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SIMULTANEOUS DETERMINATION OF α- AND β-CAROTENE

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SUMMARY

A method has been devised for the simultaneous determination of α- and β-carotene in mixtures. All measurements are done spectrophotometrically in the range of 420 nm to 480 nm. A new constant, the V-value, is introduced for characterisation of the absorption spectrum and to follow the degradation of α- and β-carotene in solution. The quantitative determination of α- and β-carotene in mixtures containing approximately 0.03 to 0.2 mg % total carotene (α + β) in differing ratios gave good and reproducible results (standard deviation 0.007 mg %).

1. INTRODUCTION

Chlorophylls and carotenoids are essential for the photosynthetic activity in green tissues (STRAIN 1965). Practical all the unicellular green algae have the same major carotenoids as the green leaves of the higher plants (BAUERNFEIND 1972, GOODWIN 1965).

The most common carotenes, α- and β-carotene, are isomeric, non polar, polyene hydrocarbons, $C_{40}H_{56}$, with eleven double bonds, all conjugated in β-carotene and only 10 conjugated in α-carotene. The xanthophylls are polar, oxygen derivatives of the carotenes (DAVIES 1965, STRAIN 1965, STRAIN & SHERMA 1969, WEEDON 1965).

β-carotene is present in all algal groups but in some of them it is subordinate to α-carotene (Bryopsidophyceae) (GOODWIN 1965, ROUND 1973, STRAIN 1965). It was not only observed in algae, indeed, some investigations on mosses revealed only α-carotene as main present carotene (CZECZUGA 1972).

The standard method of carrying out the quantitative determination of carotenoids is by spectrophotometry (DAVIES 1965, DEROCHE 1971, HERTZBERG & LIAAEN-JENSEN 1966). This method requires however that the carotenoids should be chromatographically pure. The pigments are therefore isolated by means of column chromatographic methods (CZECZUGA 1972, DAVIES 1965, GROSS et al. 1971, HERTZBERG & LIAAEN-JENSEN 1966, STRAIN 1965, STRAIN & SHERMA 1969).

In nature the carotenoids exist primarily in their all-trans form, however the cis-forms are observed (DAVIES 1965, ZECHMEISTER 1972). Too long extraction procedures, the presence of oxygen or light favorise isomerisation and oxidation (BAUERNFEIND 1972, CHICHESTER & NAKAYAMA 1965, DAVIES 1965, STRAIN 1965, STRAIN & SHERMA 1969, WEEDON 1965).

The variation in pigment composition of natural extracts makes an accurate measurement difficult if not impossible. It is consequently important to determine separately both carotenes (α- and β).

The quantitative determination of the carotenes would be less cumbersome if we had the possibility of determining them in one step. In the work reported here, we investigate upon a method that allow a simultaneous determination of α- and β-carotene by spectrophotometry. In order to obtain an estimation of the extent of deterioration and isomerisation of carotenes in solution we have introduced a new value – the V-value – calculated from measurements of the relative values of the absorption maxima.

2. EXPERIMENTAL

As standards we used α-carotene (Sigma type VCO 251) and β-carotene (Sigma type IVCO 126).

Stock solutions from 3 μg/100 μl β-carotene and 6 μg/100 μl α-carotene were made in hexane plus 2% acetone or hexane alone. The measurements were made on mixtures containing increasing (0, 20, 40... 100 μl and 0, 10... 50μl) amounts of α-carotene and decreasing (100, 80... 0 μl and 50, 40... 0 μl) amounts of β-carotene stock solution, diluted in 3 ml hexane plus 2% acetone or hexane alone. We measure between the range of 420 nm and 480 nm.

It is advisable to take a spectrophotometer of high sensitivity and accuracy since we need perform very precise measurements. Indeed the difference between both λ_{max} is very small and the bands are rather large.

