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Characterization of High Purity Lycopene from Tomato Wastes Using a New Pressurized Extraction Approach

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In this paper, a method for the extraction of high purity lycopene from tomato wastes is presented. The method is based on a pressurized extraction that uses the Extractor Naviglio, and it is performed in the 0.7−0.9 MPa range. Tomato skin, the byproduct deriving from manufacturing of tomato, in a water dispersion, are used as starting material. Lycopene is transferred, for the effect of the high pressure used, in the form of molecular aggregates into the water as a dispersion, while apolar compounds remain in the matrix. The aggregates are easily purified in a single subsequent step by using methanol, thus, obtaining lycopene at 98% chromatographic purity or higher. A new stationary phase, phenyl−hexyl silicone, and a simple water/acetonitrile gradient were used for HPLC analysis of lycopene. The extract was characterized by UV−Vis spectrophotometry, ^{1}H NMR, ^{13}C NMR, and electrospray ionization mass spectrometry. An average recovery of 2.8 mg lycopene/kg tomato waste can be obtained after 4 hours of extraction and using tap water as the extracting liquid. The recovery percentage was of about 10%. The exhausted tomato byproduct can be easily dried and used in agriculture or as feeding for animals.

KEYWORDS: Lycopene; Extractor Naviglio; tomato wastes; pressurized extraction; HPLC; UV−Vis; ^{1}H NMR; ^{13}C NMR; ESI-MS

INTRODUCTION

The first study on the chemistry of tomato pigments dates about one hundred years ago (*1*). Since then, the interest toward carotenoids has been rapidly growing; in the last fifteen years, an exponential increase in the number of scientific papers concerning tomato pigments has been observed, contemporary with the recognition of the relevant physiological role played by natural antioxidants. Lycopene (2,6,10,14,19,23,27,31-Octamethyl-dotri-aconta-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene), whose structural formula is shown in **Figure 1**, is one of the most extensively studied tomato carotenoids. The molecule contains 13 double bonds, 11 of which are conjugated, which make it one of the known compounds with the highest molar absorption coefficient value and determines its high antioxidant activity. For these reasons, lycopene is among the major components of pharmaceuticals for the treatment of prostate cancer and digestive-tract tumors (*2*–*7*); it is widely used in cosmetics formulations (*8*), and for its high solubility in fats and oils, it is widely used in the food and beverages industry and as a colorant in foodstuffs. As a consequence of its wide uses, the request for high-purity, well-characterized, low-cost lycopene is growing continuously. Unfortunately, all the synthetic pathways proposed up to now are not convenient economically, and at present, the simplest way for obtaining it is the extraction from natural sources. Among these, the most useful are certainly tomatoes that contain about 30−400 mg/kg in pulp and about 20−30 mg/kg in peels (*9*). The amount of tomato byproduct deriving from industrial processes is growing annually; in Europe, it is estimated that about 10 million tons of tomatoes are processed by the food industry, and the deriving wastes can be quantified in about 0.1 million tons. These residues can be reincorporated in low-quality tomato products, used in animal food as an ingredient, or used as a source of the purest lycopene.

The overall procedures used at present to obtain pure lycopene are based on a preliminary extraction from natural sources (e.g., tomatoes, red peppers, water melon, and papaya (*10*)) followed by a purification process. All the procedures require at least one organic solvent in the extraction step, which creates some disadvantages: (i) organic solvents could be more or less toxic,

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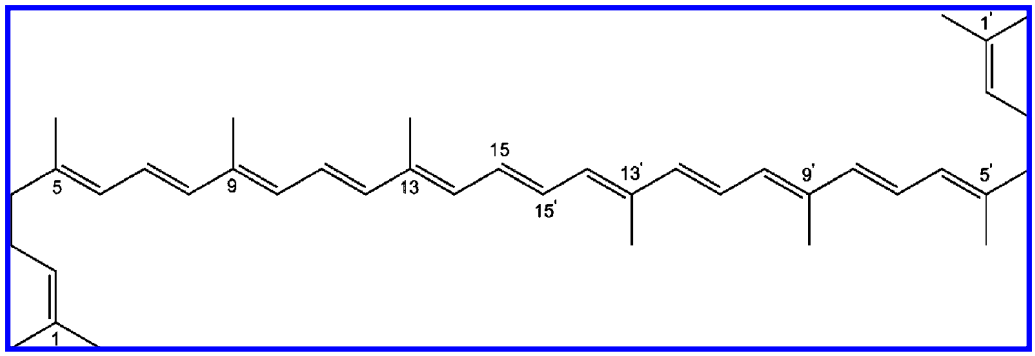

