JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 3–7 **3**

ARTICLES

Supercritical Fluid Extraction of *all-trans*-Lycopene from Tomato

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A procedure is proposed for the supercritical fluid extraction of *all-trans*-lycopene from tomato using carbon dioxide at 40 °C without modifier. The present method minimizes the risk of degradation via isomerization and oxidation of health-promoting ingredients, such as lycopene. The effect of different experimental variables on the solvating power of the supercritical fluid was evaluated in terms of both the selectivity achievable in the process and the yield of the extraction of *all-trans*-lycopene. Satisfactory separations of the *all-trans*-lycopene isomers from the *cis* counterparts were achieved using a C_{30} column. The obtained extract contained 88% *all-trans*-lycopene and 12% *cis*-lycopene.

KEYWORDS: Supercritical fluid extraction; *all-trans*-lycopene; *β*-carotene; tomato

INTRODUCTION

In the past few years, an ever increasing number of publications have reported the biological and physicochemical properties of carotenoids, mainly related to their effects as natural antioxidants and coloring agents. Of the more than 50 dietary carotenoids, *β*-carotene is the most extensively studied because it exhibits provitamin A activity. More recently, lycopene, a carotenoid found in tomatoes and tomato products which is the principal component responsible for its characteristic deep-red color, has attracted attention in different fields of research (*1*, *2*). In fact, lycopene is the most abundant carotenoid in ripe tomatoes, as it represents approximately 80−90% of the total pigment content.

The singlet oxygen-quenching properties of lycopene and, thereby, its ability to trap peroxyl radicals (*3*) result in the reduction of the risk of developing atherosclerosis and coronary heart disease, as it prevents oxidation of low-density lipoprotein (LDL) cholesterol (*4*). Moreover, there are also a rising number of clinical evidences and epidemiological studies supporting the role of lycopene to provide protection against different types of cancer (*2*, *5*−*9*).

In nature both lycopene and *β*-carotene exist predominantly in the *all-trans* form (*10*−*12*), but they may easily undergo degradation via *(trans*−*cis)* isomerization and oxidation, as they are very sensitive to light, heat, and oxygen (*13*, *14*). These reactions, which result in an array of mono- or poly-*cis* isomers, can take place during processing and may finally affect the sensorial quality of the product as well as its health benefits for consumers, since the function of these compounds is often related to the presence of a specific isomer.

Besides the well-known field of applications of *β*-carotene (*1*), lycopene is considered to be a commercially important natural pigment for the emergent market of nutraceutical products due to its function as a health-promoting ingredient. Specifically, lycopene is highly demanded not only by pharmaceutical companies but also for the food, feed, and cosmetic industries. In this respect, there is a great interest in the use of environmentally friendly processes for industrial production of lycopene-containing products.

On the other hand, the potential of supercritical fluids in food composition studies has been demonstrated by different authors (*15*−*25*), and more recently the interest of supercritical fluid extraction (SFE) for industrial scale-up production has been underlined regarding its applications in the area of nutraceuticals (*2*, *26*). However, information about the usefulness of supercritical fluids for lycopene extraction is not easily available. In fact, nondetailed references to its use have been reported in a limited number of patents, which mainly refer to supercritical conditions for the purification of the extracts obtained with liquid solvents rather than for the extraction process (*27*). Also, extraction of lycopene from tomato using SFE has only been recently reported (*28*−*31*), but the authors did not investigate the degree of isomerization during experimentation. This is, however, a critical aspect, as it has been established that extraction, storage, handling, and analysis of lycopene have to be carried out under controlled environmental factors to minimize losses of lycopene through oxidation or isomerization (*2*, *9*). Actually, it has been suggested that the bioavailability of *cis*-lycopene in foods is higher than that of *all-trans*-lycopene

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10.1021/jf0202842 CCC: $25.00
Published on Web 12/07/2002

(*2*), but apparently, in-vivo mechanisms, which are still unclear, promote isomerization of lycopene from *trans* to *cis* form (*9*). In any case, considering that lycopene typically occurs in the *all-trans* configuration, which is precisely the most thermodynamically stable form, there is clearly interest in avoiding *trans*−*cis* isomerization when lycopene is obtained for its subsequent incorporation into functional foods or nutraceuticals. Moreover, studies concerning biological functions of lycopene require the accurate determination of the degree of lycopene isomerization.

