See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/12749295

## Characterization of Paprika (Capsicum annuum) Extract in Orange Juices by Liquid Chromatography of Carotenoid Profiles

ARTICLE in JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · APRIL 1999					
Impact Factor: 2.91 · DOI: 10.1021/jf980835h · Source: PubMed					
CITATIONS	READS				
6	67				

### 3 AUTHORS, INCLUDING:



Emile M Gaydou Aix-Marseille Université

352 PUBLICATIONS 3,914 CITATIONS

SEE PROFILE

# Characterization of Paprika (*Capsicum annuum*) Extract in Orange Juices by Liquid Chromatography of Carotenoid Profiles

Pierre P. Mouly,\*,† Emile M. Gaydou,‡ and Josiane Corsetti†,‡

Centre de recherche et de valorisation des produits de la consommation, Parc Club des Aygalades, Bat. A7, ZIN 1207, 35 Boulevard du Capitaine Gèze, 13333 Marseille Cedex 14, France, and Laboratoire de Phytochimie de Marseille, Faculté des Sciences et Techniques de Saint Jérôme, Avenue Escadrille Normandie Niemen, 13397 Marseille Cedex 13, France

The carotenoid pigment profiles of authentic pure orange juices from Spain and Florida and an industrial paprika (*Capsicum annuum*) extract used for food coloring were obtained using reversed-phase liquid chromatography with a C18 packed column and an acetone/methanol/water eluent system. The procedure involving the carotenoid extraction is described. Both retention times and spectral properties using photodiode array detection for characterization of the major carotenoids at 430 and 519 nm are given. The influence of external addition of tangerine juice and/or paprika extract on orange juice color is described using the U.S. Department of Agriculture scale and adulterated orange juice. The procedure for quantitation of externally added paprika extract to orange juice is investigated, and the limit of quantitation, coefficient of variation, and recoveries are determined.

**Keywords:** Fruit juices; tangerine juice; orange juice; Citrus sinensis; paprika extract; carotenoid; adulteration; liquid chromatography

#### INTRODUCTION

The color of beverages, and particularly orange juice beverages, directly influences the consumer point of view on the flavor, consistency, and quality of these products. Addition in edible products of a coloring matter is possible to restore natural color, which may have been destroyed during industrial transformation (cooking, drying), or to enhance the aspects of the products and to mask eventual deficiencies, or to ensure a standard production (Berset, 1990). In the case of the carotenoid family, many natural coloring matters could be used to modify or increase the color of beverages from yellow to red-orange by using a single carotenoid such as  $\beta$ -carotene (orange),  $\beta$ -apocarotenal (red-orange), or lycopene (red) or by using natural complex carotenoid extracts such as extract of marigold flower (Tagetes erecta) (yellow), roucou or annatto (Bixa orellana) (yellow), paprika (Capsicum annuum) (red-orange), or citrus peel extract (Citrus sinensis) (red-orange) (Philip et al., 1989). Many of these coloring raw materials can be employed for color change of orange juices, although this practice is not allowed by European legislation. Nevertheless, adulterations have been reported in orange juices with annatto extract from the seeds of B. orellana or with  $\beta$ -carotene (Philip et al., 1989). Two physicochemical characteristics are used by industrial processors for selecting pure or frozen orange juice concentrates: (i) the ratio between 'Brix and acid content and (ii) the color intensity measured using the U.S. Depart-

ment of Agriculture's (USDA) scale method. The orange juice color values have an influence on the consumer's perception. Other reasons for the addition of coloring matter are either to increase the poor color of orange juice due to dilution with water (Perfetti et al., 1988; Nagy, 1997) or to enhance the pale yellow-orange color of some orange varieties such as Early-mid from Florida. Addition of tangerine or mandarin in orange juice (Toursel, 1996; Nagy, 1997) increases in the same case the color of orange juice; this practice is allowed up to a low percentage (10% in orange juice) in some countries such as the United States, but in Europe this practice is forbidden. Therefore, mixture of citrus species juices is widely investigated, and detection of this kind of adulteration can be easily made using a flavonoid profile (Ting et al., 1979; Dugo et al., 1994; Bronner and Galensa, 1994; Mouly et al., 1998). In the same way, detection of  $\beta$ -carotene can be easily made by photometry method (VDF, 1987; MAFF, 1991) because the range of this compound is the subject of various specifications such as RSK values (VDF, 1987) or French norms (AFNOR, 1995).  $\beta$ -Apocarotenal is easily detectable by liquid chromatography (LC) of carotenoids (Hofsommer, 1994). The addition of citrus peel extract in Valencia orange is detected by the presence of  $\beta$ -citraurin esters not present in orange juices (Philip et al., 1989). Marigold flower extract (T. erecta), which contains lutein esters (Gregory et al., 1986), can be used for enhancing the yellow color of orange juices (Philip et al., 1988). Annatto extract (B. orellana) is detected in food products by the presence of bixin and norbixin compounds (Tricard et al., 1998). Paprika extracts are widely used for coloring orange juice beverages in Europe and to give soft drinks an orange-red color similar to tangerine, citrus peel extract, or  $\beta$ -apocarotenal addition.

<sup>\*</sup> Author to whom correspondence should be addressed (telephone + 33 4 95 05 00 00; fax + 33 4 95 05 00 48; e-mail emile.gaydou@iut-chimie.u-3mrs.fr).

<sup>†</sup> Centre de recherche et de valorisation des produits de la consommation.

