Relationship between colour parameters and structure of carotenoids

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ABSTRACT

Pure carotenoids were isolated to assess the differences in their colour as a result of their chemical differences. Considering yellow and orange-yellow carotenoids it was observed that a* values clearly decreased from the carotenoids with 11 conjugated double bonds to those with 9 conjugated double bonds, although this trend reversed in the case of carotenoids with 7 conjugated double bonds. In terms of hue, it was seen that the decrease in conjugation of the molecules involved slight increases in h_{ab}. On the other hand, the aperture of the end rings or the increase in conjugation involved clear increases in hue.

1. INTRODUCTION

Carotenoids are responsible for the colour of many vegetable and animal foods. The yellowish, reddish or orange colours of these pigments are due to the presence in their molecules of an extensive double-bond chain. This light-absorbing chromophore is responsible for the UV-vis spectra of these compounds, which are very useful for analysts for identification purposes. Although the influence of chemical structure on the shape and absorption maxima of the spectra is well-known[1], it has not been studied in terms of colour coordinates. The aim of this work was to assess the differences in colour among different carotenoids as a result of their chemical differences.

2. METHOD

Pure carotenoids were obtained according to standard procedures[2]. Lutein, violaxanthin and neoxanthin were isolated from spinach leaves (*Spinacia oleracea* L.), lycopene from tomato (*Lycopersicon esculentum* Mill.), α and β-carotene from palm oil (*Elaeis guineensis* Jacq.), β-cryptoxanthin and zeaxanthin from ripe peppers (*Capsicum annuum* L.), ζ-carotene from Valencia orange juice (*Citrus sinensis* (L.) Osbeck) and lutein epoxide from petals of dandelion (*Taraxacum officinale* Weber). Antheraxanthin was obtained by treating zeaxanthin with 3-chloroperoxybenzoic acid. 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. Canthaxanthin was a gift from Hoffman-La Roche.

Absorbance measurements were made by means of a Hewlett Packard UV/Visible diode-array spectrophotometer model HP8452, using a glass cuvette (10 mm of pathlength). The whole visible spectrum (380-770 nm) was registered ($\Delta\lambda$ = 2 nm) and Illuminant D_{65} and 10° Observer were considered as references. The colour parameters corresponding to the uniform colour space CIELAB[3] were obtained by means of the software CromaLab©[4].

Carotenoids were dissolved in acetone for measurements. To minimize the influence of concentration on the colour coordinates, solutions equal in concentration, determined spectrophotometrically, were used. For this purpose the values of absorption coefficients reported in the literature[5-6] were considered. When those values were not referred to acetone, solutions at the concentration considered were prepared in suitable solvents, 2-mL aliquots were taken, concentrated to dryness and re-dissolved in 2 mL of acetone for colour measurements.

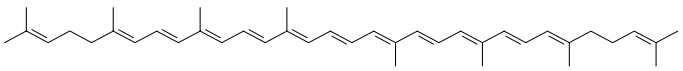
3. RESULTS

Absorption coefficients are difficult to obtain, so some reported values may have some significant level of error[6]. In the case of lutein epoxide two values of $A^{1\%}_{1cm}$ (2400 and 2800 at 441 nm in ethanol) were considered. Taking into consideration that the same concentration was considered in both cases, two spectra differing in absorbance 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 $A^{1\%}_{1cm}$ = 2400; -11.85 CIELAB units and 104.50°, respectively, for $A^{1\%}_{1cm}$ = 2800), whereas differences in b* and C^*_{ab} were higher (38.17 and 39.50 CIELAB units, respectively, for $A^{1\%}_{1cm}$ = 2400; 45.80 and 47.31 CIELAB units, respectively, for $A^{1\%}_{1cm}$ = 2800). According to this, for the assessment of the influence of the chemical structure of carotenoids on their colour, a* and h_{ab} were taken into consideration.