The purpose of this work was to investigate the possibility of using SFE to obtain the most stable isomer (*all-trans* form) of lycopene, precluding the risk of oxidative degradation and the introduction of artifactual isomers during experimentation.

MATERIALS AND METHODS

Samples and Materials. Tomatoes (*Pear* type) were purchased in commercial market and maintained at 7 °C for no more than 48 h. The tomatoes (skin and pulp without seeds) were dried in a freeze-dryer and subsequently ground, divided into 10 batches, and transferred to Teflon screw cap tubes that were flushed with nitrogen, wrapped with aluminum foil, and stored at −18 °C until the extraction was performed. Each batch was extracted at a different CO_2 density, as explained below. At least two replicates of each extraction were performed. Carbon dioxide (food quality) was acquired from Carburos Metálicos (Madrid, Spain). Methanol (HPLC-grade) was obtained from Scharlau Chimie (Barcelona, Spain) while methyl *tert*-butyl ether (HPLC-grade) and water (HPLC-grade) were purchased from Labscan (Dublin, Ireland) and dichloromethane (HPLC-grade) was obtained from SDS (Peypin, France). Lycopene used for identification purposes was obtained from Sigma Chemical (St. Louis, MO).

Supercritical Fluid Extraction. The extractions were carried out using a Hewlett-Packard 7680A extraction module fitted with a 7-mL thick-walled stainless steel thimble as the extraction cell. The instant depressurization of the supercritical fluid and the independent control of both the pressure and the supercritical fluid flow rate are achieved through a nozzle/trap assembly, which acts as a controllable variable restrictor. The extraction system is fully automated and includes an internal trap where the extracted analytes are retained once the supercritical fluid evaporates and leaves the system. Throughout the experimentation, different values for the CO_2 density (namely, 0.25, 0.35, 0.45, 0.55, 0.60, 0.70, 0.75, 0.80, 0.85, and 0.90 g/mL) were tested, with 30 min being the extraction time. Other experimental conditions were as follows: supercritical CO_2 flow, 4 mL/min; sample weight in the extraction cell, 0.5 g; extraction cell temperature, 40 °C; trap temperature, 35 °C; restrictor temperature, 45 °C. The trap was packed with Hypersil octadecylsilica (30−40 μm); after extraction, it was rinsed five times with 1 mL of dichloromethane at a rate of 2 mL/min.

HPLC Analysis. The extracts obtained were analyzed with an HPLC system comprising a Beckman (Fullerton, CA; model 126) delivery system, a manual injection valve (Rheodyne, model 125; Cotati, CA), and a 168 Beckman photodiode array detector with a wavelength range of 200−600 nm. Before the SF extract was injected onto the HPLC system, the solvent was evaporated under a stream of nitrogen and subsequently redissolved in a 1-mL volume of dichloromethane. As the mobile phase, a mixture of eluent A (methanol−water, 96:4, v/v) and eluent B (methyl *tert*-butyl ether) was used, with 1000 μL/min being the flow rate. A linear gradient was applied in 60 min from initial conditions (A−B, 83:17, v/v) to those maintained until the end of the analysis (A−B, 33:67, v/v). Before use, methanol and methyl *tert*-butyl ether were filtered and degassed with helium. All the analyses were performed with a 250 mm × 4.6 mm Develosil UG C_{30} column (Nomura Chemical, Sojo, Japan) operated at 20 °C. In all cases β-apocarotenal was used as the internal standard and the chromatograms were monitored at 285, 347, 450, and 472 nm to determine in the same run different carotenoids. An identification criterion for carotenoids was the similarity of their UV/vis absorption spectra compared to those of their respective standard compounds. The percentages of carotenoid compounds were calculated from peak area measurements.

