CO_2. Three factors were investigated to determine the solubility of carotenoids (α- and β-carotenes) in the supercritical CO_2 following a factorially designed experiment. They observed that the optimum conditions for the extraction were 50 °C, 30.40 MPa, using 10% ethanol as a cosolvent. In addition, the provitamin A activity was 7% greater in the sample extracted by supercritical CO_2 than that obtained by chemical solvent extraction (*15*).

* To whom correspondence should be addressed. Tel.: (765)494-5802. Fax: (765)494-7953. E-mail: watkins@foodsci.purdue.edu.
† Department of Food Science, Purdue University.
‡ Current address: Department of Food Science and Technology, University of Georgia, Athens, GA 30602.
§ Department of Agronomy, Purdue University.

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Cadoni et al. (*16*) extracted lycopene from tomatoes using supercritical CO_2. They tested temperatures ranging from 40 to 80 °C at a pressure of 27.58 MPa. As temperatures were increased, the amount of lycopene extracted increased 20-fold. In addition, they found that by using chloroform or hexane as a cosolvent, the percentage of lycopene extracted increased with the addition of increasingly nonpolar cosolvents.

Baysal et al. (*17*) further studied the extraction of lycopene from tomato paste waste using supercritical CO_2 at temperatures of 35, 45, 55, and 65 °C and pressures of 20, 25, and 30 MPa. Ethanol was utilized as a cosolvent at levels of 5, 10, and 15% in the experiments. Results from both studies revealed that elevating the pressure and/or temperature of the CO_2 resulted in an increased amount of lycopene extracted. This is due to the increase in the density of the supercritical CO_2, which improved the ability of the CO_2 to solubilize carotenoids. At lower densities, supercritical CO_2 performs similar to hexane as a nonpolar solvent for extraction, while at higher densities it behaves more like chloroform (*18*).

The goal of this research was to determine the effects of temperature, pressure (beyond those conditions examined in previous research), flow rate, and CO_2 volume on SFE of lycopene from a byproduct of tomato processing. The parameters for extraction were evaluated on the recovery of carotenoids and tocopherols in the tomato product and on the fatty acid composition of the lipid in the sample.

MATERIALS AND METHODS

Sample Preparation. *Tomato Seeds and Skins*. Tomato seeds and skins (51.6% dry matter) were obtained from Red Gold, Inc. (Elwood, IN) on September 13, 2000. The samples were the byproduct of steam peeling used to produce tomato sauce and collected prior to removal from the processing facility. The sample was stored at −20 °C until used and did not undergo any further preparation. The distribution of seeds and skins within 3 g of the raw material was 30.5 ± 2.2% tomato skin and 69.5 ± 2.2% tomato seeds.

Chemical Extraction. A chemical extraction of lycopene from tomato byproduct was performed to serve as a standard for the recovery of phytochemicals in the test material. A 2 g sample of seeds and skins was placed in an extraction tube, and 20 mL of chloroform was added to the tube followed by sonication for 30 min. The sample was centrifuged for 15 min at 2000 rpm (913*g*), and an aliquot was removed for analysis of lycopene content by high-performance liquid chromatography (HPLC). The extraction procedure was repeated on the sample to recover residual lycopene in the sample. Exhaustive extraction of tomato seeds and skins with additional volumes of chloroform did not result in additional recovery of lycopene.

SFE. The SFE system consisted of an Isco model 260 D syringe pump, SFX-210 extractor, and a temperature-controlled variable restrictor (Isco, Lincoln, NE). Three experiments were conducted to assess the effects of temperature, pressure, flow rate, and CO_2 volume on SFE of phytochemicals from the samples. The experiments evaluated the following: experiment 1 (exp. 1), the effect of CO_2 temperature and pressure; experiment 2 (exp. 2), the effect of CO_2 flow rate; and experiment 3 (exp. 3), the effect of CO_2 volume. Exp. 1 provided information on factors that contributed to improved solubility, exp. 2 evaluated flow rate for CO_2, and exp. 3 tested the combination of operation factors on recovery of lycopene from tomato seeds and skins.

