JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Application of Reflectance Colorimeter Measurements and Infrared Spectroscopy Methods to Rapid and Nondestructive Evaluation of Carotenoids Content in Apricot (*Prunus armeniaca* L.)

DAVID RUIZ,*,† MARYSE REICH,‡ SYLVIE BUREAU,‡ CATHERINE M. G. C. RENARD,‡ AND JEAN-MARC AUDERGON†

Unité de Génétique et d'Amélioration des Fruits et Légumes, INRA-Avignon, Domaine St. Maurice, BP-94, 84143 Montfavet, France, and UMR 408, Sécurité et Qualité des Produits d'Origine Végétale, INRA-Avignon, Université d' Avignon, 84000 Avignon, France

The importance of carotenoid content in apricot (*Prunus armeniaca* L.) is recognized not only because of the color that they impart but also because of their protective activity against human diseases. Current methods to assess carotenoid content are time-consuming, expensive, and destructive. In this work, the application of rapid and nondestructive methods such as colorimeter measurements and infrared spectroscopy has been evaluated for carotenoid determination in apricot. Forty apricot genotypes covering a wide range of peel and flesh colors have been analyzed. Color measurements on the skin and flesh (L^*, a^*, b^*, hue, chroma, and a^*/b^* ratio) as well as Fourier transform near-infrared spectroscopy (FT-NIR) on intact fruits and Fourier transform mid-infrared spectroscopy (FT-MIR) on ground flesh were correlated with the carotenoid content measured by high-performance liquid chromatography. A high variability in color values and carotenoid content was observed. Partial least squares regression analyses between β-carotene content and provitamin A activity and color measurements showed a high fit in peel, flesh, and edible apricot portion (R^2 ranged from 0.81 to 0.91) and low prediction error. Regression equations were developed for predicting carotenoid content by using color values, which appeared as a simple, rapid, reliable, and nondestructive method. However, FT-NIR and FT-MIR models showed very low R^2 values and very high prediction errors for carotenoid content.

KEYWORDS: ***Prunus armeniaca* L.; fruit quality; β-carotene; provitamin A; HPLC; color index; FT-NIR; FT-MIR; partial least-squares regression**

INTRODUCTION

Scientific interest in the carotenoid content and the distribution patterns in fruits and vegetables has increased recently since it was discovered that carotenoids are important not only because of the color that they impart but also because they express protective activity against several human cancers and degenerative diseases (*1–5*). β-Carotene is the main pigment (*6*) in apricot (*Prunus armeniaca* L.), although other carotenoids such as β-cryptoxanthin, γ-carotene, phytoene, phytofluene, lycopene, and lutein are present in lower amounts (*6*). Apricot is one of the fruits richest in carotenoids, along with papaya, mango, pittango, and prune (*7*). However, there are few studies on the carotenoids of ripe apricots (*6, 8–11*). Genotype is known to influence not only the total carotenoid content but also the proportions of each carotenoid species (*10*), as it has been observed in peaches and plums (*12*).

Long, expensive, and destructive chemical methods are currently required to evaluate carotenoids content. They are work-intensive, and they are susceptible to loss of part of the carotenoids during extraction due to pigment instability. Therefore, the development of reliable, new, rapid, and nondestructive analytical methods for the evaluation of these compounds in apricot would be a considerable advance for the apricot industry. Another important benefit would be the measurement of carotenoids directly on fruits still on the tree, a technique requiring reliable portable instruments.

Several studies have correlated the reflectance color measurements with the pigment content of different foods (*13–19*). Color measurements have been considered to be appropriate for the rapid estimation of β-carotene content and, therefore, a reliable indication of vitamin A activity (*16, 17, 19*). Little work on this matter has been carried out in apricot species, although good

* To whom correspondence should be addressed. Tel: +33 432722660. Fax: +33 432722764. E-mail: david.ruiz@avignon.inra.fr.
† Unité de Génétique et d'Amélioration des Fruits et Légumes.
‡ UMR 408, Sécurité et Qualité des Produits d'Origine Végétale.

10.1021/jf7036032 CCC: $40.75
Published on Web 06/17/2008

correlations have been found between color values and carotenoid concentration (*10*).

Recently, infrared reflectance spectroscopy has received considerable attention as a tool to assess fruit quality, especially soluble solids content and acidity, in various species. Near infrared (NIR) spectroscopy has been used as a rapid and nondestructive technique for measuring carotenoid contents in maize (*20*), tritordeum (*21*), and tomato (*22, 23*). Application of mid-infrared (MIR) spectroscopy for the determination of carotenoids content has only been reported in tomato (*23*). However, no work has been carried out in apricot species.

The objective of this work was to evaluate reflectance colorimeter measurements and infrared spectroscopy as rapid and nondestructive techniques to assess carotenoid contents in apricot fruit, by comparison with standard destructive techniques. Results will be a first step to develop portable instruments for measuring carotenoid contents, which would involve a considerable advance for the apricot industry instead of long, tedious, and costly conventional chemical methods. In addition, the large phenotypic variability of the genotypes that we selected for evaluation, especially concerning flesh and skin color, provides valuable information about carotenoid contents in the apricot species.

MATERIAL AND METHODS

Plant Material. The plant material assayed included Goldrich and Moniquí apricot cultivars, both highly contrasted for fruit color and carotenoid contents (*24*), and 38 apricot selections issued from a Goldrich × Moniquí progeny. The evaluated genotypes were chosen among the largest population to represent the variability of skin and flesh color (10 white, 10 yellow, 10 light orange, and 10 orange apricot genotypes). All of them were grown in the same experimental orchard (Amarine, Nimes, South of France, experimental orchard of INRA), according to standard apricot orchard management. All genotypes were harvested between June 20th and July 10th (2006) at commercial maturity on the basis of their skin color (degreening stage) and fruit firmness.

Chemicals. *trans*-β-Carotene and β-apo-8′-carotenal were purchased from Sigma Chemical Co. (St. Louis, MO), and β-cryptoxanthin was from Extrasynthese SA (Genay, France). All reagents and solvents were of high-performance liquid chromatography (HPLC) grade (Prolabo, Paris, France).