RESULTS AND DISCUSSION

Although the convenience of performing the supercritical fluid extraction at high temperatures to increase the solubility of lycopene has already been pointed out by other authors (*28*−*31*), they did not evaluate the effect of the proposed conditions, mainly temperature and extraction time, on *trans*−*cis*-lycopene isomerization. For that reason, we considered the minimization of the risk of undergoing *trans*−*cis* isomerization during the extraction procedure a priority of our study and, consequently, we decided to maintain all throughout the experimentation an extraction temperature that was as low as possible. The optimization of lycopene solubility was carried out by establishing an adequate experimental pressure range (i.e., density range) rather than using the solute vapor pressure increase resulting when raising the extraction temperature at constant pressure. Specifically, we observed that when we worked at 40 °C and covered a pressure range from low (77 bar) up to intermediate values (281 bar), the effect on the extraction yield of an increase of temperature would be either negative or nonsignificant due to the balance between CO_2 density and solute vapor pressure changes.

Therefore, initial experimental work was performed, under the conditions given in Materials and Methods, at 40 °C. To make easier the eventual use of the method in large-scale food and pharmaceutical applications, we discarded the addition of different modifiers (such as acetone, methanol, hexane, and dichloromethane) previously suggested by other authors (*30*) for the lycopene extraction with supercritical fluids.

The obtained results were evaluated in terms of both lycopene recoveries achieved in each case and the risk of isomerization involved. As the degree of isomerization is directly correlated with the intensity and the duration of heat processing (*32*, *33*), we considered it more adequate to use extraction times that are as low as possible. As a result, an extraction time of 30 min was finally established. Subsequently, the effect of the carbon dioxide fluid density on the yield of the extraction was studied by carrying out six extractions at different densities. As can be seen in **Figure 1**, the chromatograms (monitored at 285, 347, and 450 nm) obtained from the corresponding extracts showed that experimentation at the lowest density (i.e., 0.25 g/mL) did not allow us to extract significant amounts of carotenoids while the increase of their solubilities achieved at higher densities enabled us to detect different compounds. The enhancement of the solvating power, which implies the increase of the pressure from 77 bar ($\rho = 0.25$ g/mL) to 281 bar ($\rho = 0.90$ g/mL) while maintaining the temperature of the extraction cell at 40 °C, made possible the extraction of different carotenoids, that is, phytoene, phytofluene, β-carotene, and lycopene.

An important aspect concerning the HPLC analysis of carotenoids is the separation efficiency of the system used. In those cases in which *cis* isomers are not well separated from the *all-trans* forms, the analysis will result in a significant overestimation of the latter, because their peaks will coelute partly or totally with the *cis* isomer peaks (*34*). In this respect, the use of a polymerically synthesized C_{30} column (*35*) allowed us to obtain adequate selectivity and sensitivity for the determination of the *all-trans* isomers from the *cis* counterparts as well as acceptable separations among the individual *cis* isomers themselves.

Figure 2 gives the relative amounts of the *cis* and *all-trans* forms of lycopene (expressed as percentages of the total lycopene) obtained at the different densities investigated in the range in which lycopene was extracted (i.e., 0.55−0.90 g/mL). Relative standard deviations (RSD, $n = 2$) obtained at each

