Solubility in Supercritical Carbon Dioxide of the Predominant Carotenes of Tomato Skin

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ABSTRACT: Solubilities in supercritical carbon dioxide of the predominant carotenes in tomato skin were measured. The use of a polymeric C_{30} RP-HPLC column to analyze the tomato extract made it possible to separate several geometric isomers from each carotene extracted. The Chrastil model was used to assign a solubility equation to each extracted carotene. Different solubility behaviors in supercritical carbon dioxide were shown by carotenes depending on their nature and configuration. The most soluble carotene was all-*trans*-phytoene and the least soluble was all-*trans*-lycopene. Significant differences in solubility were observed between the *trans* and *cis* isomers of lycopene. The results indicate that a fractionation of the tomato skin carotenes can be achieved by using supercritical CO_2 extraction.

Paper no. J10111 in *JAOCS 79*, 897–902 (September 2002).

KEY WORDS: Carotenes, solubility, supercritical carbon dioxide, tomato.

Carotenoids constitute a group of natural pigments essential for human health. Epidemiological studies have shown an inverse correlation between a diet rich in carotenoid-containing foods and the incidence of certain types of cancer (1) and cardiovascular diseases (2). These health benefits are principally attributed to their antioxidant properties.

Tomato represents a good source of carotenoids, especially of lycopene. This hydrocarbon carotenoid exhibits the highest antioxidant activity of all dietary carotenoids. β-Carotene, another carotene found in tomato, has been studied extensively owing to its provitamin A activity. Apart from these two named carotenes, other carotenoids such as phytoene, phytofluene, lutein, ξ-carotene, γ-carotene, and neurosporene have been identified in tomatoes (3). These molecules have a great tendency to degrade in the presence of light, oxygen, or heat. For this reason, extraction of carotenoids usually is difficult and tedious. New methods are needed that avoid molecular damage and possible losses of carotenoids during the extraction process.

Supercritical fluid extraction (SFE) provides a possible alternative to conventional extraction methods (4,5). Supercritical fluids allow highly selective extraction as a result of their low viscosities, high diffusivities, and wide density ranges. Supercritical carbon dioxide (SC-CO_2) is the most frequently used fluid because it is neither toxic nor inflammable and has moderate critical pressure and temperature, which make it suitable for the extraction of many natural products using mild conditions (6).

SC-CO_2 has been used to extract carotenoids from natural products such as carrot (7), buriti (8), sweet potatoes (9), and microalgae (10). In these studies, optimal extraction conditions have been investigated. To assess solubility in SC-CO_2 of carotenoids from microalgae, buriti, and carrot, a modification of the Peng Robinson equation, the Sovová model, and a response surface model were used, respectively. The use of a model that describes carotenoid extraction from plant material is of great interest because it permits prediction of the carotenoid concentration in the extract.

Recent papers have shown the usefulness of SC-CO_2 for the extraction of lycopene and β-carotene from tomato (11–13). However, to our knowledge, no papers have appeared dealing with SFE of other carotenes present in tomato or with models of tomato carotene extraction. The latter are necessary in order to design and evaluate the carotene extraction process. The semiempirical model of Chrastil is very simple and provides a good correlation between experimental and calculated data (14,15); this model may be useful in predicting the total carotene content.

Another important point to study is *trans* to *cis* isomerization during SFE. Spanos *et al.* (9) reported that SC-CO_2 extraction could promote the *cis* isomerization of β-carotene extracted from sweet potatoes. As the geometric configuration of carotenes influences not only their physicochemical features but also their nutritional properties and stability, the isomer composition of the extract should be considered.

The objective of this work was to determine the solubility of carotenes in SC-CO_2 from a multicomponent system such as tomato skin by using Chrastil's model. We also correlated results with those obtained by applying the modification of this model proposed by Del Valle and Aguilera (16).

EXPERIMENTAL PROCEDURES

Reagents. Lycopene, 90–95% purity, and α-tocopherol, 99% purity, were obtained from Sigma Chemical (St. Louis, MO); and all-*trans*-β-carotene, 97% purity, and β-apo-8′-carotenal, 98% purity, were purchased from Fluka Chemical (Buchs, Switzerland). All HPLC-grade solvents, including methanol, dichloromethane, and methyl *t*-butyl ether (MTBE), were

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obtained from Labscan Ltd. (Dublin, Ireland). Praxair (Madrid, Spain) supplied carbon dioxide (99.995% pure).

