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Extraction and Analysis of Tomato Seed Oil

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Abstract Tomato seeds represent a very large waste by-product from the processing of tomatoes into products such as tomato juice, sauce and paste. One potential use for these seeds is as a source of vegetable oil. This research investigated the oil content of tomato seeds using several extraction techniques as well as an examination of the oil extracts to determine the composition of the minor constituents such as phytosterol and antioxidant composition. The oxygen radical absorbance capacity (ORAC) of the tomato seed oils were also measured and correlated with antioxidant contents. This research demonstrated that tomato seed oil yield was highest using hot ethanol and followed by hot hexane and finally SC-CO_2. The SC-CO_2 treatment, however, had the highest total phytosterol content as well as highest individual phytosterol content. Sitosterol, cycloartanol, and stigmasterol were the most abundant phytosterols present in the extracts. The highest concentrations of antioxidants were found in the hexane extract. The most abundant antioxidants found in the tomato seed oils were all-*trans*-lycopene, *cis*-3-lycopene and β-carotene. ORAC was highest for the hexane extract. Oil yield was inversely proportional to both α-tocopherol and γ-tocopherol content and positively correlated with *cis*-3-lycopene content. ORAC values were positively correlated with only all-*trans*-lycopene and *cis*-3-lycopene demonstrating their role as antioxidants in the tomato seed oil.

Keywords Tomato seed oil · Supercritical carbon dioxide · Accelerated solvent extraction · Phytosterol · Antioxidant · ORAC

Introduction

In 2002, ca. 12 million tons of tomatoes were canned and the commercial processing of tomatoes produces large amounts of waste [1, 2], with between 33 and 40% of the raw tomatoes ending up as processing waste [3, 4]. Seeds and skins constitute the main waste by-product of the tomato processing industry [5, 6]. To maximize profits, tomato processors need to find uses for these waste materials. Tomato seeds have been shown to contain ca. 20% oil [7] with a fatty acid composition of tomato seed essentially like that of low linolenic soybean oil [8]. It has been suggested that tomato seeds would be a good source of salad oil [9].

Tomato seed oil has previously been effectively extracted using supercritical CO_2 (SC-CO_2) [6, 10]. In addition, SC-CO_2 has been used to extract minor constituents from tomatoes, including phytosterols [11], tocopherols [11, 12], β-carotene [6, 11–13] and lycopene [6, 11–16]. Although tomato products have been shown to contain high levels of both the carotenoids, β-carotene and lycopene [17] and carotenoids have excellent antioxidant activity due to their ability to quench singlet oxygen and trap peroxyl radicals [18, 19], very little is known about the antioxidant capacity of tomato seed oil.

The objective of this research was to use SC-CO_2 and pressurized solvent extraction (*n*-hexane and ethanol) for

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name USDA implies no approval of the product to the exclusion of others that may also be suitable.

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the extraction of oil from tomato seeds, determine the sterol, tocopherol and carotenoid contents of the resulting tomato seed oils as well as investigate the antioxidant capacity of the oils.

Experimental

Materials

Tocopherol standards (≥95% purity), cholesterol and stigmasterol were purchased from Matreya, Inc. (Pleasant Gap, PA, USA). Campesterol, brassicasterol and cycloartenol were from Steraloids (Newport, RI, USA). Cholestane, sitosterol, sitostanol, β-carotene and lycopene were from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Each phytosterol standard was ≥97% purity. *N,O-bis*(trimethylsilyl)fluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS) was purchased from Regis (Morton Grove, IL, USA). Sodium fluorescein, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and methyl-β-cyclodextrin were purchased from Sigma Aldrich (St. Louis, MO, USA) and used as received. Potassium phosphate dibasic and sodium phosphate monobasic were obtained from Fisher Scientific.