<sup>&</sup>lt;sup>‡</sup> Laboratoire de Phytochimie de Marseille.

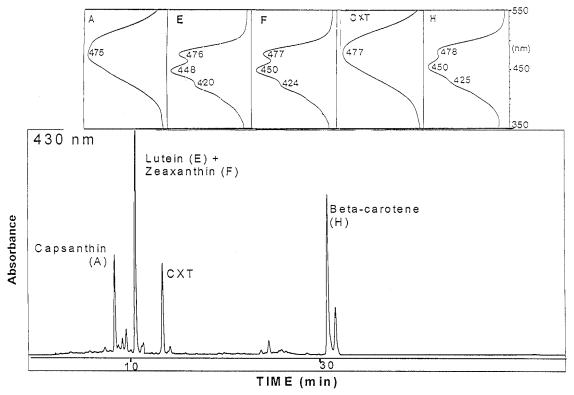


Figure 1. HPLC profile of standards and spectral characteristics, visible detection at 430 nm. CXT was used as internal standard. The column was a C18 ODS 2 (Waters),  $250 \times 4.6$  mm i.d.; see Table 1 for chromatographic conditions.

In this paper, we investigate the different carotenoid profiles obtained from orange juices, paprika extract, and orange juice adulterated with paprika extract. A quantitative study, using the determination of the paprika extract concentration in orange juice, was achieved, and a correlation has been made using the USDA scale color, also used in French specifications (AFNOR, 1995).

#### MATERIALS AND METHODS

**Samples.** Qualitative and quantitative studies were carried out on 15 orange juices, an industrial natural aqueous paprika extract commercially available, and a tangerine juice purchased at a local market. The carotenoid profile and the identification of main pigments were carried out on 15 authentic pure orange juices prepared during 1996-1997 harvesting. Seven samples from Florida (two blends of Earlymid/Valencia 60:40 and 70:30 v/v) were obtained from the Fruival Society (Valence, France), and seven from Spain (Valencia varieties) and one frozen concentrated orange juice reconstituted at 11.2 °Brix (var. Pera from Brazil) were obtained from the Bureau Couecou Society (Biarritz, France). The industrial paprika extract used for carotenoid profile determination and for mixed samples with orange juices was obtained from Lami Lutti (Bondues, France). This paprika extract, the most common used for food coloring, is soluble in water, and its carotenoid level content is defined by French legislation (7% minimum total carotenoids in paprika extract, JORF, 1997). Determination of the coefficient of variation (CV) and recovery on paprika extract quantitation in orange juice was carried out using four standard sample mixtures of orange juice (vide supra). The determination of the limit of quantification (LOQ) of paprika addition in orange juice was achieved using orange juice mixture from concentrate (blend of Earlymid/Valencia 70:30, v/v, Florida) with paprika extract at 0-0.16% level. These standard mixtures were done also for the paprika calibration curve.

Reagents. All reagents used were of HPLC grade from Carlo Erba or BdH. Internal standard, purchased from Ex-

Table 1. Gradient Profile Used in LC of Carotenoid Separations of Orange Juice and Paprika Extract

time <sup>a</sup> (min)	acetone <sup>b</sup> (% vol)	methanol <sup>b</sup> (% vol)	water <sup>b</sup> (% vol)
0	55	25	20
1	62	24	14
22	80	12.8	7.2
42	90	7.0	3.0
43	100	0	0
44	100	0	0
45	55	25	20

<sup>a</sup> Equilibrating time, 10 min, linear gradient. <sup>b</sup> Chromatographic conditions based on the work of Hofsommer (Parma, 1994). trasynthese (Genay, France), consisted of canthaxanthin

(CXT), which is a synthetic commercially available carotenoid. The stock solution of CXT can be kept for several weeks without any degradation, and its retention time does not interfere with that of other carotenoids contained in pure orange juice samples. The other standards used for retention time determinations and spectral identifications were purchased from Extrasynthese and were capsanthin, lutein, zeaxanthin, and  $\beta$ -carotene.

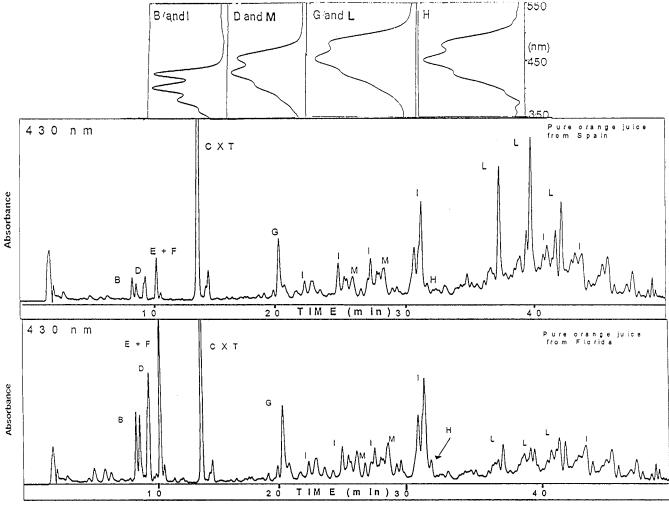
The color obtained on a mixture of orange juice/paprika extract was compared with color given by addition of various tangerine juice percentages in orange juice from Florida using the USDA scale.

