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Relationship between the colour and the chemical structure of carotenoid pigments

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Abstract

Several carotenoids were isolated and their colours were ascertained objectively to establish relationships between the chemical structures of those pigments and their actual colours, considering the coordinates of the uniform space CIELAB. The results of this study revealed that the different carotenoids surveyed could be grouped in the a^*b^* plane according to the number of conjugated double bonds. For yellowish carotenoids, it was observed that a^* values clearly decreased from those with 11 conjugated double bonds (c.d.b.) to those with 9 c.d.b., although this trend reversed in the case of carotenoids with 7 c.d.b.. In terms of hue (h_{ab}), it was seen that the decrease in conjugation of the molecules involved a slight rise in h_{ab}. On the other hand, the aperture of the end rings or the increase in conjugation involved clear increases in hue.

Keywords: Carotenoids; CIELAB; Colour; Colour–chemical structure; Colorimetry

1. Introduction

Carotenoid pigments account for the natural yellow, orange or red colours of many foods. Therefore, they are directly related to the perception of their quality, as colour does influence the consumers' preferences (Clydesdale, 1993; Meléndez-Martínez, Vicario, & Heredia, 2004a, 2005; Roth et al., 1988; Tepper, 1993). In addition to this role, it is well-known that some of them show vitamin A activity (Meléndez-Martínez, Britton, Vicario, & Heredia, 2005a; Olson, 1989; Rodriguez-Amaya, 1997). Interest in these compounds has risen considerably lately because of their likely involvement in the prevention or protection against serious human health disorders, such as heart disease, cancer and macular degeneration, among others (Fraser & Bramley, 2004; Giovanucci, 1999; Krinsky, 2001; Meléndez-Martínez, Vicario, & Heredia, 2004b; Olson, 1999).

The UV/Vis spectrum of carotenoids is of great importance for analysts, because it provides valuable information about their structure. The spectrum is due to the presence of the long chromophore of conjugated double bonds (c.d.b.). Organic molecules can absorb ultraviolet or visible light. As a consequence of the absorption of light, electronic transitions occur, which lead to a higher energy or excited state of the molecule. In the case of carotenoids, electronic transitions are from π to π^* orbitals. Due to the delocalization of the electrons along the chromophore, the excited state of the molecule is of low energy so that, in general, absorption of visible light is enough to give rise to the transitions (Britton, 1995). At least 7 c.d.b. are needed for a carotenoid to have perceptible colour. Thus, ζ-carotene, with 7 c.d.b., has a slight yellowish colour, whereas phytoene and phytofluene (3 and 5 c.d.b., respectively) are colourless (Britton, 1995; Rodriguez-Amaya, 2001).

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The influence of the chemical structure of carotenoids on the location of their absorption maxima and the shape of the spectra is well-known (Britton, 1992, 1995; Mínguez-Mosquera, 1997), although it has not been exhaustively studied in terms of colour coordinates, despite the fact that objective measurement of colour by means of colorimetry is being increasingly used for the analysis of carotenoid pigments (Arias, Lee, Logendra, & Janes, 2000; Meléndez-Martínez, Britton, Vicario, & Heredia, 2005b; Meléndez-Martínez, Vicario, & Heredia, 2003; Mínguez-Mosquera, Rejano-Navarro, Gandul-Rojas, Sánchez-Gómez, & Garrido-Fernández, 1991; Sinnecker, Gomes, Arêas, & Lanfer-Marquez, 2002). Objective measurement of colour is a non-destructive and very rapid technique that enables the analyst to obtain a series of parameters in a few seconds, which is a very useful tool for quality control of carotenoids in the industry (Meléndez-Martínez et al., 2003). The goal of this paper is to evaluate differences in the colours of several carotenoids as a consequence of differences in their chemical structures, in order to provide new data that facilitate the usage of the objective measurement of colour for the analysis of those compounds.

2. Materials and methods

2.1. Samples

Sixteen carotenoid pigments were surveyed (chemical structures in Fig. 1). α-Carotene, β-carotene, ζ-carotene, lutein, neoxanthin, violaxanthin, β-cryptoxanthin, zeaxanthin, lutein epoxide and lycopene were isolated from natural sources (Table 1) by column chromatography and/or thin-layer chromatography according to standard procedures (Britton, Liaaen-Jensen, & Pfander, 1995; Mínguez-Mosquera, 1997; Mínguez-Mosquera & Hornero-Méndez, 1993).