Tomato Seed Oil Extractions

Ground tomato seed samples were obtained from Morning Star (Los Banos, CA, USA) and were shipped frozen. The ground tomato seeds had an average particle size of 0.64 mm and a moisture content of 8.4%. Three methods of extraction were tested for comparison: supercritical carbon dioxide (SC-CO_2), and accelerated solvent extraction using *n*-hexane (Hexane ASE) and accelerated solvent extraction using ethanol (Ethanol ASE). Supercritical carbon dioxide extractions were conducted using a pilot plant extractor previously described [20]. The extraction cell held ca. 1.5 kg ground tomato seeds and the extraction conditions were as follows: 80 °C and 55.2 MPa and a flow rate of ca. 0.3 kg CO_2/min. These conditions are known to be very effective for extracting triglycerides [20]. A reduced pressure receiver (50 °C and 8.3 MPa) was used to collect the extract. A solvent:feed ratio of 8.5:1 was used to give a sample large enough for determining the chemical composition, although a higher solvent:feed would have undoubtedly given a higher yield. A Dionex Accelerated Solvent Extractor (ASE) 300 (Dionex Corp., Sunnyvale, CA) was used to extract the ground tomato seeds using *n*-hexane and absolute ethanol. The sample size was ca. 0.6 g and the extraction conditions were the same for both solvents as follows: 100 °C, 1,500 psi, 7 min static, 3 cycles, 60 s purge. Extracts were dried under a gentle stream of nitrogen to a constant weight. All yield data (g oil/100 g seeds) were based on the fresh weight of the tomato seeds. There were three replications of each extraction method.

Compositional Analyses

For phytosterol analyses, tomato seed oils (ca. 100 mg) were saponified in 2 N KOH in ethanol at 60 °C for 45 min. Phytosterols were extracted twice with 2 ml hexane. The combined hexane fractions were dried under nitrogen. Trimethylsilyl (TMS) derivatives of the phytosterols were made by adding 100 µl each pyridine and *N,O-bis* (trimethylsilyl)-fluoroacetamide with 1% trimethylchlorosilane (Regis Tech., Morton Grove, IL, USA) and heating at 60 °C for 1 h on a heating block. For quantitation, 1 µl of each sample was manually injected, in triplicate, onto a Varian (Palo Alto, CA) 3400 gas chromatograph (GC) equipped with a flame ionization detector (FID), and a Supelco (Bellefonte, PA, USA) SPB^{TM}-1701 (30 m × 0.25 mm × 0.25 µm) capillary column. Helium was used as a carrier gas, with a flow rate of 1 ml/min and a 1:50 injector split. Injector temperature was 270 °C, and detector temperature was 290 °C. The column oven initial temperature was 250 °C for 0.5 min, increased at 10 °C/min to 270°C and held for 27 min, then increased at 10 °C/min to 280 °C and held for 3.5 min. Data collection and integration were performed using Varian Galaxie Chromatography Software Ver. 1.9.3.2. Cholesterol, cholestane, brassicasterol, campesterol, stigmasterol, sitosterol, and cycloartenol were identified by comparison of their retention times (RT) and confirmed by gas-chromatography mass-spectrometry (GC-MS). Quantitation was carried out by the internal standard (IS) method using 50 µg cholestane as IS with a minimum of four levels for each standard. For phytosterols with no available commercial standard, the response factor for β-sitosterol was used for quantitation. GC–MS was used to assist in identifying sterols with no commercially available standards. GC–MS analyses were performed on an Agilent (Santa Clara, CA, USA) 6890 GC–MS equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm), a 5973 mass selective detector, and a 7683 autosampler. The transfer line from GC to the MSD was set to 280 °C. The injector and oven temperature programs were the same as described for the GC-FID instrument above. MSD parameters were as follows: scan mode, 50–600 amu, ionizing voltage, 70 eV, and EM voltage, 1,823 V. Mass spectral identification was performed using the Wiley MS database combined with comparison to literature values for RT and mass spectra (see details in "Results and Discussion").