Figure 1. Structural formula of lycopene.

so they have to be completely removed from the exhausted material through a sequence of purification steps before dumping it; (ii) after the extraction, matrixes must be dumped as "special residue", and they cannot be reused; (iii) when using organic solvents, molecules different from lycopene may also be extracted, such as carotenes, xanthophylls, and lipids. This last aspect makes the purification of lycopene by HPLC particularly laborious and time-consuming, since long time and ternary or quaternary solvent gradients must be used. In the case of tomato-waste, lycopene recovery can also be performed by supercritical extraction using carbon dioxide, which is quite a safe solvent capable to extract a greater quantity of carotenoids (*11, 12*). Unfortunately, this method is very expensive, which makes the whole process not economically suitable on a large scale, and moreover, the final product is an oleoresin containing a low percentage of lycopene. Biotechnological productions of lycopene using microorganisms have been reported too, mainly at the bench scale and in most cases with a low specific yield; recently, a new biotechnological approach that can be scaled-up to an industrial application has been proposed (*13*).

In summary, the extraction procedures proposed up to now require the use of nonaqueous solvents, are time-consuming, and last but not least they are not yet economically appealing. It is not then surprising that some recent reviews on the subject (*14–16*) pointed out the need of methods for extracting lycopene with a high yield and at a purity level higher than the one of the commercially available products.

In this paper, a new process for the extraction of lycopene from tomato wastes is presented. The method is based on a pressurized extraction that uses the Extractor Naviglio (*17, 18*) at a pressure in the 0.7−0.9 MPa range and uses as starting material tomato skin residues from tomatoes processing in a water dispersion. Lycopene, even if not soluble in water, forms molecular aggregates (*19*), which can be recovered by the pressure/depressure cycles operating in the extractor device. Through a cyclic variation of the pressure gradient between the inner and the outlet of the solid matrix, lycopene aggregates are forced to separate. In this way, the procedure allows the transfer of lycopene aggregates to water as a dispersion, while apolar compounds, insoluble in water, remain in the matrix. Afterward, the aggregates are easily purified in one step by solid phase extraction using small amounts of methanol, and lycopene at more than 98% chromatographic purity is obtained after recovery with a small quantity of acetone. The chromatographic analysis of the final product may be carried out on a phenyl−hexyl silicone reversed phase using a suitable water/acetonitrile gradient.

MATERIALS AND METHODS

Raw materials recovered from tomatoes grown in the Naples area and processed by factories in the same area were used as samples; after collection, they were kept at 277 K. All the organic solvents used (Fluka, Bucks, Switzerland) were of analytical grade. Lycopene standard was purchased from Sigma-Aldrich at a certified purity grade of 90−95%. Deionized water of HPLC grade, produced by a Milli-Q device (Millipore, Bedford, MA), was used throughout. The extractions were performed by using an Extractor Naviglio mod. 500 mL (Nuova Estrazione s.a.s., Naples, Italy).

To prevent the samples from being oxidized, all the extracts were dried under a nitrogen atmosphere by using a rotating evaporator at 303 K and were stored at 253 K, protecting them from light and atmospheric oxygen. It was in fact reported that atmospheric oxygen quite rapidly oxidizes lycopene, while light catalyzes its conversion from the all trans to the cis configuration, and both reactions determine a decrease of the biological activity of the molecule (*20*).

