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Application of a UV–vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables

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Abstract

The purpose of this paper is to optimize an HPLC method for the determination of lycopene and β-carotene in vegetables and compare it with a spectrophotometric standard method. Among the different conditions studied the most suitable ones for our samples were: extraction with hexane/acetone/ethanol (50:25:25 v/v/v), evaporation of the hexane layer, dissolution of the dry extract in THF/ACN/methanol (15:30:55 v/v/v) and injection on a C18 column with methanol/ACN (90:10 v/v) + TEA 9 μM as mobile phase (Φ = 0.9 ml/min) and $\lambda_{detection}$ = 475 nm. Samples considered for analysis were: tomato, carrot, pepper, watermelon, persimmon and medlar. The HPLC method proposed showed adequate reproducibility (RSD < 10.5%), accuracy (100–109% recovery) and sensitive detection limits (0.6 μM for lycopene; 0.3 μM for β-carotene), with a simple preparation of the samples (one step direct extraction) and short run times (10 min) for the quantification of lycopene and β-carotene.
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1. Introduction

Nowadays the major interest of carotenoids, which are found in plants, is not only due to their provitamin A activity but also to their antioxidant action by scavenging oxygen radicals and reducing oxidative stress in the organism (Rao & Honglei, 2002). There are many studies showing strong correlations between carotenoid intake and a reduced risk of some diseases, as cancer, aterogenesis, bone calcification, eye degeneration and neuronal damages (Cantuti-Castelvetri, Shukitt-Hale, & Joseph, 2000; Ferguson, 1997; Yamaguchi & Uchiyama, 2003).

Lycopene is the compound responsible for red colour in tomato, watermelon and other fruits, and it is also used as a colour ingredient in many food formulations (Richelle et al., 2002). A great interest has recently been focused on lycopene due to its preventive activity against several pathologies, such as cardiovascular disease (Rao, 2002), hepatic fibrogenesis (Kitade, Watanabe, Masaki, Nishioka, & Nishino, 2002), solar light induced erythema (Stahl & Sies, 2002), human papillomavirus persistence (Sedjo et al., 2002) and some cancer types, such as prostate, gastrointestinal and epithelial (Clinton, 1998; Livny et al., 2002; Shi & Le Maguer, 2000). Lycopene has also been recently reported to play a role in lung function (Schunemann et al., 2002) as well as in foetal growth (Sharma et al., 2003). Finally, it is also important to consider the synergic action of carotenoids with other bioactive compounds present in fruits

and vegetables (Rao & Agarwal, 2000; Sharoni et al., 2002; Sook Lee, Jae In, Hee-Kang, Khachik, & Han Yoon Park, 2003).

Carotenoid analysis in food products may be carried out by different methods: HPLC, spectrophotometry, or colour evaluation (Schoefs, 2002). Although spectrophotometry or colorimetry can be used to rapidly assess the lycopene content of products derived from tomatoes, a highly versatile, sensitive and selective method such as HPLC is needed for reliable analysis of food samples. HPLC analysis of carotenoids is usually done with C18 or C30 RP-columns, operated with isocratic or gradient elution with a wide variety of mixtures of different organic solvents as mobile phases, using UV–vis ($\lambda \approx 450$ nm) or photodiode array or MS detection (Burns, Fraser, & Bramley, 2003; Craft, 2001; Gomez-Prieto, Caja, & Santa Maria, 2002; Lin & Chen, 2003; Pichini et al., 2002; Tzouganaki, Alta-Politou, & Koupparis, 2002; Van Breemen et al., 2002). Heating the column is sometimes used to improve pigment separation as well as to standardize the separation conditions (Schoefs, 2002).

For the extraction of carotenoids from the samples, different systems can be used, like liquid–liquid extraction, solid phase extraction or supercritical fluid extraction (Gomez-Prieto, Caja, Herraiz, & Santa Maria, 2003; Kozukue & Friedman, 2003; Rozzi, Singh, Vierling, & Watkins, 2002; Tzouganaki et al., 2002). AOAC (1993) recommends methanol/tetrahydrofuran (THF) (50:50 v/v) for extracting the carotenoids, while other authors use ethyl acetate (100%) or different mixtures of ethanol/hexane, acetone/ethanol/hexane, ethyl acetate/hexane or acetone/hexane (Lin & Chen, 2003; Pichini et al., 2002).

