

Kinetics of the Decolouring of Carotenoid Pigments

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Abstract: The decolouring reaction of carotenoids was studied using β -carotene, di-esterified capsanthin and capsanthin. The study was designed to determine what effects the increase in temperature, the nature of the reaction medium and the presence of light have on the kinetics of the reaction. The reactions that take place in an anhydrous medium follow a zero-order kinetics, while those that take place in an aqueous medium follow a first-order kinetics. Light and temperature accelerate the degradation reaction without changing the formal aspects of the reactions. The values for the thermodynamic parameters (activation energy and enthalpy of activation) of each reaction were different according to the conditions used, but this variation was due to a compensation effect. However, the existence of this effect confirms that the decolouring reaction is the first step of the carotenoid degradation, and this step is identical for all of the pigments irrespective of the reaction conditions, although the formal aspects and kinetics of this first step are strongly modulated by the nature of the pigment and the experimental conditions used.

Key words: carotenoids, decolouring, kinetics.

INTRODUCTION

The carotenoids are a group of pigments with colours varying from red to yellow, that are widely distributed throughout the plant kingdom (Isler 1971; Goodwin 1976). Until recently, the use of pure forms of these pigments by industry has been rather limited and mainly restricted to the production of pharmacological preparations due to the provitamin A activity of some of the pigments (Isler 1971; Simpson 1983). At the present time, however, the production of massive quantities of these pigments has begun to be more profitable, being employed as colourants, antioxidants, etc, chiefly by the food industry (Gross 1991).

The use of carotenoids as food colorants is increasing. This group of compounds fulfils certain commercially desirable properties such as their natural origin, null toxicity and high versatility, providing both lipo- and hydro-soluble colourants with colours ranging from yellow to red. Furthermore, they have the added advantage of having a high biological value, not only because

some of them have provitamin A activity, but also because they help to prevent ulcers, degenerative processes and cancer (Olson 1989). All of these beneficial effects possibly arise out of their antioxidant properties.

Almost all carotenoids have a C_{40} skeleton with a high degree of unsaturation. The number of conjugated double bonds is normally eleven and, in general, lateral hydroxyl or ketone substitution groups are present, increasing the possibility of electronic delocalisation even more (Isler 1971). In these conditions, the energetic proximity of excited states means that the molecule can absorb energy in the visible region of the spectrum, thus behaving as a chromophore (Davies 1976).

The lability of the carotenoid molecule is inherent to its condition as a chromophore. Provision of energy can break the conjugation sequence, possibly by promoting the electrons to anti-bonding orbitals, and this brings about the loss of its chromophore properties. On the other hand, the small amount of energy that is required to remove electrons makes the carotenoids highly effective antioxidants, although if they act as such then they lose their colourant properties and possibly their therapeutic properties also (Sweeney and Marsh 1970).

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In summary, therefore, the colourant and antioxidant properties of the carotenoids are inseparable and mutually exclusive. Despite this somewhat simplified theoretical interpretation of the properties of the carotenoid molecule, there are a number of variables that can modify the kinetics of the decolouring reaction and this fact makes it difficult to apply the results and conclusions of laboratory experiments to industry.

Cole and Kapur (1957a, 1957b), considered that the presence of oxygen in the headspace does not have excessive importance for the degradation of pigments in aqueous solutions. Chou and Breene (1972), however, studying the degradation of β -carotene with a system that enabled regulation of the water activity, demonstrated a strong effect of oxygen on the degree of decolouring achieved.

One of the main points of contention concerns the kinetic model that best describes the carotenoid degradation reaction. Philip and Francis (1971) using capsanthin did not find an induction step in the reaction and neither did Chou and Breene (1972). These results were interpreted as indicating the absence of autoxidation. The results of Philip and Francis (1971) indicate that the consumption of oxygen follows zero-order kinetics.

Goldman *et al* (1983) pointed out the controversy that exists in the literature with respect to the apparent kinetics of degradation and the existence, or otherwise, of an induction period. In their study they, like Chou and Breene (1972), found that, while oxygen is a limiting factor for the decolouring reaction, the capacity of the support to adsorb oxygen is much more limiting. Goldman *et al* (1983) observed a greater consumption of oxygen per mol of pigment than did Philip and Francis (1971), and they interpreted this as indicating the existence of an oxygenated multi-reaction.