SPE-C18 cartridges (10 g) were from Restek Corp. (Bellefonte, PA). Chromatographic analyses were made using a HPLC 1100 apparatus pursued by Agilent Technologies (Santa Clara, CA), equipped with a diode array detector. A phenyl−hexyl silicon stationary phase column of 250 mm × 4.6 mm I.D., 5 μm particle size was used (Phenomenex, Torrance, CA). Membrane filters of 0.20 μm porosity were purchased by Millipore (Bedford, MA). UV−Vis spectra were recorded with a Lambda EZ201 UV/vis spectrometer (Perkin-Elmer, Waltham, MA). NMR spectra were recorded with at 400.135 MHz for ^{1}H and 100.03 MHz for ^{13}C with a Bruker DRX 400 (Bruker BioSpin Corp., Billerica, MA). Mass spectra were performed with a Micromass Quattro micro API triple quadrupole mass spectrometer equipped with an electrospray ion source (Waters, Milford, MA). The instrument settings used were as follows: capillary voltage, 3.4 kV; cone voltage, 20 V; extractor lens voltage, 2 V; source temperature, 373 K; desolvation temperature, 523 K; rf lens, 0.2 V. Nitrogen was used both as the nebulizer (60 L/h) and the desolvation (at 550 L/h) gas. The ESI-MS/MS experiments were performed by using argon as the collision gas with a collision energy of 20 eV.

Extraction and Purification of Lycopene. A preliminary extraction of lycopene from tomato peels was performed with a well-standardized method to be used as a comparison procedure for the recovery of this molecule. A quantity of 2 g of accurately weighed tomato peels were placed in an extraction tube, and 20 mL of chloroform was added. The tube was treated with ultrasounds for 30 min and then centrifuged for 15 min at 2500 rpm (913*g*). The liquid phase was analyzed by high-performance liquid chromatography (HPLC). This extraction procedure was repeated on the same sample in order to recover any residual lycopene. Exhaustive extraction of tomato seeds and skins with additional volumes of chloroform did not result in additional recovery of lycopene.

The extraction was then repeated on the same product using the Extractor Naviglio. A quantity of 100 g of tomato peels was placed in two 50 μm filtering membrane bags and placed into two 500 mL chambers of the Extractor Naviglio, filled with deionized water. Each extraction cycle consisted of a 2 min static phase, followed by a dynamic phase and a delay time of about 10−15 s before a new cycle started, and it took 4 min to be completed; total cycles were 60 for a total time of 4 h. At the end of the extraction, the bags were removed and squeezed, and the aqueous phase was recovered and loaded onto an octadecyl solid phase extraction (SPE-C18) 10 g vacuum packed column. Lycopene, present in the form of solid aggregates, was

separated on the filter column; also, a small fraction of the other pigments present in the starting material were retained on the column head. The column was washed with methanol in order to remove pigments other than lycopene, which is not soluble in this polar solvent. Lycopene was finally recovered by dissolving the deep red residue in acetone, and the extract was analyzed by UV−Vis spectrophotometry and HPLC-DAD. Recovery of lycopene in the solid−liquid extracts was quantified using a calibration curve prepared by using a purchased lycopene standard (Sigma-Aldrich, Milan, Italy) on 10 different samples furnished by several different factories, by varying the overall extraction time.

Characterization of the Lycopene Extracts. *by HPLC.* In order to determine the purity of the lycopene, the filtered extracts were analyzed by high-performance liquid chromatography (HPLC) using a diode array detector (DAD). A phenyl−hexyl silicone column was used for the separation, with a flow rate of 1 mL/min and a H_2O/CH_3CN gradient starting with 100% H_2O up to 3 min, ending in 20 min time with 100% CH_3CN. The elution of lycopene from the C18 SPE was made both in one step, using acetone as the eluent, or by washing the column with methanol before eluting with acetone.

by Spectrophotometry. The molar absorption coefficients of lycopene in the visible region of the spectrum were determined both in $CHCl_3$ (relative polarity: 0.259) and in CH_2Cl_2 (relative polarity: 0.309). Before recording the spectra, tomato waste extracts were filtered through a 0.20 μm membrane in order to eliminate any rough impurities. Clear solutions containing 1.0×10^{-5} mol/L of lycopene in chloroform and in dichloromethane, prepared starting from lycopene dried in nitrogen current using a rotating evaporator, were analyzed.