The instability of lycopene during processes of extraction, handling, and elimination of organic solvents makes the preparation of a sample for analysis an extremely delicate task, often requiring successive and complex procedures to ensure that all the carotenoids are extracted. Besides, not all the analytical methods available for carotenoid analysis in food products are suitable for lycopene-rich foods due to the low solubility of lycopene in some of the solvents employed – as in the case of methanol – and due to the fact that the use of other solvents may interfere with the mobile phases applied for carotenoid separation. There are many HPLC methods that can be applied to the determination of carotenoids. However, this kind of compounds needs a very careful and tedious manipulation due to their chemical lability. Therefore the development of new methodologies of extraction/separation is of relevance and the necessity for a reliable and rapid analysis method for lycopene in vegetable products has been recognized (Bicanic et al., 2003; Schoefs, 2002).

This work is aimed at solving the problems above mentioned with a quite simple preparation of the samples, the selection of extraction solvent mixtures more compatible with mobile phase, and short run times, by developing a suitable, reliable, rapid and simple HPLC method for lycopene and β-carotene analysis on vegetables which have very different carotenoid content, such as tomato, carrot, persimmon, watermelon, pepper or medlar, and its comparison with a reference spectrophotometric method.

2. Experimental

2.1. Reagents

Standards of all *trans*-lycopene, β-carotene and lutein were from Sigma (St. Louis, MO, USA). The HPLC grade solvents were purchased from Symta (Madrid, Spain) in the case of methanol and acetonitrile (ACN) and from Sigma (Steinheim, Germany) in the case of triethylamine (TEA) and THF stabilized with <0.025% butylated hydroxytoluene. The reagent grade solvents used in liquid–liquid extraction were supplied by Merck (Darmstadt, Germany) in the case of hexane and by Panreac (Barcelona, Spain) in the case of diethyl ether, acetone and ethanol. Doubly distilled water was obtained from a Milli-Q System (Millipore, Bedford, MA, USA).

2.2. Samples

On each harvest season, different Spanish commercial varieties of tomato, carrot, green and red pepper, watermelon, persimmon and medlar, as carotenoid-rich foods, were purchased from local markets. Fresh vegetables and fruits (a minimum of five individual fruits) were prepared as appropriate by removal of the outer leaves, peeling, etc. Larger products, such as watermelon, were quartered, cut and mixed; smaller products were cut and mixed. All samples were homogenized in a domestic blender to obtain a representative sample for carotenoid analysis.

2.3. Instrumentation

The chromatographic apparatus consisted of a Micron Analitica, S.A. (Madrid, Spain) PU II isocratic pumping system; a Jasco (Tokyo, Japan) AS-1555 autosampler; a ERC-Gecko-2000 (Riemerling, Germany) column heater; a Thermo Separation Spectra Series UV100 (San Jose, CA, USA) UV–vis detector. For data processing and analysis, Biocrom 2000 3.0 version software from Micron Analitica, S.A. (Madrid, Spain) was used. The analytical column was a μBondapack C18 (300 mm × 2 mm), 10 μm of pore size, with a μBondapack C18 precolumn (20 mm × 3.9 mm), 10 μm of pore size, both purchased from Waters (Milford, MA,

USA). A Pharmacia Ultrospec 4000 (Barcelona, Spain) spectrophotometer was used for absorbance measurements. A Büchi Labortechnik AG (Flawil, Switzerland) rotatory evaporator was used to obtain the dry extracts.

2.4. Standard carotenoid preparation

Individual stock standard solutions were freshly prepared every day adding a suitable volume of hexane to the vial containing the carotenoid standard and mixing until complete dissolution; then the solutions were transferred to a volumetric flask and the concentration was determined spectrophotometrically using Beer's law. Absorptivities were taken from Scott, Finglas, Seale, Hart, and de Froidmont-Görtz (1996). An aliquot of each individual stock standard solution was evaporated to dryness and prepared for HPLC as mentioned in Sections 2.5 and 2.6 to check purity as reported by Hart and Scott (1995), Konings and Roomans (1997) and Scott et al. (1996). Considering the purity obtained in this test, the concentration values of the above solutions were corrected according to the absorbance. *Individual working standard solutions* of around 0.5–9.5 µM were freshly prepared every day from individual stock standard solutions by diluting in hexane. For identification and quantification purposes a *mixed standard solution* was freshly prepared every day from stock standard solutions following Sections 2.5 and 2.6 procedures.

