Differentiation of Several Geographical Origins in Single-Strength Valencia Orange Juices Using Quantitative Comparison of Carotenoid Profiles

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Pure Valencia orange (*Citrus sinensis*) juices (64 samples) from Spain, Israel, Belize, Cuba, and Florida, harvested during two seasons (1996−1997 and 1997−1998), were analyzed for their carotenoid profiles. The detection of saponified carotenoid pigments has been achieved and quantitated using a photodiode array detection monitored at 350, 430, and 486 nm. Carotenoid pigments commonly found in the orange variety Valencia have been separated on a polymeric C-30 column using a ternary gradient as eluent. Pure Valencia juices from oranges grown in Mediterranean regions (Israel and Spain) have a high carotenoid content, expressed in β-carotene (5−18 and 14−35 mg L^{-1}, respectively), compared to those grown in tropical and subtropical regions (Cuba, Belize, and Florida) (4−10, 2−8, and 5−10 mg L^{-1}, respectively). Quantitative results allowed the differentiation of Valencia variety geographical origins, in particular, the Mediterranean area from tropical and subtropical areas, using multidimensional analyses of carotenoid contents.

Keywords: *Citrus sinensis; Valencia orange juices; geographical origin; carotenoids; liquid chromatography; food analysis; adulteration*

INTRODUCTION

Orange juices are probably the most recognized and globally accepted fruit juices. In 1996, worldwide orange juice consumption had reached 13 billion liters, and all of the economical forecasts indicate a constant increase in consumer demand. The chemical composition of this juice has been widely investigated (VDF, 1987; AFNOR, 1996; AIJN, 1996), particularly for professional norms and for adulteration detections, which have become increasingly sophisticated. Among all of the types of adulteration, origin adulteration or origin mislabeling can be done for economical reasons, because the cost of raw materials varies with varieties and locations. Customized labeling has introduced into the market many product ranges mentioning origin and/or variety claims, contributing to substantial price differences for the proposed juices.

Within the orange juice "not from concentrate" (NFC) market, the major origins are Florida, Israel, Spain, Central America, and the Caribbean area (Belize, Cuba, and Costa Rica). Even if Brazil produces significant quantities of single-strength orange juices, this origin has not be taken within the field of this study, because the main varieties in Brazilian groves are pera and natal. Price quotations of single-strength NFC juice are highly connected with these origins. Among the sweet oranges, the so-called Valencia variety remains the highest priced and is acknowledged as the highest quality level. The price in Europe for a Valencia NFC juice from Florida has been quoted as ∼$900 per metric ton for the 1997/1998 season. Thus, this origin remains the most expensive on the market, followed by juices from the Caribbean area. Other important sources of Valencia NFC orange juices are Israel, with a medium price in Europe ∼2-fold below the Valencia orange juice from Florida, and Spain, with a price slightly lower than that for juice from Israel. Therefore, the economical interest in origin and varietal adulterations can be clearly understood, whereas certainty of these origins and famous names has become very attractive to the customer.

Detection of orange juice mixtures of various geographical origins involves quantitation of trace metals (McHard, 1979a,b; Bayer et al., 1980; Nikdel et al., 1988; Lapierre, 1996; Fournier et al., 1996a), combined with amino acid content (Naulet et al., 1997) and the use of near-infrared spectrometry (Harting and Hölsher, 1994), NMR isotopic ratio of δ D and δ ^{18}O (Fournier et al., 1996b; Lapierre, 1996), or NMR isotopic analyses of δ ^{13}C and δ ^{15}N in orange juice pulps (Kornexl et al., 1996). These techniques, whichhave been proved to be efficient in origin identification, require sophisticated technical equipment (NMR, IRMS, ICP-MS spectrometers), generally associated with pattern recognition techniques. Any of these studies take into account variety or the crop to crop variation effects except for the Martin et al. (1997) study, which has shown significant differences

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on trace and ultratrace elemental content in orange juices between some countries and varieties.

Another way to provide origin assessment has been the utilization of orange juice color variations and/or carotenoid profiles. The subject of this paper will be to focus on this last parameter, as an origin-recognizing tool. Carotenoids are the class of orange constituents present both in peel and in juice, but 60% of the total carotenoids of the fruit are present in the peel (Hulme, 1971). Citrus carotenoid profiles have been studied extensively with different techniques on different species or varieties (Fisher and Rouseff, 1986; Gregory et al., 1986; Perfetti et al., 1988; Philip et al., 1989; Rouseff et al., 1996) such as valencia (Gross et al., 1971, 1972; Philip et al., 1988). Among all of the carotenoids present in orange juice in a complex mixture, β-cryptoxanthin is the main contributor to the orange color of the juice (Nagy et al., 1993). Valencia orange juices are famous and wanted for their deep and bright coloration. Compared to other earlier blond varieties, they can always be distinguished by their highest orange color, explained by their higher content in carotenoids (Robards and Antolovich, 1995).

Biochemical and climatic factors are responsible for these color intensity variations, as carotenoid content is linked with maturity and external temperature during the growth and final ripening of oranges. According to Praloran (1971) and Kimball (1991), it is clearly noticeable that oranges from tropical areas are much lighter in color than those from northern origins. In Belize and Cuba, for example, the flavedo remains green or green-yellow, even when the fruits are fully mature, leaving a juice typically pale orange in colored. Saunt (1990) observed in the case of the Valencia variety that the flesh and juice products in tropical regions were paler than those of Valencia produced in subtropical Mediterranean climates. The same tendency can be illustrated by the color gap generally existing between Valencia oranges from Florida and those from California, which endurre drier and cooler nights than the former. Hofsommer (1994) observed by analyzing carotenoid profiles on various orange species and varieties from the Mediterranean and Brazil differences with a pattern recognition technique in the same varieties and supposed that the difference in carotenoid patterns is defined more by the origin than by the variety. In this paper we have investigated pure orange juices for their saponified carotenoid extracts. Identification and quantitation of carotenoids were performed using liquid chromatography with a ternary gradient method on a polymeric C-30 column. Photodiode array detection plotted at 350, 430, and 486 nm was able to determine the major carotenoid pigment contents in juices. Pattern recognition analyses allowed a characterization of Valencia orange juice origins.