by NMR. Solutions of lycopene in $CDCl_3$ were analyzed by ^1H NMR and ^{13}C NMR. The chemical shifts (in ppm) corresponding to 56 protons in the ^1H NMR spectrum and to 40 carbons in the ^{13}C NMR spectrum were assigned.

by Mass Spectrometry. Lycopene fragmentation was studied by tandem mass spectrometry with an electrospray ionization source (ESI-MS/MS). Experiments were performed in positive ion mode on micromolar solutions of the analyte in 1:1 $CH_3CN/CHCl_3$ ratio. Full scan mass spectra were recorded in the 250−800 mass/charge (m/z) interval, at various cone voltages.

RESULTS AND DISCUSSION

In this work, a procedure to transfer lycopene in the form of molecular aggregates from tomato wastes in both tap potable or deionized water using the Extractor Naviglio is described. In order to overcome the problem of lycopene's insolubility in polar solvents, the method takes advantage of the possibility to suspend it in water in the form of solid aggregates (*19*). This goal is achieved by applying to the solid matrix moderate pressures, about 1000 times lower than the ones required by the recently reported methods, based on very high pressure processing (*21, 22*). The Extractor Naviglio is a rapid and dynamic solid−liquid extractor, capable of imposing to a solid−liquid dispersion suitably programmed pressure cycles in the 0.1−0.9 MPa range. The device is formed by two extraction chambers constituted of a stainless steel cylinder with a piston each. At the bottom of the extraction chambers, two porous sets let the liquid phase to flow through, while solid particles are blocked. The two extraction chambers communicate with each other through a pipe including an electro gauge, which is closed during the extraction phase and is open to collect the liquid phase once the extraction program ends. The solid raw material is placed in the chambers that are then filled with the solvent (e.g., water, organic, inorganic, or a mixture of solvents). Then, through the pistons, a suitable pressure is applied to the mixture. When the maximum value set for the pressure is reached (0.9 MPa), the device stops for a predetermined time interval, thus, allowing the solid matrix to equilibrate with the solvent (static phase). Immediately after this step, the pistons are suddenly moved, creating a negative pressure gradient between the outside and the inside of the solid matrix (dynamic phase). During this step, depending on the liquid phase composition, compounds in the solid matrix can be physically and/or chemically extracted from the solid matrix and transferred to the solvent due to the existing pressure gradient. The movement of the pistons and hence the static and dynamic steps alternate. Each extraction cycle is formed by one static and one dynamic step. The effect of several cycles, which can be performed at controlled temperature or at subambient temperature, is to transfer analytes from the solid matrix to the liquid phase both chemically, by diffusion and osmosis (depending on the solvent used), and physically (due to the negative pressure gradient between the liquid into the inner of solid phase and the solution). By carefully controlling the solvent/solid matrix combination and suitably programming the pressure cycles, each one of the above mentioned effects may be enhanced with respect to the other. This allows the increase of the extraction selectivity with respect to the selected analyte(s). Furthermore, the possibility to operate at room temperature or at a subambient temperature greatly reduces the thermal stress for any thermolabile analyte of interest, as in the case of lycopene. By repeating these cycles many times, complete exhausting of the solid matrix can be obtained in a short time and with high recovery of the analyte(s) in the liquid phase (*17, 18*).