Experimental Procedure. Immediately after harvest, fruits were transported in an air-conditioned car to the laboratory (50 km). Fruits were carefully selected to ensure that they were free of defects. According to their firmness, four homogeneous fruits from each cultivar and selection were selected for this study. After skin ground color and Fourier transform near-infrared (FT-NIR) were measured on intact fruits, two wedges were cut vertically from each side of the fruit to measure the flesh color. The remaining wedges of each cultivar and selection were peeled, and the flesh and peel were frozen separately in liquid nitrogen and kept at −80 °C until analyzed. The frozen flesh or peel was ground to an homogeneous powder in liquid nitrogen using a PM-400 ball grinder (Retsch GmbH, Germany). Fourier transform mid-infrared (FT-MIR) analysis was performed on flesh powder, and carotenoid contents were analyzed on both flesh and peel powders. Carotenoid contents in edible portions were calculated considering 9% of the peel and 91% of the flesh, which corresponds to the whole fruit except the kernel (the peel of apricots is usually eaten).

Determination of Color Values. Color values on the surface (ground skin color, color measured on the unblushed side of fruit) and after peeling on the flesh were measured using a chromameter (CR-400, Minolta, Osaka, Japan) tristimulus color analyzer calibrated with a white porcelain reference plate. The visible reflectance spectra (380−770 nm) were obtained through a silicone photo cell and a pulsed xenon lamp as a source of illumination (illuminant D65, 0° view angle, illumination area diameter 8 mm). From the spectra, the apparatus calculated and returned the color parameters. The color coordinates of the uniform color space CIELAB (*25*) L^*, a^*, b^*, hue angle ($H°$) [h_{ab} = arctg (b^*/a^*)], and chroma (C^*) [$C_{ab}^* = (a^{*2} + b^{*2})^{1/2}$] were determined in the fruit equatorial region. L^* values [from 0 (black) to 100 (white)] represent luminosity, a^* values range from −60 (green) to 60 (red), and b^* values range from −60 (blue) to 60 (yellow). The $H°$ (h_{ab}) takes values from 0 to 360° and is the qualitative attribute that allows any color to be graded as reddish, greenish, etc., and C^* (C_{ab}^*) is regarded as the quantitative attribute of colorfulness.

Extraction and HPLC-DAD Analysis of Carotenoids. Procedures used were described by Wright and Kader (*26*) based on the method of Hart and Scott (*27*). The sample of frozen fruit material (5 g) was homogenized with an Ultra Turrax T-25 (Ika, Staufen, Germany) for 2 min on ice, with 10 mL of extraction solution (methanol/hexane 1:1). The homogenates were centrifuged at 10500*g* for 15 min at 2−5 °C. The supernatant was recovered carefully to prevent contamination by the pellet. The extraction process was repeated 3−4 times with 5 mL of hexane until the disappearance of the color in the hexane layer. The pooled extracts were filtered through a bed of anhydrous Na_2SO_4 (Prolabo, France) to remove the water and evaporated to dryness in a rotary evaporator at 35 °C. The pigments were redissolved in 2 mL of acetone, filtered through a 0.45 μm Osmonics/MSI cameo Nylon filters (Fisher Scientific, Los Angeles, CA), and kept refrigerated until the analysis by HPLC (for a period not exceeding 12 h). Samples of 20 μL of extracts were injected. At the beginning of the extraction process, β-apo-8′-carotenal was added (0.6 mg/5 g of fruit material) to all samples as an internal standard (IS).

HPLC-diode array detection (DAD) analysis was performed using an Agilent 1050 (Hewlett-Packard, Palo Alto, CA). Chromatographic separation was carried out using a 250 mm × 4.6 mm i.d. 5 μm VYDAC 201TP54 C18 column (Interchim, France) with a 10 mm × 4.6 mm i.d. 5 μm Vydac guard cartridge (Interchim, France), both thermostatted at 30 °C. The mobile phase was composed of acetonitrile/methanol/dichloromethane (60/38/2, v/v) 38, 60, and 2% at a flow rate of 1 mL/min. Absorbance spectra were measured over the wavelength range 200−600 nm in steps of 2 nm. The carotenoid compounds were identified on the basis of UV/vis spectra (β-carotene, β-cryptoxanthin, γ-carotene, phytoene, and phytofluene) and retention times (R_t), in agreement with our previous works on apricot (*10, 11*).

Carotenoids were quantified by integration of their absorbance at 450 nm for β-carotene and β-cryptoxanthin, 470 nm for γ-carotene, 350 nm for phytofluene, and 290 nm for phytoene against a calibration curve obtained from dilution series of standard solution (phytoene, phytofluene, and γ-carotene were expressed in equivalent β-carotene). Carotenoid levels were expressed in mg kg^{-1} fresh weight.

FT-NIR and ATR-FTMIR Analysis. FT-NIR spectra were acquired on each intact fruit using a multipurpose analyzer (MPA) spectrometer (Bruker Optics, Wissembourg, France) fitted with an integrating sphere (diffuse reflectance). Spectra were measured between 800 and 2500 nm by accumulating 32 scans at resolution of 2 nm, and the absorbancies were recorded on a linked computer by Opus software (Bruker Optics, Wissembourg, France).

Attenuated total reflectance Fourier transform mid-infrared (ATR-FTMIR) was used to measure the MIR spectra of flesh powder. The spectrometer was a Tensor 27 FTIR spectrometer (Bruker Optics) equipped with a horizontal ATR zinc selenide crystal with six reflections. The spectra were recorded at 20 ± 1 °C, in the spectral range from 650 to 4000 cm^{-1}, by accumulating 32 scans at a resolution of 4 cm^{-1}. Between determinations, the crystal was carefully cleaned with water and dried.

The spectral data were analyzed using a MatLab software package, specifically SAISIR application (*28*) and DESIR interface (*29*). The spectral processing included the preprocessing of spectral data and the development and validation of prediction models. The FT-NIR and FT-MIR ATR reflectance data were transformed with standard normal variate (SNV) to correct multiplicative interferences, variation in baseline shift, and curvilinearity (*30*). Before the calibration, the spectra variation of the data was analyzed by principal component analysis (PCA), and defective spectra were eliminated.

