JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Rapid Assessment of Vitamin A Activity through Objective Color Measurements for the Quality Control of Orange Juices with Diverse Carotenoid Profiles

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The evaluation of the vitamin A activity of foods is important for the establishment of Dietary Reference Intakes and food composition databases, food labeling, etc. Regarding orange juice, probably the most accepted fruit product in much of the world, the vitamin A labeling has been reported to be defective and misleading, which revealed the inadequacy of the quality control system. In this study, the color and the vitamin A activity (in terms of retinol activity equivalents) of diverse orange juices were evaluated as well as the correlations existing between them. Correlation coefficients above 0.9 were found for some color parameters considered jointly and individually, so appropriate equations to assess the vitamin A activity of the samples from them were obtained. The results of the analysis of variance ($p < 0.05$) revealed that there were no differences between the data derived from the chromatographic analyses and those calculated from the color parameters, thereby validating the assessment of the vitamin A activity of the juices through objective measurements of color, whose advantages (rapidity, versatility, nondestructiveness, portability, etc.) make of it a powerful tool for quality control purposes in the food industry.

KEYWORDS: Analysis of variance (ANOVA); carotenoids; CIELAB; food labeling; multiple regression; objective measurement of color; orange juice; provitamin A; quality control; retinol activity equivalents (RAE); vitamin A

INTRODUCTION

Dietary vitamin A can be obtained through provitamin A carotenoids from plant-derived foods, such as carrots, leafy vegetables, palm oil, tomato, etc. (*1*−*4*) and from preformed vitamin A (e.g., retinol, retinyl esters, retinoic acid, retinal, and 3-dehydroretinol) from foods of animal origin (liver, dairy products, etc.), fortified foodstuffs, or supplements (*5*−*7*).

Of the more than 700 carotenoids identified hitherto, only around 60, those containing at least one unsubstituted β-ring (**Figure 1**) with an 11-carbon polyene chain, exhibit vitamin A activity. The carotenoids fulfilling this structural requirement can be centrally cleaved inside the human body to give retinal, which can be subsequently transformed into retinol, even though there seems to now be evidence that retinoids showing vitamin A activity could also be formed from eccentric cleavage of provitamin A carotenoids (*8*, *9*). Anyhow, these processes are highly regulated such that episodes of vitamin A overdoses for massive ingestion of provitamin A carotenoids-rich products are virtually impossible (*10*, *11*).

β-Carotene (β,β-carotene) is regarded as the most important provitamin A carotenoid due to its widespread presence and to the fact that it possesses two unsubstituted β-ionone rings, so its vitamin A potency is in principle higher relative to those of α- and γ-carotene (β,ϵ-carotene and β,ψ-carotene, respectively), β-zeacarotene (7′,8′-dihydro-β,ψ-carotene), α- and β-cryptoxanthin (β,ϵ-caroten-3′-ol and β,β-caroten-3-ol, respectively), β-apo-8′-carotenal (8′-apo-β-caroten-8′-al), criptoflavin (5,8-epoxy-5,8-dihydro-β,β-caroten-3-ol) (chemical structures in **Figure 1**), and the remaining carotenoids exhibiting only one "intact" β-ionone ring. However, it is important to point out at this point that the absorption of carotenoids in general depends upon diverse factors (e.g., isomeric form, ingestion of dietary fat, vitamin E and other nutrients, the existence of certain pathologies, age, food matrix, etc.) (*12*); this is one of the main reasons why we are still far from achieving a totally accurate assessment of the vitamin A potential of any source (*13*). As far as the isomeric forms of carotenoids are concerned, it has to be taken into consideration that, besides the all-*E* geometrical isomers, different *Z*-isomers of provitamin A carotenoids, which reportedly display different vitamin A activity (*10*, *14*), can occur naturally and/or after industrial treatments or cooking practices, processes that can also lead to losses of such vitamin compounds (*10*, *15*, *16*).

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10.1021/jf0635412 CCC: $37.00
Published on Web 03/24/2007