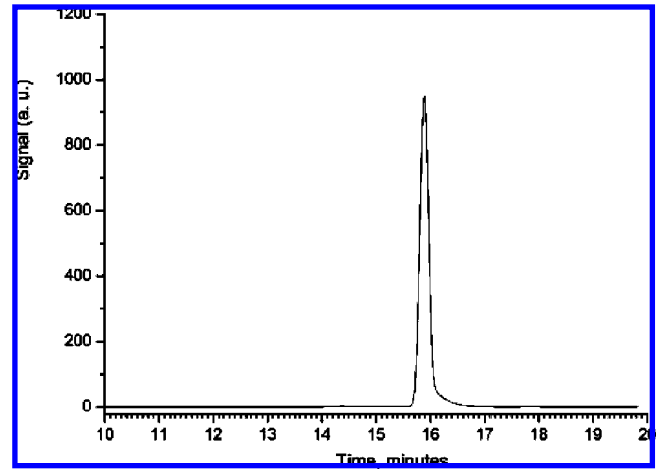

Figure 2. Chromatogram of 0.1 mg/mL purified lycopene extract in CH_2Cl_2. Mobile phase gradient: up to 3 min H_2O 100%; time 20 min, CH_3CN 100%. Flow rate: 1 mL/min. Diode array detector at 474.0 nm.

Once the analyte is removed from the tomato matrix, it may be easily purified in a single step by using small quantities of acetone or washing with methanol first and then eluting with acetone.

In the first case, low-purity lycopene was obtained because of the presence of other carotenoids (such as β-carotene and lutein) in the extract. On the contrary, washing the column with methanol before dissolving the residue with acetone allowed the recovery of lycopene at more than 98% (w/w) purity. In **Figure 2**, a typical HPLC chromatogram of the extract is presented; under these chromatographic conditions, the retention time for lycopene was about 16 min. The impurities content never exceeded 2% of the total signal intensity. Moreover, the acquisition in the wavelength range 200−600 nm did not show the presence of other colored substances in the solution. Peaks of minor components were detected, but they were well-separated from the lycopene peak. On the basis of the retention times and of the diode array spectra, the signals were attributed to cis isomers of the molecule and to β-carotene. The most common technique used for the separation of lycopene, even when in a mixture with other carotenoids as in vegetal sources,

Table 1. Lycopene Recovery (mg/kg) of Tomato Byproducts Using Tap and Deionized Water at Different Values of the Extraction Time

	lycopene content (mg/kg)				
sample	deionized water (4 h)	tap water (4 h)	tap water (2 h)	tap water (6 h)	tap water (8 h)
1	2.3	3.2	1.5	2.6	2.5
2	3.4	3.0	1.9	2.8	3.2
3	2.7	3.5	1.1	2.5	3.3
4	1.9	1.5	0.9	1.4	1.6
5	2.8	3.9	2.1	2.9	3.5
6	1.7	1.7	1.2	1.8	1.8
7	2.9	2.5	1.8	2.4	2.6
8	3.9	3.6	1.8	2.9	3.5
9	2.2	2.0	1.4	1.7	1.9
10	2.6	2.9	1.5	2.0	2.8

Table 2. Lycopene Recovery (mg/kg) from Tomato Byproducts Using Tap and Deionized Water at the Optimum Extraction Time of 4 h

sample	deionized water	tap water
1	2.3	3.2
2	3.4	3.0
3	2.7	3.5
4	1.9	1.5
5	2.8	3.9
6	1.7	1.7
7	2.9	2.5
8	3.9	3.6
9	2.2	2.0
10	2.6	2.9

is liquid chromatography. The stationary phase frequently employed is the reverse phase octadecyl silicone, and the gradients proposed are generally complex (*14, 16*). In this paper, comparable results have been obtained by using a reverse phase phenyl−hexyl silicone as the stationary phase and a very simple water−acetonitrile gradient.

Table 1 shows lycopene recovery from tomato byproduct using tap and deionized water at different values of the extraction time, while in **Table 2** a comparison of the obtained lycopene (mg/kg) in tap and deionized water, at the optimum extraction time of 4 h, is shown. The highest recoveries, about 2.6 mg (average value) lycopene/kg tomato were obtained after 4 h of extraction time. It was also possible to observe that the increase of the extraction time did not increase the recoveries, and on the other hand, degradation events could have been possible. The whole extraction procedure was repeated using tap water instead of the deionized one, and the average recoveries coincided, within the experimental uncertainty, with the ones obtained with deionized water. On a laboratory scale by using the Extractor Naviglio 500 mL, about 0.5 mg of pure lycopene was obtained from 200 g of tomato wastes in 4 h extraction time, and the recovery percentage was about 10% (w/w) with respect to 20 ± 3 mg/kg raw material (average of 10 samples analyzed) obtained using the traditional extraction. The efficiency of the proposed process is lower than the procedures that utilize organic solvents, but when considering the low cost of the tomato wastes, the use of water as the extracting solvent, the easy dumping of the exhausted starting material, and the high purity of the final product obtained, it turns out to be cost reducing.

