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Orginal paper

Photobleaching of astaxanthin and canthaxanthin

Quantum-yields dependence of solvent, temperature, and wavelength of irradiation in relation to packaging and storage of carotenoid pigmented salmonoids

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Die Lichtentfärbung von Astaxanthin und Canthaxanthin. Abhängigkeit der Quantenausbeute von Lösungsmittel, Temperatur und Wellenlänge der Bestrahlung in Relation zur Verpackung und Lagerung des Carotenoid-pigmentierten Lachs

Zusammenfassung. Die Quantenausbeute bei der Lichtentfärbung von Astaxanthin (das Carotenoid von Wildlachs) und von Canthaxanthin (das eng verwandte Carotenoid als Futterzusatz für gezüchteten Lachs) wurde bei monochromatischem Licht und unterschiedlichen Wellenlängen und in unterschiedlichen Lösungsmitteln bestimmt. Astaxanthin ist weniger lichtempfindlich als Canthaxanthin. Die Lichtentfärbung ist von der Wellenlänge stark abhängig, und die Quantenausbeute von Astaxanthin (gelöst in Chloroform) bei 22 °C beträgt bei 254 nm $3{,}2 \times 10^{-1}$ mol · Einstein^{-1}, bei 313 nm $3{,}1 \times 10^{-2}$ und bei 436 nm $1{,}6 \times 10^{-6}$. Die Quantenausbeuten sind weniger abhängig von der Art des Lösungsmittels; sie zeigen keine einfache Wechselbeziehung mit der Sauerstofflöslichkeit, das heißt, bei 366 nm Erregung von Astaxanthin gelöst in Aceton $6{,}1 \times 10^{-5}$ mol · Einstein, in gesättigtem Pflanzenöl $1{,}2 \times 10^{-4}$, in Chloroform $1{,}9 \times 10^{-4}$ und in Wasser $3{,}4 \times 10^{-4}$. Die Quantenausbeute der Lichtentfärbung liefert ein objektives Maß für die Lichtempfindlichkeit der Carotenoide in Relation zu der Entfärbung von Carotenoid-pigmentiertem Lachs und wurde von der Carotenoidkonzentration und in homogener Lösung, unabhängig von der Lichtintensität gefunden. Die Quantenausbeute von in Wasser solubilisiertem Astaxanthin steigt bei niedrigen Lichtintensitäten. Die Quantenausbeute der Lichtentfärbung für die Erregung mit sichtbarem Licht von Astaxanthin, in Wasser solubilisiert, ist von der Temperatur unabhängig, obwohl die Quantenausbeute bei einer Erregung von 313 nm bei zunehmenden Temperaturen steigt, gemäß einer Aktivierungsenergie von 28 kJ · mol^{-1}. Aus den bei der Photooxidation verfügbaren photophysikalischen Daten über β-Carotin wird für die Quantenausbeute der Carotenoide die obere Grenze von 3×10^{-5} mol · Einstein^{-1} für einen nicht-radikalen Mechanismus geschätzt. Dieses Resultat erlaubt eine Schätzung von 10^4 für die Länge der Ketten in einem von 254 nm Licht angeregten radikalen Prozeß.

Summary. The quantum yield for the photobleaching of astaxanthin (the carotenoid of wild salmonoids) and of canthaxanthin (the closely related carotenoid used as a feeding additive for farmed salmonoids) has been determined for monochromatic light at different wavelengths and in different solvents. Astaxanthin is less sensitive to light than canthaxanthin. The photobleaching is strongly wavelength dependent, and the quantum yield for astaxanthin dissolved in chloroform at 22° C is 3.2×10^{-1} mol · Einstein^{-1} at 254 nm, 3.1×10^{-2} at 313 nm, and 1.6×10^{-6} at 436 nm, respectively. The quantum yields are less dependent on the nature of the solvent and show no simple correlation with oxygen solubility, i.e. for 366 nm excitation of astaxanthin the quantum yields are 6.1×10^{-5} mol · Einstein^{-1} in acetone, 1.2×10^{-4} in saturated vegetable oil, 1.9×10^{-4} in chloroform, and 3.4×10^{-4} solubilized in water, respectively. The photobleaching quantum yield provides an objective measure of the light sensitivity of the carotenoids in relation to the discolouration of carotenoid-pigmented salmonoids. The quantum yield was also found to be independent of the carotenoid concentration and, in a homogenous solution, of light intensities. For astaxanthin solubilized in water, the quantum yield increases for low light intensities. Excitation of astaxanthin solubilized in water using visible light shows that the photobleaching quantum yield is independent of temperature, while excitation at 313 nm shows an increase in the quantum yield with increasing temperatures, corresponding to an energy of activation of 28 kJ · mol^{-1}. From the available photophysical data for β-carotene, an upper limit of 3×10^{-5} mol · Einstein^{-1} for photooxidation quantum yields for carotenoids is estimated for a limiting non-radical mechanism, providing an estimate of 10^4 for the chain length in a radical process initiated by 254 nm light.