The temperatures tested in exp. 1 ranged from 32 to 86 °C at 9 °C intervals, and the pressures ranged from 13.78 to 48.26 MPa at 3.45 MPa intervals. The flow rate of the CO_2 was maintained at 2.5 mL/min for 20 min, giving a total volume of 50 mL of CO_2. After the extraction, the sample was removed, and another 50 mL of CO_2 was passed through the extractor to ensure that any remaining lycopene was recovered. The flow rates were measured at the extraction temperature and pressure due to equipment design. The conditions were fully crossed in the factorial design, and the extractions were performed in triplicate. To prevent plugging, the restrictor temperature was maintained at 15 °C above the extraction temperature. Extracts were collected in a vial immersed in ice water to facilitate condensation of the extract. Once collected, extracts were dissolved in 5 mL of MTBE (methyl *tert*-butyl ether) for analysis by HPLC.

The extractions for exp. 2 were performed by holding the temperature, pressure, and CO_2 volume constant and then increasing the flow rate of CO_2 used for each extraction. The temperature and pressure selected for exp. 2 were the optimum conditions determined for the extraction of lycopene in exp. 1. For this experiment, flow rates from 2.5 to 15 mL/min were examined. These flow rates were tested at intervals of 2.5 mL/min. The optimum conditions determined in exp. 1 and exp. 2 were utilized in exp. 3 to determine the effect of CO_2 volume on the extraction of lycopene under optimum temperature and pressure conditions. This was accomplished by repeatedly collecting extracts from a sample at 100 mL intervals until a total of 1200 mL of CO_2 was used to perform the extraction.

Carotenoid and Tocopherol Determination. The concentrations of lycopene, β-carotene, α-carotene, α-tocopherol, γ-tocopherol, and δ-tocopherol in each extract were quantified using an HPLC equipped with an electrochemical detector (Coularray, ESA, Inc., Chelmsford, MA). Two mobile phases were used for the gradient separation [mobile phase A (pH 4.8), methanol:ammonium acetate (0.2 M) (90:10), and mobile phase B (pH 4.8), methanol:1-propanol:ammonium acetate (1.0 M) (78:20:2)]. The gradient used for this separation consisted of 100% mobile phase A at time 0, 80% mobile phase B at 10 min, 100% mobile phase B at 20 min, 100% mobile phase B at 27 min, and 100% mobile phase A from 28 to 32 min. All transitions between mobile phases were linear. The flow rate for the separation was 1.6 mL/min, which generated a pressure of 14 MPa. A Phenomenex Luna C-18(2) column was used for the separation (150 mm × 4.6 mm, 3 μm particle size) (Phenomenex, Inc., Torrance, CA). The cell potentials for the detector were set at 350−700 mV by increments of 50 mV, and the column was maintained at a temperature of 37 °C. Carotenoid standards were purchased from Sigma (St. Louis, MO), and tocopherol standards were purchased from Matraya (Pleasant Gap, PA).

Fatty Acid Determination. To assess the effect of SFE on the fatty acid composition of the samples, lipid extracts were prepared from tomato seeds and skins using either chloroform/methanol (2:1 v/v) or supercritical CO_2. The conditions tested for SFE were 32, 50, and 68 °C and 13.78, 27.57, and 41.36 MPa at a flow rate of 2.5 mL/min. The extracts were saponified, and fatty acid methyl esters (FAME) were prepared by esterification using boron trifluoride (BF_3) in methanol (14%, w/w) (Supelco Inc., Bellefonte, PA).

FAME were analyzed using a gas chromatograph (GC HP 5890 series II, auto sampler 7673, HP 3365 ChemStation; Hewlett-Packard Co., Avondale, PA) equipped with a DB 23 column (30 m, 0.53 mm i.d., 0.5 μm film thickness; J&W Scientific Co., Folsom, CA). The GC was operated at 140 °C for 2 min, and the temperature was programmed to increase at 1.5 °C/min to 198 °C and held for 7 min (*19*). The injector and flame-ionization detector temperatures were 225 and 250 °C, respectively. FAME were identified by comparison of their retention times with authentic standards [GLC-422, CLA (UC-59-A and UC-59-M)], Nu-Chek-Prep, Elysian, MN, and FAME were prepared from menhaden oil (Matreya Inc., Pleasant Gap, PA).

Statistics. The data collected were analyzed using a two way multivariate analysis of variance (MANOVA). Temperature and pressure of the supercritical CO_2 were used as predictor values in the statistical design and δ-tocopherol, γ-tocopherol, α-tocopherol, lycopene, α-carotene, and β-carotene served as the output values. The fatty acid composition data were subjected to a two way MANOVA using temperature and pressure as the predictor values and the individual fatty acids as the output values. A Duncan's Multiple Range Test was performed in conjunction with each MANOVA (*20*).

