

Effect of High-Temperature Degradative Processes on Ketocarotenoids Present in Paprika Oleoresins

Antonio Pérez-Gálvez, Manuel Jarén-Galán, and Maria Isabel Mínguez-Mosquera*

Departamento de Biotecnología de Alimentos, Instituto de la Grasa (CSIC), 41012, Sevilla, Spain

Carotenoid pigments in six commercial oleoresins, qualitatively uniform in pigment composition, were thermodegraded to determine the degradation kinetics in the two (red and yellow) isochromic fractions, structurally differentiated by the presence or absence of keto groups. The different rate of degradation of total pigment indicates that the oily medium modulates the degradation reaction differently, so that each oleoresin shows a different stability. However, in all the oleoresins, the red pigment fraction is degraded at a higher rate than the yellow. Arrhenius curves reveal an isokinetic temperature (T_{isok}) distinguishing two zones in which the degradation process has a different effect on each fraction. At temperatures above T_{isok} , degradation is preferentially toward the red fraction, while at lower temperatures, it is toward the yellow fraction. An isokinetic point between the two pigment fractions means that the thermal conditions at which the stability of the system is to be determined must be stated, as such conditions produce a different effect on each fraction.

Keywords: Arrhenius parameters; carotenoids; isokinetic effect; paprika; oleoresin; thermodegradation

INTRODUCTION

Depending on the way pepper fruits are processed, two commercial forms of carotenoid pigment concentrate are obtained: paprika and oleoresin. Because of the nature of the paprika production process, which in certain areas continues to be a craft, technological innovation in the two basic operations of drying and milling is infrequent (Mínguez-Mosquera et al., 1996). The production of oleoresin, however, includes not only drying and milling but also extraction with organic solvent, which is then removed. This requires a final heat treatment of the product for the removal of solvent residues to the statutory requirements. The thermal effects during this treatment include isomerization and degradation reactions of the carotenoid pigments (Govindarajan, 1986). To avoid degradation of the product, new extraction methods using supercritical fluids have been recently developed (Jarén-Galán et al., 1999). The final product is an oily concentrate of pigments with a polyunsaturated lipid matrix similar to that of paprika.

The aim in both cases is common to that of most technological processes—to obtain a quality product. In the case of paprika and oleoresin, quality is synonymous with color, which implicitly determines the commercial value of the product (Mínguez-Mosquera and Pérez-Gálvez, 1998). Processing can be assumed to result in loss of coloring capacity with respect to that of the raw material, but as the carotenoid pigment concentrate obtained from pepper fruit is fairly nonuniform (containing β -carotene, hydroxylated xanthophylls with β ring, epoxides of these xanthophylls, and hydroxylated xanthophylls with κ ring and keto groups, and their epoxides), such loss is not necessarily uniform or even the same under different processing conditions.

Different studies of the stability of carotenoid pigments in paprika have established a behavior pattern

in the face of different degradation processes, with the pigments of the red isochromic fraction—capsanthin and capsorubin—being more stable than those of the yellow isochromic fraction (Pérez-Gálvez et al., 1999). The stability of carotenoids in oleoresins has also been studied, but here the behavior pattern depends on the degradation conditions, in particular on the temperature used. It was established that at low temperatures the behavior pattern is the same as in paprika but that above a certain temperature (situated within the temperature range used) the pattern is inverted (Jarén-Galán and Mínguez-Mosquera, 1999).

The existence of such inversion implies an isokinetic point (IKP) that could lead mathematically to a linear relationship between the enthalpy and the entropy of activation, referred to as the “*compensation effect*” (Labuza, 1980). The determination of the isokinetic point is used as a tool in the field of structural research and reaction mechanisms. It allows groups of reactions to be distinguished that are apparently similar but which, when grouped around a physical or chemical parameter, are observed to take place by different mechanisms. Depending on the parameter in question, a particular step in the reaction may have special importance, indicated by a change in the activation energy (E_a) and preexponential factor (A).