2.5. Sample preparation

Six solvents mixtures were used for comparison of extraction efficiency: (1) methanol (100%), (2) ethyl ether (100%), (3) THF (100%), (4) methanol/THF (92:8 v/v), (5) methanol/THF (50:50 v/v) and (6) hexane/acetone/ethanol (50:25:25 v/v/v).

A sample containing between 100 and 300 µg of total carotenoids (5 g for persimmon and medlar, and 2.5–3 g for the other vegetables) was placed in a vessel, protected from light, and mixed with 100 ml of extraction solvent. The mixture was magnetically stirred during 30 min. For (1), (2), (3), (4) and (5) systems, the extracts were centrifugated to separate the supernatant, and these operations were repeated until the pulp was completely colourless. After that the extracts (1) and (4) were directly injected in the HPLC system; extract (5) was diluted to a concentration of THF lower than 10% to avoid peak broadening; extracts (2) and (3) were evaporated to dryness under N_2 flow under vacuum. For extract (6), 15 ml of water were added; the upper layer was placed in a round-bottomed flask, and an aliquot of 10 ml of the extract was evaporated to dryness.

In order to increase the sample concentration and to provide a better compatibility between the mobile phase and extraction solvent, the evaporation step was applied to extracts (2), (3) and (6). Several solvents were assayed for the dissolution of the dry extract: (1) methanol (100%), (2) THF/methanol (10:90 v/v), (3) different mixtures of ACN/methanol (4) different mixtures of THF/methanol/water and (5) different mixtures of THF/ACN/methanol.

The residue was dissolved to a final volume of 4 ml, except in the case of persimmon and medlar, for which the final volume was 1 ml. For samples with high lycopene content (absorbance at 501 nm in hexane extract was higher than 1), the final volume was 10 ml. The final solution was filtered through 0.45 µm membrane filters and 100 µL were injected for HPLC analysis.

2.6. Chromatographic conditions

Several mobile phases were assayed: (1) methanol/ACN (90:10 v/v), (2) methanol/ACN (90/10 v/v) + TEA 9 µM, and different mixtures of methanol/THF/water. The mobile phase was filtered through a 0.45 µm membrane, and degassed ultrasonically prior to use. The mobile phase flow rate was 0.9 ml/min. The column temperature was 30 °C and the absorbance was read at 475 nm.

The efficiency of the separation was evaluated by the calculation of the number of plates (N), using the width of the peak at half its maximum height. The identification of the peaks was carried out by comparing the retention times with those obtained with a mixed standard solution of all-*trans* lycopene, β-carotene and lutein (Fig. 1). The quantification was performed using calibration curves made with different injected amounts of all-*trans*-lycopene and β-carotene, in a similar proportion as in the samples.
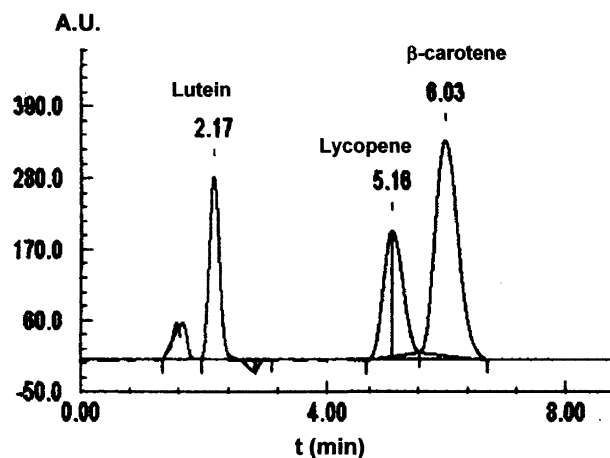

Fig. 1. Chromatogram of the carotenoid standard solution. Chromatographic conditions: µBondapack C18 column (300 mm × 2 mm), 10 µm of pore size, µBondapack C18 precolumn (20 mm × 3.9 mm), 10 µm of pore size; mobile phase methanol/ACN 90:10 (v/v) + TEA 9 µM; flow rate 0.9 ml/min; column temperature 30 °C; $\lambda_{detection} = 475$ nm.