RESULTS AND DISCUSSION

In this study, lycopene was extracted from tomato seeds and skins using supercritical CO_2. The extraction conditions were varied to examine the effect of extraction temperature, pressure, CO_2 flow rate, and volume of CO_2 used on the extraction yield. The initial lycopene content of the seeds and skins was 11.8 ±

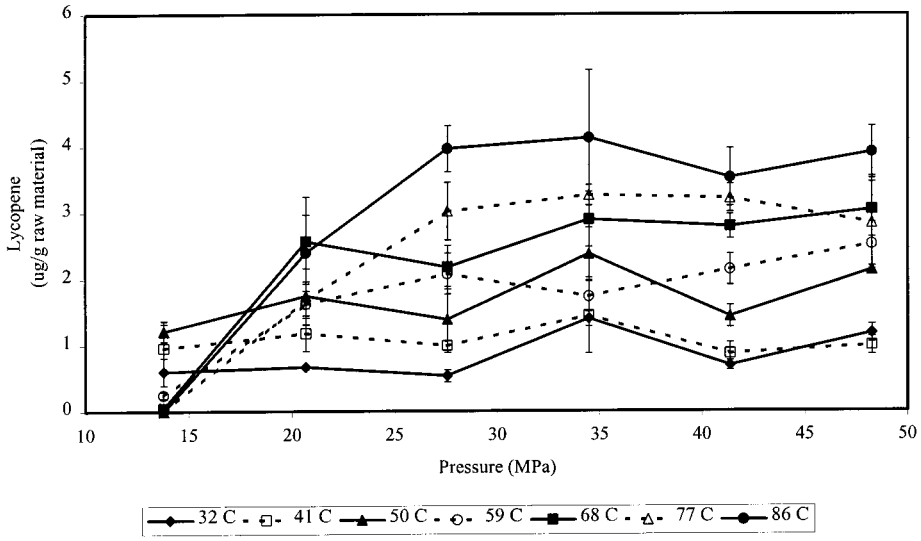

Figure 1. Concentration of lycopene extracted from tomato seeds and skins vs extraction pressure at different temperatures.

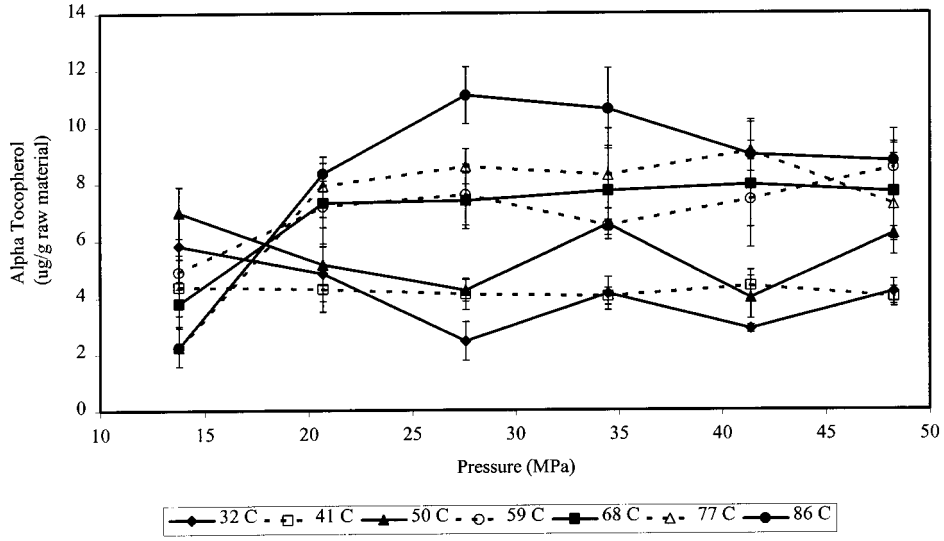

Figure 2. Concentration of α-tocopherol extracted from tomato seeds and skins vs extraction pressure at different temperatures.