3. RESULTS AND DISCUSSION

The spectra of α- and β-carotene show two maxima (λ_{max} and λ_{2ndmax}, for α-carotene: 444 nm and 473 nm; for β-carotene: 449 nm and 477 nm), an inflection between 420 nm and 430 nm and a minimum (α-carotene: 461 nm and β-carotene: 465 nm). The following values are typical and constant for both spectra:

$$\frac{A_{\lambda\ 2nd\ max}}{A_{\lambda\ max}} \text{ and } \frac{A_{\lambda\ min}}{A_{\lambda\ max}}$$

We have introduced the difference of the ratios, called the V-value

$$\left(V = \frac{A_{\lambda\ 2nd\ max}}{A_{\lambda\ max}} - \frac{A_{\lambda\ min}}{A_{\lambda\ max}}\right)$$ as a practical measure of the extent of isomerisation of carotenes in solution. This formula reflects in fact the configuration of the spectrum, although it does not give information about the wavelength position. The V-values found for standards are: α-carotene, 0.21 ± 0.01 and β-carotene, 0.06 ± 0.01. We have found that the V-value is a very sensitive indicator of deterioration of carotenes in aging solutions. (*table I*). The V-value of the α-carotene standard solution was found to decrease from 0.21 to 0.15 and the

V-value of the standard β-carotene from 0.07 to 0.05. This decrease is particularly obvious for α-carotene, less so for β-carotene. Since molecular changes in carotenes can be attributed to the effects of light. T°, O_2 etc... (BAUERNFEIND 1972, CHICHESTER & NAKAYAMA 1965, DAVIES 1965, STRAIN 1965, STRAIN & SHERMA 1969, WEEDON 1965) which induce isomerisation, any such change is rapidly detected by a V-value determination. For accurate determinations, measurements should be done in conditions (low light and temperature, absence of O_2 etc...) which minimalize isomerisation. *Table 1* shows the V-value of the aging standard solutions (α- and β-carotene 0.1 to 0.2 mg %) as a function of time.

The standards were conserved in a refrigerator in the presence of O_2. V-values were calculated from the spectra and from values given by Zechmeister. (1962).

For the simultaneous determination of α- and β-carotene we made use of the additive absorption at 444 nm (the absorption at 449 nm can also be used). Knowing that $A_{444} = A_{444\ \alpha\text{-carotene}} + A_{444\ \beta\text{-carotene}}$ and that $\varepsilon_{\alpha\ 444\ nm} = 134.\ 10^{+3}\ mol^{-1}\ cm^{-1}$, $\varepsilon_{\beta\ 449\ nm} = 134.\ 10^{+3}\ mol^{-1}\ cm^{-1}$, $\varepsilon_{\alpha\ 449\ nm} = 128.\ 10^{+3}\ mol^{-1}\ cm^{-1}$ and $\varepsilon_{\beta\ 444\ nm} = 126.\ 10^{+3}\ mo^{-1}\ cm^{-1}$, this becomes after application of Cramers rule:

$\alpha\text{-carotene} = A_{444} \times 0.07330 - A_{449} \times 0.06843$

$\beta\text{-carotene} = A_{449} \times 0.07330 - A_{444} \times 0.07002$

(The concentration is expressed as 10^{-3} molar).

Table 1. Comparison of the values calculated from the spectra and values given by ZECHMEISTER (1962) and the V-values calculated from spectra of aging standard solutions.

V-values calculated from the spectra and values given by Zechmeister (1962)		V-values calculated from spectra of aging standard solutions (in hexane plus 2% acetone)	
V-value	*α-carotene*	*V-value*	*α-carotene*
0.22	Fresh solution of the all-trans component	0.22	Fresh solution
0.20	Mixture of stereoisomers after refluxing in the solvent	0.19	Kept for 24 hours at c. 0 °C
0.08	Mixture of stereoisomers after treatment with I_2	0.18	Kept for 24 hours at c. 0 °C
		0.16	Kept for two months at c. 0 °C.
	β-carotene		*β-carotene*
0.07	Fresh solution of the all-trans component	0.07	Fresh solution
0.06	Mixture of stereoisomers after refluxing in the solvent	0.07	Kept for 48 hours at c. 0 °C
0.01	Mixture of stereoisomers after treatment with I_2	0.06	Kept for 48 hours at room temperature and in day-light
		0.03	After treatment with I_2 (15')

The overwhelming majority of plant carotenoids posses the all-trans configuration. Previous storage conditions may however be responsible (ZECHMEISTER 1962) for the occurrence of some cis-carotenoids in extracts.

The application of this formula for carotene content was checked by measurements on standard mixtures of α- and β-carotene in differing ratios. The concentration range of carotenes taken was similar to that found in extracts from fresh water microorganisms (±0.03 to 0.2 mg %). The results were found to be satisfactory with a standard deviation of ± 0.007 mg %.

These methods are now being used to determine carotene content and composition in extracts from freshwater algae.

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