Other authors have examined the effects of other variables. Thus, Carnevale *et al* (1979) studied the effects of light exposure and the presence of fatty acids on pigment degradation. Haralampu and Karel (1983) studying the degradation of pigments in dehydrated potatoes, found a correlation between the degradation of pigments and the water content of the samples.

Pesek and Warthesen (1989) established that in an aqueous medium and under conditions of strong lighting, the kinetics of β -carotene degradation were of first order, while the same authors (Pesek and Warthesen 1990) reported that the photo-isomerisation reaction followed approximately zero-order kinetics. Like Chu and Breene (1972) and Goldman *et al* (1983), Pesek and Warthesen (1989) and Pesek *et al* (1990) found the nature of the reaction medium to have a very strong influence on pigment degradation.

Therefore, a series of studies have been reported in the literature, but the results of these are not very easy to extrapolate, since there is no clear and unifying interpretation of the effects of variables external to the

reaction itself. The aim of the present study is to establish the kinetic and thermodynamic parameters that govern decolouring of carotenoid pigments under different environmental conditions. The following variables have been studied factorially; carotenoid pigments (β -carotene, capsanthin di-esterified and capsanthin), reaction media (cyclohexane, absolute ethanol and water), temperatures (15, 25, 35 and 45°C) and the presence or absence of light.

MATERIALS AND METHODS

Raw materials

The pigments used were β -carotene, di-esterified capsanthin and capsanthin. All these pigments had a purity of 95%. β -Carotene was supplied by Sigma (C-0126), while the di-esterified capsanthin and capsanthin were obtained in the laboratory from pepper following methodology described in previous publications (Mínguez-Mosquera and Hornero-Méndez 1993; Mínguez-Mosquera *et al* 1993).

A sufficient amount of each pigment was dissolved in 100 ml of cyclohexane to give stock solutions with a theoretical absorbance of 100. The stock solutions were placed under nitrogen in amber-coloured flasks which were hermetically sealed and stored in the dark at -30°C.

Reaction media

Two 0.25 ml aliquots of the stock solution of each pigment were taken and placed in separate 25 ml graduated flasks. The solvent was evaporated to dryness under a stream of nitrogen and the residues were redissolved directly in either absolute ethanol or cyclohexane. The volumes were made up to 25 ml. For preparing the aqueous solutions of the pigments, 0.125 g of Tween-20 was added to the 0.25 ml aliquots of the stock solutions prior to evaporation of the solvent. Subsequently, deionised water was added slowly with constant gentle shaking until a final volume of 25 ml was reached. The dilution performed gave pigment solutions with an absorbance close to unity with the three different solvents. All the procedures were performed under a soft green light at a room temperature of 20°C.

Environmental conditions for the reaction

A thermostatically controlled chamber was subdivided into two compartments. One was used to study the effects of light and was fitted with fluorescent tubes that produced a white light which was uniformly distributed throughout the compartment so that all points of it received an illuminance of 1000 lx. The second com-

partment was used to study the reactions of darkness. This compartment was lined in black and all joints were sealed to prevent the entry of light, achieving levels of illuminance lower than 5 lx. The temperatures used in the study were 15, 25, 35 and 45°C ($\pm 3^\circ\text{C}$).

Using the design described above, the carotenoid degradation reactions could be studied in both light and darkness simultaneously and under identical temperature conditions.

Preparation of the experiment and monitoring of the reaction

From each pigment solution prepared in the different solvents and for each temperature studied, four aliquots of 1.5 ml were taken and placed into spectrophotometric cuvettes which were then hermetically sealed. The remaining solution was discarded. Two cuvettes of each pigment and solvent were placed both under illumination and in darkness. Consequently, the experiment permits controlling 18 reactions simultaneously under the same conditions of reaction temperature and illumination.

To monitor the progress of the decolouring reaction, periodically each cuvette was removed from the chamber for 3 min for the spectrophotometric measurement. In previous controls of the experiment, it was established that in the fastest reactions (β -carotene in water at 45°C and under illumination), it was necessary to measure the absorbance each hour in order to get at least seven measurements between 1 and 0.1 units of absorbance. In the slowest reactions (capsanthin in cyclohexane at 15°C and in darkness) the measure could be performed at longer time interval. The measurement of the absorbance was always performed in duplicate and the data were expressed as percentage of retention of colour. The end of the reaction was fixed on reaching a colour retention around 10%, and in each sample, the experiments were repeated three times.