Sample preparation. Tomatoes, bought in a local market, were carefully peeled with a knife, and the skin was freeze-dried in a Virtis model FM12XL lyophilizer. The freeze-dried tomato skins were ground with a mill (0.050–0.250 mm particle size), flushed with N_2, and stored in polyethylene bottles protected from light with aluminum foil at −18°C until SFE.

SFE. SFE was carried out on a fully automated Hewlett-Packard 7680 extraction module (Wilmington, DE) with a nozzle/trap assembly that acted as a controllable variable restrictor. The extraction system included an internal trap packed with Hypersil octadecylsilica (ODS) (30–40 μm) where the analytes were retained. Carotenes were extracted from milled lyophilized tomato peel by placing 0.5 g of sample in an extraction vessel (7-mL thick-walled stainless steel thimble). All experiments were performed at a CO_2 flow rate of 4 mL/min, extraction time of 30 min, nozzle temperature of 318 K, and ODS trap temperature of 308 K. Chrastil's equation points were obtained by combining CO_2 densities between 400 and 800 g/L with extraction vessel temperatures of 313, 323, and 333 K. Each point of extraction assayed was performed in triplicate. Each extract was recovered by rinsing the trap with 3 mL of dichloromethane. The carotenoid extract was then evaporated under nitrogen, and the residue was redissolved to a final volume of 1 mL in dichloromethane just before HPLC analysis.

HPLC. The RP-HPLC separation was performed on a Beckman System Gold binary delivery system (module 126) equipped with a UV-vis photodiode array detector model 168 (Beckman Instruments, Fullerton, CA). Analytical separations were carried out on a stainless steel (250 × 4.6 mm i.d.) Develosil UG C_{30} (5-μm particle size) column (Nomura Chemical, Sojo, Japan) with a guard cartridge (Phenomenex, Macclesfield, United Kingdom) packed with ODS C_{18}. Sample injection was performed by means of a valve (Rheodyne, Cotati, CA) with a 20-μL peek loop.

Elution conditions were based on the chromatographic method developed by Sander *et al.* (17). Slight modifications in the water content and in the oven temperature were made in order to obtain adequate selectivity and sensitivity with the available HPLC system. The linear mobile phase gradient was methanol (4% H_2O)/MTBE from 83:17 to 33:67 over 60 min at a flow rate of 1 mL/min. The column was thermostated at 22°C in a Shimazu CTO-10AS (Columbia, MD) column oven. The Gold Nouveau software data system (Beckman Instruments) was used to evaluate peak areas.

Commercial standards and spectral data were used to assign carotenoid peaks. The optimal absorption wavelength for each carotene was used for calibration of standards and for integration of peaks from tomato extracts: phytoene at 285 nm, phytofluene at 347, β-carotene at 450, and lycopene at 472 nm. The concentrations of lycopene and β-carotene were calculated using response factors relative to β-apo-8′-carotenal. The internal standard was dissolved in methanol/MTBE 25:75 (vol/vol). To prepare each sample, 200 μL of internal standard solution was added to 200 μL of carotene extract. The mixture was filtered through a 0.45-μm filter, and a volume of 20 μL was injected in the HPLC system. Response factors used were 1.03 for lycopene and 1.05 for β-carotene. SFE from tomato skin (using a 313 K extraction vessel temperature and 400 g/L CO_2 density) provided an extract rich in phytoene and phytofluene and substantially free of other carotenes. Phytoene and phytofluene were quantified by means of a calibration curve over the expected range of concentration, 0.26–3.12 and 0.13–1.22 μg, respectively. A DU-70 spectrophotometer (Beckman Instruments) was routinely used to check the concentration of the working standard solutions. These concentrations were calculated using the extinction 1% (1 cm) coefficient $E^{1\%}_{1\text{cm}}$ (18).

Parameter calculation from the solubility equations. A nonlinear regression model included in the statistical package SPSS for Windows (2001; SPSS Inc., Chicago, IL) was used to calculate the different parameters from the equations of Chrastil and Del Valle–Aguilera.