For determination of tocopherol content, tomato seed oil extracts (ca. 50–60 mg) were weighed into test tubes and

1 ml of hexane added. The tubes were mixed by vortex for 30–60 s, then filtered through a 0.45 μm filter (Chrom Tech, Inc., Apple Valley, MN, USA) and these diluted extracts were immediately analyzed in triplicate by HPLC. Analyses were carried out with a Thermo Separation Products (San Jose, CA, USA) SpectraSYSTEM pump and autosampler connected to a SpectraSYSTEM FL fluorescence detector. Samples (50 μl) were injected onto a YMC-Pack-Diol-NP, 5 μm, 4.6 × 250 mm column (YMC, Wilmington, NC, USA). The mobile phase consisted of 98:2 v/v hexane: 2-propanol with a continuous flow rate of 1.5 ml/min. Tocopherols were identified based upon retention time similarity with known standards using fluorescence detection (excitation: 292 nm, emission: 344 nm). Quantitation was carried out using external calibration with pure standards injected at concentrations between 5 and 50 μg/ml for α- and γ-tocopherols, and between 0.5 and 20 μg/ml for δ-tocopherol.

For β-carotene and lycopene analyses, tomato seed oil extracts were diluted to 50 mg/ml in 50:50 (v/v) methanol:methyl *tert*-butyl ether (MTBE), and were injected onto a Shimadzu (Columbia, MD, USA) HPLC system with an LC20AT HPLC pump, DGU-20A membrane degasser, SIL-10AF autosampler and SPD-M20A diode array detector. Separation of β-carotene and lycopene was performed using the solvent gradient described by Sass-Kiss et al. [21] using a YMC (Kyoto, Japan) C30 column (3 μm, 250 × 4.6 mm i.d.) [21]. Peaks were scanned from 200 to 550 nm, the detection wavelength was 450 nm. Carotenoids were identified by retention time compared to known standards as well as by spectral characteristics. Quantitation was carried out by the external standard method using β-carotene standards solutions ranging from 13.3 ng/ml to 1.3 μg/ml, and lycopene standard solutions ranging between 0.5 and 2.5 μg/ml. Concentrations of the initial stock solutions were determined using the extinction coefficients for each standard. The lycopene standard was declared by Sigma to be ≥90% pure, however, HPLC analysis revealed that the standard had three peaks besides the all-*trans*-lycopene isomer, which accounted for 70% of the total peak area. The three other peaks were tentatively identified as *cis*-lycopene isomers based on their spectral and retention characteristics compared to other reports of the *cis*-lycopene isomer analysis by HPLC [22, 23]. Samples with peaks eluting at the same retention time and with the same spectral characteristics were identified as *cis*-isomers and their quantitation was determined using the same standard curve for the all-*trans* isomer.

Lipophilic Oxygen Radical Absorbance Capacity (L-ORAC$_{FL}$) Analyses

Lipophilic ORAC$_{FL}$ assays were performed similarly to those described by Prior and coworkers [24, 25] and the previous report by Haung et al. [26] with minor modification to allow the use of standard cuvettes instead of a microplate reader. Magnetically stirred reaction solutions in plastic cuvettes (Fisher, Cat# 14-955-130) were monitored at an excitation wavelength of 485 nm and an emission filter of 520 nm using a Varian Cary Eclipse Fluorometer (VIC, Australia) equipped with a circulating water jacket to maintain cell temperature at 37 °C. Fluorescence was followed using the Varian Cary Eclipse kinetics software package. AAPH was used as the peroxyl generator and Trolox as the standard. Final L-ORAC$_{FL}$ values are given in μmol of Trolox equivalents/g (μmol Tequiv/g) oil by using the standard curve calculated for each assay.

Extracted tomato seed oil extracts sample were accurately weighed (10.0–15.0 mg) and dissolved in 1.0 ml of acetone and then diluted with 9 ml of 50% acetone/50% water, v/v containing 7% randomly methylated β-cyclodextrin (RMCD) as a solubility enhancer. Samples were shaken at room temperature on an orbital shaker operating at 400 rpm for 1 h before use. The 7% RMCD solution was used to perform further dilutions of samples as needed, for the blank, and to dissolve the Trolox standards for the lipophilic assay.