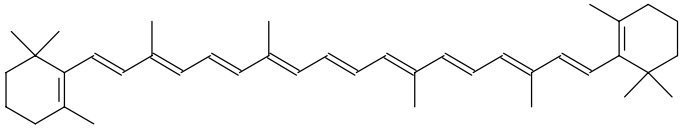
Table 1: Colour coordinates of the solutions in acetone of the carotenoids studied.

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

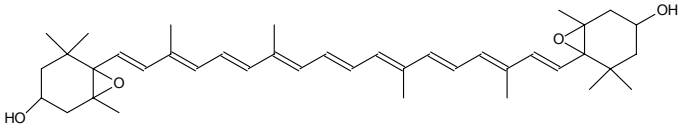
The absorption spectra of carotenoids depends largely on the number of conjugated double bonds, so that the longer the chromophore the longer the wavelength of maximum absorption. Acyclic carotenoids absorb maximally at longer wavelengths that cyclic carotenoids with the same number of conjugated double bonds, due to the fact that cyclization involves steric hindrance. Thus, the acyclic carotene lycopene is reddish (λ_{max} = 474 nm; a* = 8.60 CIELAB units; h_{ab} = 76.59°), whereas β-carotene is orange-yellowish (λ_{max} = 454 nm; a = -3.11 CIELAB units; h_{ab} = 93.96°), despite both pigments have the same number of conjugated double bonds (figure 1).



lycopene



β-carotene



violaxanthin

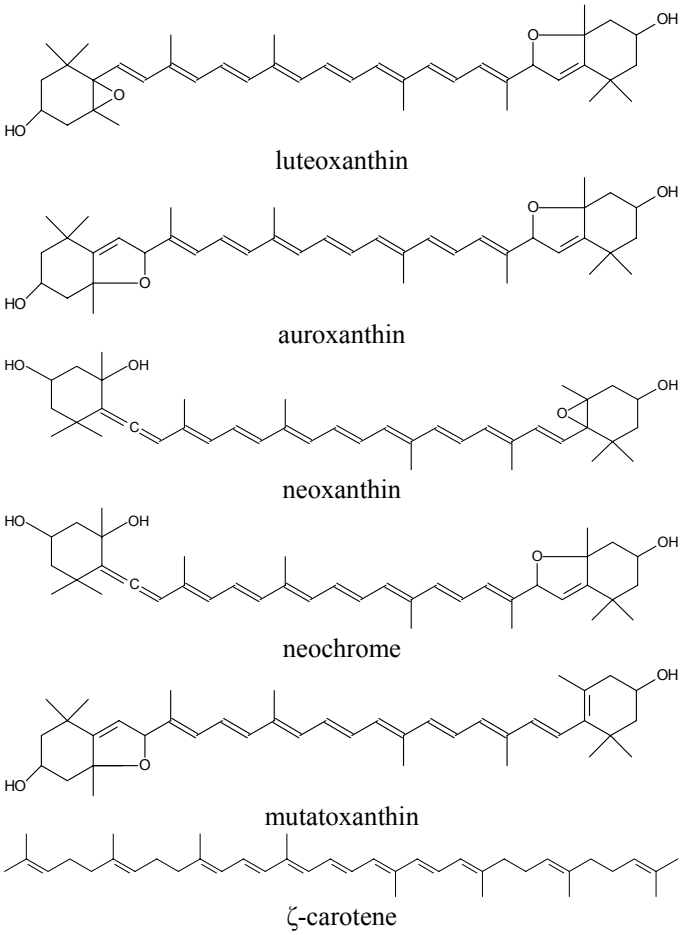
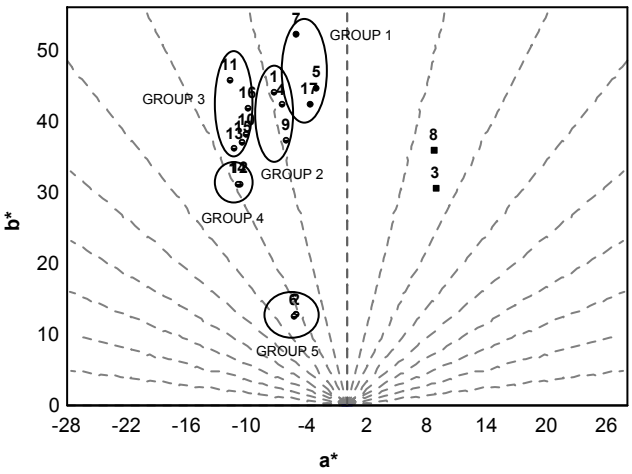


Figure 1: Chemical structures of lycopene, β-carotene, violaxanthin, luteoxanthin, auroxanthin, neoxanthin, neochrome and ζ-carotene.