After preprocessing, the spectra variation of the data was analyzed by partial least-squares (PLS) regression method (*31*), which was used to develop calibration models. Various wavelength intervals were tested

Table 1. Color Values (Reflectance Measurements L^*, a^*, b^*, h_{ab}, C_{ab}^*, and a^*/b^*) in Apricot Peel and Apricot Flesh on White, Yellow, Light Orange, and Orange Apricot Groups (10 Accessions Each) at Commercial Maturity Stage[a]

		L^*	a^*	b^*	h_{ab}	C_{ab}^*	a^*/b^*
			apricot peel				
white apricot	mean ± SD	70.2 ± 3.3 a	−0.53 ± 2.2 a	43.4 ± 5.3 a	90.8 ± 2.7 a	43.4 ± 5.3 a	0.0 ± 0.0 a
	range	66.8−77.2	−4.8−3.7	37.6−54.6	86.1−96.4	37.6−54.8	−0.1−0.1
yellow apricot	mean ± SD	69.5 ± 2.4 a	6.8 ± 2.4 b	47.9 ± 2.7 b	81.9 ± 2.8 b	48.4 ± 2.7 b	0.1 ± 0.1 b
	range	64.5−72.9	3.6−10.7	44.2−52.5	78.2−85.4	45.1−52.7	0.1−0.2
light orange apricot	mean ± SD	67.0 ± 2.4 b	12.6 ± 2.2 c	48.6 ± 3.8 b	75.6 ± 1.8 c	50.4 ± 4.1 b	0.3 ± 0.0 c
	range	64.1−70.9	9.2−15.6	42.5−53.8	73.5−77.8	43.5−55.3	0.2−0.3
orange apricot	mean ± SD	64.0 ± 2.3 c	19.4 ± 3.2 d	50.6 ± 3.6 b	69.1 ± 2.9 d	54.3 ± 3.9 c	0.4 ± 0.1 d
	range	60.6−68.0	15.3−24.9	45.6−57.7	64.7−72.3	47.9−60.5	0.3−0.5
			apricot flesh				
white apricot	mean ± SD	62.8 ± 4.3 ab	5.6 ± 2.6 a	37.4 ± 8.1 a	81.9 ± 2.7 a	37.9 ± 8.3 a	0.1 ± 0.1 a
	range	54.4−69.2	1.2−9.4	21.9−47.9	78.9−87.0	22.0−48.8	0.1−0.2
yellow apricot	mean ± SD	64.3 ± 2.3 a	11.4 ± 2.4 b	45.2 ± 3.4 b	75.9 ± 2.2 b	46.6 ± 3.7 b	0.2 ± 0.0 b
	range	61.6−68.0	7.5−16.5	39.2−50.0	71.2−79.5	40.1−51.4	0.2−0.3
light orange apricot	mean ± SD	59.4 ± 4.3 b	15.3 ± 3.1 c	46.7 ± 3.5 b	72.0 ± 2.4 c	49.2 ± 4.2 b	0.3 ± 0.1 c
	range	54.7−65.7	11.3−19.9	39.8−50.8	68.1−74.2	41.3−54.1	0.3−0.4
orange apricot	mean ± SD	61.8 ± 2.4 ab	19.7 ± 2.6 d	47.3 ± 2.2 b	67.4 ± 2.7 d	51.3 ± 2.4 b	0.4 ± 0.1 d
	range	56.8−64.9	17.1−24.0	43.6−50.5	61.4−70.0	47.8−55.9	0.4−0.5

[a] Significant differences between groups are shown with different letters according to SNK's multiple-range test ($P < 0.05$).

in both FT-NIR and FT-MIR, starting with the full spectra. Noninformative segments were tentatively purged, and the resulting performance was evaluated. The suited wavelength interval was found by this method. To determine the optimum number of factors (LVs, latent variables) for each calibration model, two statistic parameters were used, the root-mean-square error of prediction (RMSEP) and the coefficient of determination (R^2) between the predicted and the measured parameters. RMSEP was estimated as

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{N}}$$

where N = number of samples, y_i = measured value, and $\hat{y}_i$ = predicted value. RMSEP represents the average uncertainty that can be expected for the prediction of future samples.

Different calibration models were calculated depending on the number of factors taken into consideration. The model with the number of factors that produced the minimum RMSEP and the maximum coefficient of determination was selected as the most desirable model for the spectral data set.

Statistical Analysis. Statistical analyses were performed using SPSS 13.0 software for Windows (Lead Technologies Inc., Chicago, IL). Correlation coefficients were determined as the coefficient of Pearson. Analysis of variance (ANOVA) was used to test the differences among groups of genotypes (white, yellow, light orange, and orange apricots) with regard to color measurements and carotenoids content. The spectral data were analyzed using SAISIR application (*28*), DESIR interface (*29*), from MatLab software package (MathWorks, Natick, MA). The PLS regression method was used to develop prediction models. The prediction models were validated using the cross-validation method. The sample data were separated randomly into two groups: a calibration set used to develop the calibration models (60% data) and the remaining samples of the population, which were used as validation set (40% data). R^2 and RMSEP were assessed on both data sets.

RESULTS AND DISCUSSION

Color Evaluation. The color values (L^*, a^*, b^*, h_{ab}, C_{ab}^*, and a^*/b^*) in peel and flesh covered a wide range of variability on the set of apricot genotypes evaluated (**Table 1**). In general, color values were higher in peel than in flesh, except for a^* and a^*/b^* ratio. The lightness factor L^* ranged from 60.6 to 77.2 in peel and from 54.4 to 69.2 in flesh. The a^* value increased from negative values in the white genotypes (minimum values of −4.8 and 1.2 in peel and flesh respectively) to positive a^* values in the light orange and orange genotypes (maximum of 24.9 in peel and 24.0 in flesh). The b^* value and C^* (C_{ab}^*) increased in the orange genotypes as compared to the white ones (**Table 1**). The values of b^* were much higher than those of a^*, which was coherent for yellowish to orangish colors. The H° (h_{ab}) ranged from 64.7° in the peel of white genotypes to 96.4° in the orange ones, while it ranged from 61.4° to 87.0° in the flesh (**Table 1**). The range of values is quite representative of apricot species and is in agreement with results obtained previously in apricot (*10*). Among the studied color variables, the most discriminating ones are a^*, a^*/b^*, and h_{ab}, which showed significant differences among white, yellow, light orange, and orange apricots in both peel and flesh (**Table 1**). There was a high correlation between peel and flesh color, especially in the case of a^* value ($r = 0.88$) and h_{ab} value and a^*/b^* ratio ($r = 0.90$).