A 100-μM stock solution of Trolox was prepared in 75 mM phosphate buffer, pH 7, and divided into 1-ml aliquots that were stored at −20 °C until use. A new set of stock Trolox vials were removed from the freezer daily for use and Trolox calibration standards at (20, 40 60 μM) were run for each sample to obtain the necessary standard curves.

Fresh fluorescein solutions (9.6×10^{-8} M) utilized for the L-ORAC$_{FL}$ assays were prepared daily by diluting 320 μl of a stock fluorescein solution (5.9×10^{-6} M; stored at −20 °C in the dark until needed) in 20 ml of 75-mM phosphate buffer, pH 7.

A stock standard solution of AAPH (172 mg) in 75-mM phosphate buffer, pH 7 (20 ml), was prepared daily for use as the rate of peroxyl generation from AAPH is temperature sensitive.

To conduct the L-ORAC$_{FL}$ assay, 100 μl of lipophilic sample solution was added to the fluorescein stock solution (2.4 ml of 9.6×10^{-8} M) in a cuvette containing a magnetic stir bar. The solution was equilibrated for 30 min at 37 °C then initiated by the addition of AAPH (750 μl). Fluorescence was recorded immediately every minute until the reading declined to less than 5% of the initial reading (e.g. 40 min). Sample replicates were run on different days. The 7% RMCD solution was used as for the blank and the Trolox standards used to obtain standard curves. Raw data were exported from the Varian Eclipse software to a Microsoft Excel spreadsheet (Microsoft, Roselle, IL, USA) for further analyses. The fluorescence versus time curves

were normalized to the curve of the blank corresponding to the same assay by multiplying original data by the factor $\text{fluorescence}_{\text{blank},t=0}/\text{fluorescence}_{\text{sample},t=0}$. From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as

$$\text{AUC} = (0.5 + f_1/f_0 + f_2/f_0 + \cdots + f_i/f_0) \times \text{CT}$$

where f_0 = initial fluorescence reading at cycle 0, f_i = fluorescence reading at cycle i, and CT = cycle time in minutes. The net AUC corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. L = ORAC_{FL} were determined by regression relating Trolox concentrations and the net area under the kinetic fluorescein decay curve for all the samples. L-ORAC_{FL} values were expressed as Trolox equivalents by using the standard curve calculated for each assay. Final results were in μmol Tequiv/g oil.

Analyses of variance (ANOVA) were conducted on the percentage data using Statistix 7 software (Analytical Software, Tallahassee, FL, USA), main effects were tested using F tests and means were compared using least significant difference (LSD) ($P = 0.05$). Correlation coefficients were determined between individual antioxidant content and oil yield as well as ORAC.

Results and Discussion

Tomato Seed Oil Yields

The effect of the extraction method on tomato seed oil yield is shown in Fig 1. The highest yield was for the Ethanol ASE, with a yield of 23.1% followed by the Hexane ASE (20.0%) and the SC-CO_2 (17.3%). The ANOVA indicated that there was a significant extraction treatment effect on oil yield ($F_{2,6} = 378.6$, $P = 0.0000$). All three extraction treatments were statistically different from one another. Although Vági et al. [11] studied tomato pomace instead of tomato seeds and their extraction conditions were similar but not identical, they reported the same trends as were found here with slightly lower values (i.e., 20.3, 15.3, and 15.1%, respectively). The ethanol treatment may have given a slightly higher yield due to the extraction of more polar compounds than the hexane treatment would have extracted. The oil yield for the SC-CO_2 treatment was expected to be more similar to that for the hexane treatment and it is possible that the SC-CO_2 treatment may have given a higher yield if a slightly higher solvent:feed ratio had been used (i.e., greater than the 8.5:1 used here).