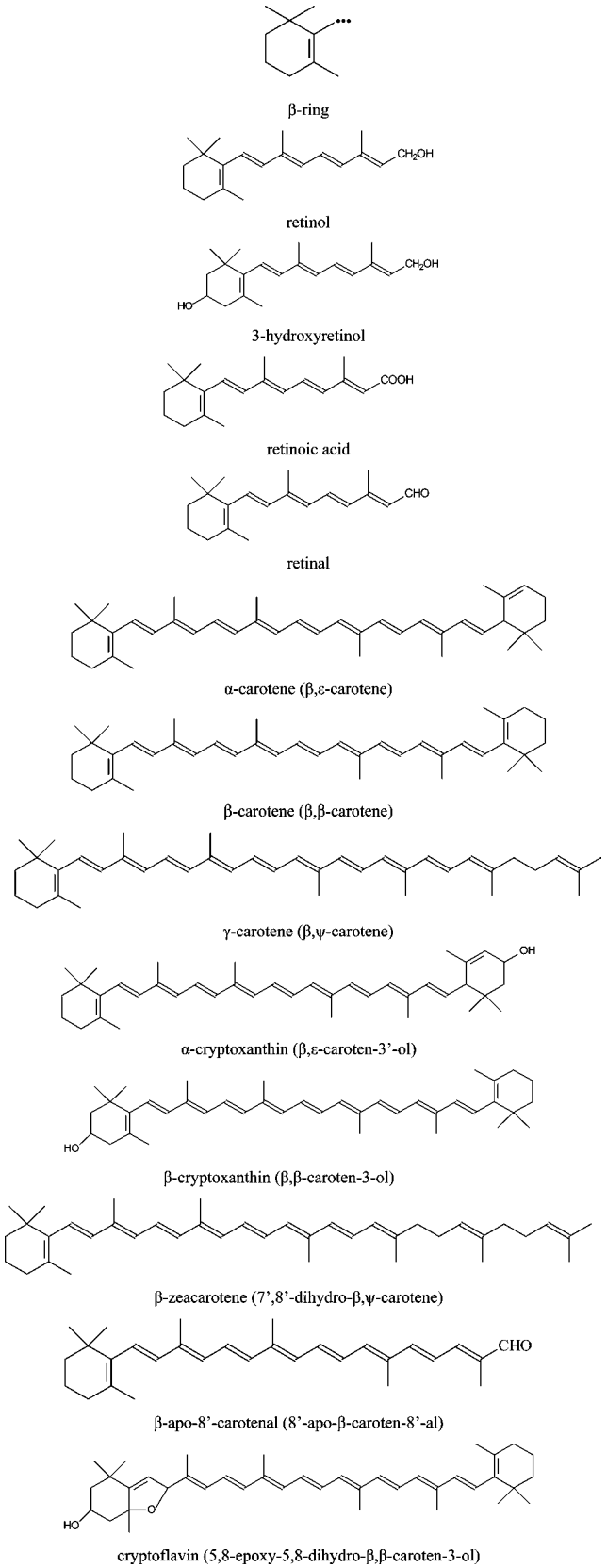

Figure 1. Chemical structures of a β-ring and different compounds exhibiting vitamin A activity.

The assessment of the vitamin A activity and the levels of nutritionally relevant constituents of foodstuffs are important for several reasons. In the case of developed countries, such determinations are being paid more attention because of the administrations and consumers' growing concern in relation to the wholesomeness of foods, which has led to a more demanding attitude toward food labeling (*17*, *18*). Contrastingly, the main concern about the assessment of provitamin A carotenoids in developing countries is aimed at eradicating vitamin A deficiency, which remains a health issue in these countries. To achieve such a goal, a number of strategies have been put into practice, such as the identification of affordable and appropriate dietary sources of vitamin A (*10*, *19*) and the genetic manipulation of crops to elevate their provitamin A carotenoid contents or to achieve that noncarotenogenic food staples, notably rice, accumulate these compounds (*8*). In any case, it is necessary that the analysts have at their disposal a certain variety of appropriate methods that enable them to tackle the analysis of provitamin A carotenoids under different circumstances, high-performance liquid chromatography (HPLC) being by far the method of choice (*20*, *21*). In relation to this need, this study was undertaken to provide insight into the assessment of the vitamin A activity of orange juices differing greatly in pigment content by means of objective color measurements, a methodology that, because of its remarkable advantages (ultrafast analysis, nondestructiveness, availability of portable instrumentation, etc.), is to be harnessed for quality control purposes.

MATERIALS AND METHODS

Orange Juice Samples. Thirty-eight orange juice samples [13 ultrafrozen orange juices (UFOJs), 11 orange juices from concentrate (OJFC), and 14 orange juices from squeezed oranges (OJFSO)] encompassing common brands retailed in Spain were surveyed. The UFOJ samples were obtained directly from the industry, while the remainder was purchased in Seville from different supermarket firms. UFOJ is a novel product that is marketed at a temperature equal to below −18 °C (*22*). OJFC were obtained by adding water to the concentrates and were subjected to different thermal treatments over their industrial processing, thereby displaying a long shelf life. As for the OJFSOs, which were not obtained from orange juice concentrates, they were retailed just as freshly squeezed juices or after they underwent thermal treatment; their shelf life was normally shorter relative to OJFC. Upon acquisition, all of the samples were stored as recommended by the manufacturer until analysis. UFOJs were thawed at room temperature.

Extraction and Saponification of Carotenoids. Carotenoids were extracted with 50 mL of the extracting solvent (methanol/acetone/hexane, 25:25:50, v/v/v, containing 0.1% BHT) and saponified by adding 25 mL of ethanolic KOH (10% w/v) as explained in detail anywhere else (*23*). The saponified extracts were eventually taken to 1 mL of a of mixture acetone:methanol (1:2, v/v, containing 0.1% BHT) and filtered through Millipore PVDF Millex filters (13 mm × 0.45 μm) (Bedford, MA) prior to their HPLC analysis.

HPLC. The HPLC procedure that was used is explained in detail anywhere else (*23*). Identification of the all-*E* isomers of α- and β-carotene and β-cryptoxanthin was carried out by comparing their chromatographic and spectroscopic characteristics with those of authentic standards isolated as explained in a previous work (*24*). To help identify the *Z*-isomer of β-cryptoxanthin occurring at low levels in some samples, an ethanolic solution of (all-*E*)-β-cryptoxanthin under an atmosphere of nitrogen was heated (80−100 °C) for 30 min in a water bath and then strongly illuminated overnight by means of a powerful incandescent lamp. As a consequence of such treatment, a mixture of geometrical isomers was obtained, whose chromatographic and spectroscopic charactersitics were compared to those of the *Z*-isomer occurring in some samples.

The levels of the provitamin A carotenoids were worked out from four-level dose−response calibration curves built with their corresponding standards, the (*Z*)-β-cryptoxanthin isomer detected in some samples being quantified by means of the same curve used for its all-*E* counterpart.