Liquid Chromatography. Separations were performed on a stainless steel column ( $250 \times 4.6$  mm i.d.) packed with C18 Spherisorb ODS 2.5  $\mu m$  (Waters, Paris, France), equipped with a precolumn ( $20 \times 4.6$  mm i.d.) filled with the same stationary phase. The gradient profile and the mobile phase composition are given in Table 1 and are in agreement with the work of Hofsommer (1994). A Waters 600 controller pump was used for analyses. Samples were introduced onto the column via an automatic injector (Waters 717) equipped with a sample loop (20  $\mu$ L). A Waters 996 diode array detector was set at 430 nm and at 519 nm; chromatographic data and UV-visible spectra were handled with a Millenium driver station. The column temperature was at 40 °C, the inlet pressure was 10 MPa, and the flow rate was fixed at 1.0 mL min<sup>-1</sup>.

Table 2. Systematic Name and Chromatographic Characteristics of Free Carotenoids Contained in Orange Juice and Paprika Extract

		occur	rence <sup>b</sup>		retention	
compd	common name <sup>a</sup>	orange	paprika	systematic name	time (min)	$R_f^c$
A	capsanthin	_	+	3, 3'-dihydroxy-β,κ-carotene-6'-one	8.6	19.3
В	auroxanthin	+	_	3,3'-dihydroxy-5,8,5',8'-tetrahydroxy-5,8,5',8'-diepoxy- $\beta$ -carotene	9.0	
C	unknown $^d$	_	+		9.7	
D	mutatoxanthin	_	+	3,3'-dihydroxy-5,8-dihydro-5,8-diepoxy-β-carotene	10.1	
$\mathbf{E}$	lutein	+	+	3,3'-dihydroxy-α-carotene	$11.0^{e}$	30.0
$\mathbf{F}$	zeaxanthin	+	+	3,3'-dihydroxy-β-carotene	$11.0^{e}$	29.5
CXT	canthaxanthin	_	_	4,4'-diketo- $\beta$ -carotene	14.0	29.0
G	$\beta$ -cryptoxanthin	+	+	3'-hydroxy-β-carotene	20.5	
H	$\beta$ -carotene	+	+	$\beta$ , $\beta$ -carotene	31.5	46.3

<sup>a</sup> See Figure 1 for structure identification. <sup>b</sup> Occurrence of the compound in either orange juice or paprika extract (+, presence; −, nd). <sup>c</sup> Response factor (ratio absorbance/concentration) at 430 nm, ×10³. <sup>d</sup> Tentatively identified as capsolutein according to Minguez-Mosquera. and Hornero-Mendez (1993, 1994) and Almela et al. (1991). <sup>e</sup> Coeluted peaks.



**Figure 2.** HPLC profile of pure orange juice from Spain (var. Valencia) and from Florida (Early-mid/Valencia, 60:40, v/v) and spectral characteristics of the principal compound pigments. See Tables 2 and 3 for compound identification, visible detection at 430 nm. CXT was used as internal standard. The column was a C18 ODS 2 (Waters),  $250 \times 4.6$  mm i.d.; see Table 1 for chromatographic conditions.

**Preparation of Standards.** All standards were diluted in methanol/acetone (2:1 v/v) to give final concentrations of 24 mg L<sup>-1</sup> for capsanthin, 40 mg L<sup>-1</sup> for lutein and zeaxanthin, and 25 mg L<sup>-1</sup> for  $\beta$ -carotene. Internal standard solution, CXT (219 mg L<sup>-1</sup>), was added (5  $\mu$ L) in 1 mL of the carotenoid standard solution before injection (Figure 1).

**Sample Preparations.** Fifty milliliter samples (orange and tangerine juices, diluted paprika extract, orange juice/tangerine juice mixtures, and orange juice/paprika extract mixtures)

were precipitated with 1 mL of an aqueous solution of  $ZnSO_4$ ·  $H_2O$  (300 g  $L^{-1}$ ) and 1 mL of  $K_4[Fe(CN)_6]\cdot 3H_2O$  (150 g  $L^{-1}$ ). After mixing, the solution was allowed to stand for 10 min before centrifugation, and the supernatant was decanted and discarded. The carotenoids contained in the precipitate were extracted 2-fold with acetone (40 and 20 mL, respectively). The precipitate/acetone mixture was stirred vigorously during 3 min with a glass rod and centrifuged during 5 min. All acetonic layers were placed into a separatory funnel containing 50 mL

Table 3. Main Free and Esterified Carotenoids Identified Using UV-Visible Spectra in Orange Juice and Paprika

			paprika	pure orange juices		this work (nm) (mobile phase)		literature (nm) (light petroleum)					
peak		common name <sup>a</sup>	extract	juice A <sup>b</sup>	juice $\mathbf{B}^c$	juice C <sup>d</sup>	max 1	max 2	max 3	max 1	max 2	max 3	ref
A	free	capsanthin	$\mathbf{P}^e$	$\mathrm{ND}^f$	ND	ND		475			474	504	В
В		auroxanthin	ND	P	P	P	381	403	427	382	402	427	В
C		unknown <sup>g</sup>	P	ND	ND	ND	420	442	472				
D		mutatoxanthin	ND	P	P	P	407	430	451		426	456	A, B
$\mathbf{E}$		$lutein^h$	P	P	P	P	420	448	476	420	447	477	В
F		zeaxanthin $^h$	P	P	P	P	424	450	477	423	451	483	В
G		$\beta$ -cryptoxanthin	P	P	P	P	428	453	477	421	451	483	A, B
Н		$\beta$ -carotene	P	P	P	P	425	450	478	421	451	478	A
I	ester	auroxanthin	ND	P	P	P	382	403	428				
J		capsorubin	P	ND	ND	ND	452	480					
K		capsanthin	P	ND	ND	ND		480	510				
L		$\beta$ -cryptoxanthin	P	P	P	P	420	443	472				
M		mutatoxanthin	ND	P	P	P	407	426	455				