To estimate the data correlation between experimental and calculated values, the average absolute deviation (AAD %) was used:

$$\text{AAD}\,(\%) = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{Y^{\text{exp}} - Y^{\text{cal}}}{Y^{\text{exp}}}\right|_i \times 100 \qquad [1]$$

RESULTS AND DISCUSSION

Identification of carotenes. Five carotenes were identified in the extract obtained from tomato skin by using SC-CO_2: phytoene, phytofluene, ξ-carotene, β-carotene, and lycopene. The chromatographic profile of these compounds is shown in Figure 1. The use of a diode-array detector together with a polymeric C_{30} column at the described experimental conditions made it possible to identify and to resolve *cis-trans* isomers from the mentioned carotenes. Small concentrations of α-tocopherol and a xanthophyll (lutein) also were identified in the extract.

All-*trans*-β-carotene and all-*trans*-lycopene were identified from the retention times and the absorption spectra of the respective standards. Peaks labeled as 7′ and 8′ in Figure 1 were associated with *cis* isomers of β-carotene and lycopene, respectively, according to the characteristics of their electronic absorption spectra and to the relative patterns of chromatographic retention obtained by other research teams working with C_{30} columns (19,20).

Phytoene and phytofluene chromatographic peaks were assigned considering previously reported data of their electronic absorption spectra (21,22). Owing to the similarities of the absorption spectra of the different peaks assigned to the geometrical isomers of phytoene and phytofluene, the criterion used to establish the carotene configuration was relative abundance. Taking into account that SFE was carried out under mild working conditions in order to avoid *trans* to *cis* isomerization, together with the fact that the major form present in nature seems to consist principally of the all-*trans* isomer, we

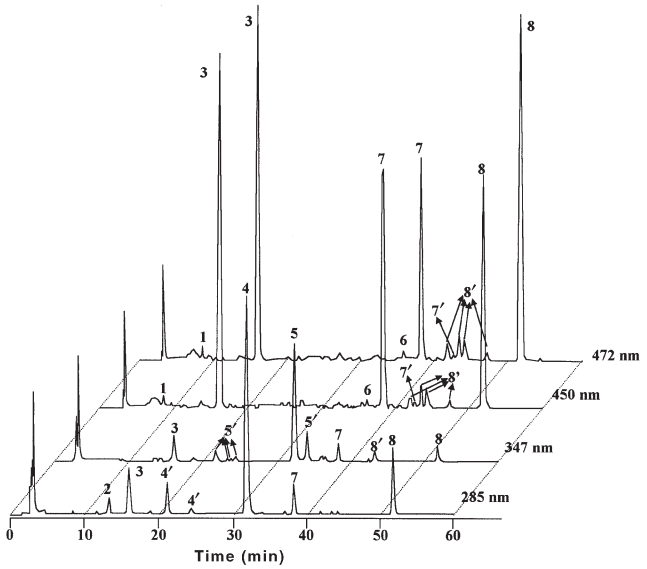

FIG. 1. HPLC chromatogram monitored at 285, 347, 450, and 472 nm of the tomato skin extract obtained by supercritical fluid extraction with CO_2 using 313 K extraction vessel temperature and 800 g/L CO_2 density. The numbered peaks are tentatively assigned to: (1) lutein; (2) α-tocopherol; (3) β-apo-8′-carotenal (internal standard); (4) all-*trans*-phytoene; (4′) *cis*-phytoene; (5) all-*trans*-phytofluene; (5′) *cis*-phytofluene; (6) all-*trans*-ξ-carotene; (7) all-*trans*-β-carotene; (7′) *cis*-β-carotene; (8) all-*trans*-lycopene; (8′) *cis*-lycopene.

assumed that the most abundant isomer must be present at the *trans* configuration. Therefore, we assigned the peaks 4 and 5 in Figure 1 to all-*trans*-phytoene and all-*trans*-phytofluene, respectively.

Quantification of carotenes. Because tentative assignments of *cis* forms could not be made, all *cis* isomers were quantified together. The fact that *cis* and *trans* isomers were considered separately made it possible to observe the solubility behavior of both configurations in SC-CO_2. This is an important point, as no solubility data were found in the literature and because a selective extraction of this compound could be interesting because of the different stability shown by the *trans* and *cis* isomers.

Lycopene and β-carotene were quantified by using β-apo-8′-carotenal as internal standard as described in the Experimental Procedures section.