Assessment of Vitamin A Activity. The vitamin A activity of the samples was expressed in terms of retinol activity equivalents (RAE),

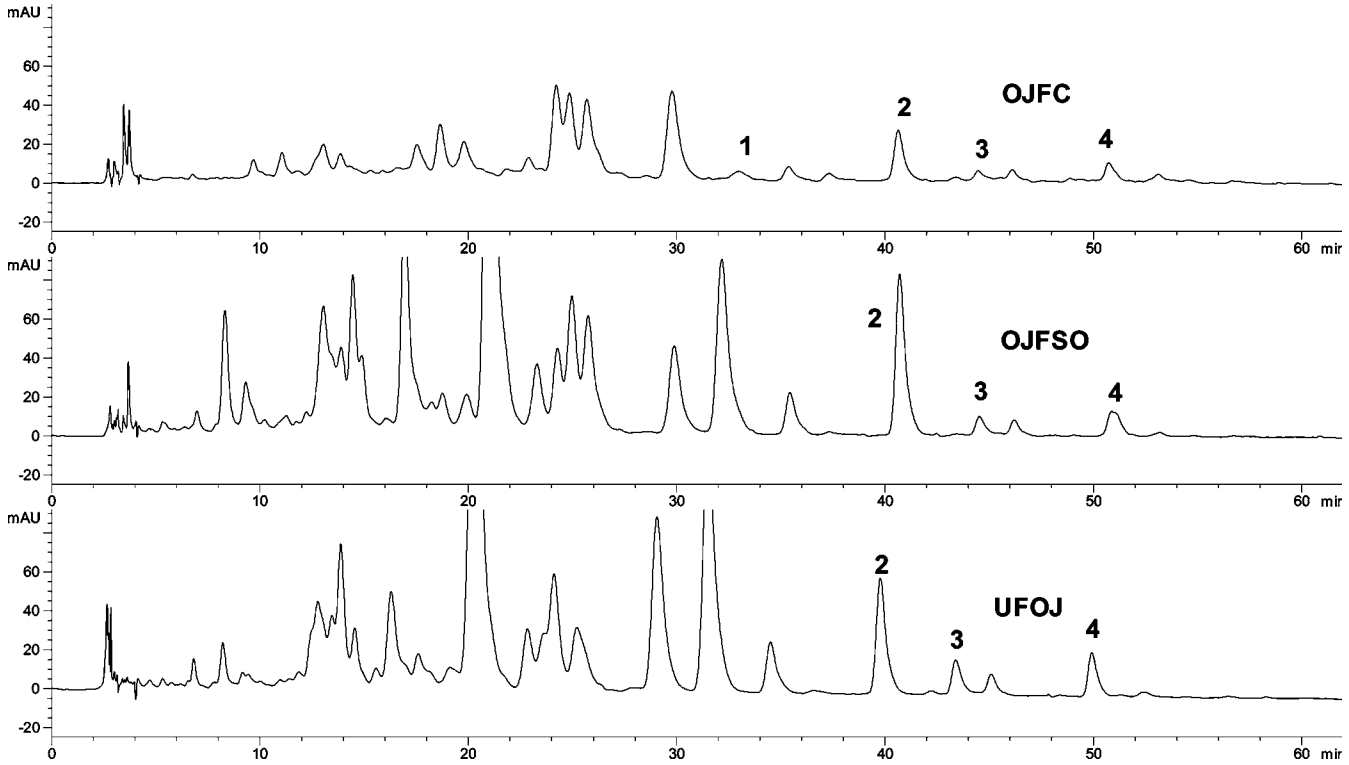

Figure 2. Chromatograms at 430 nm of OJFC, OJFSO, and UFOJ samples. Peak identification (spectroscopic and chromatographic data in **Table 1**): 1, (13*Z*)- or (13′*Z*)-β-cryptoxanthin; 2, β-cryptoxanthin; 3, α-carotene; and 4, β-carotene.

considering the equivalences 1 RAE = 12 μg of dietary all-*trans*-β-carotene = 24 μg of other dietary provitamin A carotenoids (*25*). Accordingly, the following formula was used and the results referred to 1 L of orange juice:

$$\text{RAE} = (\mu\text{g } \beta\text{-carotene}/12) + (\mu\text{g } \alpha\text{-carotene} + \mu\text{g } \beta\text{-cryptoxanthin}/24)$$

The assessment of the actual vitamin A potency of provitamin A carotenoids was very difficult, as was already stated in the Introduction section, especially as far as *Z*-isomers were concerned, so no distinction was made between the all-*E*- and the Z-isomer of β-cryptoxanthin for the calculation of RAE, the latter occurring in any case at low levels in some samples.

Objective Color Measurement. The color of the samples was objectively measured by spectroradiometry as explained in detail elsewhere (*26*). In brief, the visible reflectance spectra (380−770 nm, $\Delta\lambda = 1$ nm) were obtained through a CAS 140 B spectroradiometer (Instrument Systems, Munich, Germany) fitted with a Top 100 telescope optical probe (Instrument Systems), a Tamron zoom model SP 23A (Tamron USA, Inc., Commack, NY), and an external incandescent lamp as a source of illumination. From the spectra, the apparatus calculated and returned the color coordinates of the uniform color space CIELAB (L^*, a^*, b^*, h_{ab}, and C_{ab}^*) (*27*) considering the Illuminant D65 and the 10° Observer as references.

