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Supercritical CO_2 extraction of lycopene and β-carotene from ripe tomatoes

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Abstract

This work describes the influence of some operative parameters of supercritical carbon dioxide extraction employed for the isolation of lycopene and β-carotene from the pulp and skins of ripe tomatoes. The extractions were conducted at pressures and temperatures ranging from 2500 to 4000 psi and 40 to 80°C, respectively. The extracted product at 4000 psi and 80°C contained about 65% of lycopene and 35% of β-carotene. Lycopene and β-carotene showed a different solubility in the supercritical fluid depending on process parameters. With a proper choice of operative parameters, it has been possible to obtain a product that contained 87% lycopene and 13% β-carotene.

Keywords: Tomato lycopene extraction; Supercritical CO_2 extraction; Natural dyes; Lycopene; β-Carotene; HPLC

1. Introduction

The use of supercritical fluids has been proposed for a wide range of industrial applications, including refining of lubricant oils, synthesis of polymers, decaffeination of coffee, extraction of hop and generally that of natural products from vegetable matrices [1]. Supercritical fluid extraction is an advanced separation technique based on the enhanced solvating power of gases above their critical point. One of the most frequently used supercritical fluids is carbon dioxide. Besides the advantages of having a low critical temperature and being neither toxic nor flammable, carbon dioxide is also available at low cost and high purity; thanks to its low critical temperature, it can also be used to extract thermally labile compounds that cannot be submitted to steam distillation. On account of these characteristics, the fluid is an ideal solvent in the food, dye, pharmaceutical and cosmetic industries, where it is essential to obtain final products of a high degree of purity.

We are currently using the described technique in two research fields: i.e. the dyeing of synthetic fibres with disperse dyes, and the extraction of natural dyes from plants of the *Mediterranean maquis*.

In the past years interest in the use of natural dyes has increased. A limited number of dyes are commercially available and small firms have started exploring the use of natural dyes as a means of producing an ecological product.

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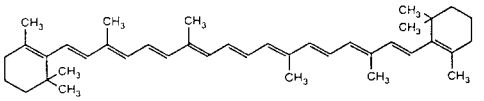
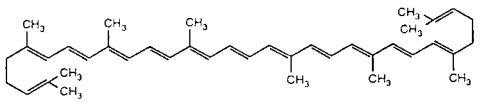
In this paper we report on the extraction of lycopene and β-carotene (**I**) from ripe tomatoes. The main pigments of ripe tomatoes are the carotenes, compounds of colours ranging between yellow and red: i.e. α, β, and γ-carotene, lycopene and xanthophyll at very low concentrations. Lycopene, which has an intense red colour, is the most abundant carotenoid in tomatoes, accounting for about 85% of the pigments present [2]. Its concentration can vary considerably: from 30 to 200 mg/kg of fresh fruit or 430 to 2950 ppm on a dry basis [3, 4].



β-carotene

(I)



Lycopene

Though carotenes are more stable than other pigments found in plants, such as chlorophyll and the anthocyanins, they tend to degrade in the presence of oxygen. A number of studies [5–9] have shown the importance of carotenoids as antioxidant compounds and precursors of vitamin A. More recent research has attributed important functions to these compounds. They include anticancer activity, regulation of the immune system, free radical inactivation and fat peroxidation inhibition [10].

Lycopene (C.I. 75125) is a red natural pigment used in the dyeing of different kinds of foodstuffs. Its importance is increasing due to a more extensive use of natural compounds in the food, cosmetic and pharmaceutical industries, following EU directives in favour of natural rather than synthetic compounds. The main problem in obtaining lycopene is its solubility, since it is insoluble in water and soluble in highly toxic organic solvents, such as benzene, chloroform, and methylene chloride. Moreover, the extraction methods reported in the literature are difficult, poorly reproducible and subject to errors on account of loss of substance during extraction. In order to overcome these difficulties and obtain both lycopene and β-carotene without traces of the solvent, we resorted to supercritical fluid extraction.

A few papers have been carried out to the extraction with supercritical carbon dioxide of β-carotene from various kinds of substracta [11–13], but supercritical fluid extraction applied to tomato products has been studied for the extraction of pigments only for analytical purposes [14].