Apart from this situation, a change may be involved not in the mechanism but in the structure of the activated complex (Boots and Bokx, 1989; Bokx and Boots, 1990; Galwey and Brown, 1997). Thus, the study of the IKP can be an excellent tool to determine whether changes take place in the reaction mechanism under different experimental conditions. With the existence of the IKP established, it is necessary to perform further experiments under different experimental conditions.

The aim of the present work is to make a kinetic study of carotenoid pigment degradation in the two (red and yellow) isochromic fractions of commercial oleoresins under reaction conditions different (higher tempera-

* To whom correspondence should be addressed. E-mail: aperez@cica.es.

Table 1. Kinetic Parameters Obtained from the Fit of the Concentration of Total Pigments to First-Order Kinetics for the Six Oleoresins and at the Three Temperatures Used

totals ^b	kinetic parameters ^a		
	($k \pm$ S.E.) $\times 10^{-4}$	origin	R
	373 K		
oleoresin 1	11.38 \pm 0.47	4.616	0.970
oleoresin 2	20.84 \pm 0.57	4.635	0.986
oleoresin 3	19.62 \pm 0.57	4.658	0.986
oleoresin 4	12.52 \pm 0.47	4.635	0.976
oleoresin 5	34.79 \pm 3.92	4.677	0.947
oleoresin 6	11.18 \pm 0.52	4.620	0.962
	383 K		
oleoresin 1	18.66 \pm 0.67	4.508	0.973
oleoresin 2	35.43 \pm 1.00	4.587	0.983
oleoresin 3	30.35 \pm 1.12	4.570	0.972
oleoresin 4	18.55 \pm 1.01	4.568	0.942
oleoresin 5	52.83 \pm 3.17	4.593	0.958
oleoresin 6	18.03 \pm 0.90	4.570	0.954
	393 K		
oleoresin 1	38.13 \pm 2.10	4.435	0.956
oleoresin 2	65.26 \pm 2.18	4.512	0.983
oleoresin 3	57.45 \pm 2.38	4.501	0.975
oleoresin 4	36.78 \pm 2.12	4.438	0.952
oleoresin 5	80.19 \pm 4.24	4.525	0.961
oleoresin 6	35.87 \pm 1.95	4.438	0.957

^a First-order kinetic model: $\ln \% \text{ ret} = 4.605 - k \times t(\text{h})$. S.E.: standard error in the determination ($p < 0.05$). ^b Totals = total carotenoid concentration.

Table 2. Kinetic Parameters Obtained from the Fit of the Concentration of Red Pigments to First-Order Kinetics for the Six Oleoresins and at the Three Temperatures Used

red ^b	kinetic parameters ^a		
	($k \pm$ S.E.) $\times 10^{-4}$	origin	R
	373 K		
oleoresin 1	12.59 \pm 0.56	4.629	0.965
oleoresin 2	22.75 \pm 0.68	4.650	0.984
oleoresin 3	20.87 \pm 0.62	4.674	0.986
oleoresin 4	12.82 \pm 0.44	4.636	0.979
oleoresin 5	41.33 \pm 4.06	4.675	0.942
oleoresin 6	12.92 \pm 0.57	4.628	0.966
	383 K		
oleoresin 1	22.86 \pm 0.72	4.535	0.980
oleoresin 2	42.47 \pm 1.21	4.639	0.983
oleoresin 3	33.51 \pm 1.18	4.583	0.974
oleoresin 4	21.34 \pm 1.10	4.596	0.948
oleoresin 5	62.74 \pm 3.08	4.607	0.968
oleoresin 6	22.14 \pm 0.99	4.616	0.962
	393 K		
oleoresin 1	51.68 \pm 2.11	4.451	0.976
oleoresin 2	85.38 \pm 2.83	4.576	0.983
oleoresin 3	70.87 \pm 2.74	4.527	0.978
oleoresin 4	45.29 \pm 2.22	4.438	0.965
oleoresin 5	95.23 \pm 4.33	4.562	0.975
oleoresin 6	47.91 \pm 2.00	4.454	0.974

^a First-order kinetic model: $\ln \% \text{ ret} = 4.605 - k \times t(\text{h})$. S.E.: standard error in the determination ($p < 0.05$). ^b Red = capsorubin + capsanthin.

tures) to those used in the earlier study to determine (from A and E_a) whether the pattern of stability is the same and if there are changes in the reaction mechanism, which would be indicated by changes in the parameters of the Arrhenius equation.