Within the uniform space CIELAB, a psychometric index of lightness (L^*) and two color coordinates, namely, a^* and b^*, were defined. L^* was an approximate assessment of luminosity, taking values within the interval 0 (black) to 100 (white). As for a^* and b^*, the former took positive values for reddish colors and negative values for greenish ones, whereas the latter took positive values for yellowish colors and negative values for bluish ones. Within this uniform color space, two psychological parameters, named hue (h_{ab}) and chroma (C_{ab}^*), were defined, which are calculated from a^* and b^* as follows:

$$h_{ab} = \arctan(b^*/a^*) \quad C_{ab}^* = [(a^*)^2 + (b^*)^2]^{1/2}$$

The hue angle (h_{ab}) takes values from 0 to 360° and is the qualitative attribute that allows any color to be graded as reddish, greenish, etc. by distinguishing it from a gray color with the same lightness. As for chroma (C_{ab}^*), which is regarded as the quantitative attribute of colorfulness, it is the attribute that allows one to determine for each hue its degree of difference relative to a gray color showing the same lightness. The color differences (ΔE^*_{ab}) between two points, i.e., colors, in the CIELAB space are worked out as the Euclidean distance between their locations in the three-dimensional space defined by L^*, a^*, and b^*. Mathematically, it is therefore calculated by applying the formula $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

The measurements were made by placing the plastic cuvette containing the samples against a white background (WB) and a black background (BB). Previously, the blank measurements were made with the cuvette filled with distilled water against the WB, specifically a reference $BaSO_4$-pressed plate (USRS-99-010, Labsphere Inc., North Sutton, NH). The color coordinates obtained from the apparatus were the average of three consecutive measurements.

Statistical Analysis. The statistical treatment of the data was carried out by means of Statistica version 5.5 software.

RESULTS AND DISCUSSION

Provitamin A Carotenoids and Vitamin A Activity. Typical carotenoid chromatograms from UFOJ, OJFSO, and OJFC are depicted in **Figure 2**, from which it can be inferred that there were large quantitative and qualitative differences in the carotenoid profiles of the different kinds of orange juices analyzed, as is the case (*28*).

The all-*E*-isomers of α- and β-carotene and β-cryptoxanthin (chromatographic and spectroscopic features in the mobile phase summarized in **Table 1**) were readily identified with the aid of appropriate standards and were detected in all of the samples surveyed. The average content of β-cryptoxanthin, the most important provitamin A carotenoid in orange juices (*24*, *29*), was 0.96 mg/L, ranging from 0.22 to 1.67 mg/L. The levels of α- and β-carotene were clearly lower, to the extent that they were hardly detectable in some samples. Specifically, the levels of α-carotene were within the range 0.02−0.24 mg/

Table 1. Chromatographic and Spectroscopic Characteristics of the Major Provitamin A Carotenoids Found in the Orange Juice Samples Analyzed

peak	t_r[a] (min)	carotenoid	absorption maxima (nm)
1	33.46	(13*Z*)- or (13′*Z*)-β-cryptoxanthin	338, 444, 466
2	41.03	β-cryptoxanthin	452, 478
3	45.69	α-carotene	424, 446, 474
4	51.07	β-carotene	452, 478

[a] Retention time.

L, averaging 0.10 mg/L. The mean content of β-carotene (0.28 mg/L; range, 0.01−0.81 mg/L) was roughly three-fold higher relative to α-carotene but yet far from the average content of β-cryptoxanthin.

As for *Z*-isomers, only (13*Z*)- or (13′*Z*)-β-cryptoxanthin (chromatographic and spectroscopic features summarized in **Table 1**) was occasionally found at detectable levels, specifically in eight samples, all of them either OJFC or OJFSO. Its average content within those eight samples was 0.15 mg/L, ranging from 0.06 to 0.31 mg/L. The location of the *cis* double bond was tentatively assigned on the basis of its spectroscopic features, considering both the intensity of the "*cis* peak" in the ultraviolet region of the spectrum (calculated as the ratio of its absorbance to that of the second absorption band in the visible region) and the hypsochromic shifts of its absorption maxima relative to those of the others isomers found in the mixture obtained by steromutation of the all-*E*-isomer (*14*). In this particular case, the intensity of the *cis* peak was intermediate between those of the isomers identified as (15*Z*)- and (9*Z*)-β-cryptoxanthin (*28*), and the absorption maximum in the visible region was located at a shorter wavelength relative to that of (15*Z*)-β-cryproxanthin, which was in agreement with the features of other (13*Z*)-isomers carried out by other authors (*30*, *31*).

The samples surveyed varied widely in their vitamin A activity from 16.88 (OJFC) to 110.83 RAE/L (UFOJ), with an average activity of 61.63 RAE/L. Considering the different groups of samples studied, it was observed that UFOJs were in general the best sources of vitamin A (mean content, 87.11 RAE/L), followed by OJFSOs (mean content, 62.58 RAE/L) and OJFCs (mean content, 30.29); the values of RAE were remarkably heterogeneous among the OJFSO studied (range, 22.32−96.44 RAE/L).

Objective Assessment of Color. Spectroradiometry has proved a useful colorimetric technique for the objective evaluation of the color of orange juices in previous studies (*26*, *32*). The color measurements were taken against a WB and a BB because some studies have revealed that the use of one or another can lead to slight differences in the results obtained when correlations with other parameters are evaluated (*26*, *33*). Typical reflection spectra of representative samples of the different sorts of orange juice analyzed are depicted in **Figure 3**.

Regardless of the kind of orange juice considered and the background used for the color readings, it was observed that the reflectance was minimum at around 450 nm due to the carotenoid pigments absorbing strongly in the proximity of such wavelength. Overall, the reflection of light was sharply higher in the case of OJFC as their carotenoid content and hence the absorption of visible light was clearly lower relative to the remaining types of orange juice surveyed. As for the latter, the reflection spectra were much similar within the interval encompassed between 380 and around 500 nm. From around that