Since standards of phytoene and phytofluene were not commercially available, these compounds were quantified by using a tomato extract obtained by SC-CO_2 extraction. This extract was achieved under mild working conditions: 313 K in the extraction vessel and 400 g/L CO_2 density. These conditions provided a fraction rich in phytoene and phytofluene and substantially free of other carotenes (Fig. 2). Absorption was measured on a spectrophotometer for total phytofluene and phytoene in petroleum ether at 347 and 285 nm, respectively, and their concentrations were calculated using the $E^{1\%}_{1cm}$. The sum of all the chromatographic peak areas that are associated with phytofluene (see peaks 1 and 1′ in Fig. 2) or phytoene (peaks 3 and 3′) was assigned to each calculated concentration in order to make the calibration curve. As small amounts of α-tocopherol present in the extract could influence the measurement of the phytoene concentration, the absorption corresponding to α-tocopherol at 285 nm was subtracted from that of the phytoene extract. In all cases the increment in the absorbance value due to the presence of α-tocopherol resulted in insignificant changes. The HPLC calibration curves showed an r^2 value of 0.9984 for phytofluene and 0.9960 for phytoene.

Although *cis*-β-carotene and all-*trans*-ξ-carotene were detected, we did not study their solubility in SC-CO_2 because of their low concentrations in the tomato extract.

Solubility study. The relationship between the amounts of *trans*-lycopene, *trans*-β-carotene, *trans*-phytoene, and *trans*-phytofluene vs. CO_2 density at 333, 323, and 313 K is shown in Figure 3. The shape of the curves depends on the solubility of each carotene extracted from tomato skin. *trans*-Lycopene exhibits an exponential curve for the seven densities assayed, whereas *trans*-β-carotene reaches a constant concentration for density values over 700 g/L. Similarly, the higher concentration values for *trans*-phytoene and *trans*-phytofluene were obtained at 550 g/L density of CO_2, and the shapes of the solubility curves for these two carotenes were very similar. The solubility behavior exhibited by the *cis* forms was very close to that of their respective *trans* forms.

An increase in temperature of the extraction vessel promoted an increase in the amount of all-*trans*-lycopene extracted at CO_2 densities greater than 550 g/L. This behavior was also observed for β-carotene, phytoene, and phytofluene at density values below the plateau of concentration. However, the temperature did not affect the density value of the concentration plateau for each carotene.

Chrastil's model relates the solubility of a solute with the density of the solvent and the working temperature. To explain his model, Chrastil assumes that a solvato complex is formed between solute and gas molecules and proposes Equation 2:

$$C = d^k \exp\left(\frac{a}{T} + b\right) \qquad [2]$$

where C (g/L) is the solute concentration in the gas, d (g/L) is the gas density, T (K) is the working temperature, k is the average number of gas molecules that form the solvato complex, a is a constant that depends on the heat of solvation, and b depends on the M.W. and m.p. of the solute and the gas. The parameters obtained by applying Chrastil's model are given in Table 1. Since Chrastil's equation is exponential, only those experimental data out of the concentration plateau were considered to calculate the parameters of solubility a, k, and b for each carotenoid.

Because the k value represents the number of gas molecules in the solvato complex, we can establish that the most soluble of all the carotenes extracted with SC-CO_2 from tomato skin is all-*trans*-phytoene, which exhibits the lowest k value. By contrast, all-*trans*-lycopene, which has the highest k value, is the least soluble carotene. The other extracted