MATERIALS AND METHODS

Starting Material and Thermodegradation Reaction.

Six commercial oleoresins were used: their pigment composition differed quantitatively, but the qualitative composition was the same. Thermal treatment was at three temperatures: 373, 383, and 393 K. Subsamples of each oleoresin were

Table 3. Kinetic Parameters Obtained from the Fit of the Concentration of Yellow Pigments to First-Order Kinetics for the Six Oleoresins and at the Three Temperatures Used

yellow ^b	kinetic parameters ^a		
	($k \pm$ S.E.) $\times 10^{-4}$	origin	R
	373 K		
oleoresin 1	9.99 \pm 0.42	4.600	0.969
oleoresin 2	18.73 \pm 0.67	4.620	0.977
oleoresin 3	17.79 \pm 0.59	4.634	0.982
oleoresin 4	11.93 \pm 0.55	4.631	0.964
oleoresin 5	24.90 \pm 4.71	4.681	0.931
oleoresin 6	9.20 \pm 0.49	4.612	0.952
	383 K		
oleoresin 1	14.23 \pm 0.69	4.478	0.954
oleoresin 2	28.60 \pm 0.89	4.534	0.980
oleoresin 3	26.00 \pm 1.11	4.551	0.963
oleoresin 4	14.71 \pm 0.93	4.527	0.923
oleoresin 5	36.46 \pm 3.42	4.576	0.933
oleoresin 6	13.35 \pm 0.85	4.513	0.928
	393 K		
oleoresin 1	27.52 \pm 2.04	4.441	0.924
oleoresin 2	51.45 \pm 2.11	4.485	0.975
oleoresin 3	44.69 \pm 2.35	4.496	0.960
oleoresin 4	27.56 \pm 1.95	4.449	0.931
oleoresin 5	48.17 \pm 4.06	4.495	0.922
oleoresin 6	25.38 \pm 1.79	4.436	0.931

^a First-order kinetic model: $\ln \% \text{ ret} = 4.605 - k \times t(\text{h})$. S.E.: standard error in the determination ($p < 0.05$). ^b Yellow = violaxanthin + cucurbitaxanthin A + zeaxanthin + β -cryptoxanthin + β -carotene.

placed in an oven at the selected temperature and removed at time intervals of 1–2 days at the beginning of the experiment and each 3 days at the end. Sampling of each oleoresin, at each time interval, was in triplicate, and the sample was kept in the freezer at 253 K.

Quantification of Carotenoid Pigments. Carotenoid composition was determined according to the method described by Mínguez-Mosquera and Hornero-Méndez, 1993. After the sample was established to be de-esterified, it was analyzed chromatographically using a Waters chromatograph fitted with a quaternary pump (model 600) and a UV–vis detector (model 996). The manual injection valve (Rheodyne, model 7125) was fitted with a 20 μL loop.

Kinetic Parameters. Data of individual composition were grouped as red and yellow isochromic fractions, for each oleoresin and temperature, expressing the concentration as percentage of retention. The order of reaction was determined by trial and error using the integral method: an order of reaction was assumed and the data were fitted to the integrated expression relating the variation in percentage of retention with time. That expression is

$$\ln \% \text{ Ret} = 4.605 - k \times t(\text{h})$$

from which the value of the kinetic constant of degradation at temperature T was obtained for each oleoresin and pigment fraction.