Antheraxanthin was obtained by treating zeaxanthin with 3-chloroperoxybenzoic acid (Barua & Olson, 2001; Meléndez-Martínez, Britton, Vicario, & Heredia, 2005c). Luteoxanthin and auroxanthin were obtained by treating violaxanthin with ethanolic HCl (0.1 M), whereas neochrome and mutatoxanthin were obtained in the same way from neoxanthin and antheraxanthin, respectively (Mínguez-Mosquera, 1997). Canthaxanthin was a gift from Hoffman-La Roche.

2.2. Colour measurement

Colour was appraised by means of an HP8452 UV/Vis diode-array spectrophotometer (Hewlett-Packard, Palo Alto, CA) with a wavelength accuracy of 2 nm. A 10 nm pathlength glass cuvette was used for the measurements and the whole visible spectrum (380–770 nm) was registered ($\Delta\lambda = 2$ nm). The colour parameters of the uniform colour space CIELAB (CIE, 1978) under CIE Illuminant D65 and 1964 Standard Colorimetric Observer were obtained by means of the software CromaLab© (Heredia, Álvarez, González-Miret, & Ramírez, 2004).

Due to the influence of the solvent on the spectra of carotenoids (Britton, 1995; Britton & Young, 1993), all the pigments were dissolved in the same solvent for the colour measurements. To minimize the influence of concentration on the colour coordinates, solutions equal in concentration (3.44×10^{-6} M), determined spectrophotometrically, were used. For this purpose the values of absorption coefficients reported in the literature were considered (Britton, 1995; Mínguez-Mosquera, 1997) (Table 2), and the corresponding molar absorption coefficients (ε) were calculated according to the formula $\varepsilon = (A^{1\%}_{1\,cm} \times P_m)/10$ (Britton, Liaaen-Jensen, & Pfander, 2004). When those values were not referred to acetone, solutions at the concentration considered were prepared in suitable solvents, after which 2 ml aliquots were taken, concentrated to dryness and re-dissolved in 2 ml of acetone for the colour measurements.

3. Results and discussion

The colour coordinates of the uniform space CIELAB corresponding to the acetone solutions of the carotenoids studied are shown in Table 3.

Absorption coefficients are difficult to obtain, so some reported values may have some significant level of error (Britton, 1995). In the case of lutein epoxide, two values of $A^{1\%}_{1\,cm}$ (2400 and 2800 at 441 nm in ethanol, Table 2) were considered. Taking into consideration that the same concentration (3.44×10^{-6} M) was considered in both cases, two spectra, differing in absorbance (0.48 and 0.56 at 441 nm, respectively), were obtained for the same pigment, which was useful to estimate to some extent the influence of absorbance, related to the actual concentration, on the colorimetric parameters. In this sense, it was seen that a^* and h_{ab} values were similar (−10.17 CIELAB units and 104.93°, respectively, for $A^{1\%}_{1\,cm} = 2400$; −11.85 CIELAB units and 104.50°, respectively, for $A^{1\%}_{1\,cm} = 2800$), whilst differences in b^* and C^*_{ab} were higher (38.17 and 39.50 CIELAB units, respectively, for $A^{1\%}_{1\,cm} = 2400$; 45.80 and 47.31 CIELAB units, respectively, for $A^{1\%}_{1\,cm} = 2800$). Accordingly, a^* and h_{ab} were mainly taken into consideration for ascertaining the influence of the chemical structure of carotenoids on their colour.

The absorption spectra of carotenoids depend largely on the number of conjugated double bonds (c.d.b.) in their molecules so, the longer the chromophore, the higher were the wavelengths of maximum absorption (Table 4). Acyclic carotenoids absorb maximally at longer wavelengths than cyclic carotenoids with the same number of c.d.b. in which conjugation extends into rings, due to the fact that, in the latter, there are steric strains (Britton & Young, 1993). Thus, as can be observed in Table 4, the dicyclic carotenoid canthaxanthin (13 c.d.b., 4 in rings) absorbs maximally in the same region as the acyclic lycopene (11 c.d.b., none in rings). Likewise, the absorption maximum of lycopene (11 c.d.b., none in rings) in acetone is located at a longer wavelength (474 nm) than that of β-carotene (11 c.d.b., 1