Analysis of results

For each pigment, solvent, temperature and illumination, a minimum of 84 data of pigment retention versus time were obtained. The percentage of retention of colour was related with time in hours (t) by the different mathematical fits in accord with a possible zero-order or first-order kinetics. In each of the 72 different reactions, the plot which gave the best correlation was selected, and the slope of the regression line was the rate constant of the degradation reaction (k_v). The integrated rate reactions expressed as percentage retention are shown in eqns (1) and (2).

$$\text{Zero order: \% retention} = 100 - k_v \times t(\text{h}) \quad (1)$$

$$t \in [0, 90/k_v]$$

$$\text{First order: } \ln(\% \text{ retention}) = 4,606 - k_v \times t(\text{h}) \quad (2)$$

$$t \in [0, 2.303/k_v]$$

For any one pigment and under identical conditions of reaction medium and light, the inverse of the reaction temperature (T) in K is plotted semi-logarithmically against the rate constants (k_v) obtained. According to the active complex theory of Arrhenius, the slope of the line corresponds to the energy of activation of the reaction (E_a) divided by the universal gas constant (R), and the ordinate at the origin is the logarithm of the reaction frequency factor ($\ln A$) (eqn 3).

$$\ln k_v = \ln A - \frac{E_a}{R} \times \frac{1}{T(\text{K})} \quad (3)$$

Plotting k_v/T against $1/T$ semi-logarithmically, the parameters corresponding to the transition state theory—the enthalpy of activation ($\Delta H^\ddagger$) and entropy of activation ($\Delta S^\ddagger$) (eqn 4)—were obtained.

$$\ln \frac{k_v}{T(\text{K})} = \ln \frac{k}{h} + \frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{RT(\text{K})} \quad (4)$$

RESULTS

Prior controls

Before starting the study it was considered important to examine two points which could be critical to the present research: the stability of the pigments in the stock solutions and the suitability of the system of measurement used.

A progressive deterioration of the stock solution would mean that the results found could not be correlated with the variables being studied since, in this situation, the stock solution itself would give rise to erroneous conclusions. Nevertheless, it was considered preferable to begin always from the same pigment solutions so that in all of the experiments, the starting point would be subject to the same variability and error. The absorbance of the pigments in each stock solution was measured at intervals, and no decrease was detected in the concentration of any of the pigments during the experimental period. The starting material can, therefore, be considered identical for all of the experiments.

The procedure used for following the reaction should be chosen after testing that it does not introduce any kind of error. The easiest operative alternative is to make the reaction take place in the spectrophotometric cuvettes where the reaction will be followed. From this point on the reaction could be monitored in two ways: (i) the reaction cuvette is periodically removed from the reaction conditions, its absorbance measured, and returned to the reaction conditions; and (ii) a battery of similar reaction cuvettes is prepared and placed under identical reaction conditions. Periodically one of them

is removed, and discarded after the measurement of absorbance. The first system could introduce an error due to the time employed for the measurement in which the sample is out of the prefixed reaction conditions. The second system does not introduce operative errors but does limit the number of reactions that can be simultaneously studied.

In order to test the suitability of these systems, solutions of β -carotene in cyclohexane, absolute ethanol and water were prepared and aliquots of 1.5 ml were taken and placed into spectrophotometric cuvettes which were then hermetically sealed. The reaction temperature was fixed at 25°C in both light and darkness. With both systems, seven determinations during the course of the reaction were made in duplicate at identical intervals of time and the results were later compared.

The kinetic parameters and the standard deviation for the decolouring reaction of β -carotene were determined by both methods and are given in Table 1. In each experiment three rate constants were obtained (one for each solvent) in the light and three in darkness. The data corresponding to the two alternative systems employed for the same reaction have been compared using analysis of variance. No statistically significant differences were found ($P < 0.05$). Consequently, for the present study the system of monitoring chosen has been

the continuous one, since, besides not introducing errors into the results, it allows studying a great number of reactions simultaneously.

Rate of decolouring of carotenoid pigments

In Tables 2, 3 and 4 are shown the kinetic parameters for the decolouring of β -carotene, di-esterified capsanthin and capsanthin, respectively, under the different experimental conditions.

Besides the usual dependence of k_d on the temperature, it can be observed that the reaction medium had a decisive effect both on the formal aspects of the reaction and on the rate of the reaction. The results show clearly that in anhydrous media decolouring of the carotenoids followed a zero-order kinetics, while in an aqueous medium the reaction was first order. These results show that the different and sometimes contradictory results reported in the literature (Goldman *et al* 1983) could be imputed to the reaction medium.