2.7. Spectrophotometric conditions

In order to validate the HPLC method, it was compared to a reference method, the spectrophotometric method of AOAC (1996). Spectra of lycopene and β-carotene standards in hexane were recorded for identifying purposes. 501 nm was selected for determining lycopene and 446 nm for β-carotene and other carotenoids.

Samples were extracted using the solvent system (6) described in Section 2.5 The quantification of lycopene and other carotenoids in the hexane layer of the extracts was performed using a calibration curve made with different working standard solutions of lycopene and β-carotene in hexane. The quantification of β-carotene in lycopene-rich samples was not possible due to the overlapping of the absorbance bands.

3. Results

3.1. Extraction of carotenoids

Considering the spectra of samples and standards on different solvents, those containing THF or hexane showed higher extraction efficiency, while the presence of methanol hampered the extraction of lycopene due to the poor solubility of this compound in methanol. From all solvents assayed, hexane/acetone/ethanol (50:25:25 v/v/v) showed the best results (obtaining the characteristic spectral profile of these compounds), with a virtually complete extraction of carotenoids. The pulp of the samples was completely colourless in only one step after 20 min extraction using this mixture, while with other solvents several steps were necessary, with high extraction volumes (low concentration of carotenoids in the extracts) and incomplete decolouration of the analyzed sample.

3.2. Optimization of HPLC method

The mixture methanol/THF/water as mobile phase gave high retention times and broad peaks. The best resolution for lycopene and β-carotene (calculated as the ratio between the difference in retention times and the average of the width of the peaks at half-maximum height) was achieved using methanol/ACN (90:10 v/v) + TEA 9 μM, as mobile phase (see Table 1). The presence of TEA improves the response of carotenoids and reduces or eliminates on-column degradation (Hart & Scott, 1995; Rodriguez, 2001). The selected mobile phase provided good retention factors (4.03), a high number of plates and tailing factors of 1 for both compounds studied. Using this mobile phase, a balance between methanol and non-polar solvent should be achieved, since a high methanol proportion can cause lycopene precipitation in the extracts, and a high non-polar solvent content can cause the deformation of the chromatographic peaks due to the great polarity difference. Therefore, a mixture of THF/ACN/methanol (15:30:55 v/v/v) was selected as the most useful combination for redissolving the residue after evaporating to dryness, because it avoided lycopene precipitation and produced suitable separation of these compounds. In these conditions, a resolution of 3.75 was obtained, which is above the minimum 1.5 recommended by Skoog, Holler, and Nieman (2001).

Saponification with KOH/methanol (40:60 v/v) at 40 °C, in similar conditions as those recommended by Müller (1997) was also assayed for samples with high chlorophyll content, as green pepper, but higher β-carotene concentrations were obtained for non-saponified samples. Saponification seems unnecessary, since HPLC provides enough separation of chlorophyll and carotenoid peaks, and some degradation and isomerization of carotenoids such as lycopene may take place due to the saponification process (Fang, Pajkovic, Wan, Gu, & van Breemen, 2003; Müller, 1997).

3.3. HPLC method evaluation

The HPLC method thus developed was evaluated in tomato samples, as a representative lycopene-rich food, by the evaluation of linearity, precision, accuracy and limit of detection (LOD) for β-carotene and lycopene, comparing with the spectrophotometric standard

Table 1
Chromatographic parameters of lycopene and β-carotene using different mobile phases

Mobile phase	P'	R	α	N	Lycopene	β-carotene	
					T	k'	T
MEOH/ACN 90:10 (v/v)	5.17	3.46	1.31	2497.36	1.00	4.21	0.85
MEOH/ACN 90:10 (v/v) + TEA 9 μM	5.17	3.75	1.29	2004.47	1.00	4.03	1.00
MEOH/THF/water 67:27:6 (v/v)	5.11	2.37	1.22	1548.33	1.50	2.45	1.00
MEOH/THF/water 64:30:6 (v/v)	5.08	2.09	1.23	1182.76	1.30	2.63	1.33
MEOH/THF/water 67:25:8 (v/v)	5.23	2.22	1.18	1668.07	0.60	4.48	0.83
MEOH/THF/water 65:27:8 (v/v)	5.21	2.79	1.16	2506.22	0.62	6.67	1.00
MEOH/THF/water 60:33:7 (v/v)	5.09	2.13	1.24	1394.18	2.00	4.47	1.25

MEOH, methanol; P', polarity index; R, resolution; α, selectivity; N, number of plates; T, tailing factor; k', retention factor.