2. Experimental

Supercritical CO_2 extractions were performed by an SFE-400 thermal pump (Supelco), equipped with a 10 cm^3 internal volume extractor. The extraction pressure could be adjusted in 100 psi increments up to a maximum pressure of 4000 psi. The temperature range was 30–200°C. The extracted substances were recovered in a vial connected to a restrictor. At each run the rate flow was maintained at about 500 cm^3/min. Several methods were used for the total or partial removal of water from the tomato samples submitted to the supercritical fluid. In all cases the samples were protected from the action of both light and oxygen in the air in order to prevent them from damaging the dye. The extractions were performed on ripe tomato pulp or dry skins, since they contain about 5 times as much lycopene as in the whole tomato pulp [15].

The extractions were initially conducted on whole fresh tomatoes, ground and filtered in a vacuum. In order to adsorb the remaining moisture, the samples were mixed with silica gel. The measurements were carried out on "camone" greenhouse tomatoes, grown in Sardinia and on field tomatoes. Extractions were subsequently carried out on the skins and dried seeds of field tomatoes.

The tomato skins and seeds were dried for 24 h in an air drier at 35°C and stored at −5°C. The product was ground before being extracted.

The extractions were run by submitting 2.5 g of fresh or dry ground samples to different pressures

(2500–4000 psi) and temperatures (40–80°C) for 30 min, both in the presence (1 ml) and absence of an entrainer. The extracts coming out of the restrictor were collected in ethanol and analysed by HPLC.

Lycopene and β-carotene were determined on a Hewlett Packard 1050 HPLC, equipped with an autoinjector and UV–visible detector, Spherisorb column C18, 5 mm, 25 cm × 4.6 mm. A mixture of methanol, THF, and water in a 67:27:6 ratio with a flow of 1 ml/min (2 μl injection volume) was used as the mobile phase; the detection was performed at 446 nm, corresponding to a maximum of lycopene. Lycopene and β-carotene were identified by comparing the retention times of the two pigments in the extraction mixture with those of their respective standard compounds (Sigma products). Lycopene was also identified by comparing the UV-visible, ^{1}H NMR and mass spectra of the extracted substance with those of standard lycopene.

Fig. 1 shows the visible absorption spectrum of the chloroform solution of the extract. The absorption spectrum practically coincides with that of standard *trans*-lycopene and shows the three characteristic maxima for *trans*-lycopene ($\lambda = 446$, 472, 515 nm). ^{1}H NMR spectra were performed at high and low fields both on standard lycopene and on the extract; the signals in the two spectra coincide and agree with those reported in the literature [16].

Fig. 2 shows the electron impact mass spectrum of the extract. In order to obtain high mass ions more abundantly, as they are the most significant in defining the structure of the compound, we monitored the ions in the 200–540 mass unit range. The mass spectrum fragmentation pattern of the extract corresponds both in mass and in abundance to the spectrum of standard lycopene. In order to calculate the concentrations of lycopene and β-carotene in the extract, calibration curves were drawn with their respective standards; five or six solutions of a known concentration were prepared for each curve by diluting a stock solution with chloroform. As a reference we used a dye of formula **II** (synthesised by us) that had λ_{max} close to that of lycopene.