β -Carotene was found to be the most labile of the pigments under almost all of the conditions tested. However, there was no clear difference between the stability of di-esterified capsanthin and that of capsanthin. Capsanthin is the pigment which is present in highest

TABLE 1
Comparison of systems for measuring the progress of β -carotene degradation at 25°C
(mean $\pm$ SD)

Conditions and reaction media	Kinetic parameters of the β -carotene decolouring reaction at 25°C			
	Reaction order	Rate constant (h^{-1})	Ordinate at the origin	R
<i>System A^a</i>				
<i>Light</i>				
Cyclohexane	0	1.011 $\pm$ 0.095	94.47 $\pm$ 7.29	0.967
Absolute ethanol	0	1.630 $\pm$ 0.130	98.96 $\pm$ 1.35	0.992
Water	1	0.020 $\pm$ 0.000	4.534 $\pm$ 0.040	0.994
<i>Darkness</i>				
Cyclohexane	0	0.596 $\pm$ 0.070	98.15 $\pm$ 0.25	0.995
Absolute ethanol	0	0.386 $\pm$ 0.023	88.18 $\pm$ 6.10	0.963
Water	1	0.003 $\pm$ 0.000	4.598 $\pm$ 0.008	0.931
<i>System B^b</i>				
<i>Light</i>				
Cyclohexane	0	1.011 $\pm$ 0.015	96.32 $\pm$ 2.47	0.986
Absolute ethanol	0	1.630 $\pm$ 0.033	97.05 $\pm$ 0.95	0.994
Water	1	0.021 $\pm$ 0.000	4.586 $\pm$ 0.025	0.994
<i>Darkness</i>				
Cyclohexane	0	0.593 $\pm$ 0.036	98.63 $\pm$ 0.25	0.997
Absolute ethanol	0	0.361 $\pm$ 0.031	93.25 $\pm$ 4.64	0.969
Water	1	0.003 $\pm$ 0.000	4.602 $\pm$ 0.004	0.957

^a System A: The same cuvette is periodically removed and measurements made.

^b System B: From a battery of cuvettes, periodically one cuvette is removed, measured and later discarded.

TABLE 2
Kinetic parameters of the β -carotene decolouring reaction obtained from the regression lines^a (mean $\pm$ SD)

Reaction media and temperature	Reaction under illumination				Reaction in darkness			
	Reaction order	Rate constant (h^{-1})	Ordinate at the origin	R	Reaction order	Rate constant (h^{-1})	Ordinate at the origin	R
Cyclohexane								
15°C	0	0.485 $\pm$ 0.062	95.07 $\pm$ 7.06	0.961	0	0.011 $\pm$ 0.000	99.01 $\pm$ 0.41	0.894
25°C	0	1.011 $\pm$ 0.095	94.47 $\pm$ 7.29	0.967	0	0.596 $\pm$ 0.070	98.15 $\pm$ 0.25	0.995
35°C	0	1.211 $\pm$ 0.092	101.34 $\pm$ 1.88	0.988	0	0.798 $\pm$ 0.022	98.07 $\pm$ 1.70	0.932
45°C	0	1.674 $\pm$ 0.090	100.83 $\pm$ 2.10	0.959	0	1.045 $\pm$ 0.037	99.13 $\pm$ 0.80	0.899
Absolute ethanol								
15°C	0	0.234 $\pm$ 0.003	100.03 $\pm$ 0.23	0.999	0	0.009 $\pm$ 0.000	100.19 $\pm$ 0.93	0.982
25°C	0	1.630 $\pm$ 0.130	98.96 $\pm$ 1.35	0.992	0	0.386 $\pm$ 0.023	88.18 $\pm$ 6.10	0.963
35°C	0	2.279 $\pm$ 0.062	99.75 $\pm$ 1.44	0.961	0	0.942 $\pm$ 0.040	94.18 $\pm$ 8.30	0.884
45°C	0	2.564 $\pm$ 0.035	94.43 $\pm$ 1.94	0.966	0	1.362 $\pm$ 0.045	94.35 $\pm$ 2.26	0.921
Water								
15°C	1	0.014 $\pm$ 0.000	4.626 $\pm$ 0.034	0.996	1	0.002 $\pm$ 0.000	4.594 $\pm$ 0.009	0.967
25°C	1	0.020 $\pm$ 0.000	4.534 $\pm$ 0.040	0.994	1	0.003 $\pm$ 0.000	4.598 $\pm$ 0.008	0.931
35°C	1	0.021 $\pm$ 0.001	4.571 $\pm$ 0.010	0.991	1	0.005 $\pm$ 0.000	4.585 $\pm$ 0.015	0.974
45°C	1	0.060 $\pm$ 0.001	4.571 $\pm$ 0.010	0.998	1	0.015 $\pm$ 0.000	4.558 $\pm$ 0.014	0.980