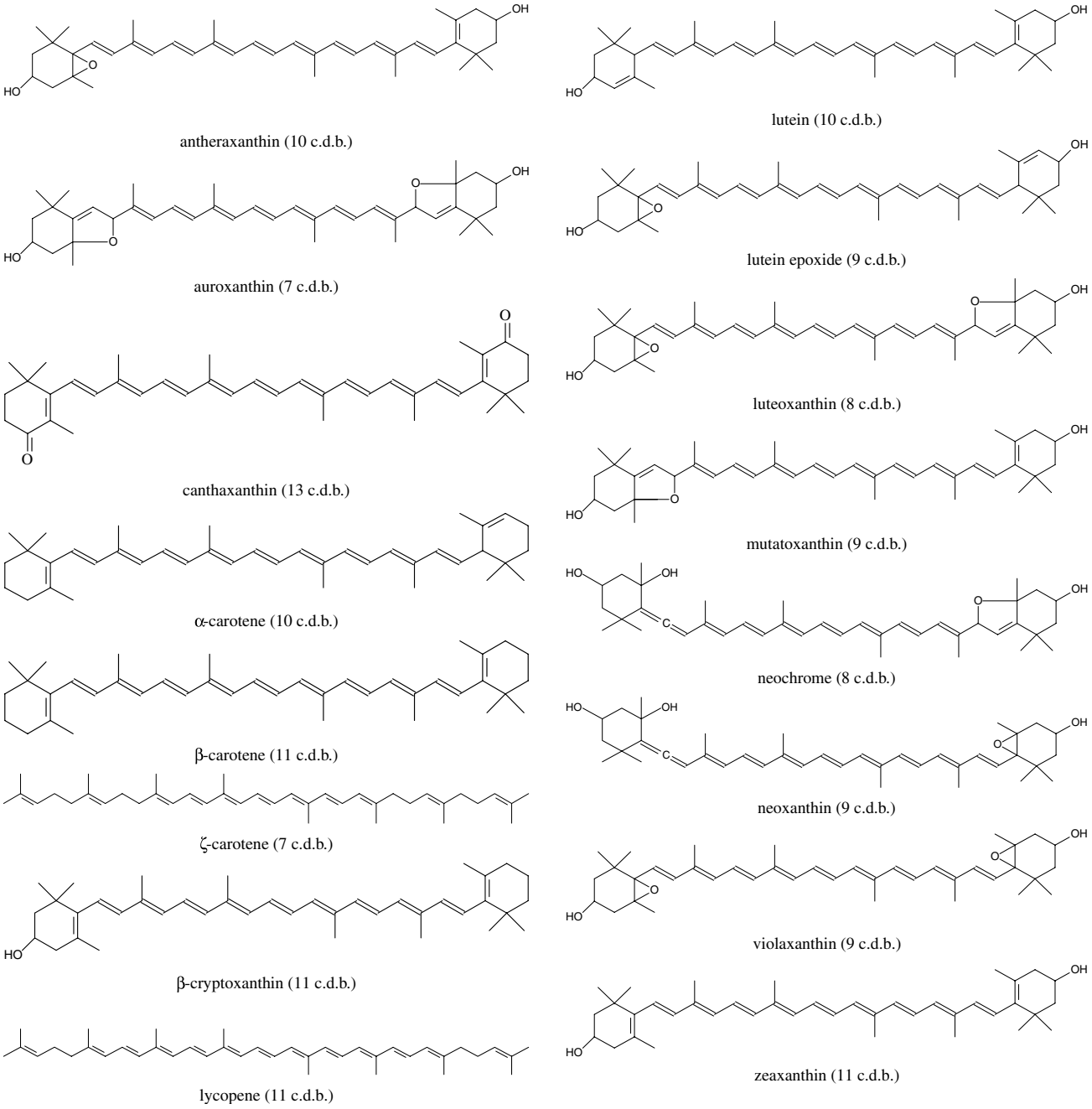


Fig. 1. Chemical structures of the carotenoids studied.

in rings) (454 nm), despite both pigments having the same number of c.d.b.