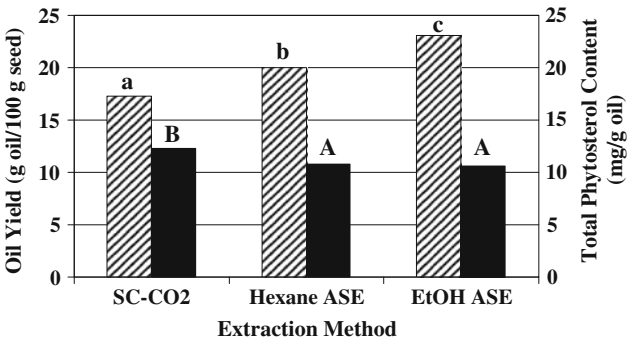

Fig. 1 Effect of extraction method on mean oil yield (*diagonal striped bars with lower case letters*) and mean total phytosterol content (*solid bars with upper case letters*). The means without letters in common differ significantly by LSD ($P = 0.05$)

Phytosterol Content

The total phytosterol contents for the extraction treatments are shown in Fig 1. The ANOVA indicated that there was a significant extraction treatment effect on total phytosterol content ($F_{2,6} = 46.2$, $P = 0.0002$). The total phytosterol content for the SC-CO_2 treatment (i.e., 12.30 mg/g oil) was significantly higher than both that for the Hexane and Ethanol ASE treatments (i.e., 10.81 and 10.55 mg/g oil, respectively) which were statistically equivalent. The 4-desmethyl, 4-monomethyl, and 4,4-dimethylsterols from tomato seed oil, along with seeds from several other solanaceous plants, have been extensively characterized by Itoh et al. [27–29], however, the absolute quantity was not determined. These amounts are relatively high compared to other vegetable oils, most of which have a phytosterol content ranging between 2 and 10 mg/g [30]. Lazos et al. [31] reported an average of 4.55 mg/g total phytosterols in tomato seed oil, or roughly half of what we found. These differences are likely due to differences in the samples, extraction conditions, and analytical methodology.

Interestingly, the oil yield data and phytosterol content data are clearly inversely proportional. This may be a result of all three extraction treatments extracting essentially all of the phytosterols but the three extraction treatments varying in the amounts of the triglycerides extracted. This resulted in the observed differences in the phytosterol concentrations between the oils. The absolute amounts of phytosterols extracted (calculated by multiplying the oil yield by the phytosterol content) are essentially the same for the SC-CO_2, Hexane ASE, and Ethanol ASE treatments (i.e., 2.1, 2.2 and 2.4 mg/g oil, respectively). This effect is also reflected in the data for the individual phytosterols.

Previous researchers have reported high levels of β-sitosterol [9, 31, 32], stigmasterol [9, 31, 32], cholesterol [31, 32], campesterol [31, 32], and brassicasterol [9, 32] in tomato seed oil. All of these phytosterols and several others were found in our tomato seed oils. The mean individual phytosterol contents of the tomato seed oils, F tests and P values from the statistical analyses (i.e., ANOVA) are

Table 1 Effect of extraction method on individual phytosterol content

Sterol	Mean phytosterol content (mg/g)[a]			$F_{2,6}$ (P value)
	SC-CO_2	Hexane ASE	Ethanol ASE	
Brassicasterol	0.0367 a	0.0433 a	0.0533 a	0.45 (0.6562)
Campesterol	0.5900 a	0.5600 a	0.5433 a	4.87 (0.0554)
Dihydrolanosterol	0.4867 a	0.4833 a	0.4467 a	1.77 (0.2483)
Cycloartenol	1.340 a	1.203 a	1.063 a	4.51 (0.0637)
Cholesterol	0.6933 b	0.5633 a	0.5800 a	58.74 (0.0001)
Cholestanol	0.1367 b	0.0767 a	0.0767 a	9.82 (0.0128)
Lathosterol	0.1300 b	0.1133 a	0.1133 a	12.50 (0.0073)
Dihydrospinasterol	0.4667 b	0.3900 a	0.4000 a	24.68 (0.0013)
Stigmasterol	1.3567 b	1.1300 a	1.1467 a	36.21 (0.0004)
Sitosterol	4.0033 b	3.6067 a	3.5733 a	28.93 (0.0008)
Cycloartenol	2.6933 c	2.3767 b	2.2767 a	60.83 (0.0001)
β-Amyrin	0.1733 b	0.0967 a	0.0967 a	16.03 (0.0039)
Citrostadienol	0.2033 b	0.1833 a	0.1867 a	10.33 (0.0114)