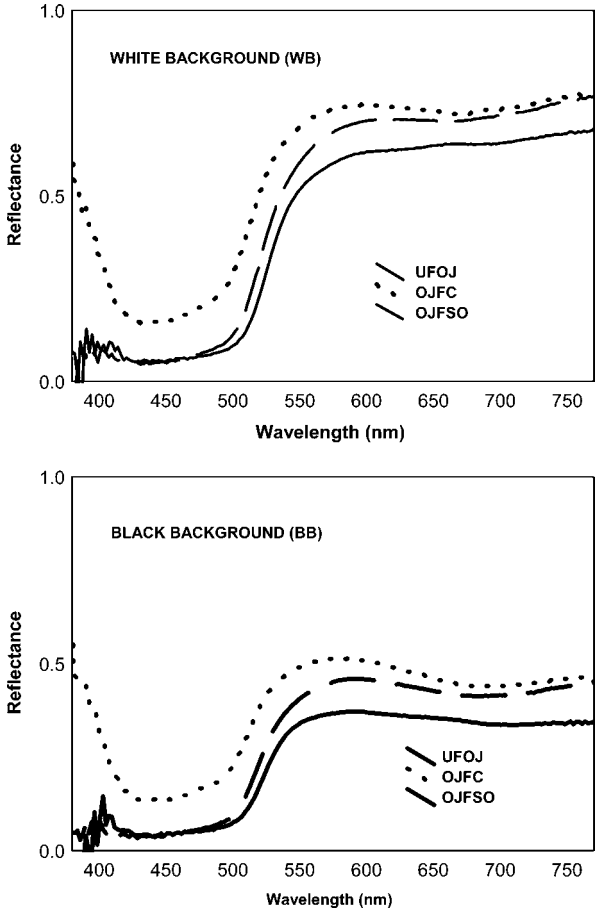

Figure 3. Visible reflection spectra (380–770 nm) of OJFC (solid lines), OJFSO (dashed lines), and UFOJ (dotted lines) samples.

wavelength onward, the reflectance observed in many OJFSO samples was distinguishable higher relative to UFOJ samples, nearing in some cases the reflectance showed by OJFC.

In terms of color coordinates, it was observed that the values of b^* were much higher than those of a^*, both of them taking positive values, which was coherent for yellowish to orangish colors. As a result of these large differences, the values of C_{ab}^* ($C_{ab}^* = [(a^*)^2 + (b^*)^2]^{1/2}$) were almost identical to those of b^* (**Table 2**). Taking into consideration the mean values of the color parameters related with the chromaticity (a^*, b^*, C_{ab}^*, and h_{ab}), it was seen that, numerically, h_{ab} was the least affected as a function of the kind of juice (around 7° of difference between extreme values, regardless of the background) or the background used for the measurements (around 3° of difference) (**Table 2**), as a consequence of it being an angular parameter. Regardless of the background used for the measurements, it was seen that OJFC showed the lowest values of a^*, b^*, and C_{ab}^* and the highest ones of h_{ab}. As for a^*, the highest mean values corresponded to the UFOJ samples, which also showed the lowest values of hue, the OJFSO showing the highest values of b^* and C_{ab}^*. As for the effect of the different backgrounds in the magnitude of the color coordinates, it was observed, as in other previous studies where the color of orange juices was assessed by spectroradiometry (*32*) or diffuse reflectance spectrophotometry (*33*), that the use of the BB yielded lower values of a^*, b^*, and C_{ab}^* and higher values of h_{ab} (**Table 2**). In other words, it can be said that the lower the amount of light reflected by these juices, the lower the values of a^*, b^*, and C_{ab}^* and the higher the values of h_{ab}.

Taking into account the different groups of orange juices studied and whatever color coordinate, it was seen that OJFSO

Table 2. Summary of the Results Obtained from the Color Measurements[a]

	L^*	a^*	b^*	C_{ab}^*	h_{ab}
			WB		
OJFC	78.92 ± 2.04 (75.99–82.86)	6.06 ± 1.37 (3.50–7.40)	58.54 ± 3.56 (49.81–62.28)	58.86 ± 3.65 (49.93–62.72)	84.13 ± 1.10 (83.06–85.99)
OJFSO	75.15 ± 3.51 (66.37–81.10)	11.92 ± 4.22 (5.70–19.37)	74.94 ± 7.25 (67.67–95.39)	75.95 ± 7.71 (68.03–97.34)	81.12 ± 2.49 (77.84–85.23)
UFOJ	73.40 ± 1.21 (71.34–74.60)	14.60 ± 2.00 (11.65–19.38)	69.11 ± 5.17 (62.67–79.82)	70.64 ± 5.41 (64.02–81.68)	78.11 ± 0.95 (76.13–80.21)
all samples	75.64 ± 3.31 (66.37–82.86)	11.14 ± 4.49 (3.50–19.38)	68.20 ± 8.71 (49.81–95.39)	69.19 ± 9.15 (49.93–97.34)	80.96 ± 2.94 (76.13–85.99)
			BB		
OJFC	67.79 ± 2.12 (64.23–71.22)	1.90 ± 0.86 (0.54–2.81)	46.93 ± 4.15 (37.47–51.52)	46.97 ± 4.17 (37.47–51.59)	87.74 ± 0.93 (86.87–89.35)
OJFSO	63.44 ± 3.22 (54.73–67.85)	6.57 ± 3.59 (1.56–12.68)	62.66 ± 11.34 (52.02–97.25)	63.07 ± 11.51 (52.07–98.07)	84.17 ± 2.74 (80.45–88.52)
UFOJ	60.24 ± 1.19 (58.03–62.12)	8.57 ± 1.56 (6.93–12.16)	54.61 ± 4.70 (48.56–64.35)	55.29 ± 4.84 (49.06–65.29)	81.12 ± 1.04 (78.90–82.90)
all samples	63.60 ± 3.81 (54.73–71.22)	5.90 ± 3.60 (0.54–12.68)	55.35 ± 9.92 (37.47–97.25)	55.75 ± 10.11 (37.47–98.07)	84.16 ± 3.20 (78.90–89.35)

[a] Means, standard deviations, and ranges are in parentheses.