<sup>a</sup> See Figure 1 for chemical structure. <sup>b</sup> Early-mid/Valencia 70:30, v/v, from Florida. <sup>c</sup> Valencia from Spain. <sup>d</sup> Frozen concentrated orange juice reconstituted at 11.2 °Brix (Pera from Brazil). <sup>e</sup> Detected in large amounts. <sup>f</sup> Not detected. <sup>g</sup> Tentatively identified as capsolutein (Minguez-Mosquera and Hornero-Mendez, 1993, 1994; Almela et al., 1991). h Coeluted peaks, spectral characteristics calculated on standards [A, Foppen (1971); B, Davies (1965)].

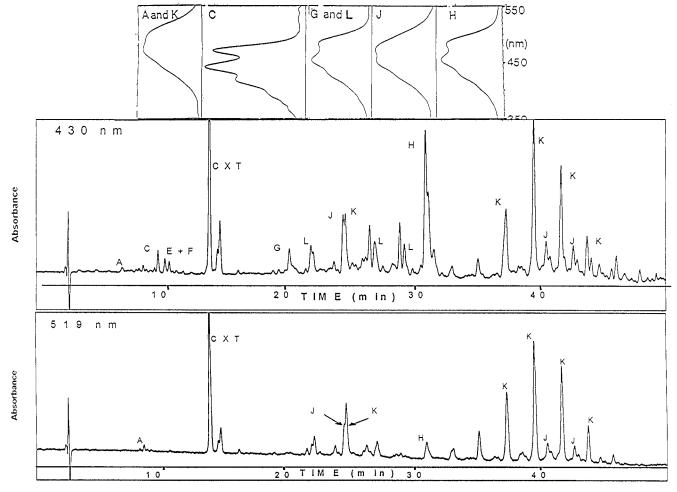
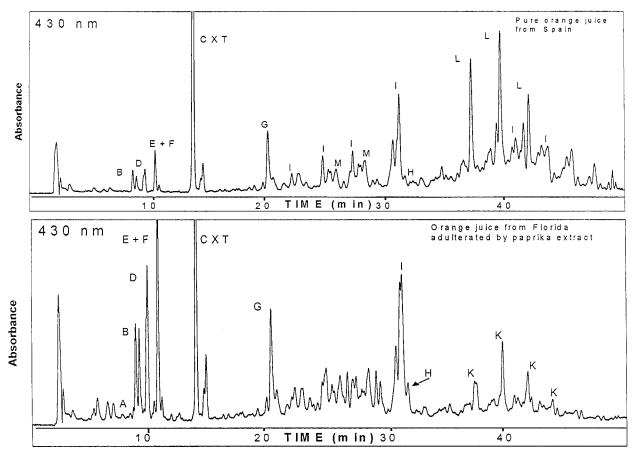


Figure 3. HPLC profile of industrial paprika extract and spectral characteristics of the principal compound pigments. See Tables 2 and 3 for compound identification, visible detection at 430 and 519 nm. CXT was used as internal standard. The column was a C18 ODS 2 (Waters),  $250 \times 4.6$  mm i.d.; see Table 1 for chromatographic conditions.

of light petroleum. The organic phase was washed with 50 mL of water. The carotenoid-petroleum phase was dried with 2 g of anhydrous sodium sulfate and centrifuged. To remove the remaining carotenoids in the desiccant, the sodium sulfate was mixed with  $\sim$ 30 mL of light petroleum. All petroleum extracts were concentrated to dryness in a rotary evaporator at 40 °C in vacuo. The carotenoids were dissolved in 500  $\mu$ L of acetone

and 1 mL of methanol. Internal standard (CXT at 219 mg L<sup>-1</sup>, 20  $\mu L$ ) was added and placed in sealed amber vials until analysis.

Calibration Curves I and II. Calibration curve I was built by successively increasing the amount of paprika extract diluted in methanol/acetone (2:1, v/v) for paprika determination in orange juice. Calibration curve II (orange juice with



**Figure 4.** HPLC profile of pure orange juice from Spain (var. Valencia) and pure orange juice from Florida (Early-mid/Valencia, 60:40, v/v) adulterated by 0.02% of industrial paprika extract. Visible detection at 430 nm. CXT was used as internal standard. See Tables 2 and 3 for compound identification. The column was a C18 ODS 2 (Waters),  $250 \times 4.6$  mm i.d.; see Table 1 for chromatographic conditions.

increasing amounts of paprika extract) was built using a method described below. The determination of orange juice adulteration with paprika extract was done using calibration curve II and was compared with results obtained using calibration curve I.

#### RESULTS AND DISCUSSION

Qualitative Analysis. The five main carotenoids encountered in orange juice, widely described in the literature, are  $\beta$ -carotene (Gross et al., 1971; Chen et al., 1994),  $\beta$ -cryptoxanthin (Fisher and Rouseff, 1986; Lin and Chen, 1995), lutein (Gross et al., 1972; Rouseff et al., 1996) and auroxanthin and mutatoxanthin (Philip et al., 1988, 1989; Rouseff et al., 1996). Figure 1 shows the separations of commercially available standards, capsanthin, lutein, zeaxanthin,  $\mathring{\beta}$ -carotene. Lutein and zeaxanthin were not resolved in these HPLC conditions. Canthaxanthin (CXT) was used as internal standard. Table 2 gives the systematic name and the spectral characteristics obtained with this eluent of the main carotenoids found in orange juices and in paprika extract (Minguez-Mosquera and Hornero-Mendez, 1994; Rouseff et al., 1996).