Table 2
Linearity parameters, precision and limit of detection of lycopene and β-carotene determinations by HPLC and spectrophotometry

	Lycopene		β-Carotene	
	HPLC (n = 10)	Spectrophotometry (n = 5)	HPLC (n = 6)	Spectrophotometry (n = 5)
Linear regression equation	Area = 1,034,311x + 5817	A = 165.78c + 0.0322	Area = 1,037,784x + 6434	A = 104.3413c + 0.06575
Correlation coefficient (r)	0.9988	0.9973	0.9989	0.9978
Slope RSD	1.7%	4.2%	2.3%	4.6%
Range of linearity (μM)	1.1–11.1	1.9–9.3	0.9–9.3	0.5–9.3
Response factors RSD	7.5%	3.2%	4.4%	8.3%
Repeatability as RSD of 9.3 μM standards (n = 6)	3.7%	0%	2.6%	0%
Intra-day reproducibility as RSD on tomato samples (n = 6)	7.8%	8.6%	10.5%	9.5%
LOD	0.6 μM	1.0 μM	0.3 μM	_[a]

A, absorbance; c, concentration (mM).
[a] Not evaluated, due to the poor recovering found for this method in mixtures with lycopene.

Table 3
Recovery percents for HPLC and spectrophotometry for lycopene and β-carotene analysis on tomato samples (n = 3)

Compound	Method	Initial (μg) (X ± SD)	Spiked (μg)	% Recovery (X ± SD)	% Mean recovery (X ± SD)
Lycopene	HPLC	177.5 ± 5.3	150	107.7 ± 3.3	108.7 ± 2.2
			300	109.7 ± 0.6	
	Spectrophotometry	127.2 ± 4.5	150	96.9 ± 26.9	98.7 ± 18.3
			300	100.4 ± 16.4	
β-Carotene	HPLC	34.1 ± 0.4	12.5	95.6 ± 12.0	100.8 ± 9.2
			25.0	98.1 ± 10.7	
	Spectrophotometry	20.6 ± 0.2	12.5	179.3 ± 1.9	175.5 ± 14.4
			25.0	169.9 ± 26.8	

X ± SD = mean ± standard deviation (n − 1).

method (Tables 2 and 3). After this, the method was applied to other fruit and vegetable samples, to evaluate the possible problems associated to different carotenoid profile of each product.

3.3.1. Linearity

In order to compare the linearity of both methods, triplicate calibration curves of lycopene and β-carotene were performed. For HPLC, lycopene and β-carotene standards were dissolved in the same conditions as the extracts and were injected in the chromatographic system, in a range between 0.9 and 11.1 μM. For spectrophotometry, the same study was performed, with calibration curves made of lycopene and β-carotene working standard solutions, in concentrations between 0.47 and 9.32 μM. Linear calibration curves were obtained for a concentration range including those of the samples analysed. The linearity of the method was confirmed by regression statistics.

As can be deduced from the calibration parameters shown in Table 2, correlation coefficients were always above 0.997, being slightly higher for HPLC method, for either lycopene or β-carotene. The RSD of the slopes were always below 4.6%, and those of the response factors were not higher than 8.3%.

3.3.2. Precision

The precision of the instrumental technique was evaluated analysing 6 standards of lycopene and β-carotene 9.3 μM each one (repeatability). For the whole procedure including sample extraction and instrumental analysis (intra-day reproducibility), six equal samples of tomato were analysed by both methods. Since carotenoid content of tomato samples cannot be maintained in its initial levels for several days, inter-day reproducibility could not be evaluated for the samples.