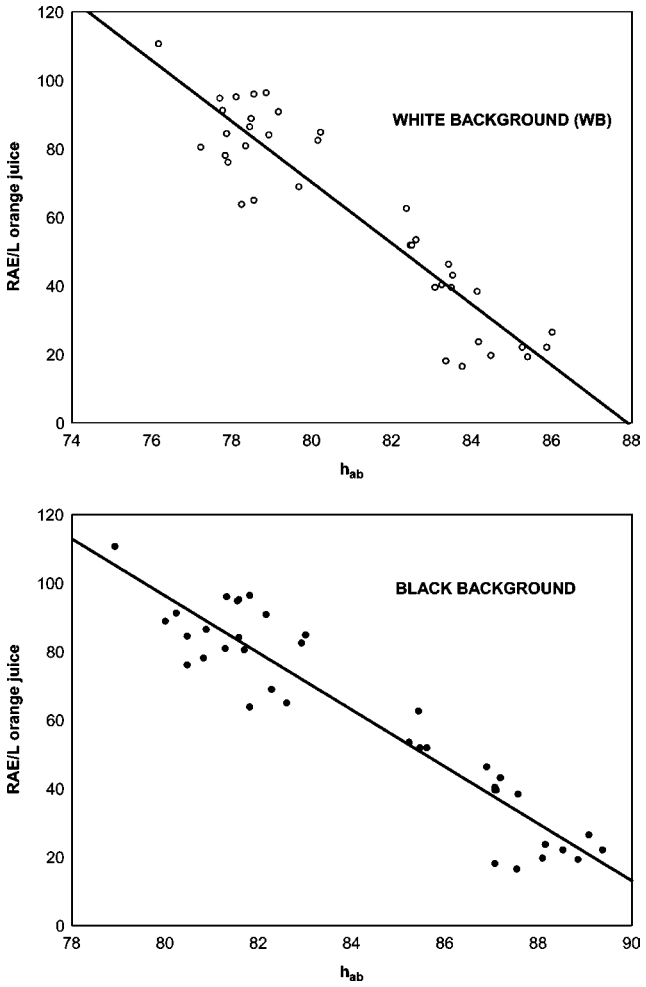

Figure 4. Representations of RAE vs h_{ab}.

was the most heterogeneous in terms of color. Considering all of the samples jointly, it was clearly observed that there were dramatic color differences among them, especially by taking into account the extreme values of chroma (C_{ab}*), the quantitative attribute of colorfulness among the samples (49.93−97.34 for measurements with WB and 37.47−98.07 for measurements with BB). Thus, the color differences between those samples were 51.01 (WB) and 62.65 (BB) CIELAB units, roughly nine and 11 times higher than the threshold of 5.6 CIELAB units from which color differences are considered to be easily distinguishable visually (*34*, *35*).

Table 3. Simple (r) and Multiple (R) Regression Coefficients and Coefficients of Multiple Determination (R^2) between the Color Parameters of CIELAB and the Vitamin A Activity of the Samples Expressed as RAE/L Orange Juice

	L^*	a^*	b^*	C_{ab}^*	h_{ab}		L^*, a^*, b^*	L^*, h_{ab}, C_{ab}^*
				WB				
r	−0.726	0.901	0.582	0.615	−0.927	R	0.924	0.932
						R^2	0.853	0.869
				BB				
r	−0.677	0.905	0.402	0.425	−0.944	R	0.935	0.946
						R^2	0.874	0.895

Table 4. Equations for the Assessment of Vitamin A Activity from Color Coordinates

equation	Vit A[a]
WB	
(1) RAE = $-8.8961h_{ab} + 781.8687$	61.62 ± 26.17
(2) RAE = $1.3171L^* + 7.8514a^* - 0.8858b^* - 65.0641$	61.63 ± 26.08
(3) RAE = $1.6945L^* + 0.4033C_{ab}^* - 9.666h_{ab} + 688.1216$	61.62 ± 26.33
BB	
(4) RAE = $-8.314h_{ab} + 761.3132$	61.62 ± 26.64
(5) RAE = $0.25334L^* + 8.81831a^* - 0.84892b^* + 40.47947$	61.62 ± 26.39
(6) RAE = $0.7649L^* + 0.1525C_{ab}^* - 8.79h_{ab} + 744.2246$	61.62 ± 26.71

[a] Average RAE/L orange juice.

Statistical Analysis. The correlation between the color parameters and the vitamin A activity of the samples was first explored by simple regression as a result of which it was concluded that regardless of the background used for the color readings, hue (h_{ab}) was the color parameter best correlated with the RAE of the samples ($r = -0.927$ and -0.944 for WB and BB, respectively) (**Figure 4**), followed closely by a^* ($r = 0.901$ and 0.905 for WB and BB, respectively). The magnitude of the values of the simple regression coefficient r for the former color coordinates was virtually identical independently of the background used, which was not the case for the remaining color parameters related to the colorfullness, b^* and C_{ab}^*, since higher simple correlations were obtained when the WB was used for

Table 5. RAE Values Obtained by Applying the Different Equations Calculated from the Different Regression Analysis Carried Out (See **Table 4** for Subscripts)[a]