Carotenoid Content of Apricot Genotypes. β-Carotene, β-cryptoxanthin, γ-carotene, phytofluene, and phytoene were identified. Traces of other carotenoids were observed in the chromatogram, but their identification was not possible as they were present in a very small amount.

Some reports have recommended concentration of the research studies on those carotenoids that show a provitamin A activity (*32*), especially β-carotene, which dominates in terms of quantity and is the main provitamin A precursor. In this study, provitamin A activity was estimated as

$$\text{IU provitamin A} = (\mu\text{g } \beta\text{-carotene}/6) + (\mu\text{g } \beta\text{-cryptoxanthin}/12)$$

Carotenoid contents and provitamin A activity were assessed in peel and flesh in the set of evaluated apricot genotypes (**Table 2**). A large variability was observed for all compounds in both peel and flesh, as expected due to the high phenotypic diversity regarding fruit color of evaluated genotypes. Thus, the data set appeared to be suitable for the calibration and validation of new analytical methods of carotenoids. The total carotenoid contents varied from 9.9 to 207.7 mg kg^{-1} in the peel and from 12.4 to 104.0 in the flesh, and significant differences were observed among groups except for white−yellow apricots in both peel and flesh (**Table 2**). The β-carotene content ranged from 2.6 to 141.7 mg kg^{-1} in the apricot peel and from 1.4 to 35.2 mg kg^{-1} in the flesh, and significant differences were found among

Table 2. Carotenoids Content and Provitamin A Activity in Apricot Peel and Apricot Flesh on White, Yellow, Light Orange, and Orange Apricot Groups (10 Accessions Each) at Commercial Maturity Stage[a,b]

		β-carotene	β-cryptoxanthin	γ-carotene	phytofluene	phytoene	total carotenoids	provitamin Ac
				apricot peel				
white apricot	mean ± SD	5.5 ± 2.2 a	0.5 ± 0.7 a	0.3 ± 0.4 a	7.9 ± 5.4 a	11.9 ± 4.6 a	26.1 ± 10.0 a	964 ± 348 a
	range	2.6−8.8	0.0−1.8	0.0−1.0	0.0−15.3	6.0−18.7	9.9−40.3	497−1474
yellow apricot	mean ± SD	14.4 ± 5.4 ab	0.8 ± 1.1 a	0.7 ± 0.3 a	5.0 ± 5.9 a	11.2 ± 4.1 a	32.1 ± 12.2 a	2478 ± 871 ab
	range	7.8−25.5	0.0−2.9	0.3−1.5	0.0−16.3	6.3−17.5	19.1−54.8	1476−4247
light orange apricot	mean ± SD	30.1 ± 16.6 b	2.1 ± 3.2 a	2.3 ± 3.2 b	9.7 ± 10.1 a	22.8 ± 10.4 b	63.9 ± 18.9 b	5188 ± 2575 b
	range	5.4−58.4	0.0−10.7	0.0−11.1	0.0−31.3	10.8−46.4	31.7−91.4	1770−9737
orange apricot	mean ± SD	71.4 ± 38.0 c	2.4 ± 2.8 a	3.1 ± 2.0 b	7.6 ± 15.0 a	28.3 ± 15.3 b	112.9 ± 48.7 c	12113 ± 6473 c
	range	19.5−141.7	0.0−9.5	1.2−6.9	0.0−47.0	11.3−59.6	59.4−207.7	3249−23834
				apricot flesh				
white apricot	mean ± SD	2.6 ± 1.0 a	0.2 ± 0.3 a	0.2 ± 0.2 a	8.9 ± 4.0 a	12.6 ± 5.5 a	24.6 ± 9.6 a	456 ± 170 a
	range	1.4−4.9	0.0−0.8	0.0−0.6	3.8−17.5	6.6−21.9	12.4−43.4	228−824
yellow apricot	mean ± SD	7.3 ± 3.6 ab	0.1 ± 0.2 a	0.3 ± 0.4 a	9.7 ± 3.7 a	13.4 ± 5.9 a	30.9 ± 12.1 a	1219 ± 595 ab
	range	3.3−13.9	0.0−0.7	0.0−0.8	4.5−17.2	6.5−26.5	14.3−56.5	549−2319
light orange apricot	mean ± SD	9.2 ± 3.5 b	0.4 ± 0.4 a	0.3 ± 0.5 a	14.4 ± 3.6 ab	22.6 ± 5.9 b	47.0 ± 10.6 b	1574 ± 594 b
	range	4.8−15.8	0.0−1.0	0.0−1.7	9.1−20.2	15.4−32.9	31.7−61.7	804−2632
orange apricot	mean ± SD	20.1 ± 10.1 c	1.1 ± 1.2 b	0.3 ± 0.3 a	16.8 ± 9.9 b	27.8 ± 13.5 b	66.1 ± 25.2 c	3448 ± 1769 c
	range	9.8−35.2	0.0−3.8	0.0−0.8	0.9−33.1	12.3−49.7	39.6−104.0	1679−6178

[a] Values in mg kg^{-1} fresh weight. [b] Significant differences between groups are shown with different letters according to SNK's multiple-range test ($P < 0.05$). [c] IU of provitamin A/mg of fresh fruit = (166.7 × mg of β-carotene + 83.3 × mg of β-cryptoxanthin) kg^{-1} fresh fruit.

Table 3. Correlation Matrix Among Carotenoid Compounds in Both Peel and Flesh[a]

peel\flesh	β-carotene	β-cryptoxanthin	γ-carotene	phytofluene	phytoene
β-carotene	0.787**	0.736**	0.150 NS	0.399*	0.440**
β-cryptoxanthin	0.317*	0.464**	0.269 NS	0.348*	0.402*
γ-carotene	0.638**	0.192 NS	0.481**	0.096 NS	0.033 NS
phytofluene	−0.089 NS	0.058 NS	−0.055 NS	0.473**	0.934**
phytoene	0.432**	0.524**	0.351*	0.524**	0.859**

[a] Values to the left of the diagonal represent correlation coefficients between the different carotenoid concentrations in peel; values above diagonal represent correlation coefficients between the different carotenoid concentrations in flesh. Values to the right of the diagonal represent correlation coefficients for each carotenoid between contents in peel and in flesh. Pearson's correlation coefficients. NS, nonsignificant; * and **, significant at $P \leq 0.05$ or 0.01, respectively.