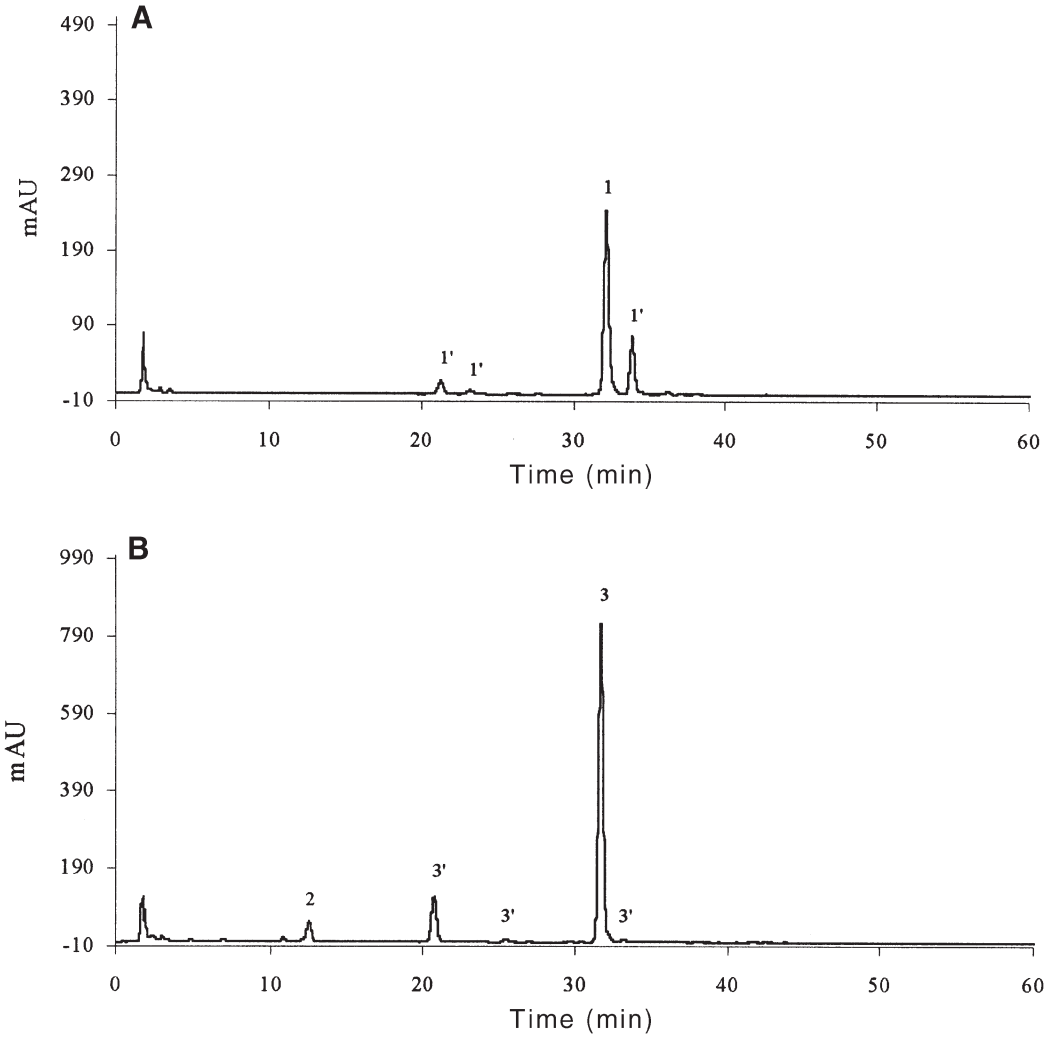

FIG. 2. Carotene contents in the extract obtained using a 313 K extraction vessel temperature and 400 g/L CO_2 density. (A) HPLC chromatogram of phytofluene monitored at 347 nm, and (B) HPLC chromatogram of phytoene peaks at 285 nm. The numbered peaks are tentatively assigned to (1) all-*trans*-phytofluene; (1′) *cis*-phytofluene; (2) α-tocopherol; (3) all-*trans*-phytoene; (3′) *cis*-phytoene.

carotenes show similar solubilities with *k* values between 4.08 and 5.67. The difference between all-*trans* and *cis* isomers of lycopene is notable. The *k* values for the *trans* and *cis* forms, 8.06 and 4.53, respectively, show that lycopene extraction with SC-CO_2 is markedly influenced by the molecular configuration. The *cis* isomer is the more soluble form in SC-CO_2 because it requires fewer CO_2 molecules to constitute the solvato complex. Consequently, the lycopene isomer separation may be achieved by using consecutive extractions.

The different solubilities exhibited by some *trans* and *cis* isomers affect the contents of geometrical isomers in the extract. When the SFE is carried out in a single step, the extract should be richer in the most soluble form during the early stage of the extraction. This fact could explain why Spanos *et al.* (9) found a great increase of *cis* forms compared with their content in the remaining extracted sample and the control. Further investigations are required to establish whether SFE can induce *trans* to *cis* isomerization.