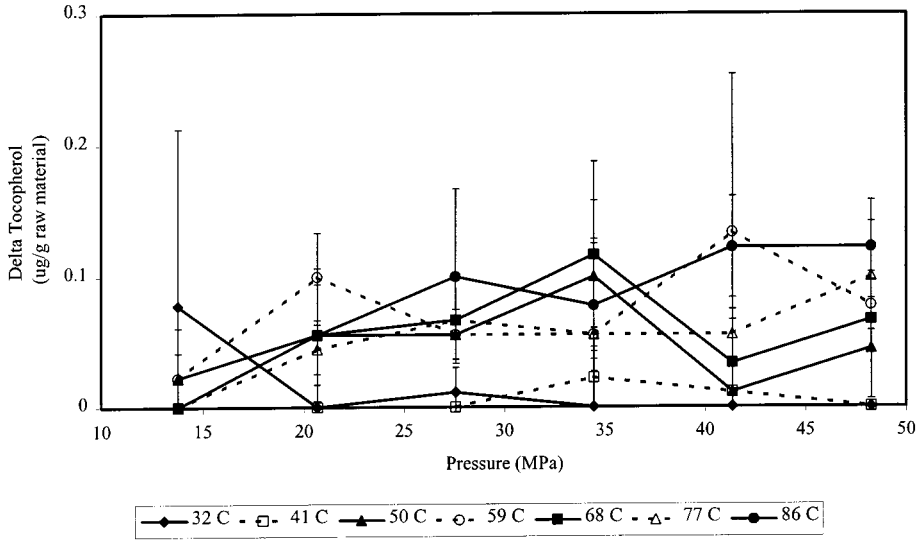

Figure 3. Concentration of δ-tocopherol extracted from tomato seeds and skins vs extraction pressure at different temperatures.

2.0 ($n = 3$) μg of lycopene per gram of raw material (wet weight) or 24.5 ± 4.0 μg of lycopene per gram of dry material (dry weight) as determined by chloroform extraction.

Effect of Extraction Conditions on Carotenoid and Tocopherol Composition. Analysis of data from exp. 1 revealed that both temperature and pressure had a significant effect

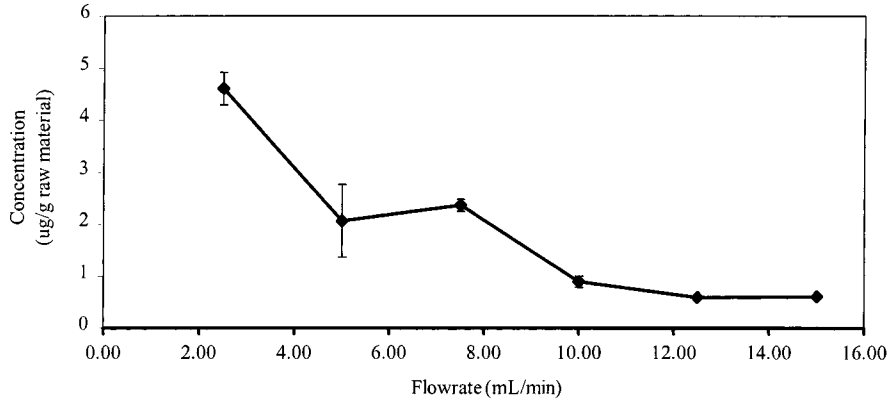

Figure 4. Concentration of lycopene extracted from tomato seeds and skins at 86 °C and 34.47 MPa vs flow rate of CO_2.

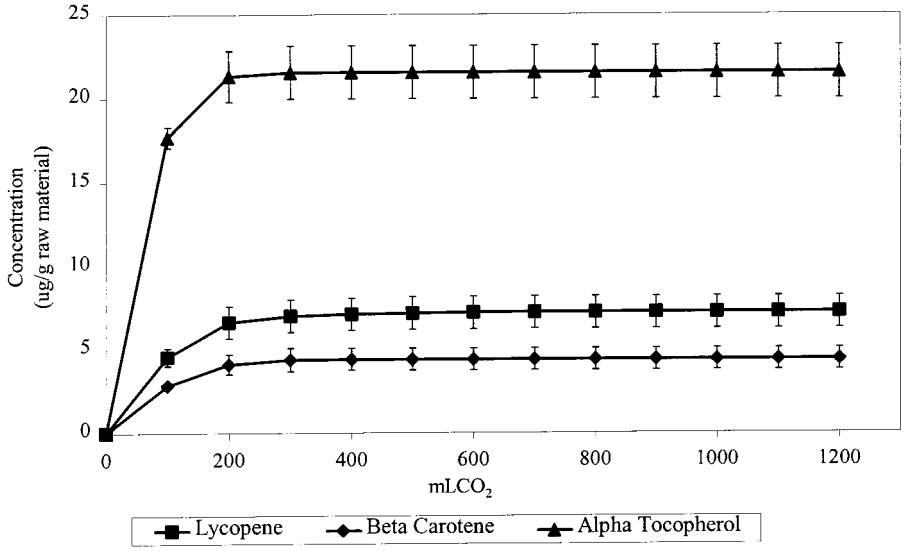

Figure 5. Concentration of lycopene extracted from tomato seeds and skins at 86 °C, 34.47 MPa, and 2.5 mL/min vs volume of CO_2.