^a Zero order: % retention = $100 - k_p \times t(h)$. First order: $\ln$ % retention = $4.606 - k_p \times t(h)$.

amounts in the red pepper and when the fruit is fully ripe it is found chiefly in the di-esterified form with mono-unsaturated fatty acids (Mínguez-Mosquera and Hornero-Méndez 1994). Esterification of the carotenoid pigments is fairly common in fruits and normally coin-

cides with the loss of chloroplast function and ripening of the fruits. De-esterification of the pigment appears to give the pigment a greater stability in aqueous medium and lower stability in non-polar media, while in an intermediate polar medium both forms of the pigment

TABLE 3
Kinetic parameters for the di-esterified capsanthin decolouring reaction obtained from the regression lines^a (mean $\pm$ SD)

Reaction media and temperature	Reaction under illumination				Reaction in darkness			
	Reaction order	Rate constant (h^{-1})	Ordinate at the origin	R	Reaction order	Rate constant (h^{-1})	Ordinate at the origin	R
Cyclohexane								
15°C	0	0.259 $\pm$ 0.019	102.56 $\pm$ 2.00	0.988	0	0.004 $\pm$ 0.000	99.48 $\pm$ 1.09	0.882
25°C	0	0.360 $\pm$ 0.024	98.80 $\pm$ 2.10	0.994	0	0.042 $\pm$ 0.002	98.69 $\pm$ 2.36	0.917
35°C	0	0.424 $\pm$ 0.019	102.04 $\pm$ 2.03	0.996	0	0.075 $\pm$ 0.005	99.23 $\pm$ 0.62	0.956
45°C	0	0.476 $\pm$ 0.023	100.35 $\pm$ 0.51	0.995	0	0.098 $\pm$ 0.009	100.00 $\pm$ 1.13	0.980
Absolute ethanol								
15°C	0	0.038 $\pm$ 0.003	99.78 $\pm$ 0.20	0.997	0	0.002 $\pm$ 0.000	98.83 $\pm$ 0.41	0.882
25°C	0	0.159 $\pm$ 0.010	96.42 $\pm$ 5.64	0.951	0	0.021 $\pm$ 0.000	92.86 $\pm$ 3.78	0.917
35°C	0	0.253 $\pm$ 0.020	98.36 $\pm$ 0.53	0.965	0	0.035 $\pm$ 0.002	98.70 $\pm$ 0.99	0.955
45°C	0	0.420 $\pm$ 0.030	99.25 $\pm$ 1.33	0.987	0	0.058 $\pm$ 0.002	96.37 $\pm$ 1.05	0.980
Water								
15°C	1	0.010 $\pm$ 0.000	4.678 $\pm$ 0.053	0.984	1	0.002 $\pm$ 0.000	4.537 $\pm$ 0.020	0.906
25°C	1	0.030 $\pm$ 0.000	4.607 $\pm$ 0.020	0.998	1	0.008 $\pm$ 0.000	4.565 $\pm$ 0.035	0.900
35°C	1	0.073 $\pm$ 0.003	4.586 $\pm$ 0.060	0.991	1	0.013 $\pm$ 0.000	4.571 $\pm$ 0.005	0.978
45°C	1	0.159 $\pm$ 0.006	4.551 $\pm$ 0.050	0.995	1	0.051 $\pm$ 0.001	4.570 $\pm$ 0.009	0.991

^a Zero order: % retention = $100 - k_p \times t(h)$. First order: $\ln$ % retention = $4.606 - k_p \times t(h)$.