Taking into account that the value of *a* depends on the heat of solvation, we conclude that the carotene most affected by the temperature of the extraction cell is all-*trans*-lycopene, which presents the highest absolute *a* value, whereas all-*trans*-β-carotene seems to be the less affected. The relationship between the absolute value of *a* and the molecular geometry is not the same for all carotenes. Whereas for lycopene the *trans* isomer presents the highest absolute *a* values, for phytoene and phytofluene the *trans* isomer exhibits the lowest absolute *a* value. The *a* value seems to be related to the nature of the carotene rather than to the molecular geometry.

From Chrastil's equation, solute solubility increases when the absolute value of *b* decreases. As expected, the ranking of *trans* isomers with regard to the absolute value of *b,* which is related to the m.p. of the carotene, is as follows for the *trans* isomers: all-*trans*-phytoene < all-*trans*-phytofluene < all-*trans*-β-carotene < all-*trans*-lycopene. According to this, the similarity between the *b* values for all-*trans*-β-carotene and all-*trans*-

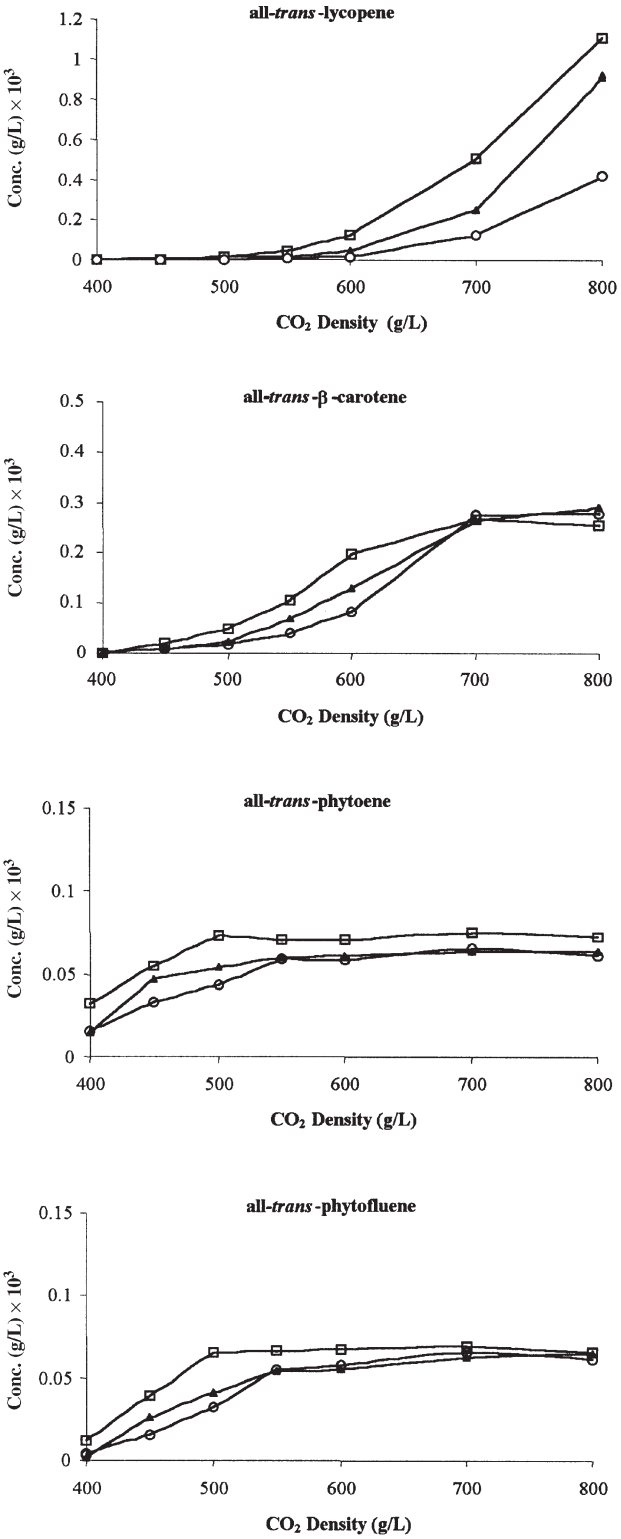

FIG. 3. Solubility curves of the predominant *trans* carotenes from tomato skin in supercritical carbon dioxide at 333 K (□), 323 K (▲), 313 K (○).

lycopene could explain why both are solids in nature, with comparable m.p. (183°C for all-*trans*-lycopene and 173°C for all-*trans*-β-carotene). On the other hand, all-*trans*-phytoene and all-*trans*-phytofluene, which can be found in nature as oils, have lower absolute *b* values than those for solid carotenes. That solid solutes are less soluble in SC-CO_2 than liquid solutes has been reported previously (23). Concerning the *cis* isomers, no differences can be observed in the *b* values. This observation may indicate that the *cis* forms have similar m.p.