Location of the solutions of the pigments in acetone in the plane a^*b^* is displayed in Fig. 2. The reddish carotenoids, lycopene and canthaxanthin, were located in the first quadrant of the plane (positive values of a^* and b^*), whereas the yellowish ones were located near the b^* axis in the second quadrant (negative values of a^* and positive values of b^*). The latter carotenoids were grouped by the number of conjugated double bonds in their molecule (Fig. 2): 11 (β-carotene, β-cryptoxanthin and zeaxanthin, group 1), 10 (α-carotene, lutein and antheraxanthin, group 2), 9 (violaxanthin, neoxanthin, lutein epoxide and mutatoxanthin, group 3), 8 (luteoxanthin and neochrome, group 4) and 7 (auroxanthin and ζ-carotene, group 5). Considering these yellowish carotenoids, it was obvious that a^* values decreased from those with 11 c.d.b. to those with 9 c.d.b. As a result of the re-arrangement of one 5,6-epoxide group in neoxanthin and violaxanthin (9 c.d.b.) to form neochrome and luteoxanthin (Fig. 1), respectively, a conjugated double bond is lost, which involves a slight decrease in a^* as well as a decrease in b^*. However, the re-arrangement of the remaining 5,6-epoxide group in luteoxanthin to form auroxanthin, with seven conjugated double bonds

Table 1
Natural sources used for the isolation of carotenoids

Carotenoid	Source
α-Carotene	Palm oil (*Elaeis guineensis* Jacq.)
β-Carotene	Palm oil (*Elaeis guineensis* Jacq.)
ζ-Carotene	Orange juice (*Citrus sinensis* (L.) Osbeck)
Lutein	Spinach leaves (*Spinacia oleracea* L.)
Neoxanthin	Spinach leaves (*Spinacia oleracea* L.)
Violaxanthin	Spinach leaves (*Spinacia oleracea* L.)
β-Cryptoxanthin	Red peppers (*Capsicum annuum* L.)
Zeaxanthin	Red peppers (*Capsicum annuum* L.)
Lutein epoxide	Petals of dandelion (*Taraxacum officinale* F. Weber ex Wiggers)
Lycopene	Tomatoes (*Lycopersicon esculentum* Mill.)

Table 2
Specific absorption coefficients ($A^{1\%}_{1\,cm}$) of the carotenoid pigments studied

Carotenoid	c.d.b. (in rings)	$A^{1\%}_{1\,cm}$	Solvent	λ (nm)
Antheraxanthin	10 (1)	2349	Acetone	444
Auroxanthin	7 (0)	1850	Acetone	402
Canthaxanthin	13 (4)	2200	Petroleum ether	466
α-Carotene	10 (1)	2800	Petroleum ether	444
β-Carotene	11 (2)	2620	Acetone	450
ζ-Carotene	7 (0)	2555	Hexane	400
β-Cryptoxanthin	11 (2)	2386	Petroleum ether	449
Lutein	10 (1)	2340	Acetone	446
Lutein epoxide	9 (0)	2400 and 2800	Ethanol	441
Luteoxanthin	8 (0)	2340	Acetone	424
Lycopene	11 (0)	3450	Petroleum ether	470
Mutatoxanthin	9 (1)	2240	Acetone	424
Neochrome	8 (0)	2270	Acetone	420
Neoxanthin	9 (0)	2050	Acetone	438
Violaxanthin	9 (0)	2400	Acetone	442
Zeaxanthin	11 (2)	2340	Acetone	452

Table 3
Colour coordinates of the solutions in acetone of the carotenoids studied

Carotenoid	c.d.b. (in rings)	L^*	a^*	b^*	C^*_{ab}	h_{ab}
Antheraxanthin	10 (1)	96.51	−7.35	44.24	44.84	99.43
Auroxanthin	7 (0)	99.23	−5.21	12.84	13.86	112.09
Canthaxanthin	13 (4)	94.03	8.72	30.73	31.95	74.16
α-Carotene	10 (1)	96.90	−6.69	42.38	42.90	98.96
β-Carotene	11 (2)	95.84	−3.11	44.89	45.00	93.96
ζ-Carotene	7 (0)	97.29	−5.43	12.74	13.85	113.07
β-Cryptoxanthin	11 (2)	94.71	−5.12	52.37	52.62	95.58
Lutein	10 (1)	93.93	−6.18	37.39	37.89	99.38
Lutein epoxide (1)	9 (0)	96.91	−10.17	38.17	39.50	104.93
Lutein epoxide (2)	9 (0)	97.35	−11.85	45.80	47.31	104.50
Luteoxanthin	8 (0)	96.89	−10.89	31.31	33.15	109.18
Lycopene	11 (0)	93.31	8.60	36.05	37.06	76.59
Mutatoxanthin	9 (1)	98.00	−11.34	36.18	37.91	107.41
Neochrome	8 (0)	97.78	−11.07	31.32	33.22	109.46
Neoxanthin	9 (0)	99.89	−10.53	37.16	38.62	105.82
Violaxanthin	9 (0)	97.42	−10.05	41.89	43.08	103.49
Zeaxanthin	11 (2)	95.87	−3.88	42.48	42.66	95.22

(Fig. 1), involved a dramatic rise in a^* and a marked drop in b^*, so that its location in the a^*b^* plane matched with that of the acyclic ζ-carotene. Thus, a^* values of these carotenoids with 7 c.d.b. were similar to those of the carotenoids with 10 c.d.b. studied, although b^* values were dramatically lower.