MATERIALS AND METHODS

Juice Samples. Authentic orange (var. Valencia) juices (64 samples), prepared from fruits harvested during the 1996−1997 and 1997−1998 seasons from five countries, were investigated. These samples came from Spain (11 samples), Israel (13), Belize (16), Florida (15), and Cuba (9). All of the juices were obtained according to standard processes involving the following steps: washing; fruit selection; grading; in-line extraction, FMC processing (low oil cups, Food Machinery Corp., Chicago, IL); finishing; pasteurization; and conditioning (either frozen drums or aseptic bins). All of the juices met U.S. Department of Agriculture (USDA) grade A quality standards.

Table 1. Gradient Profile Used in Liquid Chromatographic Separation of Free Carotenoids of Valencia Orange Juice

time[a] (min)	mobile phase (% vol)		
	MTBE[b]	methanol	water
0	5	90	5
12	5	95	0
25	11	89	0
40	25	75	0
60	50	50	0
62	5	90	5

[a] Equilibrating time, 13 min, linear gradient. [b] Methyl *tert*-butyl ether.

Reagents. All reagents used were of HPLC grade: methanol from Carlo Erba (Val de Reuil, France); water and acetone from Riedel-de-Haën (Seelze, Germany); and methyl *tert*-butyl ether (MTBE) from Sigma Aldrich (Steinheim, Germany). The other reagents used for sample preparations were pure for analyses: light petroleum from Carlo Erba (boiling range = 40−70 °C); diethyl ether, sodium sulfate, and sodium chloride from SDS (Peypin, France). The standards used for retention time determinations and spectral identifications were purchased from Extrasynthese (Genay, France) and were zeaxanthin, β-cryptoxanthin (M), and β-carotene (Q).

Liquid Chromatography. Separations were performed on a stainless steel column (250 × 4.6 mm i.d.) packed with a C30 YMC column (Hampsted, NC), 5 μm not endcapped (Rouseff et al., 1996). The gradient profile and the mobile phase composition are given in Table 1. 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 μL). A Waters 996 diode array detector was set at 350, 430, and 486 nm. Chromatographic data and UV−visible spectra were handled with a Millenium driver station. The column was set at ambient temperature, the inlet pressure was 7 MPa, and the flow rate was fixed at 1.0 mL min^{-1}.

Preparation of Standards. All standards were diluted in methanol/acetone (2:1, v/v) to give final concentrations of 25 mg L^{-1} for zeaxanthin, 20 mg L^{-1} for β-cryptoxanthin, and 5 mg L^{-1} for β-carotene.

Sample Preparation. Sample preparation was in agreement with the work of Rouseff et al. (1996): 50 mL orange juice samples were precipitated with 1 mL of an aqueous solution of $ZnSO_4 \cdot H_2O$ (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 10 min before centrifugation, following which the supernatant was decanted and discarded. The carotenoids contained in the precipitate were extracted 2-fold with acetone (40 and 20 mL). The mixture precipitate and acetone were stirred vigorously during 3 min with a glass rod and centrifuged during 5 min. All acetonic layers were placed into a 200 mL separatory funnel containing 50 mL 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. After filtration, the remaining carotenoids contained in sodium sulfate were dissolved with ~30 mL of light petroleum. All unified petroleum extracts were concentrated to dryness in a rotavapor at 40 °C in vacuo. The residue was dissolved in 6 mL of diethyl ether and 6 mL of 10% methanolic KOH. After standing for 12 h protected from light at room temperature, the methanolic KOH layer was extracted in a first step with 20 mL and then with 30 mL of diethyl ether (sum = 50 mL). An aqueous NaCl solution (100 mL of a 10% w/v) was added to the separatory funnel. After shaking, the ether layer was removed and washed with a portion of 50 mL of distilled water until free of alkali. The ether layer was dried with sodium sulfate and evaporated to dryness under vacuum. The carotenoids were dissolved in 500 μL of acetone and 1 mL of methanol and placed in sealed amber vials until analysis.

Statistical Analysis. Principal component analysis (PCA) has been performed by using the data set transformed into

centered and reduced variables (standardized PCA). The data set was composed of 56 Valencia juices (chosen at random among the 64 samples) and height carotenoid variables. Factorial discriminant analysis (FDA) has been performed to classify the pure Valencia juices into five populations based on origin. Supplementary samples composed by pure Valencia juices (8 samples from the remaining juices not used for the data set) and mixture [7 samples, mixture of two (50:50, v/v) pure juices] were used to check the database stability. Juice database and supplementary samples were processed using the STATITCF program, version 4 (ITCF, France), and with the UNISTAT program, version 3.0 a (Megalon and Unistat, England).

RESULTS AND DISCUSSION

Carotenoid Profiles. The main carotenoids have been identified by comparison of their UV−visible spectra with those given in the literature. We have developed and applied a quantitative method for the separation of carotenoid pigments and their quantitation in pure Valencia orange juices grown in five countries. These carotenoids are given in Table 2 and their corresponding chemical structures in Figure 1. Quantitation of carotenoid compounds has been realized at 350 nm for phytofluene (N), at 486 nm for antheraxanthin (B), *cis*-antheraxanthin (C), lutein (J), and *β*-cryptoxanthin (M), and at 430 nm for the other carotenoids. We have chosen these wavelengths to quantify the main carotenoid compounds contained in orange juices because they have an absorbance maximum at one of these three wavelengths, except for B and C, which have been quantified at 486 nm for a better resolution at this wavelength than at 430 nm (Table 2).