Representation on semilogarithmic coordinates of the value of the constant k versus T^{-1} , for each oleoresin and pigment fraction, gave the value of the activation energy (E_a) and the preexponential factor (A), according to the Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT} \quad (1)$$

The isokinetic point verifies that the degradation constant is the same for both pigment fractions, so that from the expression

$$\ln A(R) - \frac{E_a(R)}{RT_{\text{isok}}} = \ln A(\text{Am}) - \frac{E_a(\text{Am})}{RT_{\text{isok}}}$$

Table 4. Arrhenius Equation Parameters, Activation Energy, and Pre-Exponential Factor for the Thermodegradation Reaction by Red and Yellow Isochromic Fractions

	red fraction		yellow fraction	
	$E_a \pm \text{S.E.}^c$ (kJ/mol)	$\ln A \pm \text{S.E.}^c$ ($\text{s}^{-1} \text{K}^{-1}$)	$E_a \pm \text{S.E.}$ (kJ/mol)	$\ln A \pm \text{S.E.}$ ($\text{s}^{-1} \text{K}^{-1}$)
oleoresin 1	85.92 \pm 9.00	21.00 \pm 2.83	61.57 \pm 11.7	12.89 \pm 3.67
oleoresin 2	80.54 \pm 3.82	19.87 \pm 1.20	61.48 \pm 6.69	13.51 \pm 2.10
oleoresin 3	74.34 \pm 10.8	17.75 \pm 3.40	56.03 \pm 6.54	11.71 \pm 2.06
oleoresin 4	76.76 \pm 9.70	18.05 \pm 3.05	50.78 \pm 15.5	9.58 \pm 4.98
oleoresin 5	50.86 \pm 0.76	10.91 \pm 0.25	40.25 \pm 3.02	7.00 \pm 0.95
oleoresin 6	79.72 \pm 9.40	19.01 \pm 2.96	61.68 \pm 10.4	12.85 \pm 3.28

^a Red fraction = capsorubin + capsanthin. ^b Yellow fraction = violaxanthin + cucurbitaxanthin A + zeaxanthin + β -cryptoxanthin + β -carotene. ^c Activation energy and preexponential factor obtained from the Arrhenius equation (eq 1). S.E.: standard error in the determination ($p < 0.05$).

in which $A(R)$, $E_a(R)$, and $A(\text{Am})E_a(\text{Am})$ are the parameters of the Arrhenius equation for the red and yellow fractions, respectively, the isokinetic temperature (T_{isok}) is obtained.

Thermodynamic Parameters. The equation including the Planck and Boltzmann constants, according to the activated complex theory, was used to determine the enthalpy and entropy of activation

$$\ln k = \ln \frac{k_b T}{h} - \frac{\Delta H^*}{RT} + \frac{\Delta S^*}{R} \quad (2)$$

in which k is the degradation constant at temperature T , k_b is the Boltzmann constant, and h is the Planck constant. Representation of k versus T^{-1} gives the thermodynamic parameters ΔH^* and ΔS^* . Numerical calculations and the fitting of the experimental data to the mathematical models were performed using the program Statistica (Statsoft Inc.), version 5.1.

RESULTS

Table 1 displays the values of the degradation constants according to a first-order kinetic model, for total pigments. The fact that each oleoresin shows a different degradation rate indicates that each can be considered a different reaction medium in which the rest of the components modulate the degradation of the carotenoid pigments. The importance is thus shown of the medium and the reaction conditions on stability, determined in this case by the higher or lower rate of carotenoid degradation. The order of stability of the oleoresins studied is

$$O5 \ll O2 \cong O3 \ll O1 \cong O4 \cong O6$$

The degradation rate of the red carotenoids was higher than that of the yellow ones for all the oleoresins and at all the temperatures (data shown in Tables 2 and 3). The lipid medium dissolving the carotenoid fraction is mainly polyunsaturated and will, at the temperatures used, produce oxidative reactions, with loss in the carotenoid composition. However, each fraction will be affected differently, not in the form of the reaction but in its rate. Degradation particularly affects the red pigments—that is, those having keto groups in their structure—while the pigments making up the yellow fraction—mainly hydroxylated xanthophylls and β -carotene—are degraded more slowly. In particular, the relationship between k of the red pigments and that of the yellow ones shows a mean variation for the six oleoresins from 1.30 at 373 K to 1.77 at 393 K. With increasing temperature, the effect of the degradative process becomes greater on the red fraction. The thermodegradation reaction can be said to be selective toward the ketocarotenoids.