This extraction process could be easily scaled-up to become an industrial application for the production of very high grade of purity (>98%) lycopene, whose demand is constantly increasing. The obtained lycopene was characterized using NMR and mass spectrometry. The chemical shift are reported in ppm

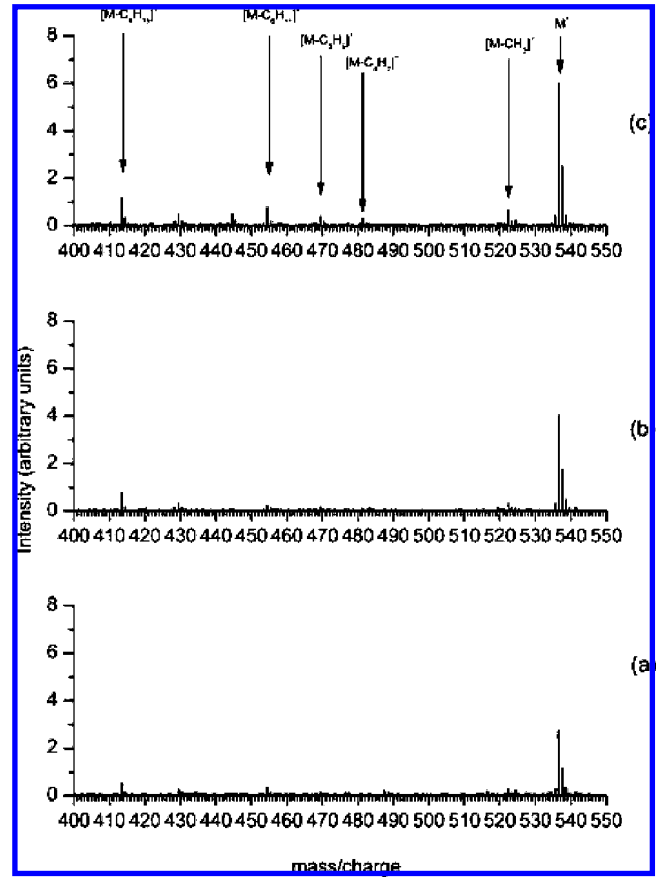

Figure 3. Full scan positive ion ESI-MS of lycopene extract at (**a**) 10 V, (**b**) 20 V, and (**c**) 30 V cone voltages.

and the $CHCl_3$ (7.26 ppm for ^1H) and $CDCl_3$ (77.23 ppm for ^{13}C) signals were used as the internal standard reference; the signals were attributed as follows: ^1H NMR ($CDCl_3$, ppm) 6.68−6.13 (14H, m), 5.954 (2H, d, J = 10.9 Hz), 5.111 (2H, bs), 2.114 (8H, bs), 1.969 (12H, s), 1.820 (6H, s), 1.690 (6H, s), 1.616 (6H, s). The value of the coupling constant of the signals centered at 5.954 ppm is typical for a trans configuration around a partial double bond, as it is the one between the C6−C7 and C6′−C7′ (**Figure 1**). ^{13}C NMR ($CDCl_3$, ppm): 139.70 (2C), 137.59 (2CH), 136.77 (2C), 136.38 (2C), 135.64 (2CH), 132.87 (2CH), 131.80 (2C), 131.79 (2CH), 130.31 (2CH), 125.97 (2CH), 125.38 (2CH), 125.02 (2CH), 124.19 (2CH), 40.46 ($2CH_2$), 26.92 ($2CH_2$), 25.92 ($2CH_3$), 17.93 ($2CH_3$), 17.18 ($2CH_3$), 13.12 ($2CH_3$), 13.02 ($2CH_3$). The attributions were confirmed with measurements performed with the DEPT (Distortionless Enhancement by Polarization Transfer) technique. **Figure 4** reports the mass spectra in the 400−550 *m*/*z* interval at 10 V (**a**), 20 V (**b**), and 30 V (**c**) cone voltage, respectively. Besides the molecular ion signal, well-characterized already at 10 V, a series of signals increasing with the cone voltage intensity were tentatively assigned. In particular, the following fragments were identified: $[M - CH_3]^+$, corresponding to the loss of a terminal methyl group; $[M - C_4H_7]^+$, due to the breaking of the bond between the C2 and C3; $[M - C_5H_9]^+$, which is determined by the loss of a isoprenic unit (break between the C3 and C4); $[M - C_6H_{11}]^+$, determined by the C4−C5 bond break; and $[M - C_9H_{15}]^+$, which is evident already at 10 V cone voltage, due to the break between the C6 and C7. The attributions were confirmed on the basis of product ion mass spectra (ESI-MS/MS) of the M^+ ion at *m*/*z* 537.