Standard FMC type extraction has been employed in each Valencia orange; oil levels are analogous in the samples and strictly under 0.025% v/v. Pasteurization conditions have only a slight effect on carotenoid profiles (Nagy et al., 1993), and all of the samples were preserved by freezing until their analysis, without pasteurization. According to Fernandez (1995), the main factor significantly affecting the carotenoid content of a single-strength juice is the finishing: indeed, these liposoluble compounds have a tendency to settle on the cellulose fragments of juice vesicles, sieved by the finisher screen. Thus, large discrepancies in fine pulp may generate quantitative carotenoid variations. We

Table 2. Chromatographic and Spectral Characteristics of Investigated Carotenoids

compound[a]	identification peak	α[b]	λ_{max}[c] (nm)		
			λ_1	λ_2	λ_3
trollichrome	A	0.18	397.1	419.3	442.8
antheraxanthin	B	0.28	421.1(S)[d]	443.2	470.7
cis-antheraxanthin	C	0.30	417.3 (S)	440.7	467.7
neoxanthin	D	0.33	415.1	437.7	467
auroxanthin A	E	0.41	379.0	399.6	423.9
auroxanthin B	F	0.44	378.9	399.2	423.5
cis-violaxanthin	G	0.45	409.0	432.7	462.1
mutatoxanthin A	H	0.54	404.2 (S)	425.1	450.5
mutatoxanthin B	I	0.56	404.1 (S)	425.3	450.5
lutein	J	0.63	423.8 (S)	449.2	477.0
isolutein + zeaxanthin	K	0.66	420.8 (S)	445.1	468.1
α-cryptoxanthin	L	0.89	421 (S)	443.5	470.9
β-cryptoxanthin	M	1.00	425.1 (S)	448.9	475.3
phytofluene	N	1.06	330.0	346.4	364.5
α-carotene	O	1.21	422.1 (S)	443.9	471.9
ξ-carotene	P	1.24	378.1	398.8	423.4
β-carotene	Q	1.29		449.9	475.8

[a] For chemical structures, see Figure 1; for peak identification, see Rouseff et al. (1996), Foppen (1971), and Gross et al. (1971). [b] $\alpha = (rt - rt_0)/(rt_{(\beta\text{-cryptoxanthin})} - rt_0)$. [c] Eluent: MTBE/methanol/water. [d] Maximum absorbance as a shoulder.

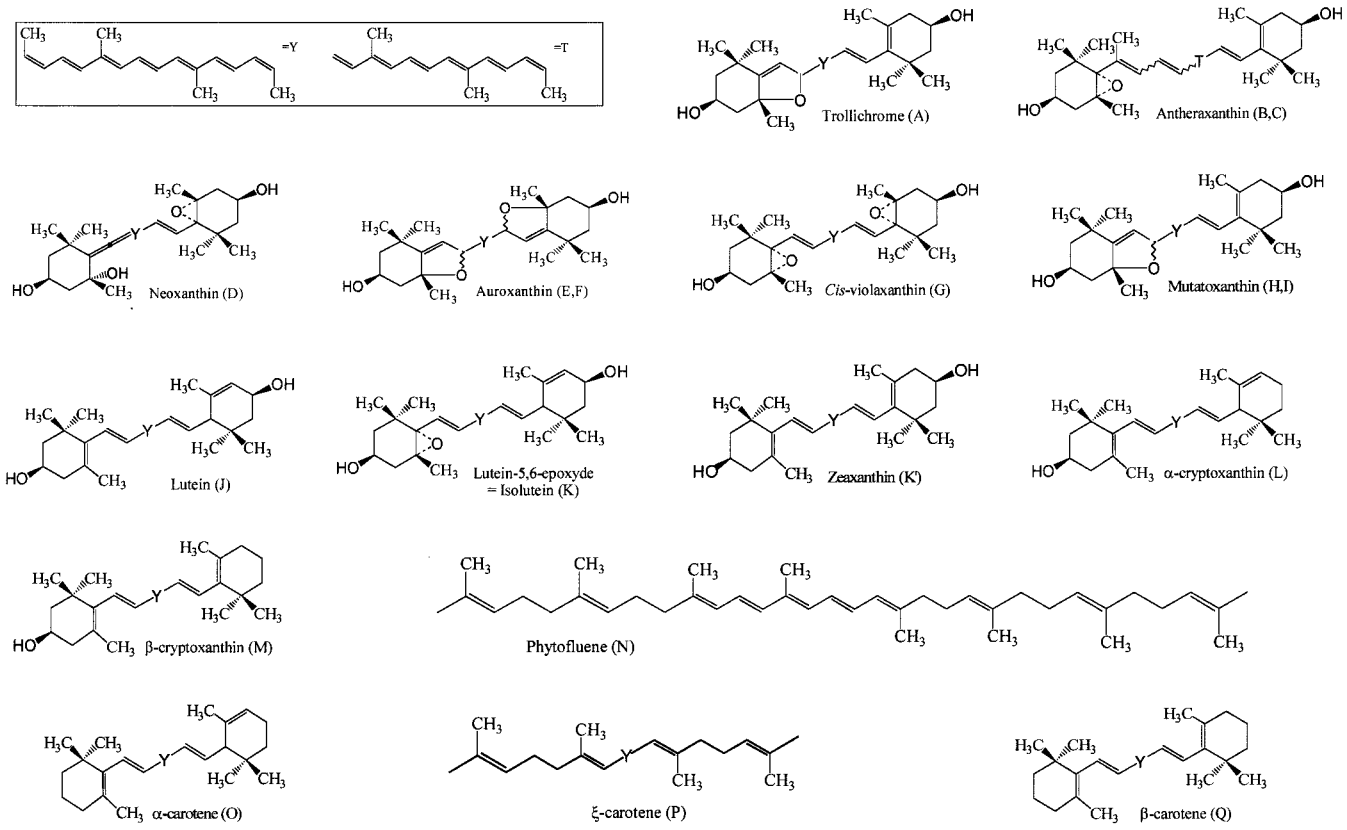

Figure 1. Carotenoid pigments commonly encountered in Valencia orange juice.