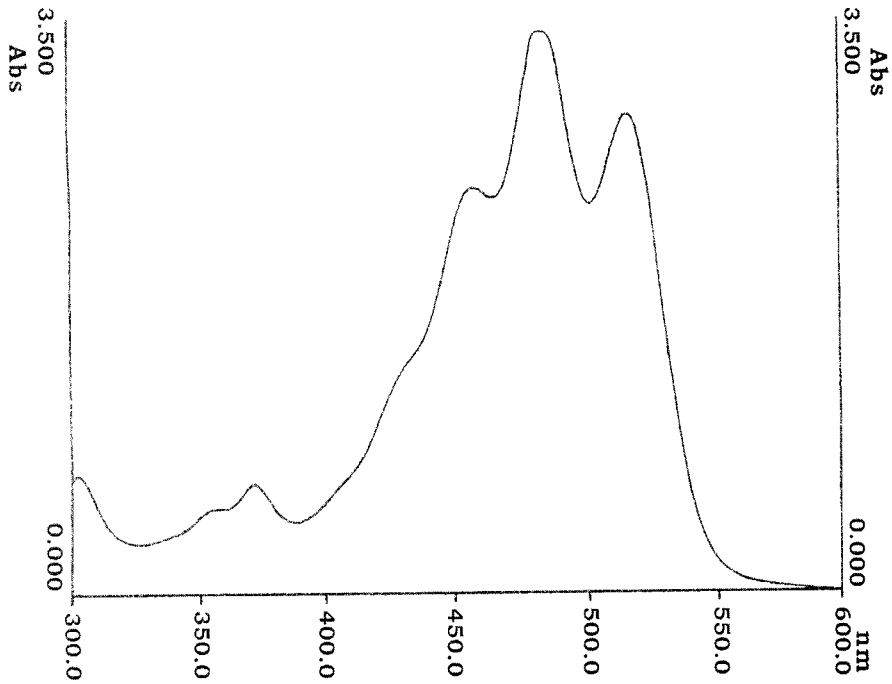


Fig. 1. Absorption spectrum of the extract in supercritical CO_2.

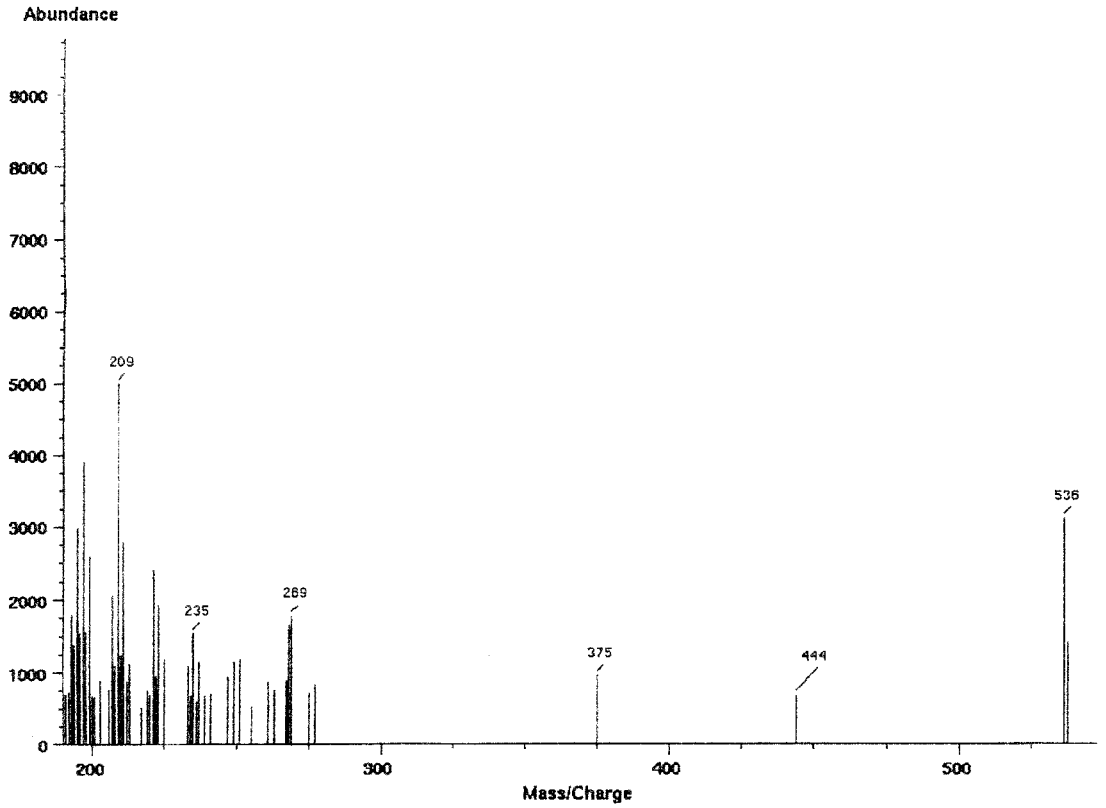
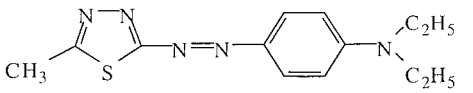


Fig. 2. Impact mass spectrum of the extract by supercritical CO_2.