All the RSD values obtained in this study (Table 2) were below the limits of 11% as maximum for substances around 1 μg/ml recommended by AOAC (1993). Slight variations on HPLC repeatability (RSD <3.7%) are due to small volume injection or integration variations. Intra-day reproducibility of samples was always below 10.5%, being similar in both methods, and lower for lycopene than for β-carotene.

3.3.3. Recovery assays

Accuracy and matrix effects of both methods were evaluated by carrying out a recovery assay adding two known amounts of lycopene and β-carotene standards to tomato samples (Table 3). Triplicate analysis were

performed for each addition level, as well as for samples without addition, using both methods.

Mean recovery percents for HPLC method ranged between 100% and 109%. All these values are in the interval accepted by AOAC (1993) for substances around 10 μg/ml (80–110%). Recovery was better for β-carotene (100.8%), probably due to the lower stability of lycopene (Bicanic et al., 2003).

For lycopene analysis, spectrophotometry showed a mean recovery percent closer to 100% than HPLC, but this method showed much higher SD than chromatography, probably because several compounds are responsible of total absorbance. For β-carotene, the accuracy of spectrophotometric method is poor in lycopene-rich samples, because absorbance at 446 nm is due not only to β-carotene, but also to lycopene, whose maximum at 447 nm overlaps. Therefore, quantification of β-carotene by spectrophotometric method at 446 nm would give rise to an overestimation of carotenoid content in lycopene-rich foods, such as tomato or watermelon, as it can be observed from the excessive recovery percent obtained for β-carotene using spectrophotometric method in tomato samples.

3.3.4. Limits of detection

The limit of detection (LOD) was defined as the concentration resulting from a signal/noise ratio of 3. The LOD obtained for HPLC method were 0.6 μM for lycopene and 0.3 μM for β-carotene and for spectrophotometry was 1.05 μM for lycopene (Table 2). The LOD for β-carotene by spectrophotometry was not evaluated due to the poor recovery found. This HPLC method was sufficiently sensitive to analyze this kind of vegetables.

3.4. Application to samples

The HPLC method was applied to several carotenoid-rich fruits and vegetables and the results are presented in Table 4. The UV–vis spectra of all the

Table 4
Lycopene and β-carotene content on different fruits and vegetables

	X ± SD		
	Lycopene (mg/100 g)	β-carotene (mg/100 g)	Total (mg/100 g)
Tomato			
Rambo	4.5 ± 0.7	1.0 ± 0.1	5.5 ± 0.7
Raf	3.1 ± 1.1	1.0 ± 0.1	4.1 ± 1.0
Cherry 1	3.4 ± 0.2	0.7 ± 0.0	4.1 ± 0.3
Cherry 2	7.1 ± 1.9	1.2 ± 0.3	8.2 ± 1.7
Canario	2.8 ± 0.1	0.6 ± 0.0	3.4 ± 0.2
RAF	3.7 ± 1.5	0.6 ± 0.1	4.3 ± 1.6
Carrot			
Nantesa	ND	9.6 ± 0.3	9.6 ± 0.4
Mokum	ND	6.3 ± 0.2	6.3 ± 0.2
Pepper			
Clovis (red)	ND	1.0 ± 0.2	1.0 ± 0.2
Lamuyo (red)	ND	0.5 ± 0.0	0.5 ± 0.0
Fantastic (green)	ND	0.2 ± 0.0	0.2 ± 0.0
Watermelon			
Dulce Maravilla	7.3 ± 1.0	1.1 ± 0.1	8.4 ± 1.0
Seedless	6.5 ± 0.1	0.1 ± 0.0	6.6 ± 0.2
Persimmon			
Rojo Brillante 1	0.4 ± 0.0	0.1 ± 0.0	0.5 ± 0.0
Rojo Brillante 2	0.3 ± 0.0	0.1 ± 0.0	0.4 ± 0.0
Sharoni	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1
Medlar			
Argelino 1	ND	0.9 ± 0.0	0.9 ± 0.0
Argelino 2	ND	1.0 ± 0.0	1.0 ± 0.0

X ± SD = mean ± standard deviation ($n = 3$); ND, non-detected.

extracts of the samples were similar to that of β-carotene standard (as the total contribution of common carotenoids), or to that of lycopene standard in the case of tomato or watermelon, as lycopene-rich products. This fact was in agreement with the composition found by HPLC. Figs. 2 and 3 show the chromatograms for the six different vegetables analyzed.