groups except for white−yellow and yellow−light orange apricots in both peel and flesh (**Table 2**). Very low contents of β-cryptoxanthin and γ-carotene were found in the apricot flesh, and only the peel of colored genotypes showed an appreciable quantity of these compounds. Significant differences among white, yellow, and light orange apricots were not found for these carotenoid compounds, and only orange apricots showed significantly higher β-cryptoxanthin contents in flesh and higher γ-carotene contents in peel. Obtained values for β-carotene, β-cryptoxanthin, and γ-carotene are in agreement with the range of results found previously in apricot (*7, 9, 10, 33*). Phytofluene and phytoene contents were very variable in both peel and flesh. Goldrich and Moniquí cultivars are characterized by a high content of these carotenoids (*24*); therefore, high values also were found in some genotypes issued from their crossing. For phytofluene content, significant differences were not observed among groups in peel, while orange apricots showed significantly higher contents in flesh. In the case of phytoene content, white and yellow apricots showed significant differences with light orange and orange apricots in both peel and flesh (**Table 2**).

The provitamin A activity in apricot peel varied from 497 IU in white apricots to 23834 IU in orange apricots, while the provitamin A content in apricot flesh ranged from 228 IU in the flesh of white genotypes to 6178 IU in the orange ones (**Table 2**). Significant differences were observed among groups except for white−yellow and yellow−light orange apricots in both peel and flesh.

The β-carotene content and provitamin A activity was 2−3 times higher in the peel than in flesh, while phytoene and phytofluene concentrations were similar in peel and flesh. The peel of apricot is consumed as an edible portion in contrast with other fruits such as peaches. The β-carotene content in edible portion ranged from 1.5 to 43.9 mg kg^{-1} edible portion, while the total carotenoid contents varied from 12.1 to 113.3 mg kg^{-1}. The provitamin A activity in the edible portion also showed a wide range of variability, from 252 to 7684 IU. The carotenoid content in apricot is higher than in other stone fruits such as nectarine, peach, and plum (*12*).

Correlations between concentrations of different carotenoid compounds were calculated in both peel and flesh (**Table 3**). Significant correlations were found between β-carotene and γ-carotene in peel and β-cryptoxanthin in flesh, while concentrations of β-carotene on the one hand and phytoene and phytofluene on the other hand varied independantly (**Table 3**). There were significant correlations between phytoene and phytofluene concentrations in both peel and flesh. This can be linked to the biosynthetic pathway of carotenoids. Phytoene is directly concerted into phytofluene by phytoene desaturase, and γ-carotene is converted into β-carotene by lycopene-β-cyclase, while these two couples of compounds, colorless and colored, are separated by two symmetric dehydrogenation steps and two cyclization steps (*34*). Therefore, colored carotenoids are closely related, and the same is true for colorless carotenoids.

Significant correlations were found for individual carotenoids between their concentrations in peel and in flesh (**Table 3**). They were especially high for β-carotene and phytoene. This means that estimating β-carotene and phytoene contents by reflectance measurements, which are limited to superficial tissues, is relevant for the whole fruit.

Relationships between Color and Carotenoids Content. Correlation coefficients between color parameters and carotenoid

Table 4. Correlation Matrix Among Carotenoids Content (mg kg^{-1} Fresh Weight) and Color Values (Reflectance Measurements L^*, a^*, b^*, h_{ab}, C_{ab}^*, and a^*/b^*)[a]

	L^*	a^*	b^*	h_{ab}	C_{ab}^*	a^*/b^*
			apricot peel			
β-carotene	−0.714**	0.812**	0.452**	−0.781**	0.624**	0.793**
β-cryptoxanthin	−0.218 NS	0.363*	0.264 NS	−0.360*	0.323*	0.363*
γ-carotene	−0.458**	0.575**	0.236 NS	−0.570**	0.371*	0.574**
phytofluene	−0.021 NS	0.020 NS	−0.201 NS	−0.063 NS	−0.155 NS	0.062 NS
phytoene	−0.304 NS	0.591**	0.307 NS	−0.581**	0.429**	0.583**
total carotenoids	−0.643**	0.817**	0.418**	−0.796**	0.598**	0.806**
provitamin A	−0.708**	0.811**	0.460**	−0.779**	0.629**	0.791**
			apricot flesh			
β-carotene	−0.311 NS	0.786**	0.418**	−0.802**	0.536**	0.813**
β-cryptoxanthin	−0.289 NS	0.394*	0.108 NS	−0.439**	0.187 NS	0.442**
γ-carotene	−0.246 NS	0.222 NS	0.120 NS	−0.242 NS	0.153 NS	0.245 NS
phytofluene	−0.023 NS	0.488**	0.361*	−0.470**	0.408**	0.461**
phytoene	−0.245 NS	0.542**	0.377*	−0.526**	0.432**	0.513**
total carotenoids	−0.315*	0.715**	0.448**	−0.710**	0.536**	0.706**
provitamin A	−0.313*	0.778**	0.409**	−0.796**	0.527**	0.806**

[a] Pearson's correlation coefficients. NS, nonsignificant; * and **, significant at $P \leq 0.05$ or 0.01, respectively.

Table 5. Statistics of PLS Models for Carotenoid Prediction by Using Color Values

			calibration		cross-validation	
	color coordinates	equation	R^2	RMSEC[a] (%)	R^2	RMSEV[b] (%)
		apricot peel				
ln β-carotene	L^*, h_{ab}, C_{ab}^*	$10.411 - 0.098L^* - 0.048h_{ab} + 0.061C_{ab}^*$	0.89	11.59	0.89	10.38
ln provitamin A	L^*, h_{ab}, C_{ab}^*	$14.499 - 0.089L^* - 0.043h_{ab} + 0.064C_{ab}^*$	0.91	3.30	0.89	4.70
ln total carotenoids	a^*	$3.022 + 0.074a^*$	0.81	6.44	0.77	10.78
		apricot flesh				
ln β-carotene	a^*/b^*	$-0.011 + 6.790a^*/b^*$	0.85	13.54	0.91	15.48
ln provitamin A	a^*/b^*	$5.158 + 6.788a^*/b^*$	0.91	3.79	0.79	3.79
ln total carotenoids	L^*, h_{ab}, C_{ab}^*	$7.118 - 0.032L^* - 0.044h_{ab} + 0.038C_{ab}^*$	0.68	8.26	0.75	7.68
		edible portion				
ln β-carotene	L^*, a^*, b^* (peel)	$8.071 - 0.096L^* + 0.064a^* - 0.002b^*$	0.81	12.15	0.81	16.19
ln provitamin A	L^*, h_{ab}, C_{ab}^* (peel)	$18.391 - 0.109L^* - 0.0514h_{ab} + 0.007C_{ab}^*$	0.84	4.54	0.78	5.38
ln total carotenoids	a^* (peel)	$3.008 + 0.081a^*$	0.77	7.67	0.82	7.94
ln β-carotene	a^*/b^* (flesh)	$-0.052 + 7.529a^*/b^*$	0.90	12.51	0.87	15.25
ln provitamin A	L^*, h_{ab}, C_{ab}^* (flesh)	$15.772 - 0.004L^* - 0.116h_{ab} + 0.008C_{ab}^*$	0.91	3.56	0.91	3.48
ln total carotenoids	L^*, h_{ab}, C_{ab}^* (flesh)	$6.504 - 0.002L^* - 0.045h_{ab} + 0.035C_{ab}^*$	0.70	7.89	0.71	7.26