(II)

The following equation was used:

$$C_x = F(Ax/Ar)Cr + I$$

where:

C_x	sample concentration
F	straight line angle coefficient
Ax	peak area of the sample
Ar	peak area of the internal reference
Cr	concentration of the internal reference
I	intercept

Fig. 3 shows the calibration curves for lycopene and β-carotene respectively. The amount of total extractable pigments was determined after extraction with acetone:hexane for 6 h using a Soxhlet apparatus.

3. Results and discussion

Initially the extractions were performed at 40°C to avoid pigment degradation. But at this temperature, even with the pressure at 4000 psi, the fluid did not extract lycopene except in trace amounts. In order to increase the solving power of the fluid we added several solvents (entrainers) on the sample charged in the extraction vessel: i.e. hexane, ethyl ether, ethyl alcohol and chloroform. The only entrainer that gave satisfactory results was chloroform and, to a lesser extent, *n*-hexane. Fig. 4 shows an HPLC analysis of the extract obtained at 40°C, at a pressure of 4000 psi in the presence of chloroform. The following compounds

were identified in the extract: *trans*-lycopene, and β-carotene. Table 1 shows the lycopene and β-carotene yields obtained with chloroform or hexane as entrainer. The results show that lycopene yields are good, especially in the presence of chloroform.

Unfortunately GC/MS analysis on the extract showed presence of traces of chloroform. For this reason we attempted to optimise the extraction without the help of the entrainer by varying the temperature between 40 and 80°C and maintaining a constant pressure of 4000 psi.

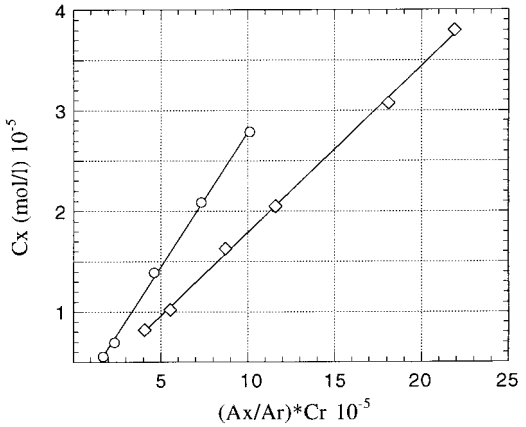


Fig. 3. Calibration curves of lycopene (◇) and β-carotene (○).

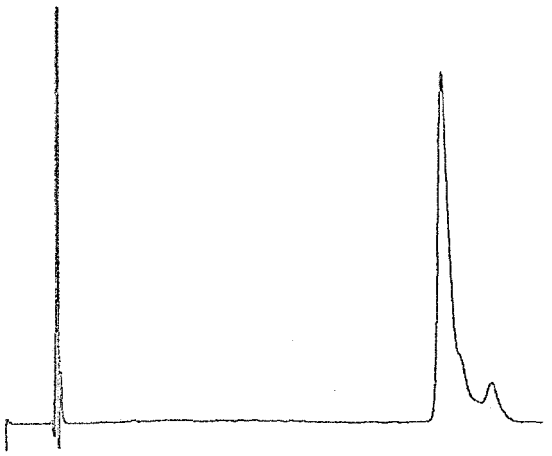


Fig. 4. HPLC of the extract at 40°C and 4000 psi with the entrainer. The identified components are in the order: first peak (retention time 2.60 min) = reference; second peak (rt 22.69 min) = trans-lycopene; 3rd peak (rt 25.22 min) = β-carotene.

This was done on dried ripe tomato skins and seeds, and was aimed at a possible utilisation of the by-products of the tomato industry. In fact, the by-products of the food industry, such as wastes from the production of peeled tomatoes and tomato concentrate could be an excellent source, especially of lycopene, but also of β-carotene. Table 2 shows the percentage yields of the two pigments obtained in supercritical CO_2 from ripe tomato skins by varying the temperature and keeping the pressure constant.