Location of the carotenoids studied within the a*b* plane is shown in figure 2.



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

Figure 2: Location of the carotenoids within the a*b* diagram.

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 (Figure 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 and orange-yellowish carotenoids it was clearly seen that a* values decreased from those with 11 conjugated double bonds to those with 9 conjugated double bonds. As a result of the re-arrangement of one 5,6-epoxide group in neoxanthin and violaxanthin (9 conjugated double bonds) to form neochrome and luteoxanthin (figure 1), respectively, a conjugated double bond is lost, which involved 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 7 conjugated double bonds (figure 1), involved a dramatic increase in a* and a marked decrease in b*, so that its location in the a*b* plane matched with that of the acyclic ζ-carotene.

In terms of hue, distinction between the red carotenoids, canthaxanthin and lycopene (h_{ab} = 74.16º and 76.59º, respectively) and the yellowish and orange-yellowish ones, with hue values ranging from 93.96º to 113.07º, was clear. This fact revealed that the aperture of the end rings or the increase in conjugation involved clear increases in hue. Considering the carotenoids located in the second quadrant of the a*b* plane it was observed that the loss of a conjugated double bond led to a slight increase in hue. Thus, hue values for the carotenoids with eleven conjugated double bonds, with λ_{max} in acetone around 454 nm, ranged between 93.96° and 95.58º, whereas those corresponding to the carotenoids with nine conjugated double bonds and none of them in rings (violaxanthin, lutein epoxide and neoxanthin), with λ_{max} in acetone around 440 nm, ranged between 103.49º and 105.82º. Mutatoxanthin, with one of its nine conjugated double bonds in an end ring (figure 1), absorbed maximally in acetone at a shorter wavelength, 428 nm, showing a slight higher hue, 107.41º. Hue values of the carotenoids with seven conjugated double bonds, auroxanthin and ζ-carotene, with λ_{max} in acetone at 402 nm, were 112.09 and 113.07º, respectively.

4. CONCLUSIONS

The relationship between the chemical structure of carotenoids and their visible spectra is well-known, although it has not been studied in terms of colour coordinates. The results of this study have revealed that the hypsochromic shift of the absorption maxima of carotenoids due to the loss of conjugation involved increases in hue. In terms of a* and b*, the different carotenoids studied could be grouped in the a*b* plane according to the number of conjugated double bonds.

References

1.G. Britton, “Carotenoids” in Methods in Plant Biochemistry, vol. 7, P.M. Dey & J.B. Harborne, eds. (Academic Press, London, UK, 1991), pp. 473-518.
2. G. Britton, S. Liaaen-Jensen and H. Pfander, “Carotenoids. Volume 1A: Isolation and analysis”, Birkhäuser, Basel (1995).
3. CIE, “Colorimetry”, 2nd edn., publication CIE No. 15.2, CIE Central Bureau, Vienna (1986).
4. F. J. Heredia, C. Álvarez, M. L. González-Miret, A. Ramírez (2004). “CromaLab, análisis de color”, Registro de la Propiedad Intelectual, SE-1052-04. Sevilla, Spain.
5. G. Britton, “UV/visible spectroscopy” in Carotenoids: Spectroscopy, vol. 1B, G. Britton, S. Liaaen-Jensen & H. Pfander eds. (Birkhäuser, Basel, Switzerland, 1995), pp. 13-63.
6. M. I. Mínguez-Mosquera, “Clorofilas y carotenoides en tecnología de los alimentos”, Secretariado de publicaciones de la Universidad de Sevilla, Sevilla (1997).