[a] RMSEC, root mean square error of calibration. [b] RMSEV, root mean square error of validation.

compounds were assessed in peel and flesh (**Table 4**). The color parameters a^*, h_{ab}, and a^*/b^* ratio showed the highest correlation coefficients with carotenoids in both peel and flesh. The β-carotene content and provitamin A activity were highly correlated ($r = 0.81$) with a^* value in peel and a^*/b^* ratio in flesh. A very high correlation ($r = 0.82$) was found between a^* value and total carotenoids in peel. The correlation coefficient was slightly lower for total carotenoids in flesh ($r = 0.71$). Previous work (*10*) found similar correlation coefficients with β-carotene content and total provitamin A carotenoids in apricot peel using h_{ab} value and a slightly higher correlation values in the case of apricot flesh. The a^* color value also was found to be the best correlated color parameter with β-carotene content in orange and yellow flesh sweet potatoes (*13*). Contrary to our results, the b^* value was the best correlated color parameter with β-carotene in white-fleshed sweet potatoes (*14*), wheat (*17, 18*), and ultrafrozen orange juices (*16*).

Significant correlations were also observed between color parameters and β-cryptoxanthin and γ-carotene contents, although correlation coefficients were relatively low (**Table 4**) as compared with values obtained previously (*10*), probably due to low concentrations.

As expected for colorless carotenoids, low or no correlations were found between phytoene and phytofluene contents and color parameters in both peel and flesh (**Table 4**). The correlations between these carotenoid compounds and β-carotene were also low (**Table 3**). Therefore, the prediction of phytoflene and phytoene content is not possible by means of color measurements.

According to the exponential relationship between carotenoids content and color values, a logarithmic transformation on carotenoids data was carried out prior to regression analysis between carotenoids content measured by HPLC and color values in peel and flesh. PLS regression analyses were established between color parameters and β-carotene content, provitamin A activity, and total carotenoids. The sets of color coordinates L^*, a^*, and b^* and L^*, h_{ab}, and C_{ab}^* were considered together as predictor variables due to the three-dimensional nature of color. Models for assessing vitamin A activity using these sets of color measurements have been developed in orange juices with good prediction results (*16, 19*). Prediction models for β-carotene content, provitamin A activity, and total carotenoids were developed in peel, flesh, and edible portions using the most informative color measurements, and a series of equations for their assessment are given (**Table 5**). For each model, R^2 values and RMSEP for calibration and cross-validation were calculated (**Table 5**).

In the apricot peel, the regression analyses between the set of color coordinates L^*, h_{ab}, C_{ab}^*, and ln(β-carotene content) or ln(provitamin A activity) gave a high fit on calibration (R^2

= 0.89 and 0.91, respectively) and cross-validation ($R^2 = 0.89$). These values were higher than obtained in a previous study in apricot (*10*) without logarithmic transformation on carotenoids data, and they are close to those obtained in orange juice for vitamin A activity (*19*). The relationship between a^* value and ln(total carotenoids) in the peel showed a lower R^2 (**Table 5**). The regression analyses between a^*/b^* ratio in the flesh and both ln(β-carotene content) and ln(provitamin A activity) showed the highest R^2 values ($R^2 = 0.85$ and 0.91 on calibration and cross-validation, respectively, for β-carotene content and $R^2 = 0.91$ and 0.79 for provitamin A activity). Previous work on tomato also found a good fit between a^*/b^* ratio and lycopene content (*15*), and equations to calculate the lycopene content of tomatoes based on the color readings were reported. The set of color coordinates L^*, h_{ab}, and C_{ab}^* was the best correlated with total carotenoids in the flesh, although R^2 values were relatively low (**Table 5**). Regarding the edible portion, results of calibration and cross-validation models showed high R^2 for ln(β-carotene content) and ln(provitamin A activity), especially by using flesh color parameters, while R^2 was lower in the case of ln(total carotenoids) (**Table 5**). Prediction of total carotenoids by color measurements was not satisfactory. This is linked to the presence of colorless compounds such as phytoene and phytofluene.

We obtained a very low prediction error on both calibration and cross-validation for the assessment of ln(provitamin A activity) in peel, flesh, and edible portion (RMSE between 3.30 and 5.38%). The prediction error was also low for ln(total carotenoids) (RMSE between 6.44 and 10.78%), and it was acceptable in the case of ln(β-carotene content) (**Table 5**).

Therefore, it should be possible to predict β-carotene content and provitamin A activity in apricots fairly accurately using a portable chromameter tristimulus color, with a possible field usage application. For example, β-carotene content and provitamin A activity in the edible portion could be estimated by nondestructive color measurements in the apricot peel by means of regression equations (**Table 5**).

Prediction models by color measurements can provide a useful diagnostic for rapid screening of apricot cultivars for carotenoid content and provitamin A activity. It could be very useful for the apricot industry and apricot breeding programs especially for grouping fruit or apricot genotypes into different ranges of values. However, these models should be checked in other apricot cultivars to verify their accuracy and improve their robustness.