TABLE 4
Kinetic parameters for the capsanthin decolouring reaction obtained from the regression lines^a (mean $\pm$ SD)

Reaction media and temperature	Reaction under illumination				Reaction in darkness			
	Reaction order	Rate constant (h^{-1})	Ordinate at the origin	R	Reaction order	Rate constant (h^{-1})	Ordinate at the origin	R
Cyclohexane								
15°C	0	0.404 $\pm$ 0.008	99.21 $\pm$ 0.80	0.991	0	0.023 $\pm$ 0.001	100.64 $\pm$ 2.33	0.966
25°C	0	0.461 $\pm$ 0.020	95.78 $\pm$ 2.30	0.996	0	0.035 $\pm$ 0.001	95.87 $\pm$ 2.40	0.948
35°C	0	0.528 $\pm$ 0.015	96.29 $\pm$ 1.82	0.998	0	0.060 $\pm$ 0.002	93.20 $\pm$ 3.41	0.969
45°C	0	0.601 $\pm$ 0.017	98.35 $\pm$ 0.96	0.997	0	0.081 $\pm$ 0.002	98.31 $\pm$ 1.27	0.981
Absolute ethanol								
15°C	0	0.110 $\pm$ 0.011	99.99 $\pm$ 1.34	0.972	0	0.002 $\pm$ 0.000	100.64 $\pm$ 0.15	0.920
25°C	0	0.119 $\pm$ 0.010	93.98 $\pm$ 3.20	0.960	0	0.028 $\pm$ 0.000	95.57 $\pm$ 2.96	0.981
35°C	0	0.150 $\pm$ 0.019	99.14 $\pm$ 0.60	0.983	0	0.030 $\pm$ 0.001	98.20 $\pm$ 1.33	0.947
45°C	0	0.204 $\pm$ 0.008	99.36 $\pm$ 0.41	0.996	0	0.034 $\pm$ 0.001	98.31 $\pm$ 0.66	0.990
Water								
15°C	1	0.011 $\pm$ 0.000	4.635 $\pm$ 0.009	0.995	1	0.001 $\pm$ 0.000	4.540 $\pm$ 0.005	0.815
25°C	1	0.014 $\pm$ 0.000	4.566 $\pm$ 0.020	0.996	1	0.006 $\pm$ 0.000	4.588 $\pm$ 0.021	0.995
35°C	1	0.019 $\pm$ 0.000	4.586 $\pm$ 0.020	0.997	1	0.006 $\pm$ 0.000	4.566 $\pm$ 0.009	0.989
45°C	1	0.027 $\pm$ 0.001	4.568 $\pm$ 0.018	0.989	1	0.015 $\pm$ 0.000	4.603 $\pm$ 0.013	0.999

^a Zero order: % retention = 100 - $k_v \times t(h)$. First order: $\ln$ % retention = 4.606 - $k_v \times t(h)$.

have a similar degree of stability. This fact could be important on using these pigments as natural colourants, due to the different physico-chemical characteristics of the foods in which they will be used.

Influence of temperature on the rate of reaction

The correlation between these two parameters was studied. Using the activated complex theory the activation energy of each reaction and its frequency factor

was determined (Table 5). Applying the transition state theory $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were obtained (Table 6).

The results obtained after applying both theories show that temperature affected in a different way all the reactions studied and there was no clear trend. For any one reaction, both the activation energy and the enthalpy of activation were lower if the reaction occurred in the light than if it occurred in darkness. At the same time, this lower energy value coincided with a lower frequency factor or entropy. Theoretically, a lower energy

TABLE 5
Activation energy ($kcal\ mol^{-1}$) for the decolouring reaction of carotenoid pigments (mean $\pm$ SD)