TABLE 1
Calculated Parameters from Chrastil's Equation

	k	*a*	*b*
All-*trans*-lycopene	8.06	−4336.06	−47.63
cis-Lycopene	4.53	−3006.07	−29.91
All-*trans*-β-carotene	5.67	−978.15	−42.36
All-*trans*-phytoene	2.53	−1999.14	−19.38
cis-Phytoene	4.08	−2872.99	−29.04
All-*trans*-phytofluene	4.31	−2302.07	−29.78
cis-Phytofluene	4.59	−3556.56	−28.86

Del Valle and Aguilera (16) reported that Chrastil's equation could be improved by introducing an empirical modification to compensate for the variation of the heat of vaporization and the temperature. From this consideration, they proposed Equation 3,

$$\ln C = k \ln d + \frac{n}{T} + \frac{n'}{T^2} + b' \qquad [3]$$

where *k*, *n*, *n′*, and *b′* are constants. Table 2 shows the parameter values obtained from Del Valle–Aguilera equation. The new solubility parameters derived from this equation are difficult to relate to those of Chrastil's. As a result, all considerations made in this work to Chrastil's parameters are invalid for Del Valle–Aguilera parameters. The relationship between solubility parameters and physical properties of solutes and solvent seems to be more complex in Del Valle–Aguilera's model than in Chrastil's model.

In order to evaluate the solubility study, experimental data were correlated with values calculated by both Chrastil's equation and Del Valle–Aguilera's equation (see Table 3). The percentage of the average absolute deviation (AAD%) was calculated considering all the concentration values different from zero. The results obtained revealed that the two equations described the solubility behavior of the tomato skin carotenes in SC-CO_2 with high accuracy, and only for all-*trans*-lycopene were the correlation results achieved by using the Del Valle–Aguilera's method better than those obtained by Chrastil's method.

TABLE 2
Calculated Parameters from Del Valle–Aguilera's Equation

	k	*n*	*n′*	*b′*
All-*trans*-lycopene	8.16	159497.08	−26588080.75	−300.55
cis-Lycopene	4.53	−15284.01	1987802.83	−10.98
All-*trans*-β-carotene	5.68	−20309.73	3121859.68	−12.52
All-*trans*-phytoene	2.53	−32346.46	4905121.27	27.48
cis-Phytoene	4.09	−46119.31	6996756.97	37.70
All-*trans*-phytofluene	4.32	−75116.32	11772123.28	82.68
cis-Phytofluene	4.61	−72531.70	11165336.52	77.51

TABLE 3
Correlation of the Experimental and Calculated Solubility Data by Using Chrastil's Equation and Del Valle–Aguilera's Equation

	AAD (%)[a] Chrastil	AAD (%)[a] Del Valle–Aguilera
All-*trans*-lycopene	4.4	3.6
cis-Lycopene	2.4	2.4
All-*trans*-β-carotene	3.5	3.6
All-*trans*-phytoene	2.1	2.2
cis-Phytoene	2.4	2.4
All-*trans*-phytofluene	3.2	3.3
cis-Phytofluene	2.9	3.0

[a]Average absolute deviation percentage.

By using Chrastil model, it was possible to predict the amount of lycopene, β-carotene, phytoene, and phytofluene extracted over a wide range of CO_2 densities. From the solubility data obtained in this study, it seems possible to perform a SC-CO_2 extraction in consecutive steps to separate and isolate tomato skin carotenes. Considering the different solubility between *trans*- and *cis*-lycopene isomers, an extract substantially free of the *cis* form, and consequently more stable, may be successfully obtained this way.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Marta Herraiz for her valuable advice and Aurora Olmedillas for her technical support.

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[Received October 1, 2001; accepted May 16, 2002]