The high purity lycopene obtained was examined by VIS-spectrophotometry to determine the molar absorption coefficient

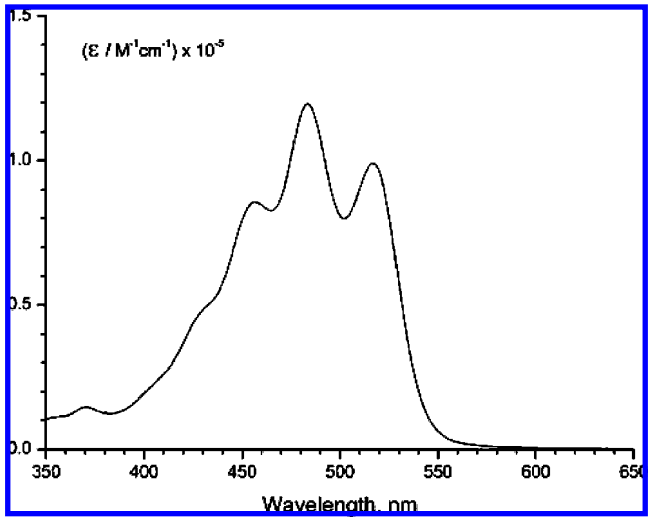

Figure 4. Visible spectrum of purified lycopene extract in $CHCl_3$. Temperature: 303 K. Scan speed: 100 nm/min. Band width: 2 nm.

considering the poor reproducibility of the molar absorption coefficient values for lycopene reported in the literature that may be attributed to the fact that it is not easy to obtain this molecule at an high enough purity. **Figure 3** reports the spectrum of lycopene at 303 K in $CHCl_3$; the extinction coefficient maximum ($\log \varepsilon = 5.15$) appeared at 483.6 nm. The spectrum of the dichloromethane solution presented exactly the same extinctions as the one in **Figure 3**, but it showed a weak hypsochromic shift, with the maximum absorption at 480.2 nm. The data obtained are in accordance with results reported in the literature within the limits of the experimental deviations (*9*).

Considering that at present the best purity obtained for lycopene is about 90−95%, the high purity of the obtained lycopene could make the process very appealing, and the pure product obtained makes it possible to use it, at a known dosage, as a drug and not only as a food integrator. Moreover, the costs of production are lower compared with the cost of a conventional solvent or supercritical fluid phase extraction procedure. On a semi-industrial scale, the 98% purity lycopene obtained by using the procedure presented here could be sold at about $^1/_{10}$ of its current commercial price. Moreover, this process produces no special byproduct to be wasted, since only water, which can also be recovered by filtration and reused, is employed in large quantities; the exhausted tomato wastes after the extraction can be easily dried without any appreciable degradation and possibly used in agriculture or as feeding for animals. The quantities of organic solvents used in the purification process (e.g., methanol and acetone) are negligible and do not compromise the economical advantage and interest of the whole process.

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