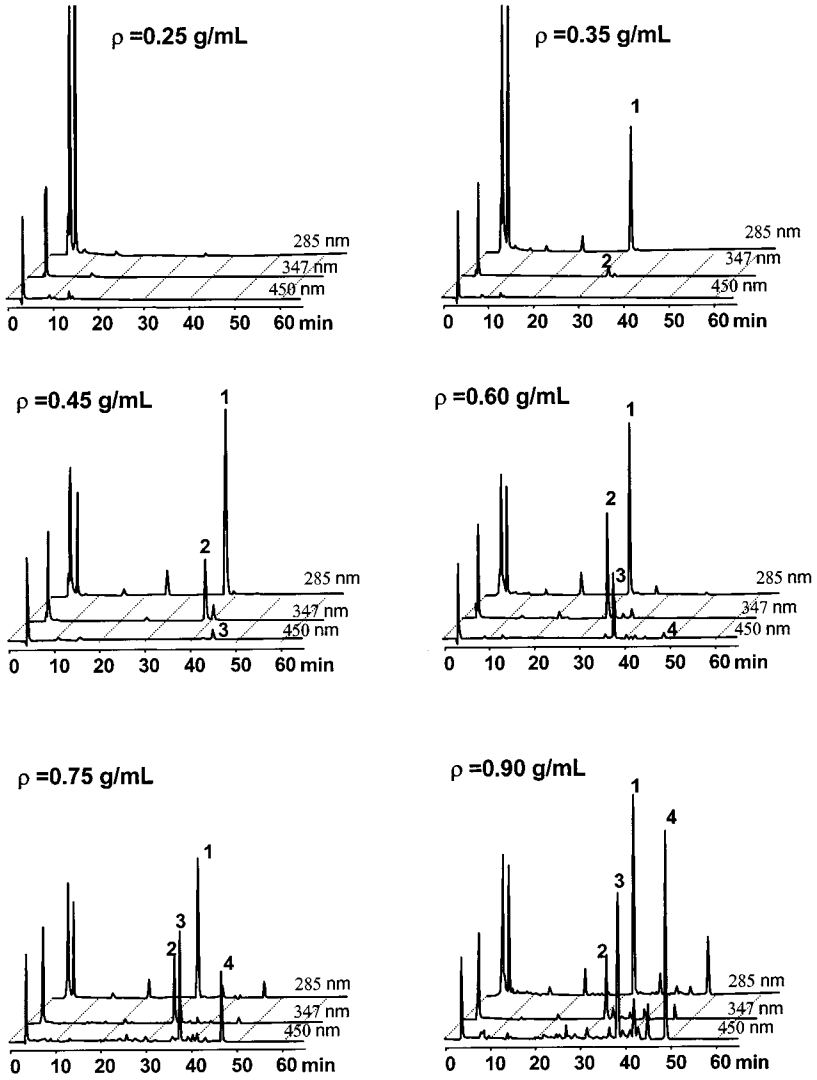

Figure 1. HPLC analysis (monitored at 285, 347, and 450 nm) of the tomato extracts resulting from supercritical fluid extractions performed at six different CO_2 densities, namely, 0.25, 0.35, 0.45, 0.60, 0.75, and 0.90 mg/L. Identification peak number: 1, phytoene; 2, phytofluene; 3, β-carotene; 4, lycopene.

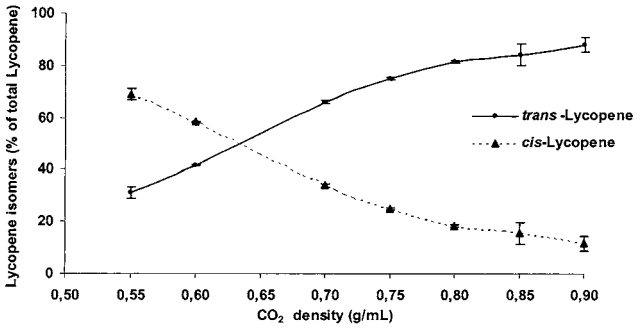

Figure 2. *all-trans*-Lycopene and *cis*-lycopene contents (expressed as percentage of the total lycopene) in the tomato SF extracts performed in the 0.55–0.90 g/mL density range. Error bars are indicated at each density of CO_2 used.

density of CO_2 used are indicated with the corresponding error bars. As it is shown, the amount of the *trans* form extracted rises (and the *cis* form content decreases) if the extraction pressure becomes greater due to the consequent increase of the supercritical CO_2 density. From the obtained results, it seems clear that the enhancement of the fractionation of *trans*-lycopene requires a proper choice of the density value at which the experimentation is performed.

Although all the experimentation was performed maintaining the temperature as low as 40 °C in the extraction cell, the presence of *mono*- and *poly-cis* forms could be observed most probably due to the ease (e.g., exposition to oxygen and light) with which lycopene can undergo *trans*-to-*cis* isomerization. In fact, when performing the SF extraction at $\rho = 0.55$ g/mL, the percentage of *cis*-lycopene detected in the obtained extract is as high as 69% of the total lycopene content. When these data are compared with those obtained when the extraction is accomplished with petroleum ether (i.e., 9% of *cis*-lycopene and 91% of *trans*-lycopene), the observation made by Spanos et al. (*36*) concerning the possibility of promoting isomerization under supercritical conditions seems understandable. However, the most predominant geometrical isomer in fresh tomatoes, and also the most thermodynamically stable form (i.e., the *all-trans* isomer of lycopene), contributes 88% to the total lycopene, provided that the extraction is carried out at $\rho = 0.90$ g/mL (**Figure 2**). Therefore, it can be stated that differences in solubility in supercritical CO_2 of *cis* and *trans* forms rather than their trend to undergo isomerization may explain the obtained results.