The paprika extract was obtained from ripe fruits of different varieties of pepper (Capsicum annuum). The red color is mainly due to the carotenoids capsanthin and capsorubin (Benedek, 1958; Zachariev et al., 1991). Paprika is also rich in other xanthophylls such as zeaxanthin (F) (Fisher and Kocis, 1987; Zachariev et al., 1991),  $\beta$ -cryptoxanthin (G) (Almela et al., 1991), lutein (E) (Minguez-Mosquera and Hornero-Mendez, 1993,

1994), and  $\beta$ -carotene (H) (Kanner et al., 1979; Ittah et al., 1993). The capsanthin and capsorubin fatty acid esters are the main components of paprika pigments; they represent 50% of the pigments. Capsanthin and capsorubin absorb at different wavelengths and, in particular, at 519 nm, which corresponds to the second shoulder of maximum absorption (Biacs et al., 1989).

Table 2 gives the response factor obtained at 430 nm and the retention time for the free carotenoids investigated. We can observe that the internal standard is eluted after the last xanthophyll and does not interfere with other carotenoid peaks. Table 3 shows the principal carotenoids and their spectral characteristics, compared with the literature, that have been encountered in this study in orange juice varieties and paprika extract. The coelution of lutein and zeaxanthin is due to the similar structures (Rouseff et al., 1996) and therefore similar spectral characteristics (Table 3). Spectral characteristics reported in Table 3 have been achieved starting from lutein and zeaxanthin standards. Identification of carotenoids in orange juice and in paprika extract was realized using the 3D maxima spectra obtained compared to the maxima obtained in literature work.

LC of unsaponified extract presents several advantages, among them a rapid method of preparation of samples in comparison with a saponified method. The chromatograms obtained give more information, particularly an ester of xanthophylls fingerprint in the different pure orange juices. The major disadvantage is an incomplete separation of every peak, in particular, peaks corresponding to carotenoid esters present in

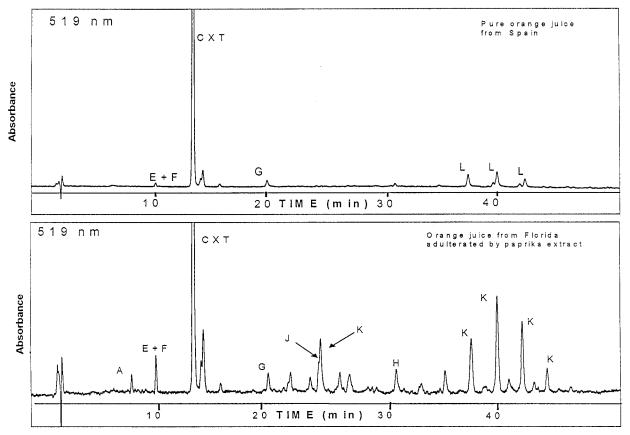


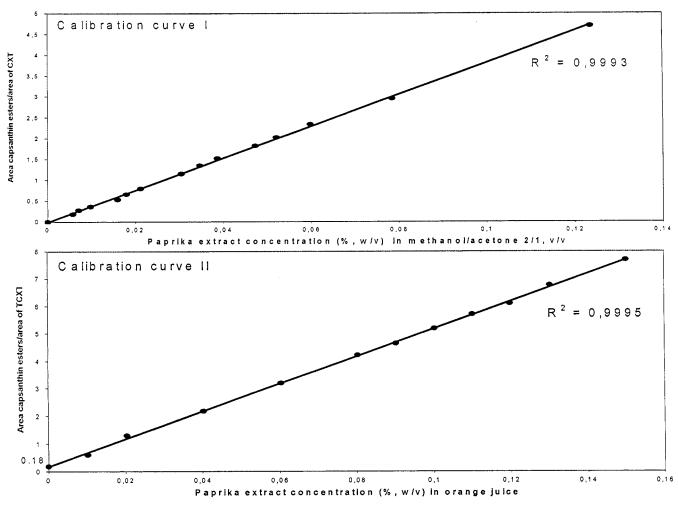
Figure 5. HPLC profile of pure orange juice from Spain (var. Valencia) and pure orange juice from Florida (Early-mid/Valencia, 60.40, v/v) adulterated with 0.02% of industrial paprika extract. Visible detection at 519 nm. CXT was used as internal standard. See Tables 2 and 3 for compound identification. The column was a C18 ODS 2 (Waters),  $250 \times 4.6$  mm i.d.; see Table 1 for chromatographic conditions.

small amounts. Therefore, we identified only the main carotenoid peaks or carotenoid peak not overlapped with another and present in the different fractions of orange juices and paprika extract.

