

Thermal Degradation Kinetics of Neoxanthin, Violaxanthin, and Antheraxanthin in Virgin Olive Oils

Ramón Aparicio-Ruiz and Beatriz Gandul-Rojas*

Chemistry and Biochemistry Pigment Group, Department of Food Biotechnology, Instituto de la Grasa, CSIC, Avenida Padre García Tejero 4, 41012 Sevilla, Spain

ABSTRACT: A first-order kinetic mechanism was appropriate for describing the thermal degradation of epoxy xanthophylls in virgin olive oil (VOO). Consecutive reactions that involve reorganization of 5,6-epoxide groups to 5,8-furanoxide groups and subsequent rupture of the polyene chain occurred in the degradation pathways. Thermal stability was significantly affected by changes in the chemical structure (epoxy to furanoid structure), being the greatest stability for neoxanthin. A true kinetic compensation effect was found in a series of similar reactions, that is, the degradation of 5,8-furanoxides into colorless products. An isokinetic study in different VOO matrices showed that the oily medium did not significantly affect the reaction mechanisms. Consequently, the kinetic parameters obtained as temperature functions according to the Arrhenius model can be used to develop a prediction mathematical model for 5,8-furanoxide xanthophylls in VOO over time. The potential usefulness of the parameter neoxanthin/neochrome ratio is discussed as a chemical marker of heat treatment in VOO.

KEYWORDS: virgin olive oil, carotenoids, isomerization, xanthophylls, thermal degradation, kinetics, Arrhenius parameters, isokinetic effect, thermal stability

■ INTRODUCTION

The main biological function of carotenoids in photosynthetic organisms is energy transfer in photosynthesis and photo-protection.¹ Among the carotenoids, in addition to β -carotene and lutein, 5,6-epoxy xanthophylls such as neoxanthin and violaxanthin are widely distributed in the photosynthetic organs of higher plants.² In mammals, which can incorporate carotenoids only through the diet, the only so far known biological function of some carotenoids is their role as vitamin A precursors. The nutritional importance of this biological function has been studied for years and is still of interest today.^{3,4} Certain physiological responses following the ingestion of food or dietary supplements rich in carotenoids have been observed. These responses are known as biological activities, which have raised the interest of the scientific community in the context of improving health through diet and developing functional foods. These include antioxidant activity and its associated benefits in preventing degenerative diseases.⁴

Carotenoids must be bioavailable to express these biological activities in tissues; that is, they must be transferred from the food matrix to the bloodstream to be metabolized and/or stored by the body. In addition to the individual's physiological factors, many dietary factors will determine their bioavailability.⁵ These include the characteristics of the food matrix⁶ and the various technological alternatives for obtaining and/or preserving food, which may influence the type and proportion of carotenoid derivatives formed.

Virgin olive oil (VOO) is considered to be a healthy fat. Its beneficial properties are attributed mainly to its proper fatty acid composition. Recently, however, benefits from other minor compounds in VOO with vitamin E (tocopherols) and provitamin A (β -carotene and β -cryptoxanthin) functions have been reported, and other potential biological activities as antioxidants (phenols, carotenoids, chlorophylls, squalene) or

hypolipemiant (β -sitosterol) have been suggested.⁷ Virgin olive oil is obtained from the olive fruit using only physical procedures under conditions, especially thermal, which do not involve alteration of the oil.⁸ Thus, the composition of bioactive compounds that are transferred from the fruit remain potentially intact in virgin olive oil. In terms of carotenoids, VOO mainly contains lutein and β -carotene, although there are also β -cryptoxanthin and 5,6-epoxy xanthophylls such as neoxanthin, violaxanthin, antheraxanthin, and their furanoxides.⁹

Carotenoids are susceptible to some reactions such as isomerization (*trans* to *cis*) and oxidation during food processing and storage due to the carbon–carbon double bonds of the polyene chain. Therefore, they react easily with acids, light, heat, and oxygen, causing loss of color and reduction of biological activity.^{10,11} Thus, these factors should be properly controlled to maximize carotenoid retention during storage. In the case of isomerization, the *trans* isomers are more common and stable in natural foods, whereas *cis* isomers are usually formed during food processing.¹² Organic acids liberated during the processing of fruit juices are strong enough to promote rearrangements of 5,6-epoxide groups to 5,8-furanoxide groups of carotenoids.^{13,14} Therefore, the stability of carotenoids in foods varies greatly.¹⁵