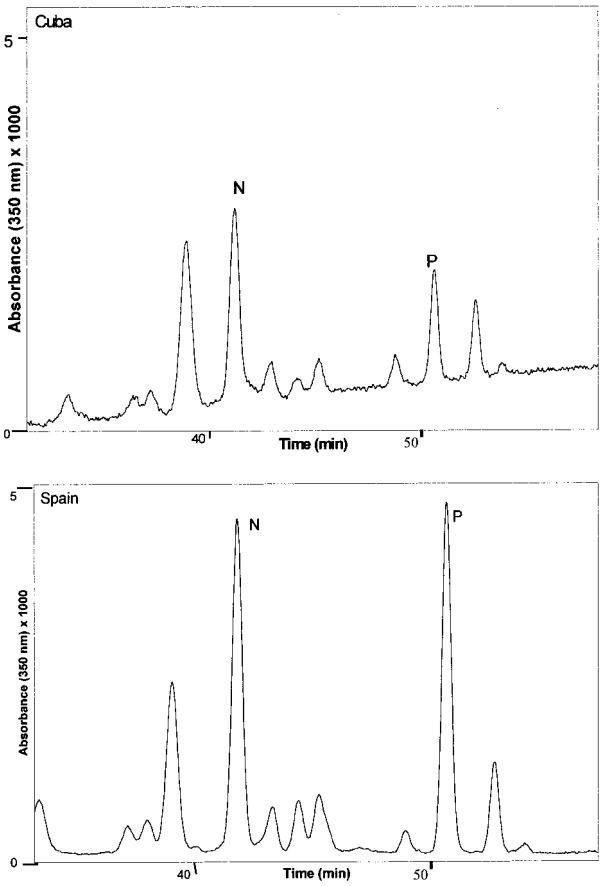

Figure 2. Carotenoid profiles of pure Valencia orange juice from Cuba and Spain plotted at 350 nm. For chromatographic conditions see Experimental Procedures and Table 1. Peaks: phytofluene (N); ξ-carotene (P).

have not taken into account here this hypothesis, because every industrial product tested had a spin-down pulp content ranging between 8 and 12% v/v (after centrifugation at 200*g* for 10 min).

Figures 2, 3, and 4 show the carotenoid profiles monitored at 350, 430, and 486 nm, respectively, on Valencia orange juices from Cuba, Belize, Florida, Israel, and Spain. These five countries can be grouped into two world climatic areas, the tropical and subtropical area (Belize, Cuba, and Florida) and the Mediterranean subtropical area (Israel and Spain). The main differences in carotenoid profiles between Mediterranean Valencia orange juices appeared with phytofluene (N), which was higher in the sample from Spain and Israel than in the sample from other areas (Figure 2). A low amount of lutein (J) and a high content of ξ-carotene (P) in the Mediterranean Valencia origin oranges compared to the other ones (Figure 3) are observed. Through these regions, we can observe many differences in the carotenoid profile function to the origin; pure Valencia orange juice from Spain can be differentiated from the others by a high content of *cis*-violaxanthin (G). Oranges originating in Israel were differentiated by low amounts of G, a low content of K (isolutein + zeaxanthin), and high contents of auroxanthin A (E), auroxanthin B (F), mutatoxanthin A (H), and mutatoxanthin B (I) (Figure 3). Furthermore, Belize and Cuba origins (tropical area) can be differentiated from the subtropical area (Florida, Israel, and Spain) by a high content of K and a low content of α-cryptoxanthin (Figure 3). Florida Valencia juice origin can be

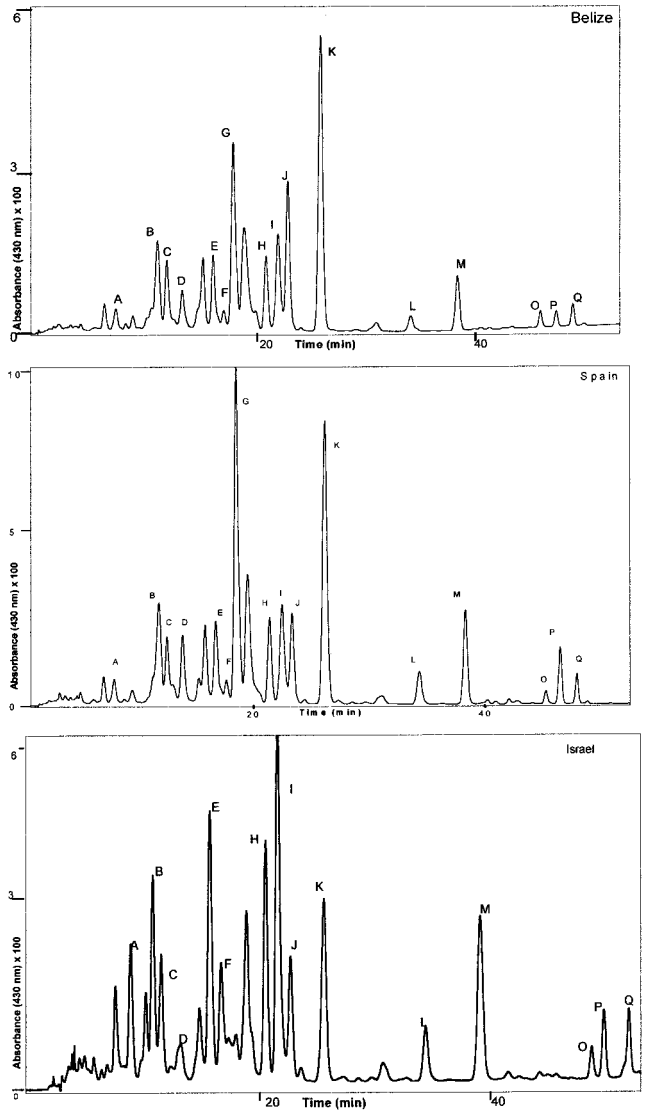

Figure 3. Carotenoid profiles of pure Valencia orange juice from Belize, Spain, and Israel plotted at 430 nm. For chromatographic conditions see Experimental Procedures and Table 1. For compound identification see Table 2.

differentiated from tropical Valencia area (Belize and Cuba) by a high content of β-cryptoxanthin (Figure 4).