From an analysis of the data it can be inferred that the concentration of lycopene increases on increasing the temperature, while the concentration of β-carotene remains practically constant in the range 60–80°C. Though the structure of the two pigments is very similar, they present a different solubility in the fluid. In fact, at 40°C and 4000 psi β-carotene is extracted almost exclusively. When the temperature is increased, the concentration of β-carotene remains practically constant, while the concentration of lycopene increases significantly (Fig. 5). The reason for this could be sought both in the different concentration of the two pigments in the various parts of the vegetable

Table 1
% yields of lycopene and β-carotene obtained from *camone* tomatoes and field tomatoes at 40°C and 4000 psi

Tomatoes	Entrainer	Lycopene (mg/100 g)	*β*-carotene (mg/100 g)
Camone	Chloroform	14.92 ± 0.12	1.94 ± 0.06
Camone	*n*-Hexane	2.35 ± 0.07	0.31 ± 0.04
Field	Chloroform	3.71 ± 0.05	0.53 ± 0.05

Table 2
% yields of lycopene and β-carotene obtained by supercritical CO_2 at 4000 psi and by Soxhlet

Temperature (°C)	Lycopene (mg/100 g)	*β*-carotene (mg/100 g)
40	3.80 ± 0.05	15.45 ± 0.55
50	12.42 ± 0.30	24.61 ± 0.16
60	18.76 ± 0.52	32.42 ± 0.68
70	48.10 ± 0.07	33.50 ± 1.52
80	64.41 ± 0.12	34.88 ± 0.42
Soxhlet	77.08 ± 0.07	37.76 ± 0.37

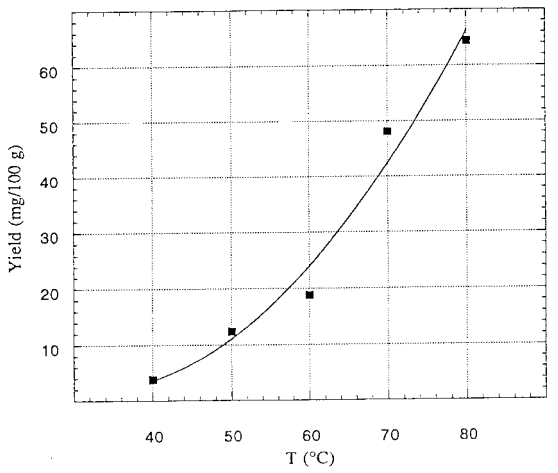


Fig. 5. Lycopene yields of the extractions conducted at different temperatures and at 4000 psi.

tissues and in the fact that lycopene crystallises as long needles [4]. Its solubilisation could therefore be more difficult at low temperatures. In order to obtain an extract richer in lycopene, the extraction must be carried out at high temperature. From the data it is also inferred that when the process is performed at 80°C and 4000 psi, 93% of β-carotene and 84% of lycopene are extracted compared to the amount of product Soxhlet extracted. The extracted product contains 65% lycopene and 35% β-carotene.

We tried to optimise the method so as to obtain a product that should be richer in lycopene and free from impurities. A first extraction was made at 40°C and 4000 psi followed by a second extraction on the same sample at 80°C and 4000 psi, and since the β-carotene was extracted in large amounts in the first extraction, the obtained product was richer in lycopene. Thus the final extract contained 87% lycopene and 13% β-carotene.

The concentration of lycopene in tomatoes is quite variable and depends on both the type of tomato and the maturation temperature. The quantity of lycopene in the berries can vary significantly depending on various factors, the most important being the climate trend. Lycopene is the last carotenoid to appear during maturation and its formation is practically inhibited by temperatures higher than 30–32°C [2], while the synthesis of the other cartenoids does proceed at temperatures above 30°C. For this reason if the temperature is maintained at high values, the tomatoes present a yellow-orange colour. In fact the field tomatoes that gave the results reported in Table 3 were the produce of 1998, which was a particularly warm year in Sardinia, and could have inhibited the formation of lycopene. In confirmation of the above, in the study on the previous year's crop of field tomatoes (Table 1), the obtained product contained about 92% lycopene and 8% β-carotene.

Acknowledgements

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