[a] Mean ($n = 3$) in the same row without letters in common differ significantly by LSD ($P = 0.05$)

shown in Table 1. The ANOVAs indicated a significant extraction treatment effect (i.e., $F_{2,6} > 5.14$) for most individual phytosterols with the exceptions of brassicasterol, campesterol, dihydrolanosterol, and cycloartenol. The three extraction treatments gave similar results, although the SC-CO_2 tended to give the highest contents of any given phytosterol. Phytosterols identified were β-sitosterol ($\sim$33%), cycloartanol (22%), stigmasterol (10.4–11%), cycloartenol (10–11%), cholesterol (5.2–5.6%), campesterol (4.8–5.2%), dihydrolanosterol (and lophenol, 3.9–4.5%), unknown (3.6–3.8%), citrostadienol (1.7%), β-amyrin (1–1.4%), lathosterol (1%), cholestanol (0.7–1%), and brassicasterol (0.3–0.5%). The identification of β-sitosterol, stigmasterol, cycloartenol, cholesterol, campesterol, brassicasterol, and cholestanol were confirmed by RT and by GC–MS of known standards, and confirm previous reports of their presence in tomato seed oil [27, 28]. Lathosterol was identified by GC–MS comparison to the Wiley database. Dihydrolanosterol, β-amyrin, and citrostadienol were identified based on their mass spectrums and RT compared to the literature [33], confirmed by the fact that these sterols have previously been identified in tomato seed oil [28]. Several ions within the dihydrolanosterol mass spectrum, including *m/e* 472, 382, 367, and 269, indicated that this peak may also have contained a small amount of lophenol, which elutes at the same retention time [33], and is confirmed by the fact that this was previously found to be the most prominent 4-dimethylsterol in tomato seed oil [29]. The cycloartanol peak matched the relative retention time and mass spectra reported by Farines [33], and has also been shown to be the most prominent 4,4 dimethylsterol in tomato seed oil [28], followed by cycloartenol. Cycloartenol elutes at the same RT as Δ5-avenasterol, which has been reported by others in tomato seed oil [27, 31, 34]. It is possible that these two peaks were co-eluting, but there were no detectable molecular ions characteristic for Δ5-avenasterol seen in the mass spectra. An unknown peak representing approximately 3.6–3.8% of the sterols eluted at an RRT of 0.71 (relative to the retention time of β-sitosterol). GC–MS analysis revealed that the TMS–ether of this sterol was of the same molecular weight as β-sitosterol (M^+ 486). There are no known 4-desmethylsterols of this molecular weight that elute at this relative retention time, and there were none of the characteristic molecular ions for either Δ5 (*m/e* 129) or Δ7 (*m/e* 229 or 255) desmethylsterols. Based on the GC–MS data and comparison to values for sterol peaks of the same molecular weight in tomato seed oil, the unknown peak likely consists of one or more of the following 4-monomethylsterols: 31-norcycloartanol, 31-norlanost-8-enol, or 31-norlanost-9[11]-enol [29].