Quantitative Determinations. Tables 3 and 4 show the quantitative composition of the main carotenoid contents in the various Valencia orange juice origins. Results are expressed in relative percentage of total peak area taken into account and also in milligrams per liter of β-carotene. Using relative percentages, we can observe lower contents of trollichrome (A) (0.8%), auroxanthin B (0.6%), and phytofluene (N) (0.4%) in Belize origin compared to the other areas (1.7, 2.0, and 0.9%, respectively, for Florida; 1.6, 1.6, and 0.8%, respectively, for Cuba; 2.3, 1.1, and 1.5%, respectively, for Spain; and 4.8, 3.6, and 1.6%, respectively, for Israel). A low content of β-carotene (Q) (1.6%) is observed in the Spanish Valencia juices compared to the other origins (from 2.1 to 2.8%). Using absolute value (expressed in milligrams per liter of β-carotene), a high content of total carotenoids is observed with Mediterranean origins (21.1 mg L^{-1} for Spain and 13.9 mg L^{-1} for Israel, compared to tropical and subtropical values of 4.8 mg L^{-1} for Belize, 8.2 mg L^{-1} for Florida, and 6.5 mg L^{-1} for Cuba). These results are in agreement with the work of Saunt (1990),

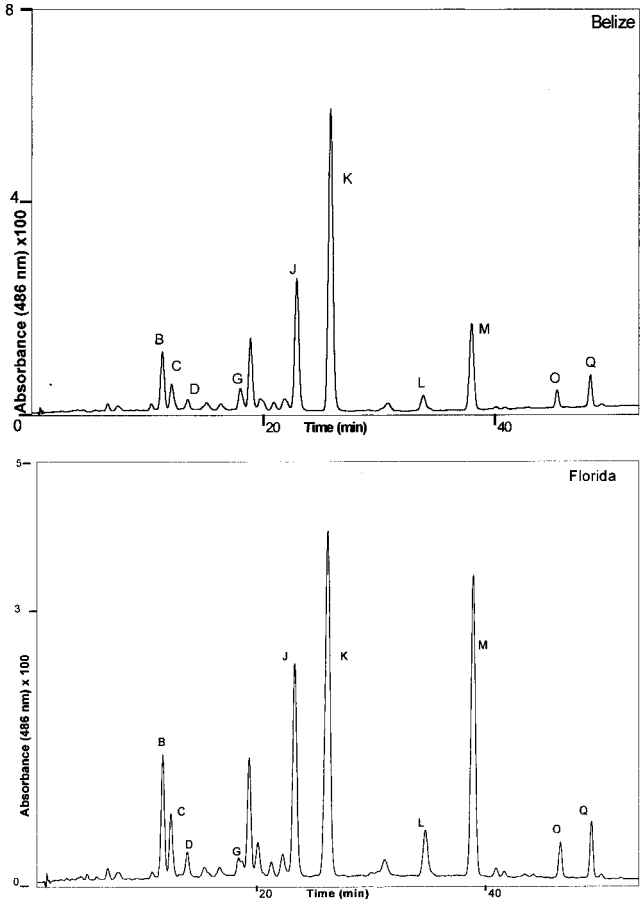

Figure 4. Carotenoid profiles of pure Valencia orange juice from Belize and Florida plotted at 486 nm. For chromatographic conditions see Experimental Procedures and Table 1. For compound identification see Table 2.

who had observed a color difference between Valencia juices produced in the Mediterranean area and those produced in the tropical area (Belize and Cuba). A high relative value in β-cryptoxanthin (M) is observed with Florida origin samples (15.5%), compared to the other origins (mean of 6.7% for Belize, 11.3% for Cuba, 10% for Spain, and 12.7% for Israel). The good juice color of this Valencia origin, usually observed, despite a low content of total carotenoids (mean of 8.2 mg L^{-1}), could be explained by the red absorbance of this compound.

Multivariate Analysis. *Principal Component Analysis (PCA).* Using PCA, we have reduced the number of variables by elimination of redundant variables as well as those having a poor effect shown by the correlation circle. The remaining variables (eight) were used for the final PCA and the FDA [trollichrome (A), mutatoxanthin B (I), lutein (J), isolutein + zeaxanthin (K), β-cryptoxanthin (M), phytofluene (N), β-carotene (Q), and the total carotenoid content, expressed in milligrams per liter]. Table 5 shows the correlation of these variables with principal components and discriminant functions. The correlation matrix in PCA shows a highly positive correlation between A and I ($r = 0.81$) and highly negative correlations between K and A ($r = -0.87$), between K and I ($r = -0.81$), between K and M ($r = -0.67$), and between K and N ($r = -0.75$). The three first principal components represent 83% of the cumulated variance divided in 52% for PC1 loaded with A, I, K, M, and N, 24% for PC2 loaded with J, K, and total carotenoids, and 7% for PC3 loaded with M (Table 5). The graphical representation on the two first PCs provides major visual differentiation and more particularly shows a differentiation of the Israel Valencia group on the positive axis of PC1 and the Belize Valencia group on the negative part of PC1. The beginning of differentiation between the Cuba and Florida groups is shown on this axis. Differentiation of Spanish origin is effective using PC2 (Figure 5). The differentiation of the Spanish origin may be explained by the high total carotenoid content (14−35 mg L^{-1}) and at the high relative percentage in N (mean of 1.5%) compared to those of Belize (0.4%), Cuba (0.8%), and Florida (0.9%) origins. The Israel group was well separated from the others by a high relative percentage in A (mean of 4.8% vs 2.3, 0.8, 1.7, and 1.6% for Spain, Belize, Florida, and Cuba, respectively) and in I (mean of 16.8% vs 8.4, 7.0, 9.8, and 8.8% for Spain, Belize, Florida, and Cuba,