FT-NIR and FT-MIR Models for Carotenoid Determination. Results of calibration and cross-validation FT-NIR models for all carotenoid compounds in the peel and flesh are summarized in **Table 6**. The spectral range used for developing NIR models was from 1111 to 2500 nm, which was the most suitable wavelength interval linked with carotenoids content. The number of LV or factors obtained in developed PLS models for each carotenoid compound ranged from 5 (γ-carotene in flesh) to 11 (β-carotene in peel and total carotenoids in peel, flesh, and edible portion). Calibration models showed high R^2 for all carotenoid compounds except for γ-carotene in flesh, as well as an acceptable calibration error (**Table 6**). However, cross-validation of NIR models showed very low R^2 and very high prediction error in all cases (**Table 6**), which makes the prediction of carotenoids content impossible. Therefore, we can assert that application of FT-NIR technique is inappropriate for determination of carotenoids content in apricot, probably due to their low concentrations and relatively less intense absorption bands in their wavelength region. Studies in apricot fruit should be continued to improve the FT-NIR spectroscopy efficiency, which means at least an optimization of the spectral range including the use of the visible spectroscopy, as well as the improvement of the accuracy in secondary metabolite detection.

Table 6. NIR PLS Regression Statistics of Calibration and Cross-Validation for Different Carotenoids in Apricot

			calibration		cross-validation	
carotenoid compound	λ range (nm)	LV	R^2	RMSEC	R^2	RMSEV
apricot peel						
β-cryptoxanthin	1111−2500	9	0.87	1.07	0.01	4.75
γ-carotene	1111−2500	8	0.81	1.13	0.25	5.37
β-carotene	1111−2500	11	0.94	8.09	0.26	44.02
phytofluene	1111−2500	9	0.86	4.29	0.04	19.13
phytoene	1111−2500	8	0.96	2.24	0.04	20.93
total carotenoids	1111−2500	11	0.97	7.59	0.33	72.20
provitamin A	1111−2500	9	0.93	716.59	0.17	11200.09
apricot flesh						
β-cryptoxanthin	1111−2500	6	0.67	0.54	0.20	0.99
γ-carotene	1111−2500	5	0.33	0.33	0.12	0.37
β-carotene	1111−2500	8	0.83	3.14	0.01	18.74
phytofluene	1111−2500	7	0.61	3.77	0.13	10.14
phytoene	1111−2500	9	0.95	1.47	0.27	17.39
total carotenoids	1111−2500	11	0.99	2.05	0.02	24.40
provitamin A	1111−2500	10	0.92	367.97	0.06	2707.95

Table 7. MIR PLS Regression Statistics of Calibration and Cross-Validation for Different Carotenoids in Apricot Flesh

			calibration		cross-validation	
carotenoid compound	λ range (cm^{-1})	LV	R^2	RMSEC	R^2	RMSEV
β-cryptoxanthin	940−1200	11	0.95	0.14	0.22	0.88
γ-carotene	940−1200	5	0.32	0.26	0.10	0.40
β-carotene	940−1200	9	0.81	4.08	0.44	9.02
phytofluene	940−1200	8	0.68	4.23	0.26	7.63
phytoene	940−1200	8	0.75	5.52	0.16	17.42
total carotenoids	940−1200	11	0.87	7.62	0.33	25.18
provitamin A	940−1200	9	0.90	288.63	0.30	1533.97

Calibration and cross-validation PLS models obtained by using FT-MIR spectroscopy technique in apricot flesh are shown in **Table 7**. After testing different wavelength intervals, the spectral range from 940 to 1200 cm^{-1} was selected for developing prediction models. The number of LVs in developed PLS models also ranged from 5 (γ-carotene) to 11 (β-cryptoxanthin and total carotenoids). As in NIR models, calibration results for developed MIR models showed high R^2 for all carotenoid compounds except for γ-carotene and an acceptable calibration error (**Table 7**). In accordance with cross-validation results, we found low coefficients of determination in PLS models developed for FT-MIR analysis of carotenoid compounds, although R^2 values were in general higher than in the case of NIR models. The MIR models also showed a high validation error (**Table 7**). So, prediction of carotenoids content in apricot is not possible by using FT-MIR technique, probably for the same reasons as for NIR, that is, relative intensity of absorption bands as compared to major compounds such as sugars and acids.

ABBREVIATIONS USED

IS, internal standard; R_t, retention time; H°, hue angle; C^*, chroma; FT-NIR, Fourier transform near-infrared; FT-MIR, Fourier transform mid-infrared; ATR, attenuated total reflectance; RMSEP, root-mean-square error of prediction; LVs, latent variables; RMSEC, root-mean-square error of calibration; RMSEV, root-mean-square error of validation.

LITERATURE CITED

(1) Nishino, H.; Tokuda, H.; Satomi, Y.; Masuda, M.; Bu, P.; Onozuka, M.; Yamaguchi, S.; Okuda, Y.; Takayasu, J.; Tsuruta, J.; Okuda, M.; Ichiishi, E.; Murakoshi, M.; Kato, T.; Misawa, N.; Narisawa, T.; Takasuka, N.; Yano, M. Cancer prevention by carotenoids. *Pure Appl. Chem.* **1999**, *71*, 2273–2278.

(2) Olson, J. A. (1999). Carotenoids and human health. *Arch. Latinoam. Nutr.* **1999**, *49*, 7–11.

(3) Stahl, W.; Ale-Agha, N.; Polidori, M. C. Non-antioxidant properties of carotenoids. *J. Biol. Chem.* **2002**, *383*, 553–558.

(4) Stahl, W.; Sies, H. Antioxidant activity of carotenoids. *Mol. Aspects Med.* **2003**, *24*, 345–351.

(5) Voutilainen, S.; Nurmi, T.; Mursu, J.; Rissanen, T. H. Carotenoids and cardiovascular health. *Am. J. Clin. Nutr.* **2006**, *83*, 1265–1271.

(6) Curl, A. L. The carotenoids of apricots. *Food Res.* **1960**, *24*, 190–196.

(7) Ben-Amotz, A.; Fishler, R. Analysis of carotenoids with emphasis on 9-*cis* β-carotene in vegetables and fruits commonly consumed in Israel. *Food Chem.* **1998**, *62*, 515–520.

(8) Brokmann, H. Die carotinoide der aprikose (*Prunus armeniaca*). *Z. Physiol. Chem.* **1933**, *216*, 45.

(9) Katayama, T.; Nakayama, T. O. M.; Lee, T. H.; Chichester, C. O. Carotenoid transformations in ripening apricots and peaches. *J. Food Sci.* **1971**, *36*, 804–806.