Figure 2 shows authentic orange juice profiles obtained at 430 nm and spectra corresponding to the four main carotenoids, the hydrocarbon  $\hat{\beta}$ -carotene (H), the alcohols auroxanthin (B), mutatoxanthin (D),  $\beta$ -cryptoxanthin (G) and their corresponding fatty esters I, M, and L, and lutein (E) plus zeaxanthin (F) (Philip et al., 1988, 1989). The use of a chromatographic separation of unsaponified extract led to a different repartition of the corresponding esters, although the major non-ester carotenoid contents in these two samples are similar. In these chromatographic conditions the profiles obtained are similar among the seven samples on orange juice from Florida and among the seven samples of pure orange juice from Spain. Figure 2 shows a different profile between a pure orange juice from Florida (blend of Early-mid/Valencia, 60:40, v/v) and a pure orange juice from Spain (Valencia). As shown in this figure,  $\beta$ -cryptoxanthin esters represent the main components in orange juice from Spain. The poor content of  $\beta$ -cryptoxanthin esters in orange juice from Florida may be explained by the lower content of this carotenoid compound in Early-mid variety compared to Valencia varieties.

The paprika extract (Figure 3) is mainly composed of fatty esters of capsanthin (K), capsorubin (J), and  $\beta$ -cryptoxanthin (G). Away the free carotenoids we have characterized capsanthin (A) (Biacs et al., 1989, 1993)  $\beta$ -carotene (H),  $\beta$ -cryptoxanthin (G), lutein (E), and

zeaxanthin (F). These results are in agreement with the works of Minguez-Mosquera and Hornero-Mendez (1993, 1994). The paprika extracts are widely used for coloring citrus juice beverages such as  $\beta$ -carotene,  $\beta$ -apocarotenal, lutein, lycopene, or roucou for increasing the color of edible products. In another way these carotenoids may be employed for orange juice adulteration when poorly colored juices are produced. Figure 4 shows two carotenoid profiles, a pure orange juice and an orange juice adulterated with paprika (0.02%, w/v), at 430 nm. Detection of paprika addition in orange juice is done at 430 nm, due to the characterization of carotenoid pigments of paprika (mainly composed of capsanthin esters) in the area of fatty esters of orange juice carotenoid pigments. Therefore, adulteration of orange juice by paprika can be easily detected by the presence of capsanthin fatty esters mainly present in paprika (Figure 3) and not detectable in orange juice. Working at 519 nm (second shoulder maxima of this compound), the major pigments of orange juice do not absorb and the chromatograms revealed only the carotenoids of paprika extract, which makes it possible to detect easily this adulteration (Figure 5). We have quantified the paprika percentage added in orange juice with the four principal fatty esters of capsanthin contained in extract. In fact, we have not a knowledge of the carotenoid compositions in all paprika products used for food coloring. We can suppose that the relative percentages of the main carotenoids in paprika are not the same between first matters; nevertheless, we have chosen for the quantitative study the four major fatty esters of capsanthin (K) (Figure 3) for quantitation of paprika



**Figure 6.** Calibration curves for determination of paprika extract concentration t in methanol/acetone (calibration curve I) and in orange juice made from concentrate (Early-mid/Valencia, 70:30, v/v) (calibration curve II). Calculation was based on the ratio of the four principal esters of capsanthin (K) on internal standard CXT.

Table 4. Comparison of the USDA Color of Orange Juice Mixed with Paprika Extract and with Tangerine Juice

			orange juice	
USDA		,	v/v) of a added	X% (v/v) of tangerine juice added
scale	visual color	$\mathbf{A}^{a}$	$\mathbf{B}^{b}$	$\mathbf{B}^{b}$
OJ5	yellow		0	0
OJ4	dark yellow	0	0.02	5
OJ3	pale orange	0.03	0.04	10
OJ2	orange	0.05	0.07	
OJ1	red orange	0.07		

 $^a$  Pure orange juice from Spain (Valencia).  $^b$  Pure orange juice from Florida (Early-mid/Valencia, 70:30, v/v).

extract added in orange juice because their relative amounts are relatively stable with different products, 31.7–38.1% (area percentage; Fisher and Kocis, 1987).

**Quantitative Analysis.** Table 4 shows the USDA color used for color quality characterization of orange juice, obtained with authentic orange juices from Florida and Spain, compared to color obtained, on the one hand, with orange juices adulterated with increasing paprika extract concentrations and, on the other hand, with orange juice from Florida mixed with increasing content of tangerine juice (*C. reticulata*) is authorized in the United States (10%, v/v, maximum) but not in Europe. The presence of mandarin in orange juice is easy to detect, considering the flavonoid profile (Mouly et al.,

1998) or using the percentage determination of cryptoxanthin esters in total carotenoids, characterized by a high content in tangerine juice (Philip et al., 1989). Table 4 shows that USDA characteristics are similar when orange juice mixtures from Florida are prepared with tangerine juice at 5 and 10% (v/v) levels or with paprika extract at 0.02–0.07% (w/v) levels. The USDA color of pure orange juice from Spain is the same as the USDA color of orange juice from Florida adulterated with 0.02% (w/v) paprika extract or the same as the USDA color of orange juice from Florida adulterated with 5% (v/v) tangerine juice.