Table 5. Activation Enthalpy and Entropy in the Degradation of Carotenoid Pigments, by Red and Yellow Isochromic Fractions

	red pigments ^a		yellow pigments ^b	
	ΔH^{*c} (kJ/mol)	ΔS^{*c} (J/molK)	ΔH^* (kJ/mol)	ΔS^* (J/molK)
oleoresin 1	82.738	148.910	58.393	216.204
oleoresin 2	77.355	158.223	58.295	211.050
oleoresin 3	71.155	175.831	52.851	226.068
oleoresin 4	73.581	173.335	47.609	243.785
oleoresin 5	67.456	132.547	37.743	223.865
oleoresin 6	76.539	165.329	58.499	216.557

^a Red fraction = capsorubin + capsanthin. ^b Yellow fraction = violaxanthin + cucurbitaxanthin A + zeaxanthin + β -cryptoxanthin + β -carotene. ^c Activation enthalpy and entropy (eq 2, $p < 0.05$).

This may seem to contradict earlier results indicating that the ketocarotenoids are more stable in such processes, while other pigments, such as β -carotene and zeaxanthin, and those of the yellow fraction in general, tend to be degraded faster (Pérez-Gálvez et al., 1999). The results obtained in this study follow the opposite line, and may even be self-contradictory, considering the values of E_a and A for each fraction shown in Table 4.

With these values, according to the activated complex theory, the energy requirement (E_a) for a ketocarotenoid pigment (capsanthin and capsorubin) to become an activated complex is well above that of carotenes and hydroxylated xanthophylls. However, once energy is supplied to the reaction system (in this case an oily medium in which both pigment fractions are dissolved), it is the other parameter of the Arrhenius equation, A , that determines the degree to which the reaction proceeds. The result is that for the red fraction, the number of molecules able to form the activated complex is higher than for the yellow fraction. Thus, for these reaction conditions, there is a degradative effect on the red pigments.

If the degradation conditions were less aggressive (lower temperatures), the reaction would still take place, but would be less extended and possibly situated in a zone where the degradation affected the yellow fraction preferentially. Figures 1 and 2 show, on semilogarithmic coordinates, the values of k for the red and yellow fractions versus $1/T$ in the temperature range 298–400 K. In the six oleoresins studied, three different zones can be observed. In zone 1, $k_R > k_A$; in zone 2, $k_R < k_A$; and in the zone between 368 K for oleoresin 4 and 326 K for oleoresin 5, $k_R = k_A$. This common zone of intersection of the Arrhenius curves for the six oleoresins is mathematically equivalent to a linear relationship between the activation energy and the preexponential factor or between the terms activation enthalpy ΔH^* and activation entropy ΔS^* shown in Table 5.