(10) Ruiz, D.; Egea, J.; Tomás-Barberán, F. A.; Gil, M. I. Carotenoids from new apricot (*Prunus armeniaca* L.) varieties and their relationship with flesh and skin color. *J. Agric. Food Chem.* **2005**, *53*, 6368–6374.

(11) Marty, I.; Bureau, S.; Sarkissian, G.; Gouble, B.; Audergon, J. M.; Albagnac, G. Ethylene regulation of carotenoid accumulation and carotenogenic gene expression in colour-contrasted apricot varieties (*Prunus armeniaca*). *J. Exp. Bot.* **2005**, *56*, 1877–1886.

(12) Gil, M. I.; Tomás-Barberán, F. A.; Hess-Pierce, B.; Kader, A. A. Antioxidant capacity, phenolic compounds, carotenoids and vitamin C of nectarine, peach and plum cultivars from California. *J. Agric. Food Chem.* **2002**, *50*, 4976–4982.

(13) Takahata, Y.; Noda, T.; Nagata, T. HPLC determination of β-carotene content of sweet potato cultivars and its relationship with color values. *Jpn. J. Breed.* **1993**, *43* (3), 421–427.

(14) Ameny, M. A.; Wilson, P. W. Relationship between hunter color values and β-carotene contents in white-fleshed African sweetpotatoes (*Ipomoea batatas* Lam). *J. Sci. Food Agric.* **1997**, *73*, 301–306.

(15) Arias, R.; Lee, T. C.; Logendra, L.; Janes, H. Correlation of lycopene measured by HPLC with the *L**, *a**, *b** color readings of a hydroponic tomato and the relationship of maturity with color and lycopen content. *J. Agric. Food Chem.* **2000**, *48*, 1697–1702.

(16) Meléndez-Martínez, A. J.; Vicario, I. M.; Heredia, F. J. Application of tristimulus colorimetry to estimate the carotenoids content in ultrafrozen orange juices. *J. Agric. Food Chem.* **2003**, *51*, 7266–7270.

(17) Humphries, J. M.; Graham, R. D.; Mares, D. J. Application of reflectance colour measurement to the estimation of carotene and lutein content in wheat and triticale. *J. Cereal Sci.* **2004**, *40*, 151–159.

(18) Fratianni, A.; Irano, M.; Panfili, G.; Acquistucci, R. Estimation of color of durum wheat. Comparison of WSB, HPLC, and reflectance colorimeter measurements. *J. Agric. Food Chem.* **2005**, *53*, 2373–2378.

(19) Meléndez-Martínez, A. J.; Vicario, I. M.; Heredia, F. J. Rapid assessment of vitamin A activity through objective color measurements for the quality control of orange juices with diverse carotenoid profiles. *J. Agric. Food Chem.* **2007**, *55*, 2808–2815.

(20) Brenna, O. V.; Berardo, N. Application of near-infrared spectroscopy (NIRS) to the evaluation of carotenoids content in maize. *J. Agric. Food Chem.* **2004**, *52*, 5577–5582.

(21) Atienza, S. G.; Avila, C. M.; Ramirez, M. C.; Martin, A. Application of near infrared spectroscopy to the determination of carotenoid content in tritordeum for breeding purposes. *Aust. J. Agric. Res.* **2005**, *56*, 85–89.

(22) Pedro, A. M. K.; Ferreira, M. M. C. Nondestructive determination of solids and carotenoids in tomato products by near-infrared spectroscopy and multivariate calibration. *Anal. Chem.* **2005**, *77*, 2505–2511.

(23) Baranska, M.; Schütze, W.; Schulz, H. Determination of lycopene and β-carotene content in tomato fruits and related products: comparison of FT-Raman, ATR-IR, and NIR spectroscopy. *Anal. Chem.* **2006**, *78*, 8456–8461.

(24) Bureau, S.; Chahine, H.; Gouble, B.; Reich, M.; Albagnac, G.; Audergon, J. M. Fruit ripening of contrasted apricot varieties: Physical, physiological and biochemical changes. *Acta. Hortic.* **2006**, 511–516.

(25) CIE Recommendations on Uniform Color Spaces, Color-Difference Equations, Psychometric Color Terms, CIE Publication No. 15 (E-1.3.1) 1971, Supplement 2; Bureau Centralde la CIE: Vienna, Austria, 1978.

(26) Wright, K. P.; Kader, A. A. Effect of controlled-atmosphere storage on the quality and carotenoid content of sliced persimmons and peaches. *Postharvest Biol. Technol.* **1997**, *10*, 89–97.

(27) Hart, D. J.; Scott, K. J. Development and evaluation of an HPLC method for the analysis of carotenoids in foods and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chem.* **1995**, *54*, 101–111.

(28) Bertrand, D. Unité de Sensométrie et de Chimiométrie ENITIAA-INRA, Rue de la Géraudi-ere-BP 82 225, 44322, Nantes, France, 2007.

(29) Lecomte, A. Plantes et Systèmes de culture Horticoles, INRA-Avignon, Domaine St. Paul, 84914, Avignon, France, 2007.

(30) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Standard normal variate transformation and detrending of near infrared diffuse reflectance spectra. *Appl. Spectrosc.* **1989**, *43*, 772–777.

(31) Martens, H.; Naes, T. Multivariate calibration by data compression. In *Near-Infrared Technology in the Agricultural and Food Industries*; Williams, P., Norris, K., Eds.;Am. Assoc. Cereal Chemists: St. Paul, MN, 1987.

(32) Müller, H. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Z. Lebensm. Unters. Forsch.* **1997**, *204*, 88–94.

(33) Dragovic-Uzelac, V.; Levaj, B.; Mrkic, V.; Bursac, D.; Boras, M. The content of polyphenols and carotenoids in three apricot cultivars depending on stage of maturity and geographical region. *Food Chem.* **2007**, *102*, 966–975.

(34) Hirschberg, J. Carotenoid biosynthesis in flowering plants. *Curr. Opin. Plant Biol.* **2001**, *4*, 210–218.

Received for review December 12, 2007. Revised manuscript received March 13, 2008. Accepted March 28, 2008. We are grateful to the Spanish Ministry of Education and Science for a grant to D.R. (reference EX2006-1870).

JF7036032