Pigment and reaction medium	Reaction under illumination			Reaction in darkness		
	Activation energy ($kcal\ mol^{-1}$)	$\ln A$	R	Activation energy ($kcal\ mol^{-1}$)	$\ln A$	R
β-Carotene						
Cyclohexane	7.07 $\pm$ 1.31	11.78 $\pm$ 2.18	0.967	25.68 $\pm$ 10.91	41.50 $\pm$ 18.19	0.857
Absolute ethanol	13.80 $\pm$ 5.10	23.21 $\pm$ 8.51	0.886	29.21 $\pm$ 9.29	47.34 $\pm$ 15.50	0.912
Water	7.82 $\pm$ 2.75	9.31 $\pm$ 4.59	0.895	11.66 $\pm$ 2.38	14.06 $\pm$ 3.98	0.961
Di-esterified capsanthin						
Cyclohexane	3.61 $\pm$ 0.52	5.02 $\pm$ 0.87	0.979	18.62 $\pm$ 5.70	27.64 $\pm$ 9.51	0.917
Absolute ethanol	13.93 $\pm$ 2.57	21.39 $\pm$ 4.29	0.967	19.37 $\pm$ 5.24	28.21 $\pm$ 8.74	0.933
Water	16.56 $\pm$ 0.45	24.46 $\pm$ 0.75	0.999	18.33 $\pm$ 2.31	25.96 $\pm$ 3.86	0.984
Capsanthin						
Cyclohexane	2.38 $\pm$ 0.06	3.26 $\pm$ 0.10	0.999	7.77 $\pm$ 0.47	9.84 $\pm$ 0.78	0.966
Absolute ethanol	3.71 $\pm$ 0.76	4.22 $\pm$ 1.27	0.960	15.81 $\pm$ 7.48	22.17 $\pm$ 12.48	0.830
Water	5.37 $\pm$ 0.46	4.86 $\pm$ 0.78	0.992	14.74 $\pm$ 4.01	19.23 $\pm$ 6.69	0.933

TABLE 6
Thermodynamic parameters for the decolouring reaction of carotenoid pigments (mean $\pm$ SD)

Pigment and reaction medium	Reaction under illumination			Reaction in darkness		
	Enthalpy of activation (kcal mol ⁻¹)	Entropy of activation (cal mol ⁻¹ K ⁻¹)	R	Enthalpy of activation (kcal mol ⁻¹)	Entropy of activation (cal mol ⁻¹ K ⁻¹)	R
β -Carotene						
Cyclohexane	7.71 $\pm$ 1.35	5.95 $\pm$ 0.72	0.970	26.33 $\pm$ 11.1	64.92 $\pm$ 25.03	0.857
Absolute ethanol	14.44 $\pm$ 5.24	28.59 $\pm$ 8.33	0.889	29.91 $\pm$ 9.58	76.64 $\pm$ 22.59	0.910
Water	8.51 $\pm$ 2.73	1.26 $\pm$ 0.35	0.910	12.39 $\pm$ 2.32	10.79 $\pm$ 1.98	0.966
Di-esterified capsanthin						
Cyclohexane	3.22 $\pm$ 0.55	-7.52 $\pm$ 0.58	0.983	19.29 $\pm$ 5.88	37.48 $\pm$ 10.6	0.918
Absolute ethanol	14.61 $\pm$ 2.68	25.12 $\pm$ 3.97	0.967	20.05 $\pm$ 5.41	38.68 $\pm$ 9.96	0.934
Water	17.56 $\pm$ 0.57	31.38 $\pm$ 0.95	0.998	19.09 $\pm$ 2.30	34.47 $\pm$ 4.01	0.985
Capsanthin						
Cyclohexane	3.00 $\pm$ 0.05	-10.98 $\pm$ 0.08	0.999	8.43 $\pm$ 0.49	2.17 $\pm$ 0.10	0.996
Absolute ethanol	4.34 $\pm$ 0.74	-9.02 $\pm$ 1.01	0.971	16.43 $\pm$ 7.64	26.51 $\pm$ 11.6	0.835
Water	6.02 $\pm$ 0.43	-7.72 $\pm$ 0.47	0.994	14.83 $\pm$ 4.10	20.90 $\pm$ 5.48	0.936

required suggests a greater sensitivity to temperature, but at the same time this coincides with the fact that there is a lower proportion of molecules able to take part in the reaction. This means that it is impossible to obtain one single thermodynamic parameter to predict stability as a function of temperature.

DISCUSSION

The results of the kinetic study suggest that the decolouring reactions of the carotenoid pigments behave as independent reactions in each case and that variables such as the reaction medium or changes in the environmental conditions make it impossible to compare the reactions.

β -Carotene is more labile than capsanthin as can be seen from the rates of the decolouring reaction. However, this difference in lability is not reflected in thermodynamic parameters, since both the energy and enthalpy of activation of the decolouring reaction of β -carotene in the light are greater than, or the same as, those of the capsanthin decolouring reaction. The fact that the rates of decolouring reaction of β -carotene are always higher than that of capsanthin, together with similar thermodynamic parameters, could be interpreted as an intrinsic higher lability of β -carotene but a different effect on both reactions by the temperature.