Table 3. Carotenoid Contents in Mediterranean Valencia Orange Juices

	origin							
	Spain				Israel			
	area %[b]		mg L^{-1} [c]		area %[b]		mg L^{-1} [c]	
compound[a]	**mean**[d]	min−max	**mean**[d]	min−max	**mean**[e]	min−max	**mean**[e]	min−max
trollichrome	**2.3**	1.4−3.6	**0.48**	0.2−1.26	**4.8**	4.0−6.0	**0.67**	0.09−1.05
antheraxanthin	**2.6**	1.8−3.3	**0.53**	0.30−1.15	**3.0**	2.5−4.3	**0.41**	0.14−0.62
cis-antheraxanthin	**1.3**	0.7−2.2	**0.28**	0.11−0.61	**2.0**	1.8−2.5	**0.28**	0.05−0.35
neoxanthin	**4.6**	2.1−6.6	**0.95**	0.48−1.85	**1.0**	0.6−1.7	**0.15**	0.06−0.29
auroxanthin A	**6.2**	3.1−8.5	**1.31**	0.52−2.81	**10.8**	8.5−12.6	**1.48**	0.37−2.31
auroxanthin B	**1.1**	0.1−2.3	**0.27**	0.02−0.70	**3.6**	2.0−5.2	**0.50**	0.08−0.96
cis-violaxanthin	**22.4**	8.5−34.8	**4.56**	1.98−9.82	**1.6**	0.6−3.3	**0.26**	0.08−0.43
mutatoxanthin A	**6.7**	3.2−10.5	**1.41**	0.48−3.23	**13.4**	11.4−15.2	**1.85**	0.48−2.78
mutatoxanthin B	**8.4**	5.7−10.3	**1.77**	0.94−3.61	**16.8**	15.6−18.8	**2.30**	0.74−3.16
lutein	**4.0**	1.5−6.0	**0.87**	0.25−2.11	**4.8**	3.4−6.2	**0.68**	0.39−1.04
isolutein + zeaxanthin	**20.6**	16.3−23.5	**4.31**	2.72−6.51	**12.2**	10.6−14.4	**1.71**	1.26−2.08
α-cryptoxanthin	**2.9**	1.7−4.2	**0.65**	0.28−1.48	**4.2**	3.5−4.9	**0.58**	0.07−0.82
β-cryptoxanthin	**10.0**	6.5−14.8	**2.03**	1.27−2.69	**12.7**	10.6−14.5	**1.75**	0.58−2.29
phytofluene	**1.5**	0.7−2.6	**0.28**	1.19−4.37	**1.6**	0.8−2.4	**0.22**	0.07−0.30
α-carotene	**1.0**	0.6−1.6	**0.19**	0.1−0.63	**1.8**	1.0−2.8	**0.25**	0.06−0.51
ξ-carotene	**3.1**	1.7−4.7	**0.69**	0.24−1.64	**3.0**	1.6−3.8	**0.41**	0.10−0.56
β-carotene	**1.6**	0.8−2.2	**0.33**	0.17−0.58	**2.8**	1.7−3.3	**0.38**	0.10−0.51
sum of carotenoids			**21.1**	14.0−35.1			**13.9**	5.1−18.5

[a] For chemical structures, see Figure 1. [b] Expressed in percent of total peak area taken in account. [c] Expressed in mg L^{-1} of β-carotene. [d] Mean of 11 samples. [e] Mean of 13 samples.

Table 4. Carotenoid Contents in Tropical and Subtropical Valencia Orange Juices

	origin											
	Belize				Florida				Cuba			
	area %[b]		mg L^{-1} [c]		area %[b]		mg L^{-1} [c]		area %[b]		mg L^{-1} [c]	
compound[a]	**mean**[d]	min−max	**mean**[d]	min−max	**mean**[e]	min−max	**mean**[e]	min−max	**mean**[f]	min−max	**mean**[f]	min−max
trollichrome	**0.8**	0.1−1.7	**0.04**	tr[d]−0.13	**1.7**	0.6−3.1	**0.16**	0.03−0.25	**1.6**	0.9−3.4	**0.10**	0.04−0.15
antheraxanthin	**2.3**	1.9−3.3	**0.12**	0.05−0.19	**2.5**	1.4−3.6	**0.21**	0.16−0.27	**2.9**	2.5−3.5	**0.19**	0.10−0.30
cis-antheraxanthin	**0.9**	0.4−1.3	**0.05**	0.02−0.07	**1.3**	0.4−2.0	**0.12**	0.02−0.20	**1.0**	0.6−1.2	**0.07**	0.03−0.1
neoxanthin	**3.8**	1.2−5.5	**0.19**	0.02−0.37	**3.7**	1.0−6.6	**0.28**	0.08−0.50	**4.5**	2.9−5.7	**0.29**	0.19−0.57
auroxanthin A	**5.7**	3.9−8.8	**0.28**	0.14−0.49	**7.4**	5.7−8.8	**0.57**	0.29−0.87	**7.4**	6.5−8.7	**0.49**	0.26−0.65
auroxanthin B	**0.6**	0.01−1.6	**0.03**	tr−0.09	**2.0**	0.6−3.5	**0.19**	0.06−0.35	**1.6**	0.4−2.6	**0.11**	0.01−0.18
cis-violaxanthin	**18.6**	5.9−28.3	**0.92**	0.11−1.59	**11.1**	2.7−21.6	**0.67**	0.20−1.11	**16.5**	13.4−20.3	**1.06**	0.70−1.75
mutatoxanthin A	**5.6**	3.6−9.8	**0.28**	0.09−0.43	**8.4**	6.9−11.8	**0.67**	0.25−0.99	**6.5**	4.9−7.6	**0.43**	0.19−0.57
mutatoxanthin B	**7.0**	0.7−12.9	**0.36**	0.04−0.59	**9.8**	6.3−12.5	**0.84**	0.44−1.31	**8.8**	7.1−10.1	**0.58**	0.20−0.87
lutein	**7.0**	5.6−8.6	**0.36**	0.14−0.71	**6.1**	3.8−8.0	**0.51**	0.36−0.64	**5.8**	5.3−6.7	**0.39**	0.21−0.61
isolutein + zeaxanthin	**33.5**	30.4−36.8	**1.67**	0.63−3.19	**21.1**	17.0−25.4	**1.69**	0.95−2.25	**25.3**	21.6−28.6	**1.64**	1.00−2.60
α-cryptoxanthin	**1.6**	0.8−2.4	**0.08**	0.02−0.2	**2.6**	2.0−3.6	**0.23**	0.11−0.33	**1.5**	0.9−2.1	**0.10**	0.03−0.14
β-cryptoxanthin	**6.7**	5.1−8.8	**0.33**	0.13−0.64	**15.5**	10.6−21.3	**1.34**	0.63−1.94	**11.3**	8.3−14.3	**0.74**	0.40−1.00
phytofluene	**0.4**	0.3−0.7	**0.02**	0.01−0.03	**0.9**	0.4−1.6	**0.07**	0.04−0.12	**0.8**	0.4−0.9	**0.05**	0.04−0.06
α-carotene	**1.8**	1.3−2.6	**0.09**	0.03−0.18	**1.7**	1.0−2.8	**0.14**	0.07−0.21	**1.2**	1.0−1.4	**0.08**	0.05−0.12
ξ-carotene	**1.2**	1.0−1.6	**0.06**	0.02−0.13	**1.9**	1.1−2.5	**0.15**	0.07−0.21	**1.2**	0.8−1.6	**0.08**	0.03−0.13
β-carotene	**2.5**	1.6−4.6	**0.12**	0.04−0.23	**2.5**	1.6−4.1	**0.22**	0.11−0.33	**2.1**	1.6−2.7	**0.14**	0.07−0.24
sum of carotenoids			**4.79**	1.81−7.67			**8.21**	5.02−10.51			**6.52**	3.82−9.94