Table 1. Summary of Conditions Studied on Lycopene Extraction by Supercritical CO_2

variable	condition	total lycopene (μg/g raw material)	total lycopene (μg/g dry weight)	% recovery of lycopene
CO_2 temp, pressure, and flow rate	86 °C, 34.47 MPa, and 2.5 mL/min	4.59	9.48	38.8
CO_2 volume	500 mL	7.19	14.86	61.0

($p < 0.05$) on the amount and composition of the extracted material. **Figures 1** and **2** illustrate the effect of temperature and pressure on the amount of lycopene and α-tocopherol extracted, respectively. There was a positive correlation between increases in temperature and/or pressure and the amount of lycopene and α-tocopherol extracted. The data also demonstrate that at the higher temperature and pressure the recovery of all of the carotenoids and tocopherols measured increased except for δ-tocopherol. **Figure 3** shows that extraction conditions had a minor effect on removal of δ-tocopherol and that the data were extremely variable (large standard deviations). The SFE result was consistent with the data on chemically extracted samples where the standard deviations of the latter samples were 86% of the mean value (0.15 ± 0.13 μg δ-tocopherol/g raw material).

To determine if temperature and pressure influenced the extraction of δ-tocopherol, a Duncan's Multiple Range Test was performed on the δ-tocopherol data. The results indicated that the effects of temperature and pressure on the extraction of δ-tocopherol were small and that the data could be segregated into four groups for interpretation, based on the range in temperature and pressure. For the effect of temperature, the data were grouped into a low-temperature group (32 and 41 °C) and high-temperature group (all other temperatures), and similar effects were observed for pressure with a low-pressure group (13.78 and 20.68 MPa) and a high-pressure group.

Figure 2 also shows that SFE at low pressures and decreasing temperature improved the extraction of compounds with low volatility. That is, more α-tocopherol was extracted at 50 °C as compared to 86 °C but the opposite was true when the pressure increased from 13.78 to 20.68 MPa. This response is weakly related to pressure in that at lower pressures, the amount of each compound extracted decreased with increased temperatures, while at higher pressures the amount of each compound extracted increased with elevated temperatures. The difference in the effect of temperature that took place at 13.78 MPa as compared to 20.68 MPa can be related to the difference in density of the supercritical CO_2 at 50 and 86 °C. At 13.78 MPa and 50 °C, the density of CO_2 is 0.42 g/mL, while at 86 °C the density of the CO_2 is 0.22 g/mL. At 13.78 MPa, the increase in

density corresponds to an increase in the solvent power of the fluid. At pressures above 20.68 MPa, the increase in the vapor pressure of the solute with increasing temperatures has a much greater effect on solubility than density. Similar trends have been observed in studies on the solubility of oleic acid in ethylene and caffeine in CO_2 (*18*).

The effect of temperature and pressure on the extraction of phytochemical compounds can be related to changes in the density of CO_2. At lower densities, the polarity of supercritical CO_2 is more like that of hexane, while at higher densities, it is more like that of chloroform (*18*). Carotenoids display much higher solubility in chloroform as compared to hexane (*21*), possibly explaining why solubility increases with elevating temperature and pressure. It also can be noted that as the pressure and temperature increased, the amount of lycopene extracted reached a maximum near 86 °C and 34.47 MPa (**Figure 1**). This effect also holds true for other compounds and implies that an optimum extraction condition can be reached (*22*). If the extraction conditions exceed the optimum conditions, the result may be a decrease in the efficiency or capacity to extract the compound(s). This optimum, which is not at the maximum pressure tested, could be attributed to a decrease in diffusivity of supercritical CO_2 as its density increases. The decrease in diffusivity could limit the ability of the CO_2 to diffuse through the sample and dissolve more of the solute. Another possibility is that the increase in pressure leads to compaction of the sample that also would limit the diffusion of CO_2 throughout the sample (*22*).