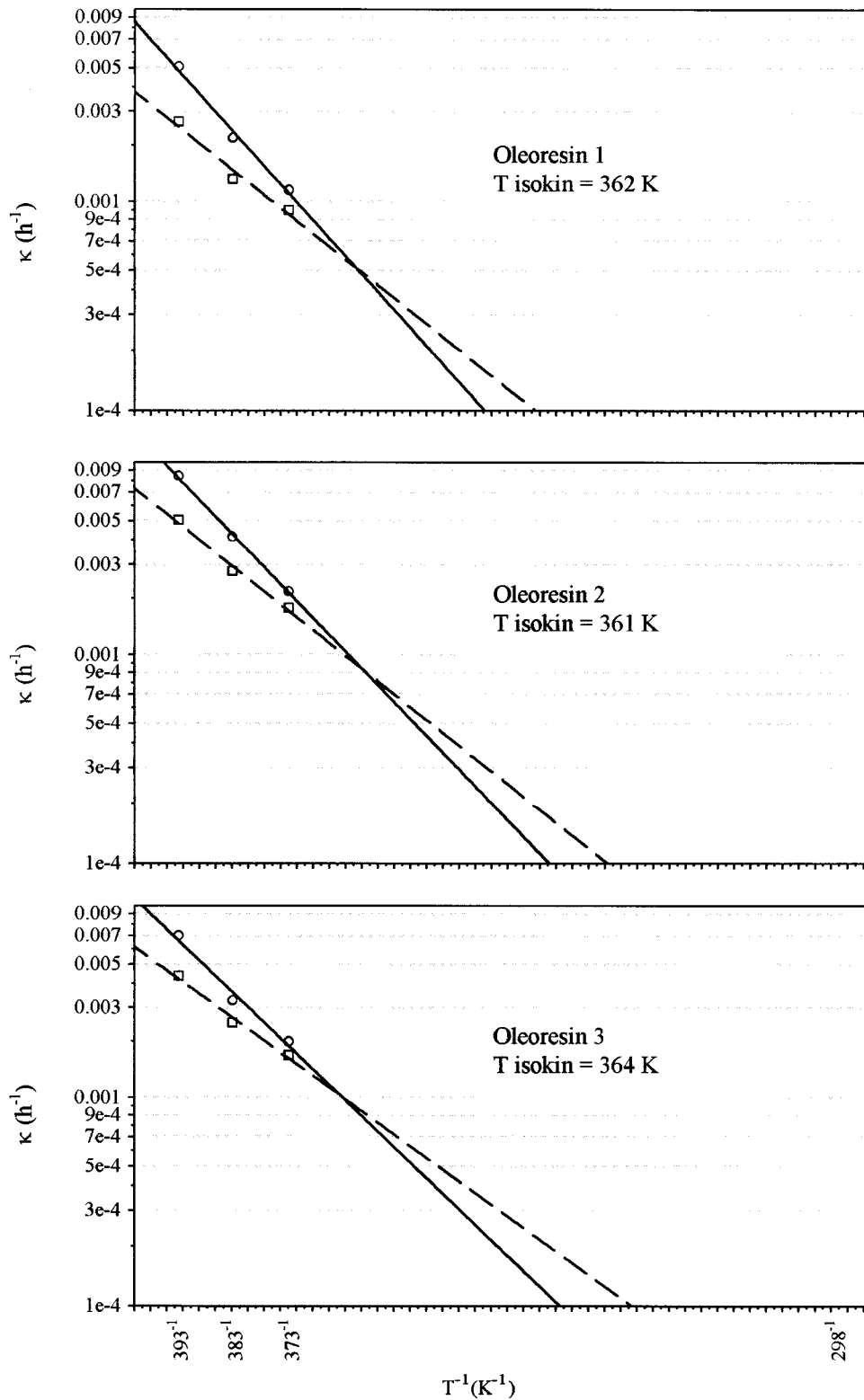


Figure 1. Arrhenius plot for the thermal degradation of red (—) and yellow pigments (---) in the oleoresins (1, 2, and 3) studied.

The values of the Arrhenius equation parameters vary by roughly the same margin, implying that there is no change in the reaction mechanism from one oleoresin to another. Furthermore, if they had differed from the results obtained in an earlier study at lower temperatures (Jarén-Galán and Mínguez-Mosquera, 1999), it could be concluded that either the mechanism or the structure of the activated complex had been altered by increasing the study temperature. That is not the case.

It is interesting that the isokinetic temperature, which for the six oleoresins studied has a mean value of 356 K, is below the experimental range (373–393 K). This implies that with increasing temperature of the degradative process the change in reaction enthalpy more than exceeds the change in entropy, and the selectivity of the reaction increases with temperature. In this case, the effect of the thermodegradative process on the ketocarotenoids is greater at high temperatures.