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Introduction

It has been widely recognized that carotenoids are important antioxidants in living organisms [1]. For photobiological systems, this function includes the physical quenching of singlet oxygen by carotenoids [2]. In general, the extended conjugation makes the carotenoids susceptible to oxidation, and the rapid chemical reaction of carotenoids with radicals and activated oxygen species prevents oxidative damage to other cellular constituents [3, 4]. Oxidation and photodegradation of man-made polymers is likewise retarded by the incorporation of naturally occurring carotenoids, as has been demonstrated for β-carotene and for carotenoids isolated from paprika [5]. A similar post-harvest and post-mortem protection of certain foods by carotenoids is likewise expected, both as a result of scavenging of radical intermediates formed in lipid oxidation during storage and as a result of quenching of photochemically generated singlet oxygen on exposure to light during retail display.

Frozen salmonoids are an example of a carotenoid-pigmented food susceptible to oxidation during storage. The reddish hue of the flesh of the wild salmonoids is caused mainly by the dietary intake of astaxanthin-containing crustaceans. For farmed fish, astaxanthin and the closely related canthaxanthin (Fig. 1) are added to the feed, on which the fish are raised [6]. Salmonoid flesh has a high content of polyunsaturated lipids, and frozen salmonoid steaks are thus a product in which a high and uniformly distributed content of carotenoids may improve the oxidative stability. An expected increase in the lag period for development of rancidity may thus result in a longer practical storage life. In a storage experiment with frozen steaks of wild salmon and farmed rainbow trout, rancidity was found to develop more slowly in products with a high astaxanthin content [7]. Notably, the apparent protection of the lipids by carotenoids was most significant for the light-induced oxidation occurring during frozen storage in an illuminated display cabinet. Quantitative information concerning the photobleaching of carotenoids is of interest for the selection of optimal packaging and storage conditions in relation to the colour stability of salmonoids during freezer storage. An objective measure of the light sensitivity is the photobleaching quantum yield, as previously determined for the carotenoids lutein and β-carotene for monochromatic excitation with light of several wavelengths [8]. The results reported in this paper involve similar photochemical investigations of astaxanthin and canthaxanthin dissolved in different solvents which serve as food models.

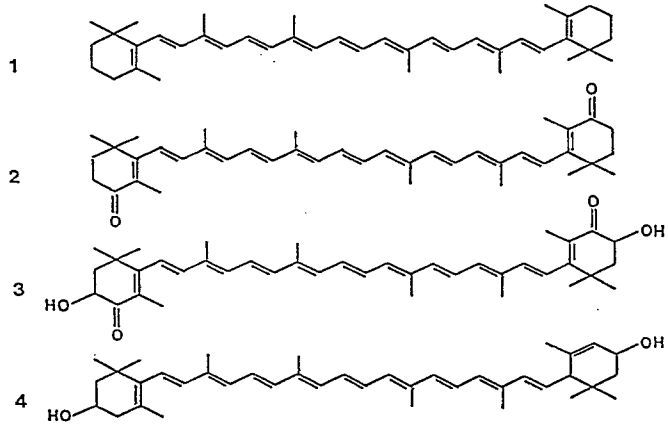

Fig. 1. Chemical structure of (*1*) β-carotene, (*2*) canthaxanthin, (*3*) astaxanthin and (*4*) lutein

Materials and methods

All-*trans*-astaxanthin and all-*trans*-canthaxanthin were obtained under nitrogen in sealed ampoules from Roche (Copenhagen) and were used without further purification. Viscoleo extra, a high purity vegetable oil with a viscosity of 30 cP and with a peroxide value less than 0.2 mEq O_2/kg (medium chain length, saturated fatty acids, iodine value less than 1) was obtained from DS Industries (Copenhagen). Tween-20 (Sigma, St. Louis, MO., USA) and other chemicals were of analytical grade. Chloroform was purified for acidic impurities by passage through an aluminium oxide column (10 × 3 cm).

Photolysis solutions.

Stock solutions of carotenoid in chloroform, acetone, vegetable oil and ethanol were kept at −18° C for up to 1 week. Dilution to the appropriate concentrations was carried out daily, and the solutions were protected against light prior to photolysis. Concentrations (approximately 1×10^{-5} *M*) were estimated spectrophotometrically using the following values for ε_{max} (in $L \cdot mol^{-1} \cdot cm^{-1}$) based on the literature values for specific absorptivities and assuming that the specific absorptivity of astaxanthin and canthaxanthin are identical. This is evidenced when combining the results obtained for acetone and cyclohexane solutions [10, 11]: chloroform: $\varepsilon_{asta} = 1.25 \times 10^5$ [9], $\varepsilon_{cantha} = 1.19 \times 10^5$; acetone: $\varepsilon_{asta} = 1.31 \times 10^5$ [10], $\varepsilon_{cantha} = 1.24 \times 10^5$ [11]; vegetable oil: $\varepsilon_{asta} = 1.23 \times 10^5$ [12], $\varepsilon_{cantha} = 1.16 \times 10^5$. For the vegetable oil used in the present study, the molar absorptivities were estimated using the empirical relationship between λ_{max} and ε_{max} demonstrated by Chen and Meyers [12] for astaxanthin dissolved in a series of vegetable oils and fish oils:

$$E^{1\%}_{1cm} = 7.44 \times 10^{13} \cdot (\lambda_{max})^{-3.93}.$$

Astaxanthin was solubilized in water using the procedure previously used for β-carotene and lutein [8]. Astaxanthin (25 mg) and Tween-20 (1.0 ml) were dissolved in chloroform to a total volume of 25.0 ml. This stock solution was stored at −18° C for up to 2 weeks. Aliquots (0.5 ml) were evaporated to dryness at reduced pressure, and the residue was dissolved immediately in a 0.10 *M* citrate buffer at pH 5.5. The aqueous solution was prepared daily and protected against light prior to photolysis. Concentrations were estimated spectrophotometrically ($\lambda_{max} = 476$ nm, $\varepsilon = 9.4 \times 10^4$ $l \cdot mol^{-1} \cdot cm^{-1}$, as determined by dissolution of known quantities of astaxanthin). Carotenoid concentrations in the photolysis solutions were approximately 1×10^{-5} *M*.

Salmonoid extracts

Carotenoid was extracted from the flesh of atlantic salmon (*Salmo salar*) and from the flesh of rainbow trout (*Salmo gairdneri*) using acetone. This was followed by subsequent isolation on silica and elution with ethanol [13]. The resulting ethanolic solutions were used as photolysis solutions and compared with a solution of astaxanthin in ethanol, after HPLC identification of the carotenoid present in the salmonoid extracts.

Photolysis

The photolysis solutions were irradiated with monochromatic light, and the extent of photodegradation was monitored at regular intervals by spectrophotometric measurements (Cary Varian 219 spectrophotometer). Light intensities were determined by ferrioxalate

actinometry. The photolysis solutions were air-saturated and were in contact with air and stirred by means of a Teflon-coated magnetic bar during photolysis. Monochromatic light was selected from an Osram HBO 100/2 high pressure mercury lamp, mounted as part of an optical train (Spindler and Hoyer, Göttingen, FRG), which also included a light condenser, a heat filter, an interference filter, a shutter connected to an electronic timer and lenses focusing the light into a thermostated (±0.5° C) quartz cell (L=2 cm) containing the photolysis solution. An Oriel 6035 low-pressure mercury-argon penlight equipped with an Oriel 6041 short-wave filter mounted in a thermostated cell-holder unit (±0.5° C) was used as a diffuse light source for irradiation at 254 nm.

Calculations

The photodegradation quantum yield was calculated from the degree of bleaching of the colour of the photolysis solution:

$$\Phi^{irr} = \frac{\text{Number of carotenoid degraded}}{\text{Number of photons absorbed by carotenoid}} = \frac{(A(t_0)-A(t_i))/A(t_0)}{Q_{car}(t_i)}, \quad (1)$$

where N_A is Avogadro's number, V is the volume (L), and A_{irr} is the maximum of the carotenoid prior to exposure to light and at time t_i, respectively. $Q_{car}(t_i)$, the number of photons absorbed by the carotenoid, was calculated from the light intensity I'_0 (quanta · s^{-1}) by adding the light absorbed in small, but finite time intervals, $t_i - t_{i-1}$ for a solution with a total carotenoid concentration, c_0:

$$Q_{car}(t_i) = \frac{1}{c_0}\sum_i \left(\frac{I'_0}{N_A V}\right)(1-10^{-A_{irr}})(t_i - t_{i-1}), \quad (2)$$

where N_A is Avogadro's number, V is the volume (1), and A_{irr} is the absorption at the wavelength of irradiation at a time $1/2(t_i - t_{i-1})$.

Thermal reactions during the time span of photolysis were monitored spectrophotometrically for solutions prepared as the photolysis solutions, but excluded from light. Thermal reactions in all of the lipophilic solvents used in the present investigation were found to be insignificant. However, for experiments with visible light (405 nm), the thermal reaction amounted to approximately 10% of the photoreaction for astaxanthin solubilized in water, and the determined quantum yield for this set of conditions may be regarded as the upper limit.