Lycopene was only detected in tomato, watermelon and persimmon. Tomato and watermelon were found as high lycopene sources, with contents ranging between
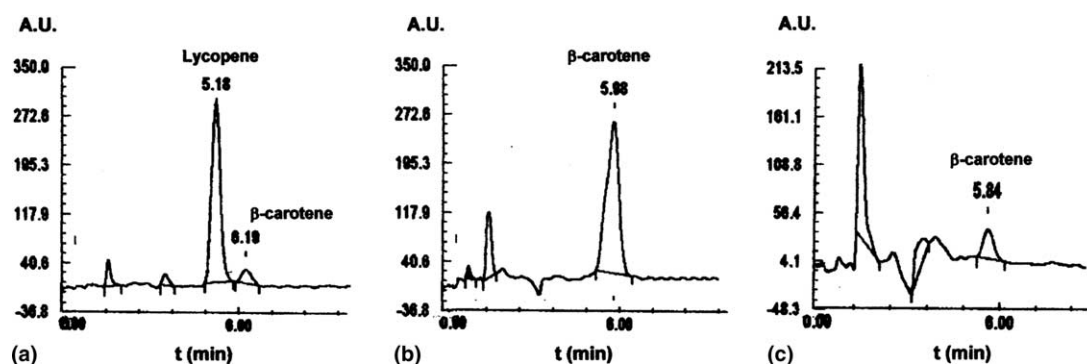

Fig. 2. Chromatograms of different vegetables: (a) tomato extract; (b) carrot extract; (c) pepper extract. Chromatographic conditions: μBondapack C18 column (300 mm × 2 mm), 10 μm of pore size, μBondapack C18 precolumn (20 mm × 3.9 mm), 10 μm of pore size; mobile phase methanol/ACN 90:10 (v/v) + TEA 9 μM; flow rate 0.9 ml/min; column temperature 30 °C; $\lambda_{detection}$ = 475 nm.

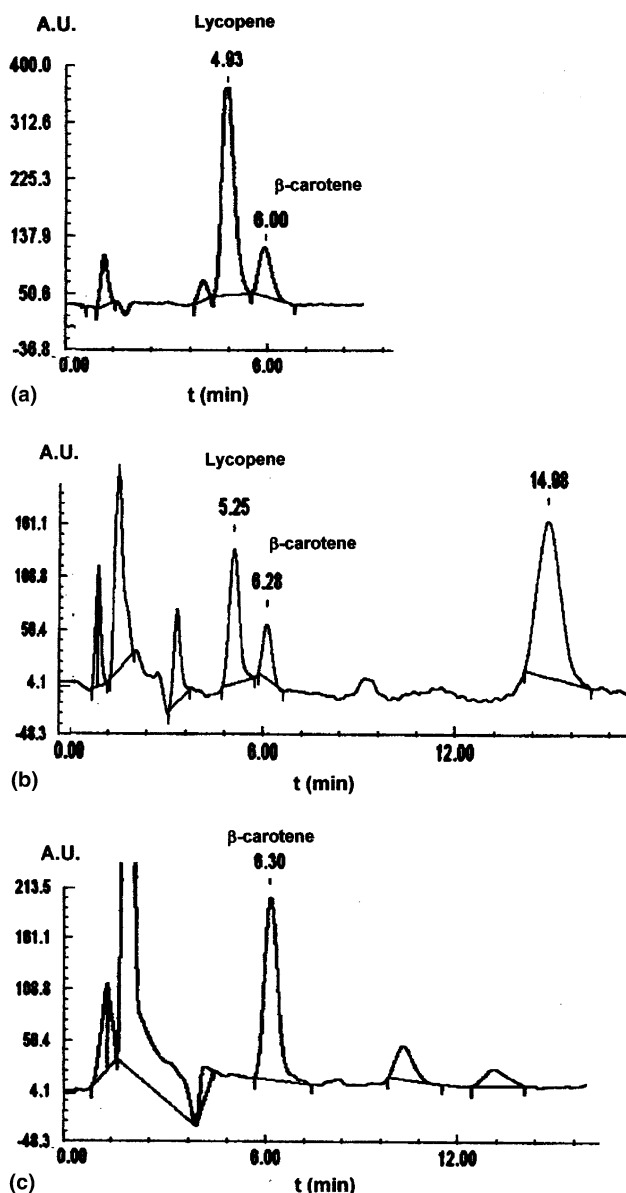Fig. 3. Chromatograms of different fruits: (a) watermelon extract; (b) persimmon extract; (c) medlar extract. Chromatographic conditions: µBondapack C18 column (300 mm × 2 mm), 10 µm of pore size, µBondapack C18 precolumn (20 mm × 3.9 mm), 10 µm of pore size; mobile phase methanol/ACN 90:10 (v/v) + TEA 9 µM; flow rate 0.9 ml/min; column temperature 30 °C; $\lambda_{detection} = 475$ nm.