[a] For chemical structures, see Figure 1. [b] Expressed in percent of total peak area taken in account. [c] Expressed in mg L^{-1} of *β*-carotene. [d] Mean of 16 samples. [e] Mean of 15 samples. [f] Mean of 9 samples.

Table 5. Correlation between Variables and Principal Component and Discriminant Axes

	axes in PC[b]			axes in DF[c]		
carotenoid[a]	1	2	3	1	2	3
A	**−0.89**	−0.07	0.01	**−0.92**	−0.37	0.11
I	**−0.85**	−0.34	0.20	**−0.79**	**−0.61**	0.03
J	0.54	**−0.59**	−0.30	**0.86**	−0.49	0.01
K	**0.97**	0.03	0.18	**0.94**	0.26	0.21
M	**−0.58**	−0.45	**−0.58**	−0.44	−0.48	**−0.76**
N	**−0.83**	0.14	0.12	**−0.99**	0.06	0.04
Q	−0.21	**−0.79**	0.46	0.04	**−0.94**	0.09
sum of carotenoids	−0.59	**0.61**	−0.05	**−0.81**	0.57	−0.01

[a] See Table 2 for identification. [b] Principal component. [c] Discriminant functions.

respectively). The Belize group was separated from the Spain and Israel groups by a high relative percentage in J (5.6−8.6% vs 1.6−6.0% for Spain and 3.4−6.2% for Israel); the Belize group was also differentiated from all other countries by a high relative content in K (mean of 33.5% vs 21.1, 25.3, 20.6, and 12.2% for Florida, Cuba, Spain, and Israel, respectively). The Florida group was separated from the Israel, Spain, and Belize groups by a high relative percentage in M (mean of 15.5% vs 6.7, 10.0, and 12.7% for Belize, Spain, and Israel, respectively). Nevertheless, two origins (subtropical for Florida and tropical for Cuba) were not well differentiated, which may be explained by the geographical proximity of these two areas, which may have been affected by similar climatic conditions during these two seasons.

Factorial Discriminant Analysis (FDA). Using the different data sets, FDA was applied for the characterization of discriminant carotenoids. Each Valencia origin was classified as follows: 100% for Spain, Israel, and

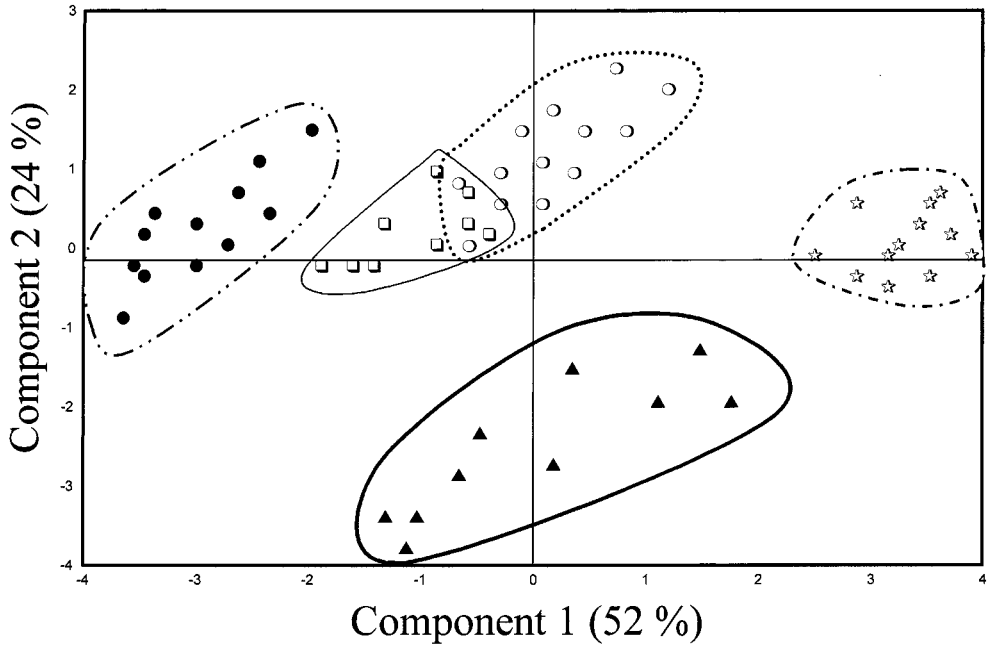

Figure 5. Pure Valencia orange juice origin differentiation using PCA of carotenoid contents: (●) Belize; (○) Florida; (□) Cuba; (☆) Israel; (▲) Spain.