Results

The colour of carotenoids is mainly determined by a single broad absorption band in the visible range caused by allowed $\pi \rightarrow \pi^*$ transitions in the conjugated polyene structure [14]. The position and the intensity of this absorption band are slightly dependent on the solvent, as seen for astaxanthin in Fig. 2. Astaxanthin and canthaxanthin were both found to be bleached by exposure to light when dissolved in a series of air-saturated lipophilic solvents. In general, astaxanthin was found to be less susceptible to light as compared to canthaxanthin, as seen from the photobleaching quantum yields for irradiation with monochromatic light given in Table 1. The quantum yields for visible light are low, as found in a previous investigation on the carotenoids lutein and β-carotene solubilized in water [8]. However, for both lutein and β-carotene a strong wavelength dependence on the photobleaching yield was demonstrated, corresponding to an exponential increase with decreasing wavelength of excitation

$$\Phi^{irr} = A_\Phi \cdot e^{-\alpha \cdot \lambda_{irr}}. \quad (3)$$

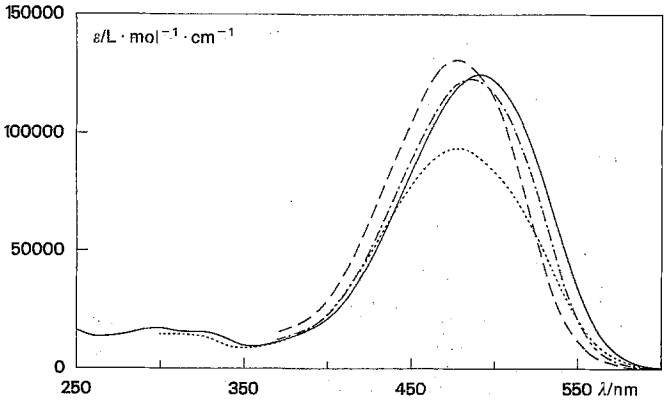

Fig. 2. Absorption spectra of astaxanthin dissolved in lipophilic solvent and solubilized in water: — chloroform; —— acetone; —·—· vegetable oil; ---- water

A similar relationship exists for the photobleaching of astaxanthin and canthaxanthin for light with wavelengths longer than approximately 300 nm, as shown in Fig. 3, for the latter two carotenoids dissolved in chloroform and for astaxanthin solubilized in water (Table 2). This wavelength dependence, which has important mechanistic implications [8], seems to be a general result for the photooxidation of carotenoids and independent of the

Table 1. Quantum yields (mol · einstein^{-1}) for photodegradation of astaxanthin and canthaxanthin dissolved in lipophilic air-saturated solvents at 22 °C

	λ_{irr} (nm)[a]	Astaxanthin	Canthaxanthin
Chloroform	254	3.2×10^{-1}	7.2×10^{-1}
	313	3.1×10^{-2}	5.0×10^{-2}
	366	1.9×10^{-4}	3.8×10^{-4}
	405	4.6×10^{-6}	4.0×10^{-5}
	436	1.6×10^{-6}	4.6×10^{-6}
Acetone	366	6.1×10^{-5}	1.1×10^{-4}
	405	5.5×10^{-6}	1.4×10^{-5}
	436	1.0×10^{-6}	7.2×10^{-6}
Oil	366	1.2×10^{-4}	8.1×10^{-5}
	405	2.9×10^{-6}	8.9×10^{-6}
	436	2.3×10^{-7}	3.8×10^{-7}

[a] Wavelength of irradiation. Average light intensity in experiments (einstein $l^{-1}s^{-1}$); $^{254}I_0 = 3.6\times10^{-7}$, $^{313}I_0 = 5.5\times10^{-7}$, $^{366}I_0 = 1.1\times10^{-5}$, $^{405}I_0 = 8.0\times10^{-6}$ and $^{436}I_0 = 7.0\times10^{-6}$. $^{irr}I_0 = (I'_0/N_A \cdot V)$ in Eq. (2)

Table 2. Temperature dependence of quantum yields (mol · einstein^{-1}) for photodegradation of astaxanthin solubilized[a] in air-saturated, aqueous solution (pH 5.5, citrate buffer)

λ_{irr} (nm)[b]	10° C	20° C	30° C
313[c]	1.6×10^{-3}	2.5×10^{-3}	3.4×10^{-3}
366	3.2×10^{-4}	3.4×10^{-4}	3.0×10^{-4}
405	9.1×10^{-5}	8.5×10^{-5}	8.0×10^{-5}

[a] Solubilized using Tween-20

[b] Wavelength of irradiation; for light intensities, see Table 1

[c] Corresponding to an apparent energy of activation of 28 kJ · mol^{-1}, as calculated from the Arrhenius equation: $\ln \Phi^{irr} = A_\Phi - (E_a/R) \cdot T^{-1}$