The results of exp. 2 indicate that as the flow rate increased from 2.5 to 15 mL/min, the amount of lycopene extracted decreased (**Figure 4**). The lycopene yield extracted remained at or below 1.0 μg/g raw material or a recovery of less than 8% for flow rates greater than 10 mL/min. In comparison, the recovery of lycopene extracted with CO_2 at 86 °C and 34.47 MPa with a flow rate of 2.5 mL/min was 38.8% (4.59 μg/g raw material). The lower amount of lycopene extracted at the higher flow rates could be linked to the reduced amount of time the solvent was in contact with the seeds and skins. In addition, the lower amount of lycopene extracted at the higher flow rate may also be a result of a channeling effect, where the solvent is forced through the sample at such a high rate that it passes only around the solid matrix and is not able to diffuse through the pores within the sample. The increased flow also could cause the sample to compact and restrict CO_2 movement into and out of the sample, reducing the amount of CO_2 that comes in contact with the sample (*22*).

The maximum amount of lycopene that can be extracted from a byproduct of tomato processing was determined by utilizing the most favorable temperature, pressure, and flow rates (86 °C, 34.47 MPa, and 2.5 mL/min.) determined in exp. 1 and exp. 2 and extracting the samples with 1200 mL of CO_2 (**Figure 5**). It can be seen that the amount of lycopene extracted improved with increasing amounts of CO_2 used until reaching a plateau for the amount of lycopene extracted. The SFE parameters used revealed that most α-tocopherol and β-carotene were extracted in the first 200−300 mL of CO_2, while the amount of lycopene extracted increased until 500 mL of CO_2 was used in the extraction process (**Figure 5**). The differences in the patterns of extraction for lycopene and α-tocopherol can be related to the unique solubility of each compound in supercritical CO_2. Exp. 3 resulted in an extract that contained 7.19 ± 0.95 μg/g raw material or a recovery of 61.0%.

The percentage recovery for the extraction conditions was calculated and is presented in **Table 1**. The highest recovery (61.0%) was obtained by performing the extractions at a flow rate of 2.5 mL/min, temperature of 86 °C, and pressure of 34.47 MPa. These results are in excess of what was obtained by Baysal et al. (*17*) who only recovered 20% of the lycopene present without the use of cosolvents. Although the percentage extracted is high for our products, the concentration of lycopene in our extracts was still much lower than that obtained by Cadoni et al. (*16*). The reason for this discrepancy may be due to higher amounts of lycopene in their raw material. Cadoni et al. (*16*) found that the lycopene content of their raw material was 778 μg/g dry wt, while the raw material used in this study contained only 24.5 μg/g dry wt.

Effect of Extraction Conditions on Lipid Composition. To further characterize the extracts produced by SFE, the fatty acid composition of the extracts produced by different extraction conditions was tested. Those conditions were 32, 50, and 68 °C and 13.78, 27.57, and 41.36 MPa at a flow rate of 2.5 mL/min. The fatty acid composition of the extracts produced by SFE varied little from the extracts produced by chemical extraction. The predominant fatty acids in the seeds and skins were 16:0 (13.51% ± 1.42) and 18:2*n*-6 (57.18% ± 0.43) as determined by GC analysis after chemical extraction. The same fatty acids were predominant in the extracts produced by SFE with greater variation (16:0, 21.58% ± 2.52 and 18:2*n*-6, 47.19% ± 8.44). Analysis of the data indicated that there was no statistically significant ($p \geq 0.05$) difference between the samples extracted by supercritical CO_2 and the chemically extracted control. Although there was greater variation within the samples prepared by SFE, there was still no statistically significant ($p \geq 0.05$) effect of temperature and pressure on the extract composition. The reason for the variation within the extract produced by supercritical CO_2 extraction was due to the changes in the solubility of the two fatty acids with the changing extraction conditions. As the temperature and pressure of the extraction increased, the solubility of the longer chain 18:2*n*-6 (linoleic acid) increased relative to 16:0 (palmitic acid). The increase in the amount of 18:2*n*-6 present in the extract is likely due to the increased solubility of 18 carbon fatty acids as previously reported (*23*) and influenced by the greater standard deviation of values obtained from the SFE procedure.

CONCLUSIONS

The results indicate that lycopene can be extracted with substantial success from a byproduct of the tomato processing industry with SFE using CO_2 without the use of cosolvents. It was determined that both temperature and pressure had an effect on the extraction of lycopene and that an optimum temperature and pressure combination, 86 °C and 34.47 MPa, resulted in extracting 61.0% of the lycopene present in the sample using 500 mL of CO_2 at a flow rate of 2.5 mL/min. The information obtained in this study can be used for scale-up of the extraction process.

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