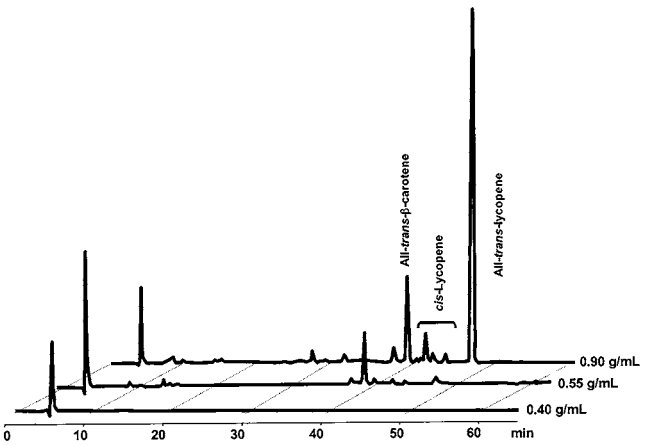

Figure 3. HPLC analysis (monitored at 450 nm) of the tomato extracts resulting from a three-step supercritical fluid extraction performed at different densities on the same sample.

As can be observed in **Figure 3**, a first extraction carried out at a density of 0.40 g/mL, followed by a second extraction on the same sample at 0.55 g/mL and a third extraction, again on the same sample, at 0.90 g/mL, provided extracts richer in different carotenoids, with 30 min being the extraction time in each of the three steps. A nearly 1.5-fold variation was found when comparing the total lycopene content in the extract achieved in the second step ($\rho = 0.55$ g/mL) with that of β-carotene while the ratio of *all-trans*-lycopene to *cis*-lycopene was 0.75. However, to obtain an extract richer in *all-trans*-lycopene, the extraction must be accomplished at the highest density (i.e., $\rho = 0.90$ g/mL), as the ratios of total lycopene to β-carotene and *all-trans*-lycopene to *cis*-lycopene resulted to be 4.5 in both cases.

Under these conditions, the relative standard deviation values ($n = 2$) obtained in the second step for β-carotene and lycopene were 10.3% and 2.6%, respectively, while 4.4% and 2.5% were the values estimated for the same compounds in the third step of the extraction.

Working at the mentioned density (0.90 g/mL), the yield of the supercritical fluid extraction of total lycopene with respect to that obtained from a standard method involving the use of liquid solvents was 42.0% (RSD = 2.5%), while values higher than 98% were obtained for other carotenoids (i.e., β-carotene, phytoene, and phytofluene). When the experiment was performed at lower densities of CO_2 (i.e., 0.85, 0.80, 0.75, 0.70, 0.60, and 0.55 g/mL), the yields obtained of total lycopene were 23.6, 14.5, 8.6, 4.5, 0.6, and 0.2%, respectively, with RSD values ($n = 2$) of 4.2, 0.4, 0.6, 0.6, 0.4, and 2.3%, respectively.

In this regard, it is interesting to emphasize that any attempt to improve the yield of the lycopene extraction process should not involve the increase of *trans*−*cis* isomerization. For that reason, the optimization of lycopene yield for specific large-scale applications of supercritical fluid extraction should include a study to establish the solubility parameters of both *all-trans* and *cis* forms at the experimental conditions, mainly temperature and extraction time, required in each particular case.

Summarizing, data obtained confirm the potential of supercritical fluid extraction for developing environmentally friendly methods suitable to extract *all-trans*-lycopene and suggest the possibility of taking advantage of very economical and natural sources (e.g., surplus of tomato production and waste during tomato processing) to obtain high-added-value products.

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Received for review March 5, 2002. Accepted October 4, 2002. Financial support from the Ministerio de Ciencia y Tecnología (Project PTR95-0501-OP) is gratefully acknowledged.

JF0202842