The high content of cholesterol, relative to other vegetable oils that usually only contain trace amounts of this sterol [30], is characteristic of phytosterols from the seeds from the Solanaceae [27, 28]. Also, while many vegetable oils contain primarily 4-desmethylsterols (i.e., sitosterol, stigmasterol, campesterol, cholesterol), and very low to trace amounts of 4-monomethyl, and 4,4 dimethyl sterols [35], tomato seed oil samples had significant quantities of 4-mono-, and 4-dimethylsterols (i.e., citrostadienol, dihydrolanosterol, cycloartenol, cycloartanol, β-amyrin, and lathosterol).

Tocopherols

The total tocopherol content ranged from 0.94 mg/g in the Ethanol ASE tomato seed extracts to 1.08 and 1.11 mg/g in Hexane ASE and SC-CO_2 extracts of the tomato seeds, respectively (Table 2). These values are in the same range as most crude vegetable oils such as soybean and sunflower oil [30]. Gamma-tocopherol was the dominant homologue, followed by much lower amounts of α- and δ-tocopherol. Lazos et al. [31] reported a similar amount of total tocopherols (i.e., ca. 1.26 mg/g), however they reported a much higher amount of δ-tocopherol. The ANOVAs indicated that there were no significant extraction treatment effects for any of the tocopherols.

β-Carotene and Lycopene

The concentrations of β-carotene and lycopene for the three extraction treatment s are shown in Table 2. The ANOVAs indicated that there were significant extraction treatment effects for β-carotene and all lycopene isomers.

Table 2 Effect of extraction method on individual antioxidant content

Antioxidant	SC-CO_2	Hexane ASE	Ethanol ASE	$F_{2,6}$ (P value)
Mean antioxidant content (mg/g)[a]				
α-Tocopherol	0.05 a	0.04 a	0.03 a	2.72 (0.1445)
γ-Tocopherol	1.05 a	1.03 a	0.90 a	4.93 (0.0541)
δ-Tocopherol	0.01 a	0.01 a	0.01 a	1.50 (0.2963)
Mean antioxidant content (μg/g)[a]				
β-Carotene	0.93 a	4.53 b	4.06 b	10.13 (0.0119)
Lycopene *cis* 1	0.00 a	1.34 b	0.58 a	11.55 (0.0088)
Lycopene *cis* 2	0.00 a	3.02 c	1.06 b	42.55 (0.0003)
Lycopene all *trans*	0.00 a	12.07 b	7.50 b	18.69 (0.0026)
Lycopene *cis* 3	0.00 a	9.43 b	7.85 b	13.62 (0.0059)

[a] Mean ($n = 3$) in the same row without letters in common differ significantly by LSD ($P = 0.05$)

β-carotene concentrations were highest for the Hexane ASE and Ethanol ASE treatments with a very low content for the SC-CO_2 extraction. Similarly, lycopene content was highest for the Hexane ASE treatment, followed by the Ethanol ASE with no lycopene detected in SC-CO_2 extracts. Four lycopene peaks were identified in the ASE extracts, and their profile was similar between the two solvents. The all-*trans* lycopene peak which eluted at 20.11 min was the most predominant in most of the samples, but there was nearly an equal amount of the *cis* isomer that eluted at 20.51 min, and less abundant content of the two other *cis* isomers. The predominant form of lycopene in tomato products is the all-*trans* isomer [22], but analysis of human serum samples reveals that after consumption lycopene is converted predominantly to various *cis*-isomers. Determination of the various isomers of lycopene in tomato seeds has never been published, so it is not clear if the high content of *cis*-isomers in these tomato seed oil samples is a result of the natural ratio in the seeds or due to storage and extraction conditions. Ishida et al. [23] showed that in pure *trans*-lycopene isolated from tomatoes, *cis*-isomers formed during storage. Isomerization occurred even with storage at −20 °C, but was accelerated at higher temperatures.