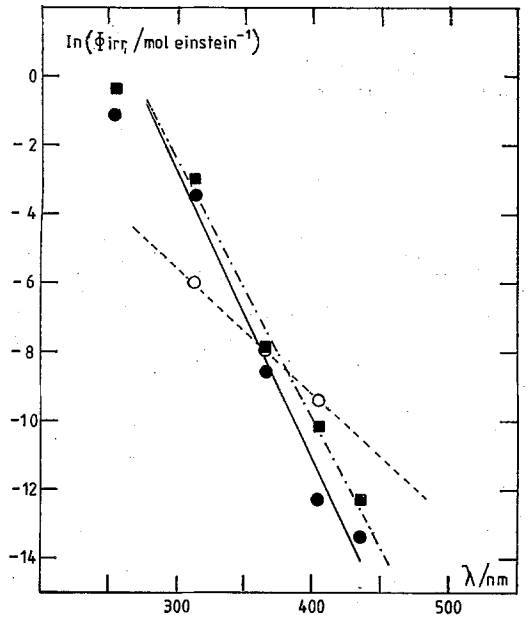

Fig. 3. Wavelength dependence of the photooxidation quantum yield for astaxanthin and canthaxanthin dissolved in chloroform and for astaxanthin solubilized (Tween-20) in water; cf. Tables 1 and 2. Lines calculated by regression analysis according to $\ln \phi^{irr} = \ln A_{\phi} - \alpha \cdot \lambda_{irr}$. Similar logarithmic dependence exists for acetone and oil as solvents. ● Astaxanthin in $CHCl_3$; ○ astaxanthin solubilized in H_2O; ■ canthaxanthin in $CHCl_3$

exact nature of the carotenoid and of the solvent. The quantum yields for photooxidation of astaxanthin dissolved in the lipophilic solvents (Table 1) and solubilized in water (Table 2) are not very different and show no simple correlation with oxygen solubility [15]. In a more qualitative study on the stability of β-carotene in relation to exposure to white light, Inamura et al. [16] concluded that β-carotene solubilized in water was less stable then β-carotene dissolved in solvents such as hexane, benzene

Table 3. Quantum yields (mol · einstein^{-1}) for photodegradation of astaxanthin, lutein and β-carotene solubilized[a] in airsaturated, aqueous solution at 20° C (pH 5.5, citrate buffer)

λ_{irr} (nm)[b]	Astaxanthin[c]	Lutein[d]	β-Carotene[d]
313	2.5×10^{-3}	7.8×10^{-3}	4.7×10^{-3}
366	3.4×10^{-4}	5.1×10^{-4}	3.9×10^{-4}

[a] Solubilized using Tween-20
[b] Wavelength of irradiation; for light intensities, see Table 1
[c] The quantum yields are the limiting yields for high intensity illumination (see Fig. 4)
[d] From [8]

and acetone. As can be seen from Fig. 3, the relative sensitivity to light for astaxanthin dissolved in chloroform and solubilized in water is dependent on the wavelength of irradiation, which requires knowledge of the actual wavelength distribution of light for a quantitative description of the light sensitivity of carotenoids in food systems and in food models.

In relation to carotenoid-pigmented foods, the solubilized carotenoids are more realistic models. In solubilized systems, the three different food-related carotenoids investigated have similar sensitivity to monochromatic light, although the differences in photobleaching quantum yield (astaxanthin < β-carotene < lutein, Table 3) were found to be larger for 313 nm light as compared to 366 nm light. However, the distribution of carotenoids between different phases together with micellar effects presents further complications to the photochemistry of the carotenoids, as illustrated by the effect of varying light intensities. For astaxanthin dissolved in chloroform, the photobleaching resulting from exposure to light for a fixed interval of time was found to be proportional to the light intensity, as illustrated in Fig. 4A. Deviation from this simple proportionality, which is the basis for the use