			WB			BB		
		RAE_{HPLC}	$RAE_{(1)}$	$RAE_{(2)}$	$RAE_{(3)}$	$RAE_{(4)}$	$RAE_{(5)}$	$RAE_{(6)}$
1	OJFC	19.28	22.34 (0.03)	28.86 (0.10)	23.39 (0.04)	22.86 (0.04)	29.98 (0.11)	24.50 (0.05)
2	OJFC	26.72	16.94 (0.10)	27.41 (0.01)	17.54 (0.09)	20.72 (0.06)	31.49 (0.05)	19.76 (0.07)
3	OJFC	22.43	18.14 (0.04)	19.29 (0.03)	14.75 (0.08)	18.46 (0.04)	21.96 (0.00)	17.32 (0.05)
4	OJFC	16.88	36.91 (0.20)	33.58 (0.17)	31.31 (0.14)	33.64 (0.17)	35.12 (0.18)	31.08 (0.14)
5	OJFC	18.15	40.41 (0.22)	37.49 (0.19)	36.31 (0.18)	37.53 (0.19)	38.39 (0.20)	35.58 (0.17)
6	OJFC	19.78	30.44 (0.11)	31.14 (0.11)	29.17 (0.09)	29.10 (0.09)	32.75 (0.13)	28.26 (0.08)
7	OJFC	39.87	39.25 (0.01)	38.39 (0.01)	38.64 (0.01)	37.38 (0.02)	37.66 (0.02)	38.39 (0.01)
8	OJFC	40.42	41.46 (0.01)	41.58 (0.01)	42.41 (0.02)	37.61 (0.03)	38.21 (0.02)	39.64 (0.01)
9	OJFC	43.41	39.02 (0.04)	38.00 (0.05)	37.55 (0.06)	36.66 (0.07)	36.97 (0.06)	38.70 (0.05)
10	OJFC	46.60	39.89 (0.07)	41.33 (0.05)	41.10 (0.05)	39.04 (0.08)	39.18 (0.07)	41.70 (0.05)
11	OJFC	39.60	42.96 (0.03)	43.93 (0.04)	43.96 (0.04)	37.54 (0.02)	38.53 (0.01)	37.40 (0.02)
12	OJFSO	84.17	79.98 (0.04)	84.06 (0.00)	83.25 (0.01)	83.18 (0.01)	88.41 (0.04)	87.11 (0.03)
13	OJFSO	84.63	89.38 (0.05)	91.46 (0.07)	89.82 (0.05)	92.46 (0.08)	95.04 (0.10)	93.58 (0.09)
14	OJFSO	96.44	80.62 (0.16)	82.33 (0.14)	80.53 (0.16)	81.31 (0.15)	85.44 (0.11)	83.64 (0.13)
15	OJFSO	86.64	84.25 (0.02)	90.10 (0.03)	89.61 (0.03)	89.09 (0.02)	87.81 (0.01)	92.11 (0.05)
16	OJFSO	65.15	83.35 (0.18)	89.95 (0.25)	80.86 (0.16)	74.81 (0.10)	83.60 (0.18)	75.24 (0.10)
17	OJFSO	52.13	48.31 (0.04)	46.81 (0.05)	49.47 (0.03)	50.85 (0.01)	48.51 (0.04)	51.16 (0.01)
18	OJFSO	53.52	47.33 (0.06)	47.33 (0.06)	50.11 (0.03)	52.78 (0.01)	50.78 (0.03)	53.53 (0.00)
19	OJFSO	22.32	23.70 (0.01)	26.06 (0.04)	29.37 (0.07)	25.38 (0.03)	20.10 (0.02)	27.05 (0.05)
20	OJFSO	62.76	49.26 (0.14)	50.12 (0.13)	53.74 (0.09)	51.23 (0.12)	49.91 (0.13)	53.82 (0.09)
21	OJFSO	38.48	33.67 (0.05)	29.77 (0.09)	31.15 (0.07)	33.39 (0.05)	31.73 (0.07)	30.32 (0.08)
22	OJFSO	23.67	33.14 (0.09)	27.66 (0.04)	33.21 (0.10)	28.50 (0.05)	19.30 (0.04)	26.79 (0.03)
23	OJFSO	51.99	47.94 (0.04)	47.04 (0.05)	50.28 (0.02)	49.73 (0.02)	47.43 (0.05)	49.87 (0.02)
24	OJFSO	84.94	68.42 (0.17)	69.28 (0.16)	70.85 (0.14)	71.41 (0.14)	71.18 (0.14)	72.00 (0.13)
25	OJFSO	69.31	73.10 (0.04)	73.96 (0.05)	74.99 (0.06)	77.45 (0.08)	78.45 (0.09)	79.16 (0.10)
26	UFOJ	110.83	104.62 (0.06)	112.38 (0.02)	106.83 (0.04)	105.35 (0.05)	109.88 (0.01)	104.91 (0.06)
27	UFOJ	91.25	90.34 (0.01)	97.93 (0.07)	95.05 (0.04)	94.27 (0.03)	98.91 (0.08)	95.36 (0.04)
28	UFOJ	95.04	90.86 (0.04)	87.24 (0.08)	87.22 (0.08)	83.36 (0.12)	81.54 (0.13)	81.51 (0.14)
29	UFOJ	91.16	77.88 (0.13)	75.45 (0.16)	76.02 (0.15)	78.43 (0.13)	76.97 (0.14)	76.89 (0.14)
30	UFOJ	80.83	95.15 (0.14)	96.42 (0.16)	97.47 (0.17)	82.28 (0.01)	77.65 (0.03)	79.11 (0.02)
31	UFOJ	96.25	83.18 (0.13)	77.65 (0.19)	79.70 (0.17)	85.35 (0.11)	81.73 (0.15)	82.78 (0.13)
32	UFOJ	89.17	83.88 (0.05)	81.73 (0.07)	83.27 (0.06)	96.28 (0.07)	95.30 (0.06)	97.18 (0.08)
33	UFOJ	78.33	89.57 (0.11)	85.83 (0.08)	89.05 (0.11)	89.46 (0.11)	85.78 (0.07)	89.01 (0.11)
34	UFOJ	82.50	68.87 (0.14)	65.50 (0.17)	67.45 (0.15)	72.04 (0.10)	69.94 (0.13)	71.22 (0.11)
35	UFOJ	64.17	85.92 (0.22)	78.52 (0.14)	82.01 (0.18)	81.29 (0.17)	76.24 (0.12)	78.70 (0.15)
36	UFOJ	95.42	87.20 (0.08)	79.89 (0.16)	81.62 (0.14)	83.22 (0.12)	80.65 (0.15)	82.47 (0.13)
37	UFOJ	81.25	84.95 (0.04)	81.69 (0.00)	84.42 (0.03)	85.63 (0.04)	80.62 (0.01)	83.35 (0.02)
38	UFOJ	76.25	89.05 (0.13)	84.62 (0.08)	88.22 (0.12)	92.54 (0.16)	88.58 (0.12)	91.45 (0.15)