Lipophilic Oxygen Radical Absorbance Capacity (L-$ORAC_{FL}$) Analyses

The mean ORAC values (μmol TE/g) for the three extraction methods are shown in Table 3. The ANOVA indicated that there was a significant extraction treatment effect on ORAC ($F_{2,6} = 9.27$, $P = 0.0146$). Although the Hexane ASE gave the highest ORAC value (i.e., 1.47), it was statistically equivalent to that for the Ethanol ASE (i.e., 1.17). The ORAC value for the SC-CO_2 treatment (i.e., 0.96) was significantly lower than those for both the Hexane ASE and the Ethanol ASE treatments. The correlation coefficients for antioxidant content and oil yield as well as antioxidant content and ORAC are shown in Table 4. Only five of the 16 possible correlation coefficients were statistically significant. Both α-tocopherol (−0.721) and γ-tocopherol (−0.761) were inversely correlated with oil yield, suggesting that the extraction of the triglyceride oil and these tocopherols are quite different. It is likely that the triglyceride oil is more easily extracted than the more polar tocopherols. On the other hand, lycopene *cis*-3 was positively correlated with oil yield, indicating that these two compounds are extracted in very similar manners or that the presence of the triglyceride oil enhances the solubility of the lycopene. Interestingly, triglyceride oils have been used in conjunction with SC-CO_2 to assist with the extraction of lycopene. Vasapollo et al. [36] describes the use of vegetable oil as a co-solvent with SC-CO_2 for the extraction of lycopene from ground sun-dried tomatoes. The presence of the vegetable oil as a co-solvent with SC-CO_2 improved both the yield as well as the stability of the lycopene extracts. Similarly, Ciurlia et al. [37] combined the extraction of hazelnut triglycerides with the extraction of lycopene from tomatoes.

The ORAC values were only statistically correlated with lycopene *cis* (0.748) and lycopene all *trans* (0.672). This suggests that the antioxidant capacity of tomato seed oil is mainly due to the presence of these two lycopene isomers. Although tomato products have been shown to contain high levels of both the carotenoids, β-carotene and lycopene [17], lycopene is a stronger antioxidant than β-carotene and the antioxidant capacity of tomatoes is reported to be related to the lycopene content [38, 39].

In summary, this research demonstrated that tomato seed oil yield was highest using hot ethanol followed by hot hexane and finally SC-CO_2. Although the SC-CO_2 treatment gave a slightly lower oil yield, it gave the highest total phytosterol content as well as for each individual phytosterol content. Sitosterol, cycloartanol, and stigmasterol were the most abundant phytosterols present in the extracts. The highest concentrations of antioxidants were found in the hexane extract with all-*trans*-lycopene, *cis*-3-lycopene and β-carotene the most abundant antioxidants present in the extracted oils. Oxygen radical absorbance capacity was highest for the Hexane extract. Oil yield was

Table 3 Effect of extraction method on oxygen radical absorbance capacity (ORAC)

Extraction method	Mean ORAC[a] (μmol Trolox equiv/g)
SC-CO_2	0.96 b
Hexane ASE	1.47 a
Ethanol ASE	1.17 a

[a] Mean ($n = 3$) without letters in common differ significantly by LSD ($P = 0.05$)

Table 4 Correlation coefficients for antioxidant content, oil yield and ORAC

Antioxidant	Oil yield	ORAC
α-Tocopherol	−0.721	n.s.
γ-Tocopherol	−0.761	n.s.
δ-Tocopherol	n.s.	n.s.
β-Carotene	n.s.	n.s.
Lycopene *cis*-1	n.s.	n.s.
Lycopene *cis*-2	n.s.	0.748
Lycopene all-*trans*	n.s.	0.672
Lycopene *cis*-3	0.668	n.s.

n.s. denotes Pearson's correlation coefficient not significant at $P = 0.05$

inversely proportional to both α-tocopherol and γ-tocopherol content and positively correlated with lycopene *cis* 3 content. ORAC values were positively correlated with only *cis*-2-lycopene and all-*trans*-lycopene demonstrating their role as antioxidants in the tomato seed oil. These results indicate that tomato seed oil is a good source of a vegetable oil with beneficial properties due to its high amounts of phytosterols and antioxidants.

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