Table 4
Absorption maxima in acetone and number of conjugated double bonds of the carotenoid pigments studied

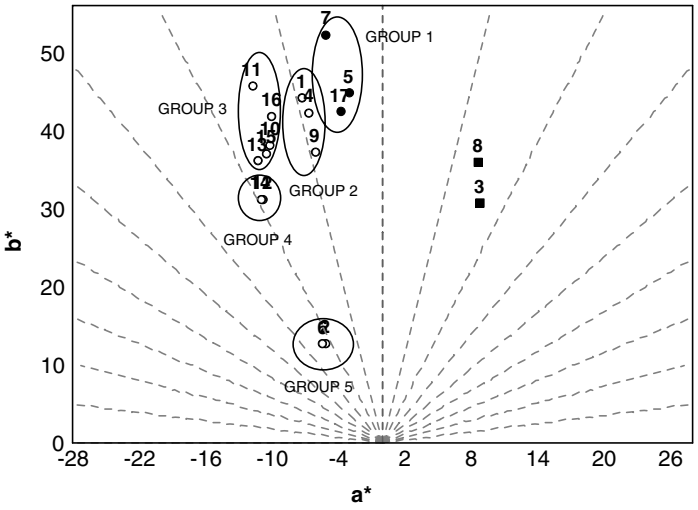
Carotenoid	c.d.b. (in rings)	Absorption maxima (nm)		
Canthaxanthin	13 (4)		472	
Lycopene	11 (0)	446	474	504
β-Carotene	11 (2)		454	480
β-Cryptoxanthin	11 (2)		452	478
Zeaxanthin	11 (2)		454	480
α-Carotene	10 (1)	424	448	476
Lutein	10 (1)	424	448	476
Antheraxanthin	10 (1)	424	448	476
Violaxanthin	9 (0)	420	442	472
Lutein epoxide	9 (0)	416	440	468
Neoxanthin	9 (0)	416	438	466
Mutatoxanthin	9 (1)	406	428	452
Luteoxanthin	8 (0)	402	424	450
Neochrome	8 (0)	400	424	450
ζ-Carotene	7 (0)	380	402	426
Auroxanthin	7 (0)	382	402	428

In terms of hue (h_{ab}), the qualitative attribute of colour, distinction between the reddish carotenoids, canthaxanthin and lycopene ($h_{ab} = 74.16°$ and 76.59°, respectively) and the remainder, with hue values ranging from 93.96° to 113.07°, was clear. In general, it was seen that the aperture of the end rings or the increase in conjugation involved clear rises in hue.

Differences in hue among the acyclic carotenoid lycopene ($h_{ab} = 76.59°$) and the cyclic ones, β-carotene, β-cryptoxanthin and zeaxanthin ($h_{ab} \approx 105°$), all of them with 11 c.d.b., are due to the presence of two c.d.b. in rings in the latter. As a result of this, the c.d.b. in rings are not coplanar with the main polyene chain and steric strain take place, so that their absorption maxima ($\lambda_{max} \approx 454$ nm) are located at shorter wavelengths than those of acyclic carotenoids with the same number of c.d.b. (Britton, 1991, 1995), in this case lycopene ($\lambda_{max} = 474$ nm). This fact also explains that the hue of canthaxanthin, with 13 c.d.b. and 4 of them in rings (Fig. 1), was similar to that of lycopene.

Taking into consideration the remaining carotenoids surveyed, it was obvious that the loss of a c.d.b. led to a slight increase in hue. Thus, hue values of carotenoids with 10 c.d.b. (α-carotene, lutein and antheraxantin) and 9 c.d.b. (violaxanthin, lutein epoxide, neoxanthin and mutatoxanthin) were around 99° and 105°, respectively, whilst those of carotenoids with 8 c.d.b. (luteoxanthin and neochrome) and 7 c.d.b. (auroxanthin and ζ-carotene) were around 109° and 112–113°, respectively.