For quantitative analysis, we have used a calibration curve (Figure 6) of the four major esters of capsanthin contained in the paprika extract at different concentrations in methanol/acetone (2:1, v/v) (calibration curve I) and in orange juice (calibration curve II). The quantification method used is based on calibration using an internal standard (CXT). This method was successfully used by Baranyai et al. (1982) for quantitative evaluation of carotenoids in various native paprika and other products using CXT as internal standard. Figure 6 shows that the calibration curves, obtained in two solutions (solvents and orange juice), are linear with a good  $R^2$  ( $R^2$  is a calculated value from linear regression analysis effected to make calibration curves I and II) (0.9993 with calibration curve I and 0.9995 with calibration curve II) for a concentration of paprika added

**Table 5. Quantitative Evaluation Test of Paprika Added** in Orange Juices

	$R_f^c$	CV <sup>d</sup> (%)	Re (%)
orange juice Aa	1015	3.4	100.4
orange juice $\mathrm{B}^b$	1001	3.4	101
orange juice $C^b$	1007	1.0	100.5

 $^a$  Pure orange juice from Spain (var. Valencia).  $^b$  Pure orange juice from Florida (var. Early-mid/Valencia, 60:40, v/v).  $^c$  Ratio of the total area of the four principal esters of paprika peaks onto the paprika extract concentration (mg L $^{-1}$ ) at 519 nm.  $^d$  Coefficient of variation, mean of five repetitions.  $^e$  Recovery of paprika added in orange juice (mean of 10 determinations of paprika added at various concentrations.

ranging from 0 to 0.16%. The presence of  $\beta$ -cryptoxanthin esters in orange juices, which have a slice absorbance at 519 nm (Figure 5), is partially overlapped with capsanthin esters and is taken into account for the determination of the percentage of paprika in orange juice, which has a maximum absorption at 517 nm (Table 3), which explains that at 0% level of paprika, the ratio of capsanthin esters/CXT equals 0.18. Therefore, the limit of quantitation (LOQ) of the four main capsanthin esters is defined by this interference and was equal to 1.8 mg L<sup>-1</sup> (expressed in capsanthin) and the LOQ of paprika percentage addition in orange juice equal to  $3 \times 10^{-4}$ %. The LOQ is very much lower than the minimum addition of paprika in orange juice necessary to change the color from OJ5 to OJ4 (0.02%). Table 5 shows the CV and the percent recovery (R). We have obtained a good repeatability (CV = 2.6%) and a good recovery (R = 100.6%) at 519 nm for the determination of the paprika percentage added in orange juice.

Conclusion. The utilization of photodiode array detection is a valuable tool for characterization of carotenoids contained in industrial paprika extract and in various orange juices. Using the rapid procedure described, the major carotenoids have been characterized from spectral and retention time data obtained with authentic standards or literature values. Utilization of unsaponified extract gave more information on the nature of orange juice and in particular on variety appartenance. The increase of orange juice color is already known by tangerine juice addition, forbidden in Europe, which is easy to detect by flavonoid profiles. Paprika extract addition increases also orange juice color. The quantification of paprika extract added in orange juice has been achieved by using a judicious choice of wavelength having a poor interference with orange juice carotenoids.

#### ACKNOWLEDGMENT

We thank L. Lapierre, Couecou Society, Mme. M. Bouyer, Fruival Society, for authentic orange juice samples and Lami Lutti Society for a gift of the industrial paprika extract.

#### LITERATURE CITED

- AFNOR (Association Française de Normalisation). *Jus de Fruits et de Légumes, Spécifications et Méthodes d'Analyses*, 2nd ed.; AFNOR: Paris, France, 1996; pp 33–47.
- Almela, L.; Lopez-Roca, J. M.; Candela, M. E.; Alcazar, M. D. Carotenoid composition of new cultivars of red pepper for paprika. J. Agric. Food Chem. 1991, 39, 1606–1609.
- Baranyai, M.; Matus, Z.; Szabolcs, J. Determination by HPLC of Carotenoids in Paprika Products. *Acta Aliment.* **1982**, *11*, 309–323.