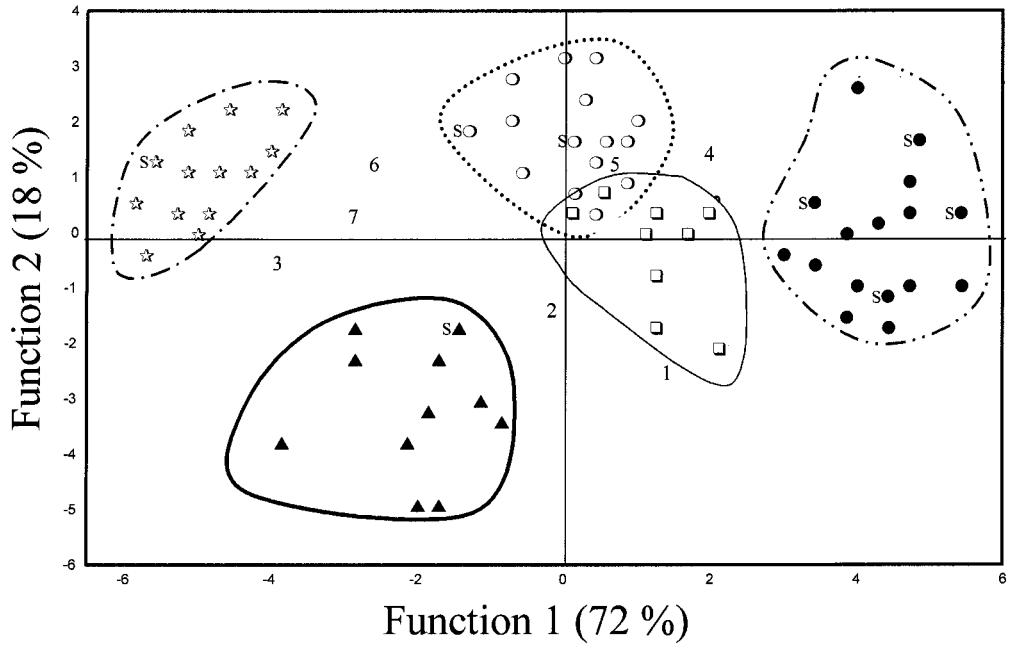

Figure 6. Pure Valencia orange juice origin differentiation using FDA of carotenoid contents: (●) Belize; (○) Florida; (□) Cuba; (☆) Israel; (▲) Spain; (S) supplementary samples of pure Valencia juices. Mixtures (50:50, v/v): 1, Spain/Belize; 2, Spain/Cuba; 3, Spain/Israel; 4, Florida/Belize; 5, Florida/Cuba; 6, Florida/Israel; 7, Cuba/Israel.

Table 6. Mahalanobis Distances between the Supplementary Origin Mixtures (50:50, v/v) and the Four Proximate Groups

mixture (50:50, v/v)	sample[a]	Mahalanobis distance from Spain	Belize	Florida	Israel	Cuba
Spain/Belize	1	2.14	**0.89**	1.75		**1.34**
Spain/Cuba	2	**0.41**	2.77	1.81		**0.88**
Spain/Israel	3	**1.38**		1.52	**0.67**	2.02
Florida/Belize	4		**1.72**	**0.78**	2.58	2.17
Florida/Cuba	5		2.04	**1.68**	2.14	**0.47**
Florida/Israel	6		2.87	**1.53**	**1.07**	2.44
Cuba/Israel	7		2.34	2.09	**0.86**	**1.58**

[a] See Figure 7 for sample identification.

Belize, 78% for Cuba (22% were classified in the Florida group), and 90% for Florida (10% were classified in the Cuba group). FDA leads to 96% correct classification. The graphical representation of samples is given in Figure 6. The discriminant power of axis 1, which represents 72% of the total variance and which is loaded with A, I, J, K, N, and total carotenoid content (Table 5), gives a fine separation of Mediterranean origins (Spain and Israel) on the negative part of FD1 and of Belize origin on the positive part of FD2. Axis 2, which is loaded with I and Q (Table 5), represents 18% of the total variance and gives both separations of Valencia juices from Spain and Israel (Figure 6).

Classification of supplementary samples constituted by eight pure Valencia juices (S) gives a correct classification for the pure Valencia juices. The Mahalanobis distances from the proximate groups for seven mixtures prepared using 50:50, v/v, of two pure juices are given in Table 6. The classification gives the smallest Mahalanobis distance of the respective groups composing the binary mixture for 2, 3, 4, 5, 6, and 7 (Figure 6).

Conclusion. Separation and quantitation of the main carotenoids contained in Valencia orange juices from five countries using liquid chromatography allows us to show, on the one hand, a major difference in the total carotenoid contents between the two world regions (Israel and Spain origins and Florida, Belize, and Cuba origins) and, on the other hand, a particular carotenoid profile of Valencia juices from Israel, which are easily differentiated from oranges from the other areas. Pattern recognition analyses (PCA and FDA) were able to differentiate pure Valencia juices from Belize, Spain, and Israel from juices from Cuba and Florida. Recognition of supplementary samples has been achieved with pure juices and some origin blends. Nevertheless, the Valencia juices from Cuba and from Florida were not well resolved. An increase of the Valencia juice database together with additional chemical analyses is needed to achieve the separation of Florida and Cuba Valencia juices.

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