To correlate type of chromophore, absorption maxima and colour, in terms of hue, the visible spectra of ζ-carotene, lycopene, β-carotene, canthaxanthin, antheraxanthin, violaxanthin, mutatoxanthin, luteoxanthin and auroxanthin were taken into account, to avoid redundant information (Fig. 3).



1.Antheraxanthin (10 c.d.b.)
2.Auroxanthin (7 c.d.b.)
3.Canthaxanthin (13 c.d.b.)
4.α-Carotene (10 c.d.b.)
5.β-Carotene (11 c.d.b.)
6.ζ-Carotene (7 c.d.b.)
7.β-Cryptoxanthin (11 c.d.b.)
8.Lycopene (11 c.d.b.)
9.Lutein (10 c.d.b.)
10.Lutein epoxide (1) (9 c.d.b.)
11.Lutein epoxide (2) (9 c.d.b.)
12.Luteoxanthin (8 c.d.b.)
13.Mutatoxanthin (9 c.d.b.)
14.Neochrome (8 c.d.b.)
15.Neoxanthin (9 c.d.b.)
16.Violaxanthin (9 c.d.b.)
17.Zeaxanthin (11 c.d.b.)

Fig. 2. Location of the carotenoids studied within the a^*b^* plane.

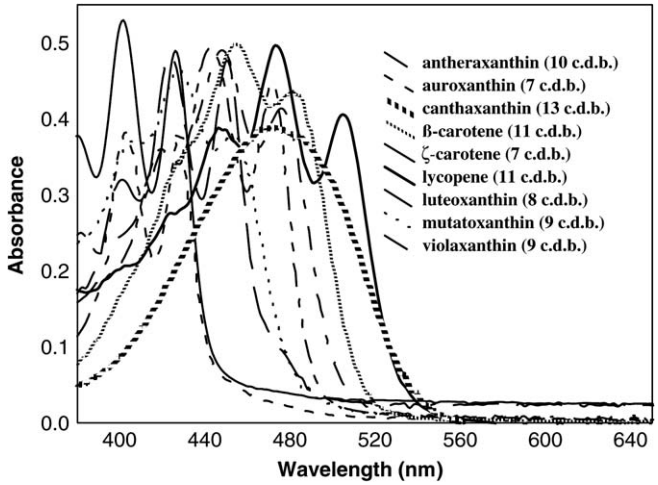


Fig. 3. Detail of the visible spectra of ζ-carotene, lycopene, β-carotene, canthaxanthin, antheraxanthin, violaxanthin, mutatoxanthin, luteoxanthin and auroxanthin.

The augmentation in the number of c.d.b. from ζ-carotene (7 c.d.b., $\lambda_{max} = 402$ nm, $h_{ab} = 113.07°$) to lycopene (11 c.d.b., $\lambda_{max} = 474$ nm, $h_{ab} = 76.59°$) involves a bathochromic shift of the absorption maxima of 72 nm. In terms of hue, that increase in conjugation involves a fall of around 37°. Thus, ζ-carotene is light-yellowish and lycopene is reddish. Cyclization of lycopene (11 c.d.b., $\lambda_{max} = 474$ nm, $h_{ab} = 76.59°$) to give β-carotene (11 c.d.b., 2 in rings, $\lambda_{max} = 454$ nm, $h_{ab} = 93.96°$) involves a hypsochromic shift of 20 nm and an increase of h_{ab} of about 18°. The presence of a hydroxyl group in β-cryptoxanthin does not affect the chromophore with respect to that of β-carotene, so both compounds have almost identical spectra, as is well-known (Britton, 1995).