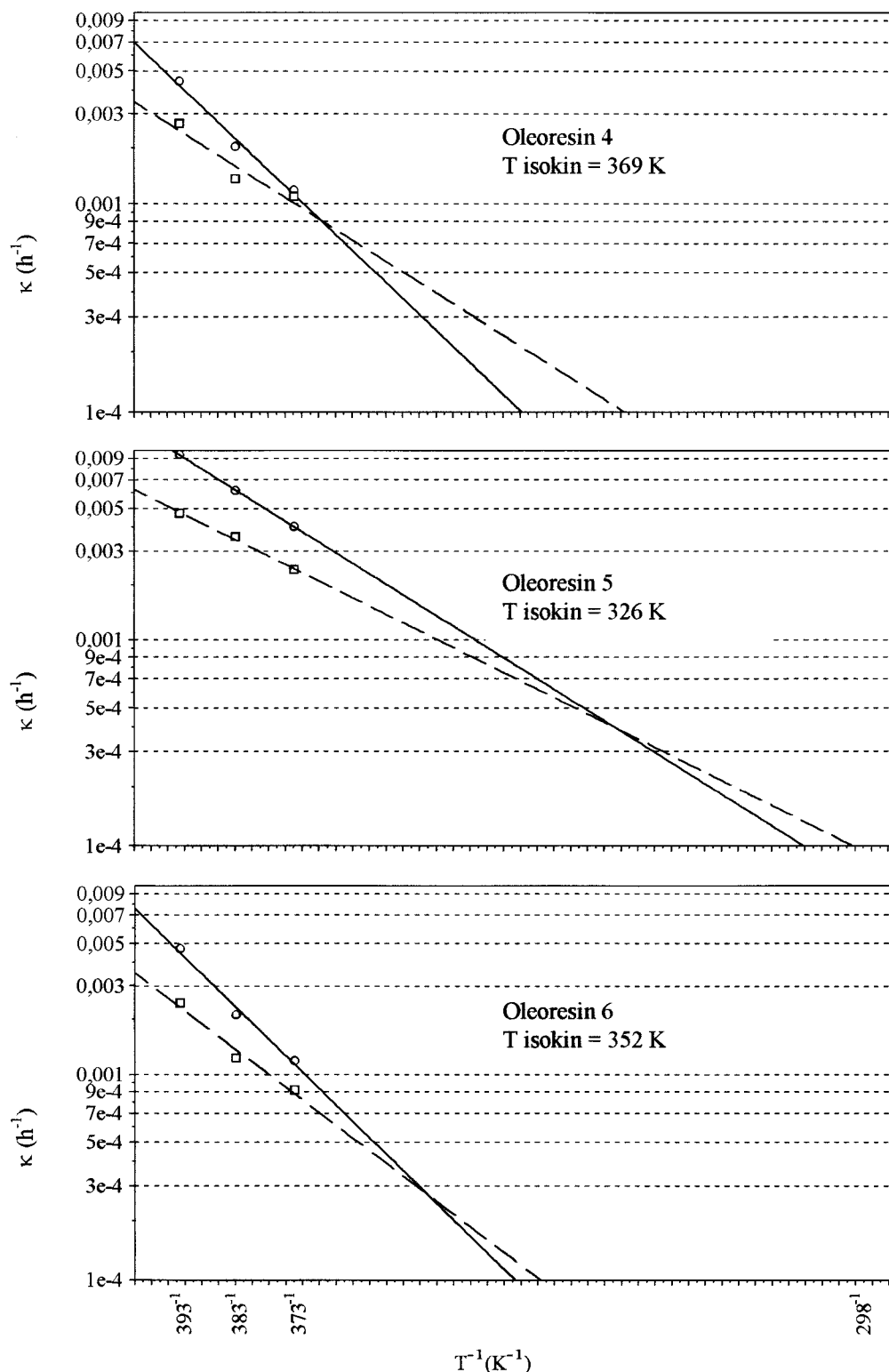


Figure 2. Arrhenius plot for the thermal degradation of red (—) and yellow pigments (---) in the oleoresins (4, 5, and 6) studied.

The expression of the reaction is different below T_{isok} , when the thermodegradation affects the yellow pigments.

The existence of this isokinetic point means that it is possible to modulate the effect of a particular process on a group of related substances present in the same reaction medium, since—depending on the conditions (in this case the temperature) used—the effect of the process will vary.

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