Assuming that in the degradation reaction of carotenoids, decolouring is the first step and implies only the loss of conjugation in the series of double bonds of the molecule, it is rather surprising that external factors have such a strong effect as to make the reactions, which are in principle quite similar, non-comparable. This apparent contradiction occurs as a result of solvent and reaction medium effects, which produce a reversal of the theoretical order of lability. Such a phenomenon has been well-described in the literature on heterogeneous catalysis and is known as the compensation effect (Cremer 1955; Galwey 1977). In summary, the increase in the energy or enthalpy of activation often does not provoke the expected decrease in the rate constant of the reaction since there is normally a simultaneous increase in the frequency factor or entropy of activation.

This effect is clearly illustrated when the energy of activation is plotted against the frequency factor or the enthalpy of activation against the entropy of activation (Fig. 1). In this figure the values of all of the reactions have been included. As can be seen, an isokinetic line is obtained, which supports the hypothesis that there is one single reaction in all cases, although external factors have a strong effect on the reaction.

Canjura *et al* (1991) found a clear compensation effect in the degradation reactions of chlorophylls and chlorophyllids, which explains the lack of agreement between the kinetic data in the literature on the degra-

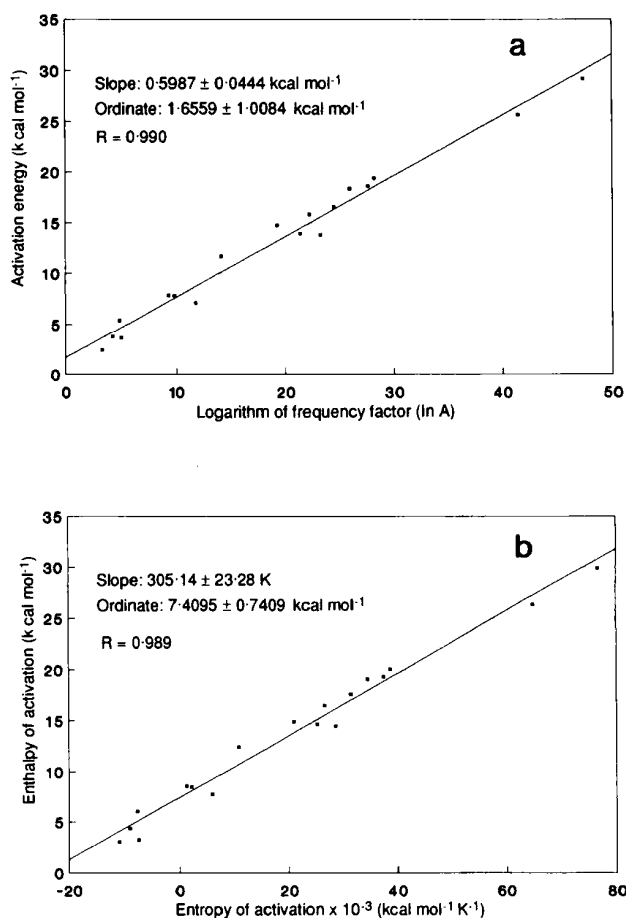


Fig. 1. Regression lines between the thermodynamic parameters of the carotenoid decolouring reaction. (a) Activation energy versus logarithm of frequency factor. (b) Enthalpy of activation versus entropy of activation.

dation of chlorophyllic compounds. Those authors distinguish between a true compensation and an apparent compensation effect, and they attribute the appearance of the second one to the existence of experimental errors. To test the validity of the compensation effect, Rhim *et al* (1989) propose the comparison of the isokinetic temperature with the harmonic mean temperature. If they are significantly different, there exists a true compensation effect.

The results found in the present study place the harmonic temperature (302.5 K) within the range calculated for the isokinetic temperature (Fig 1(b)), which could be interpreted, according to Canjura *et al* (1991), Rhim *et al* (1989) and Rhim *et al* (1990), as false compensation effect. The hypothesis employed by these authors is that in the same reaction the appearance of a false compensation effect shows experimental errors. In this study the argument should be employed in the contrary direction. If several reactions, in principle all of them different, show a compensation effect, then, in all cases the reaction is the same and necessarily the effect

would be 'false', since all the reactions are performed under different conditions (different pigment, reaction media, etc). The 'experimental errors' cannot be employed as the only explanation of 'false compensation effect'. This effect could appear due to changes introduced into the reaction conditions, as suggested by Galwey (1976).

It could be concluded that the existence of the compensation effect makes it impossible to extrapolate the experimental results to other products or conditions. These differences produce their own specific situations that are not easily predictable.

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