As a result of the presence of two keto groups in the end rings of canthaxanthin (13 c.d.b., 4 in rings, $\lambda_{max} = 472$ nm, $h_{ab} = 74.16°$), its absorption maximum is located at a wavelength 18 nm longer than that of β-carotene. The increase in conjugation involves a drop in h_{ab} of about 20° with respect to β-carotene, so canthaxanthin is reddish whilst β-carotene is yellowish. Antheraxanthin (10 c.d.b., 1 in rings, $\lambda_{max} = 448$ nm, $h_{ab} = 99.43°$) has 1 c.d.b. less than β-carotene, so its absorption maximum is located at a wavelength 6 nm shorter and its hue is around 5° higher. In the case of α-carotene and lutein, the loss of one c.d.b. with respect to β-carotene is due to the presence of a ε ring, so the changes in their absorption maxima and hue values are analogous to those of antheraxanthin. The main difference between the chromophores of β-carotene and violaxanthin (9 c.d.b., $\lambda_{max} = 442$ nm, $h_{ab} = 103.49°$) is the loss of two c.d.b. in the latter one, so violaxanthin does not have c.d.b in rings, which involves a hipsochromic shift of its absorption maxima of 12 nm and an increase in h_{ab} of about 10°, changes similar to those observed in lutein epoxide and neoxanthin. Due to the isomerization of violaxanthin and antheraxanthin to form luteoxanthin (8 c.d.b., $\lambda_{max} = 424$ nm, $h_{ab} = 109.00°$) and mutatoxanthin (9 c.d.b., 1 in rings, $\lambda_{max} = 428$ nm,

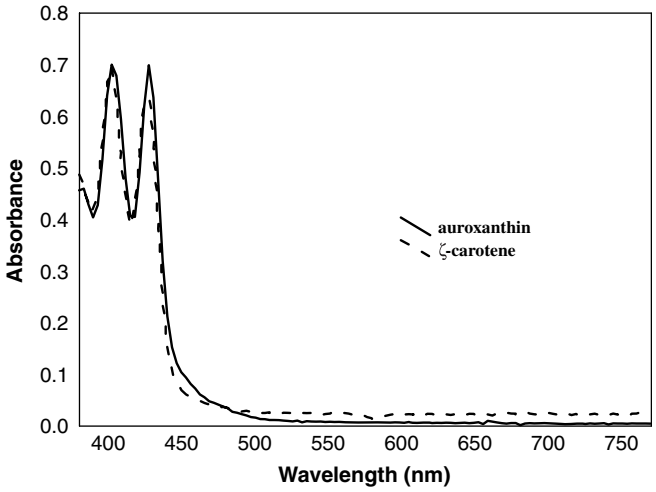


Fig. 4. Visible spectra of auroxanthin and ζ-carotene in acetone.

$h_{ab} = 107.41°$), respectively, a c.d.b. is lost and hipsochromic shifts of their absorption maxima are observed (18 and 20 nm, respectively). In the case of luteoxanthin, the increase in h_{ab}, due to the loss of one c.d.b. with respect to violaxanthin, is of about 6°. In the case of mutatoxanthin, the increase with respect to antheraxanthin is higher, about 8°, since the chromophore of mutatoxanthin extends into a ring. As for neochrome (8 c.d.b., $\lambda_{max} = 424$ nm, $h_{ab} = 109.00°$), the 5,8-epoxyderivative of neoxanthin, the changes are analogous. As a result of the isomerization of the two 5,6-epoxy groups of violaxanthin, auroxanthin (7 c.d.b., $\lambda_{max} = 402$ nm, $h_{ab} = 112.09°$) is formed, and a hipsochromic shift of the absorption maxima of 40° and an increase in hue of 10° are observed. Fig. 4 shows the great similarity between the spectra of auroxanthin and the acyclic carotenoid ζ-carotene, which corroborates that rings in which there are no c.d.b. have little effect on the spectra or, therefore, on the colour coordinates.

References

Arias, R., Lee, T.-C., Logendra, L., & Janes, H. (2000). 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 lycopene content. *Journal of Agricultural and Food Chemistry, 48*, 1697–1702.

Barua, A. B., & Olson, J. A. (2001). Xanthophyll epoxides, unlike β-carotene monoepoxides, are not detectibly absorbed by humans. *The Journal of Nutrition, 131*, 3212–3215.

Britton, G. (1991). Carotenoids. In P. M. Dey & J. B. Harborne (Eds.), *Methods in plant biochemistry* (pp. 473–518). London: Academic Press.

Britton, G. (1992). Carotenoids. In G. A. F. Hendry & J. D. Houghton (Eds.), *Natural food colorants* (pp. 141–182). Glasgow and London: Blackie.

Britton, G. (1995). UV/visible spectroscopy. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids. Spectroscopy* (Vol. 1B, pp. 13–62). Basel, Switzerland: Birkhäuser.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (1995). *Carotenoids. Isolation and analysis* (Vol. 1A). Basel, Switzerland: Birkhäuser.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (2004). *Carotenoids. Handbook*. Basel, Switzerland: Birkhäuser.