a lycopene content more than 2-fold higher than β-carotene) and orange in "Sharoni" (with similar amounts of both compounds). Other compounds were detected in these extracts, whose identification was not possible due to the absence of commercial standards of other carotenoids different from lycopene, β-carotene or lutein. The major one could be some non-identified cryptoxanthin ester, as reported by Daood, Biacs, Czinkotai, and Hoschke (1992). These authors did not find lycopene in persimmon, while Wright and Kader (1997) reported this compound in levels similar to those found in the present study.

β-Carotene was found in all the analyzed samples, being the only carotenoid detected in carrots, medlar and peppers. It showed the highest levels in carrot (6.3–9.6 mg/100 g), in agreement with Hart and Scott (1995) and Granado et al. (1992); levels in medlar and pepper ranged between 0.2 and 1 mg/100 g. In the case of pepper, levels of carotenoids were higher for the two varieties of red peppers studied (0.5–1.0 mg/100 g) than for green ones (0.2 mg/100 g). Red colour of pepper is probably due to capsantin or other compounds different from lycopene, as the profile of the UV–vis spectrum of pepper was different from those of lycopene or other common carotenoids (β-carotene or lutein). Other authors also indicate the absence of lycopene in red pepper and the higher levels of carotenoids in red peppers compared to green fruits (Granado et al., 1992; Hart & Scott, 1995).

Lycopene contents evaluated by HPLC or spectrophotometry at 501 nm were coincident in all the analyzed samples, but not for total carotenoids at 446 nm in tomato and watermelon, whose real content of other carotenoids different from lycopene (β-carotene) was lower than the content found by spectrophotometry.

We can conclude that both spectrophotometric and HPLC methods have shown acceptable linearity, precision, accuracy and LODs for lycopene analysis on different fruits and vegetables. However, for total carotenoids expressed as β-carotene, spectrophotometric data are not suitable for products containing lycopene, because of higher absorbances obtained at 446 nm as a consequence of the absorption of lycopene, providing poor accuracy, with an overestimation of β-carotene content in the samples.

The election of the analytical method depends on the particular interest of the analysis and the kind of product analyzed. For routine analysis of products with lycopene as the major compound, spectrophotometry can be a simple and quick alternative; however, HPLC should be applied when the interest of the analysis is focus on the carotenoid composition of the product. The proposed method, extracting the samples with hexane/acetone/ethanol (50:25:25 v/v/v), followed by evaporation of hexane layer, dissolution in THF/ACN/methanol (15:30:55 v/v/v) and injection on a C18 column with

2.8 and 7.3 mg/100 g, which represented 82–87% of the total carotenoid content in tomato fruit and 87–99% in watermelon. Contents of lycopene and β-carotene in tomato are coincident with those of Hart and Scott (1995), Müller (1997) and Granado, Olmedilla, Blanco, and Rojas-Hidalgo (1992) who reported a wide range of lycopene content in tomato fruits (2–62 mg/100 g), depending on the varieties.

Persimmon varieties ranged between 0.2 and 0.4 mg/100 g of lycopene, which was in agreement with the colour of the fruit, red in the "Rojo Brillante" variety (with

methanol/ACN (90:10 v/v) + 9 μM TEA as the mobile phase, flow rate of 0.9 ml/min and $\lambda_{detection} = 475$ nm, provides reliable results, with a one-step direct extraction, a quite simple preparation of the samples, and short run times (10 min) for the quantification of lycopene, β-carotene or other compounds that could be present in the product.

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