[a] Deviation in absolute terms regarding the standard values (RAE_{HPLC}) in parentheses.

the measurements (**Table 3**). In any case, all of the regression coefficients obtained were significant at $p < 0.05$.

Because of the fact that color is a three-dimensional property, and hence either the sets of coordinates L^*, C_{ab}^*, h_{ab} or L^*, a^*, b^* must be taken into consideration together to define completely the CIELAB color space, the relationships existing between the vitamin A activity of the samples and the color parameters were further evaluated by multiple regression analysis. The values of the multiple regression coefficient R found when the sets "L^*, a^*, b^*" and "L^*, C_{ab}^*, h_{ab}" were considered were 0.924 and 0.932, respectively, for measurements with WB and 0.935 and 0.946, respectively, for measurements with BB (**Table 3**). Comparing the values of R and r (**Table 3**), it was seen that the joint consideration of the sets of color coordinates improved the correlations relative to a^* or h_{ab} considered individually, which is obvious as more variables are taken into account. Nonetheless, it was also observed that such gains were not substantial in absolute values, when compared with the values of r corresponding to h_{ab} (WB: $r_{h_{ab}} = -0.927$, $R_{L^*h_{ab}C_{ab}^*} = 0.932$; BB: $r_{hab} = -0.944$, $R_{L^*h_{ab}C_{ab}^*} = 0.946$).

From the different regression analyses performed, a series of equations for the assessment of the vitamin A activity of the samples from the color coordinates were inferred. These equations along with the average RAE/L calculated from them are shown in **Table 4**. The individual values of vitamin A activity obtained by applying the different formulas calculated are displayed in **Table 5**, as well as the values obtained considering the HPLC data, which were considered as the standard ones. To ascertain whether the latter were statistically different from those worked out through the color measurements, the corresponding analyses of variance (ANOVA) for repeated measurements ($p < 0.05$) were performed, considering the measurements made with each background all of the possible pairs (RAE by HPLC vs RAE by h_{ab}; RAE by HPLC vs RAE by L^*, a^*, b^*; RAE by HPLC vs RAE by L^*, C_{ab}^*, h_{ab}; RAE by h_{ab} vs RAE by L^*, a^*, b^*; RAE by h_{ab} vs RAE by L^*, C_{ab}^*, h_{ab}; RAE by L^*, C_{ab}^*, h_{ab} vs RAE by L^*, a^*, b^*). The p values obtained (Table 6) ranged from 0.995 to 0.999, which indicated that there were no significant differences ($p < 0.05$) in the values of vitamin A activity obtained with independence of the procedure of calculation.

Table 6. p Values from the ANOVA Analyses for Repeated Measurements Performed

	WB	BB
HPLC vs h_{ab}	0.999	0.998
HPLC vs L^*, a^*, b^*	0.999	0.999
HPLC vs L^*, C_{ab}^*, h_{ab}	0.999	0.999
h_{ab} vs L^*, a^*, b^*	0.995	0.995
h_{ab} vs L^*, C^*_{ab}, h_{ab}	0.997	0.995
L^*, C_{ab}^*, h_{ab} vs L^*, a^*, b^*	0.996	0.997

This study builds upon a previous one carried out in our laboratory in which the applicability of the objective measurement of color to estimate the vitamin A activity of a quite homogeneous sort of orange juice in terms of color and carotenoid profile, specifically UFOJ from the same season, was tested (*32*). Thus, it is now safe to claim that it is feasible to achieve a very good estimation of the vitamin A activity of orange juices differing greatly in carotenoid content, and hence in color, through objective color measurements. In this sense, it has been observed that the best results are obviously achieved when the groups of color coordinates L^*, a^*, b^* or L^*, C_{ab}^*, h_{ab} are considered jointly, although these are not statistically different from those obtained by solely taking into account the hue values, which makes the assessment simpler and even faster.

As for the possible practical applications of this novel procedure for assessing vitamin A activity, they all revolve around the peculiarities of the objective measurement of color (rapidity of analysis, easy automation, availability of portable apparatus, nondestructiveness, etc.), which make it an amenable technique for quality control purposes. The implementation of such technique within the food industry could, for instance, provide the analyst with a powerful tool for performing a more realistic quality control in relation to the vitamin A labeling of foods, which has been reported to be very defective in several studies (*36*, *37*), by allowing the samples to be analyzed for vitamin A almost instantaneously over production, storage, retailing, etc., at the same time that the color itself, which is a very important quality index in the particular case of orange juice (*38*), is also objectively assessed.

ACKNOWLEDGMENT

We thank the company Zumos Vitafresh (Almonte, Huelva, Spain) for kindly supplying the UFOJs analyzed in this study.

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Received for review December 7, 2006. Revised manuscript received February 19, 2007. Accepted February 20, 2007.

JF0635412