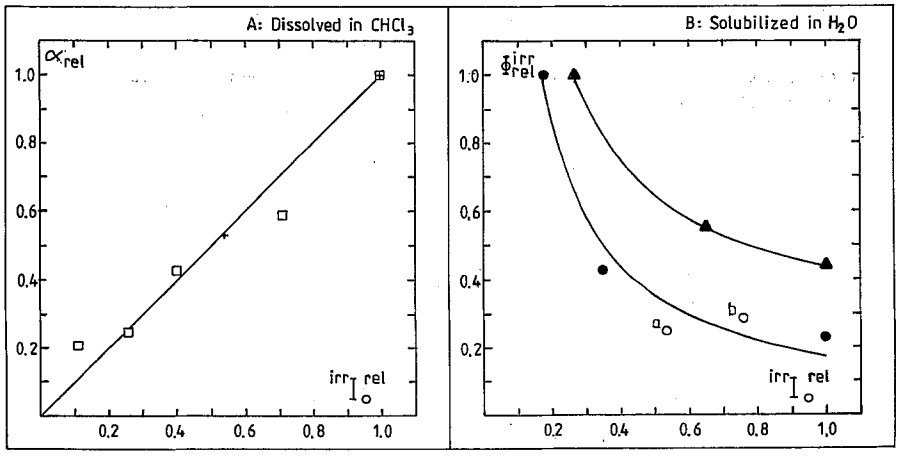

Fig. 4 A, B. Effect of light intensity on photobleaching of astaxanthin at 20° C. **A** Relative photobleaching after 51 min of light exposure to light at 366 nm of varying intensity ($^{366}I_0^{rel} = 1$ corresponds to 1.3×10^{-5} Einstein · $l^{-1} \cdot s^{-1}$; $\alpha_{rel} = 1$ corresponds to 41% photobleaching). For light at 436 nm; $^{436}I_0^{rel} = 1$ corresponds to 5.5×10^{-6} Einstein · $l^{-1} \cdot s^{-1}$. **B** Relative quantum yield for photobleaching for varying light intensities. For excitation at 313 nm: $^{313}I_0^{rel} = 1$ corresponds to 7.1×10^{-7} Einstein · $l^{-1} \cdot s^{-1}$, and $\phi_{rel}^{irr} = 1$ corresponds to 0.011 mol · Einstein^{-1}. Concentration of astaxanthin 1.0×10^{-5} mol · l^{-1}, except *a* (1.5×10^{-5} mol · l^{-1}) and *b* (4×10^{-6} mol · l^{-1}). For excitation at 405 nm: $^{405}I_0^{rel} = 1$ corresponds to 5.8×10^{-6} Einstein · $l^{-1} \cdot s^{-1}$, and $\phi_{rel}^{irr} = 1$ corresponds to 2.0×10^{-4} mol · Einstein^{-1}. The full lines were calculated by regression analyses: $\phi^{313} = 0.172/^{313}I_0^{rel} + 0.0081$; and $\phi^{405} = 0.207/^{405}I_0^{rel} + 0.231$. □ 366 nm irradiation, + 436 nm irradiation; ● and ○ 313 nm irradiation; ▲ 405 nm irradiation

Table 4. Quantum yields (mol · einstein^{-1}) for photobleaching resulting from irradiation[a] with 254 nm monochromatic light of astaxanthin dissolved in ethanol and for ethanolic carotenoid extracts of salmon and trout

	Astaxanthin in ethanol	Salmon extract[b]	Trout extract[c]
Φ^{254}	1.4×10^{-3}	7.2×10^{-5}	7.7×10^{-5}

[a] For light intensity, see Table 1
[b] Ethanolic extract of atlantic salmon (*Salmo salar*); carotenoid identified by HPLC [13] as astaxanthin
[c] Ethanolic extract of rainbow trout (*Salmo gairdneri*); carotenoid indentified by HPLC as astaxanthin with traces of canthaxanthin

of quantum yields as defined in Eq. (1) as a measure of photoreactivity, was, however, seen for astaxanthin solubilized in water. It should be noted that the quantum yields were not found to be dependent on the carotenoid concentration ($4\times10^{-6}<c<1.5\times10^{-5}$). For irradiation at both 313 nm and 405 nm, the quantum yield has a limiting value for high light intensities, but increases for decreasing light intensities (Fig. 4 B):

$$\Phi^{irr}=\frac{b}{I_0}+\Phi^{irr}_{lim}. \quad (4)$$

Such behaviour is indicative of radical processes initiated by light, as discussed below.

Reaction quantum yields are not expected to be sensitive to moderate changes in temperature. For excitation of solubilized astaxanthin with visible light, corresponding to the main absorption band (Fig. 2), the slight decrease noted for the photobleaching quantum yield is hardly significant, whereas for irradiation at 313 nm, a significant increase was found with increasing temperature (Table 2). The value of 28 kJ · mol^{-1} for the apparent energy of activation, as calculated by direct substitution of ϕ^{irr} into the Arrhenius equation, seems to indicate that different excited states are involved for the two different wavelength regions.

For a comparison between the well-characterized solution of purified carotenoids (Tables 1–3) with the carotenoids present in fish, two ethanolic salmonoid extracts were photolysed with monochromatic light at 254 nm. The photobleaching quantum yields of salmon extract and trout extract were both smaller than the quantum yield for the photobleaching of astaxanthin dissolved in ethanol (Table 4). However, in relation to food systems, the quantum yields determined provide information on the relative harmfulness of light of different wavelengths in relation to light-induced degradation of astaxanthin and canthaxanthin. For transparent packaging of frozen steaks of trout and salmon, packaging materials with a UV barrier are thus expected to reduce the photodegradation of the fish pigment significantly, resulting in a product with better colour stability and with better oxidative stability. We are currently testing this prediction in a series of storage experiments with frozen trout steaks packed in materials with different light permeability and stored under different sets of conditions.

Discussion

In view of the importance of carotenoids as natural protectors against photooxidation of biomolecules, relatively little is known about the mechanism of the photooxidation of carotenoids [17]. Such mechanistic information, requiring both detailed photophysical and photochemical investigations, is also important for an understanding of the protection yielded by carotenoids against light-induced oxidation in biological systems. The observation of very weak fluorescence from β-carotene in fluid solution at ambient temperature has now been confirmed [17–19] and provides key information in relation to excited state chemistry. The results from these emission studies and other recent characterizations of excited state dynamics [20] seem to warrant a more mechanistic interpretation of the photooxidation quantum yields determined for β-carotene and lutein [8] and for astaxanthin and canthaxanthin under different set of conditions.