- Benedek, L. Untersuchungsverfahren zur bestimmung des farbstoffgehaltes in paprikamahlgut. **1958**, *107*, 228–232. Berset, C. Etats des Recherches sur les Colorants Alimentaires Naturels. *IAA* **1990**, Nov. 1163–1166.
- Biacs, P. A.; Daood, H. G.; Pavisa, A. Hajdu, F. Studies on the Carotenoids Pigments of Paprika (*Capsicum annum* L. var. Sz 20). *J. Agric. Food Chem.* **1989**, *37*, 350–353.
- Biacs, P. A.; Daood, H. G.; Huszka, T. T.; Biacs, P. K. Carotenoids and carotenoid esters from new cross-cultivars of paprika. *J. Agric. Food Chem.* 1993, 41, 1864–1867.
- Bronner, M.; Galensa, R. Detection of authenticity and adulterations of orange juices by HPLC-determination of flavonoids. In *Progress in the Authenticity-Assurance for Fruit Juices*; Report on Sgf-Symposium, Parma, Italy, 1994; pp 85–105.
- Chen, B. H.; Chen, T. M.; Chien, J. T. Kinetic model for studying the isomerization of  $\alpha$  and  $\beta$ -carotenes during heating and illumination. *J. Agric. Food Chem.* **1994**, *42*, 2391–2397.
- Davies, B. H. Analysis of carotenoids pigments. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: New York, 1965; pp 489–532.
- Dugo, P. On the Genuineness of Citrus Essential Oils. Part XLVI. Polymethoxylated Flavones of the Non-Volatile Residue of Italian Sweet Orange and Mandarin Essential Oils. *Flavour Fragrance* **1994**, *9*, 105–111.
- Fisher, C.; Kocis, J. A. Separation of Paprika Pigments by HPLC. *J. Agric. Food Chem.* **1987**, *35*, 55–57.
- Fisher, J. F.; Rouseff, R. L. Solid-Phase Extraction and HPLC Determination of  $\beta$ -Cryptoxanthin and  $\alpha$  and  $\beta$ -Carotenes in Orange Juice. *J. Agric. Food Chem.* **1986**, *34*, 985–989.
- Foppen, F. H. Tables for the Identification of Carotenoid pigments. *Chromatogr. Rev.* **1971**, *14*, 133–298.
- Gregory, G. K.; Chen, T.-S.; Philip, T. Quantitative Analysis of Lutein Esters in Marigold Flowers (*Tagetes erecta*) by High Performance Liquid Chromatography. *J. Food Sci.* **1986**, *51*, 1093–1094.
- Gross, J.; Gabai, M.; Lifshitz, A. Carotenoids in Juice of Shamouti Orange. *J. Food Sci.* **1971**, *36*, 466–472.
- Gross, J.; Gabai, M.; Lifshitz, A. A comparative Study of the Carotenoid Pigments in Juice of Shamouti, Valencia and Washington Oranges, Three Varieties of *Citrus sinensis*. *Phytochemistry* **1972**, *11*, 303–308.
- Hofsommer, H. J. Determination of anthocyans and carotinoids in fruit juices. In *Progress in the Authenticity-Assurance for Fruit Juices*; Report on SGF Symposium, Parma, Italy; 1994; pp 47–83.
- Ittah, Y.; Kanner, J.; Granit, R. Hydrolysis study of carotenoid pigments of paprika (*Capsicum annuum* L. Variety Lehava) by HPLC/photodiode array detection. *J. Agric. Food Chem.* **1993**, *41*, 899–901.
- JORF (Journal Officiel de la République Française). Additifs pouvant être employés dans la fabrication des denrées destinées à l'alimentation humaine. '1997, Oct 2, 1–387.
- Kanner, J.; Mendel, H.; Budowski, P. Carotene oxidizing factors in red pepper fruits. J. Food Sci. 1979, 43, 709– 712.
- Lin, S. D.; Chen, A. O. Major Carotenoids in Juices of Ponkan Mandarin and Lieucheng Orange. *J. Food Biochem.* **1995**, *18*, 273–283.
- MAFF (Ministery of Agriculture, Fisheries and Food). Orange Juice: Comparison of Methods for the Detection of Added Substances, MAFF Publications: London, U.K., 1991.
- Minguez-Mosquera, M. I.; Hornero-Mendez, D. Separation and Quantification of the Carotenoid Pigments in Red peppers (*Capsicum annum* L.) Paprika and Oleoresin by Reverse Phase. *J. Agric. Food Chem.* **1993**, *41*, 1616–1620.
- Minguez-Mosquera, M. I.; Hornero-Mendez, D. Comparative Study of the Effect of Paprika Processing on the carotenoid in Peppers (*Capsicum annum*) on the Bola and Agridulce Varieties. *J. Agric. Food Chem.* **1994**, *42*, 1550–1560.
- Mouly, P.; Gaydou, E. M.; Auffray, A. Simultaneous Separation of Flavanone Glycosides and Polymethoxylated Flavones in Citrus Juices Using Liquid Chromatography. *J. Chromatogr. A* **1998**, *800*, 171–179.

- Nagy, S. Economic Adulteration of Fruit Beverages. *Fruit Process.* **1997**, *4*, 125–131.
- Perfetti, G. A.; Joe, F. L., Jr.; Fazio, T.; Page, S. W. Liquid Chromatographic Methodology for the Characterization of Orange Juice. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 469– 473
- Philip, T.; Chen, T. S.; Nelson, D. B. Liquid Chromatography Profiles of Major Carotenoid Esters in Commercially Processed California Navelines and Valencia Orange Juice Concentrates. J. Chromatogr. 1988, 442, 249–265.
- Philip, T.; Chen, T. S.; Nelson, D. B. Detection of Adulteration of California Orange Juice Concentrates with Externally Added Carotenoid by Liquid Chromatography. *J. Agric. Food Chem.* **1989**, *37*, 91–95.
- Rouseff, R.; Railey, L.; Hofsommer, H. J. Application of Diode Array Detection with a C-30 Reverse Phase Column for the Separation and Identification of Saponified Orange Juice Carotenoids. J. Agric. Food Chem. 1996, 44, 2176–2181.
- Ting, S. V.; Rouseff, R. L.; Dougherty, M. H.; Attaway, J. A. Determination of Some Methoxylated Flavones in Citrus Juices by High Performance Liquid Chromatography. *J. Food Sci.* **1979**, *44*, 69–71.

- Toursel, P. Adultération des Jus de Fruits: un Sucre peut en Cacher un Autre. *Process* **1996**, *1115*, 54–55.
- Tricard, C.; Cazabeil, J. M.; Medina, B. Identification de colorants naturels ajoutés aux aliments. *Sci. Aliments* **1998**, *18*, 25–40.
- VDF (Verband der Deutschen Fruchtsaftindustrie). RSK-Values, the Complete Manual; Bielig, H.-J., Faethe, W., Fuchs, G., Koch, J., Wallrauch, S., Wucherpfenning, K., Eds.; Schönborn, Germany, 1987.
- Zachariev, Gy.; Kiss, I.; Szabolcs, J.; Toth, Gy.; Molnar, P.; Matus, Z. HPLC-analysis of carotenoids in irradiated and ethylene oxide treated red pepper. *Acta Aliment.* **1991**, *20*, 115–122.

Received for review July 30, 1998. Revised manuscript received November 27, 1998. Accepted December 22, 1998. This work was supported in part by the Regional Council of Provence-Alpes-Côte d'Azur (France) (registry no. 970171).

JF980835H