Britton, G., & Young, A. (1993). Methods for isolation and analysis of carotenoids. In A. Young & G. Britton (Eds.), *Carotenoids in photosynthesis* (pp. 409–458). London: Chapman & Hall.

CIE (1978). *Recommendations on uniform color spaces, color-difference equations, psychometric color terms, CIE Publication No. 15 (E-1.3.1) 1971, Supplement 2*. Viena: Bureau Central de la CIE.

Clydesdale, F. M. (1993). Color as a factor in food choice. *Critical Reviews in Food Science and Nutrition, 33*, 83–101.

Fraser, P. D., & Bramley, P. M. (2004). The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research, 43*, 228–265.

Giovanucci, E. (1999). Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *Journal of National Cancer Institute, 91*, 317–331.

Heredia, F. J., Álvarez, C., González-Miret, M. L., & Ramírez, A. (2004). CromaLab, análisis de color.

Krinsky, N. I. (2001). Carotenoids as antioxidants. *Nutrition, 17*(10), 815–817.

Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., & Heredia, F. J. (2005a). Identification of zeinoxanthin in orange juices. *Journal of Agricultural and Food Chemistry, 53*(16), 6362–6367.

Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., & Heredia, F. J. (2005b). Color and carotenoid profile of Spanish Valencia late ultrafrozen orange juices. *Food Research International, 38*, 931–936.

Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., & Heredia, F. J. (2005c). Identification of isolutein (lutein epoxide) as *cis*-antheraxanthin in orange juice. *Journal of Agricultural and Food Chemistry, 53*(24), 9369–9373.

Meléndez-Martínez, A. J., Vicario, I. M., & Heredia, F. J. (2003). Application of tristimulus colorimetry to estimate the carotenoids content in ultrafrozen orange juices. *Journal of Agricultural and Food Chemistry, 51*(25), 7266–7270.

Meléndez-Martínez, A. J., Vicario, I. M., & Heredia, F. J. (2004a). Correlation between visual and instrumental colour measurements of orange juice dilutions. Effect of the background. *Food Quality and Preference, 16*, 471–478.

Meléndez-Martínez, A. J., Vicario, I. M., & Heredia, F. J. (2004b). Importancia nutricional de los pigmentos carotenoides. *Archivos Latinoamericanos de Nutrición, 54*(2), 149–154.

Meléndez-Martínez, A. J., Vicario, I. M., & Heredia, F. J. (2005). Instrumental measurement of orange juice colour: a review. *Journal of the Science of Food and Agriculture, 85*, 894–901.

Mínguez-Mosquera, M. I. (1997). *Clorofilas y carotenoides en Tecnología de los Alimentos*. Sevilla: Secretariado de publicaciones de la Universidad de Sevilla.

Mínguez-Mosquera, M. I., & Hornero-Méndez, D. (1993). Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika, and oleoresin by reversed-phase HPLC. *Journal of Agricultural and Food Chemistry, 41*, 1616–1620.

Mínguez-Mosquera, M. I., Rejano-Navarro, L., Gandul-Rojas, B., Sánchez-Gómez, A. H., & Garrido-Fernández, J. (1991). Color–pigment correlation in virgin olive oil. *Journal of the American Oil Chemists Society, 68*(5), 332–336.

Olson, J. A. (1989). Biological actions of carotenoids. *Journal of Nutrition, 119*, 94–95.

Olson, J. A. (1999). Carotenoids and human health. *Archivos Latinoamericanos de Nutrición, 49*(1-S), 7–11.

Rodriguez-Amaya, D. B. (1997). *Carotenoids and food preparation: the retention of provitamin A carotenoids in prepared, processed and stored foods*. Washington, DC: OMNI/USAID.

Rodriguez-Amaya, D. B. (2001). *A guide to carotenoid analysis in foods*. Washington, DC: ILSI Press.

Roth, H. A., Radle, L., Gifford, S. R., & Clydesdale, F. M. (1988). Psychophysical relationships between perceived sweetness and color in lemon and lime flavored beverages. *Journal of Food Science, 53*, 1116–1162.

Sinnecker, P., Gomes, M. S. O., Arêas, J. A. G., & Lanfer-Marquez, U. M. (2002). Relationship between color (instrumental and visual) and chlorophyll contents in soybean seeds during ripening. *Journal of Agricultural and Food Chemistry, 50*(14), 3961–3966.

Tepper, B. J. (1993). Effects of a slight color variation on consumer acceptance of orange juice. *Journal of Sensory Studies, 8*, 145–154.