For the symmetrical all-*trans* carotenoids (Fig. 1), the excited state initially populated by the absorption of visible light is the $S_2(1\,^1B_u^+)$ state [21], and the transition to this singlet state ($1\,^1B_u^+ \leftarrow 1\,^1A_g^-$) is fully allowed. This is in contrast to the transition from the ground state to the lower S_1 state, which is symmetry forbidden ($2\,^1A_g^- \leftarrow 1\,^1A_g^-$). The S_1 state is populated as the result of a very efficient transition from the initially populated, higher energy S_2 state. Weak fluorescence has been observed from carotenoids and a small Stokes shift and a high fluorescence anisotropy has been used to identify the $1\,^1B_u^+$ singlet as the emitting state [18, 19]. The very low emission yield has been rationalized on the basis of a competing efficient transition to the non-emitting $2\,^1A_g^-$ state. Both the S_1 and the S_2 singlet states in carotenoids are short lived (less than 1 ps for S_2 and approximately 10 ps for S_1 at ambient conditions [18, 19]). These very short lifetimes, together with both a low photobleaching quantum yield in the presence of oxygen [8], and a corresponding low fluorescence quantum yield, provide evidence for a very efficient non-radiative deactivation to the ground state without any significant competitive processes. Based on the results from an investigation of the temperature effect and the deuterium isotope effect on transient absorption changes for β-carotene following a 0.4 ps laser flash, low-amplitude C–C stretching along the carbon backbone of the carotenoid has been suggested to contribute most significantly to the fast vibronic decay of the excited state [20]. In agreement with this assignment of the non-radiative decay mechanism in carotenoids to vibronic coupling involving C–C stretching modes, molecular orbital calculations have shown that the lengthening of the polyene double bonds and the concomitant shortening of the carbon/carbon single bonds in the excited state increase on moving from the cyclohexene rings towards the centre of the carotenoid molecule [20].

According to the results obtained in photophysical investigations, the double bonds in the carotenoid molecule elongate on the absorption of a light quantum, and the neighbouring carbon atoms get a partial radical character, which makes it susceptible to attack by a variety of reactants. Notably, the effect is most pronounced for the

central double bonds, and non-sensitized photooxygenation of β-carotene has, in agreement herewith, been shown to yield products such as β-ionone, in which the ring and the double bond adjacent to the ring is unaltered [22]. This is in clear contrast to the sensitized photooxygenation, which leads to photooxygenation products in which the cyclohexene rings are also oxidized [22].

Based on these results and on the strong wavelength-dependent photobleaching quantum yields previously demonstrated for β-carotene and lutein [8] and confirmed for astaxanthin and canthaxanthin in the present study, the mechanism for non-sensitized photooxygenation of carotenoids seems to involve the following sequence of events: (i) an initial light absorption resulting in the population of an excited singlet state (S_2 and higher energy states); (ii) a lengthening of the central double bonds providing a partial radical character of the central carbon atoms; (iii) reaction of the diradical with triplet oxygen in competition with non-radiative transition to the lower S_1 singlet state and in competition with radiative transition to the S_0 ground state (Fig. 5).

The light absorption step is fast and yields the excited molecule with the partial diradical character. Notably, the molecular orbital calculations have shown that the central carbon/carbon double bonds are elongated more than three times as much in the higher energy $S_2(1\,^1B_u^+)$ than in the lower energy $S_1(1\,^1A_g^-)$ states [20]. This clearly points towards a reaction with oxygen in the initially populated, fluorescent S_2 state and in higher energy S_n singlet states, in which the bond lengthening and the diradical character are expected to be even more pronounced. The gradual increase in diradical character with increasing excitation energy provides the rationale on the molecular level for the experimental observation for the

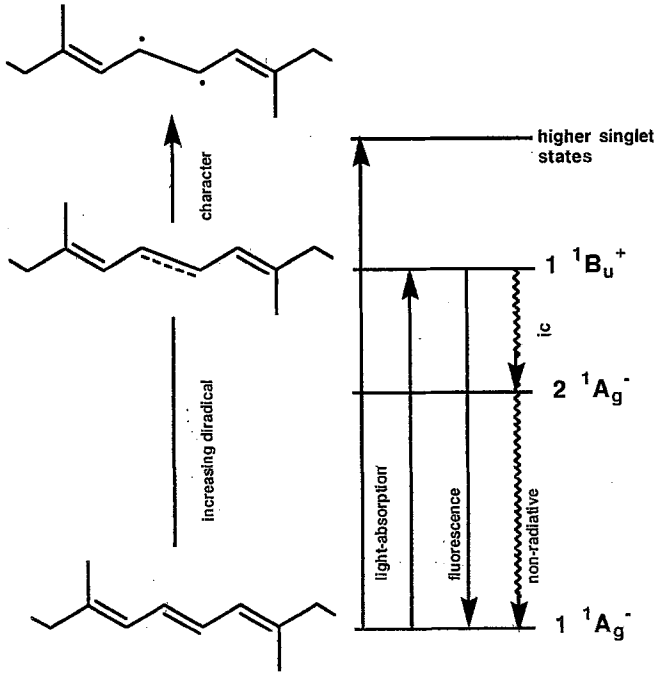

Fig. 5. Excited state diagram for a symmetrical all-*trans* carotenoid. The central double bound in the emissive $1\,^1B_u^+$ state is significantly elongated, and for higher singlet states the partial diradical character increases

wavelength dependence of the photobleaching quantum yield given in Eq. (3), which now has been confirmed for four different carotenoids and in a number of different solvents (Fig. 3) [8]. The lack of the temperature dependence for the 366 nm and 405 nm excitation is in agreement with the lifetime of the reactive singlet states, which are too short for thermal equilibration. In contrast, the photobleaching resulting from excitation with 313 nm monochromatic light shows a significant temperature dependence. It should be noted that light at 313 nm corresponds to the separate absorption band in all-*trans*-carotenoid spectra which, due to its low intensity, is normally assigned to a symmetry-forbidden transition [14]. This higher energy transition becomes symmetry-allowed in *cis*-carotenoids and is accordingly referred to as the *cis* band. Based on the difference in the temperature dependence, this excited state may be sufficiently long-lived to attain a certain degree of thermal equilibration prior to deactivation or reaction.

The photophysical information available in relation to the singlet states populated by excitation in the symmetry-allowed absorption band also provides the basis for a discussion of the excited-state reaction kinetics. Assuming that the fluorescent S_2, state is reacting with the ground state molecular oxygen:

$$\mathrm{Car}(S_2)+{}^3O_2 \xrightarrow{k_2} \text{oxidation products} \tag{5}$$

in competition with radiative decay to the ground state and non-radiative decay to the S_1 state, the steady-state condition for the $[\mathrm{Car}(S_2)]_{ss}$ is:

$$\frac{d[\mathrm{Car}(S_2)]}{dt}=0$$
$$={}^{irr}I_0(1-10^{-A_{irr}})-k_2[\mathrm{Car}(S_2)]_{ss}\cdot[{}^3O_2]$$
$$-k_{fl}[\mathrm{Car}(S_2)]_{ss}-\Sigma k_{nr}[\mathrm{Car}(S_2)]_{ss}\,, \tag{6}$$

where k_{fl} is the rate constant for fluorescence decay, and k_{nr} the rate constants for the non-radiative deactivation of the S_2 state. The photobleaching rate is proportional to the fraction of the light absorbed

$$v(\text{bleaching})={}^{irr}I_0\cdot(1-10^{-A_{irr}})\cdot\Phi^{irr} \tag{7}$$

and is related to the excited state rate constants through

$$v(\text{bleaching})=k_2[\mathrm{Car}(S_2)]_{ss}\cdot[{}^3O_2] \tag{8}$$

substitution of the steady-state concentration for $[\mathrm{Car}(S_2)]_{ss}$ into Eq. (8), and combining this latter equation with Eq. (7), yields

$$\Phi^{irr}=\frac{k_2[{}^3O_2]}{k_2[{}^3O_2]+k_{fl}+\Sigma k_{nr}}=k_2[{}^3O_2]\tau\,. \tag{9}$$

A direct comparison of the results from the photophysical and the photochemical experiments can be made by the use of Eq. 9, in which τ is the measured lifetime of the reactive singlet state. τ has been determined for β-carotene and is less than 1 ps [18, 19]. The oxygen solubility in chloroform is 3×10^{-3} mol/L [15], and taking the limiting second-order rate constant for diffusion controlled reactions in chloroform to be 1×10^{10} l · mol^{-1} · s^{-1} [23], an upper limit of $\phi^{irr}<3\times10^{-5}$ mol · Ein-

stein^{-1} is obtained. A similar calculation for the other aprotic solvents gives similar results, and the quantum yield determined for photobleaching resulting from excitation with visible light ($\lambda > 350$ nm) approaches this limit. In view of the many approximations involved, the qualitative agreement is encouraging. However, for irradiation with UV light and for astaxanthin solubilized in water, the quantum yields are significantly higher than predicted by Eq. 9, and the ratio between the observed quantum yield and the limiting quantum yield may provide a crude estimate of the length of the chain-propagating steps in the radical processes initiated by light absorption

$$\text{Chain length} = \frac{\Phi^{\text{irr}}}{\Phi^{\text{irr}}_{\text{lim}}}. \quad (10)$$

For irradiation at 254 nm; a chain length of approximately 10^4 is indicated. While the following photoinitiation process is likely

$$\text{Car} + h\nu \rightarrow \text{Car}(S_2), \quad (11)$$

$$\text{Car}(S_2) + O_2 \rightarrow \text{CarO}\cdot + \text{HO}\cdot. \quad (12)$$

the propagation steps are more uncertain and are likely to involve many different radicals including Car· (formed through hydrogen-atom abstraction). A more detailed discussion will have to await further experimental results. We are currently investigating the photochemistry of crocin, the water soluble carotenoid from saffron, with special emphasis on the role of oxygen radicals in its photodegradation.

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