During the mechanical process of extracting virgin olive oil, a total transfer of carotenoids from the fruit to the oil does not occur despite their lipophilic character. A high percentage remains in the *alperujo* (a subproduct from the olive oil extraction process), whereas some of it undergoes oxidation to

Received: August 3, 2011

Revised: April 18, 2012

Accepted: April 18, 2012

Published: April 18, 2012

colorless products.¹⁶ The other structural changes of carotenoids associated with the processing are, however, of special importance, because they generate colored products and these compounds leave a “footprint” in the oil, which is used as a tracking parameter. These reactions are mostly mediated by the release of acid into the medium, the greater accessibility of enzymes and substrates, and the oxygenation that occurs during the milling of the fruit and the beating of the paste. In the fraction of xanthophylls, of note is the partial transformation of 5,6-epoxy xanthophylls to their corresponding 5,8-furanoxides.¹⁷

Kinetic models are becoming more popular for studying the changes in the chemical composition of food. These models are capable of predicting shelf life in keeping with the different variables that can affect the degradation of the food item. Studies describing the kinetics of carotenoids in fruit- and vegetable-based products are rather limited, although this information would be very useful and industrially relevant for predicting changes in functional compounds during fruit and vegetable processing.¹⁸ In those studies, analysis of kinetic data suggested a first-order model to describe the thermal degradation of carotenoids as in paprika oleoresins,¹⁹ citrus juice,¹³ or carrot puree.¹⁸ The thermal and oxidative degradation of lycopene, lutein, and 9-*cis*- and *all-trans*- β -carotene has been studied in an oil model system²⁰ to determine their relative stabilities. The degradation kinetics also followed a first-order model, and the thermodynamic parameters indicated a kinetic compensation effect between all of the carotenoids, with lutein being the most stable to degradation. A higher thermal resistance of lutein than β -carotene has been suggested by Achir et al.²¹ in model systems with two different frying oils reporting the influence of the oil initial composition in all degradation rates.

There are numerous experimental works in the literature describing VOO degradation, but recently the kinetic performance in oxidation parameters as peroxide value (PV), and absorbance at 232 nm (K_{232}) and 270 nm (K_{270}) has been described.²² The first kinetic and thermodynamic study of pigment thermodegradation products in VOO is referred to chlorophylls and was reported in 2010.²³

Our most recent research in this field has been aimed at the kinetic study and characterization of the thermodynamic parameters governing the thermal degradation reactions of carotenoids in VOO, to advance our understanding of the thermal stability of these compounds in an oily matrix, and to establish for the first time mathematical models enabling the prediction of the degradation of this pigment during VOO storage and/or thermal processing. This study necessarily had to be separated into two parts due to the large amount of data. Recently the results for lutein, β -carotene, and β -cryptoxanthin have been reported,²⁴ and in this work the results concerning 5,6-epoxide xanthophylls are presented.

MATERIALS AND METHODS

Chemicals and Standards. Tetrabutylammonium acetate and ammonium acetate were supplied by Fluka (Zwijndrecht, The Netherlands). HPLC reagent grade solvents were purchased from Teknokroma (Barcelona, Spain), and analytical grade solvents were supplied by Panreac (Barcelona, Spain). For the preparation, isolation, and purification of carotenoid pigments, analytical grade reagents were used (Panreac). The deionized water used was obtained from a Milli-Q 50 system (Millipore Corp., Bedford, MA, USA). Reference samples of neoxanthin, violaxanthin, and antheraxanthin were obtained from a pigment extract of fresh spinach saponified with 3.5 M KOH in

methanol and isolated by TLC on silica gel GF254 (0.7 mm thickness) on 20 × 20 cm plates using petroleum ether (65–95 °C)/acetone/diethylamine (10:4:1).²⁵ Luteoxanthin, auroxanthin, neochrome, and mutatoxanthin were obtained by acidification with 1 M HCl in ethanol.²⁶ All standards were purified by TLC using different eluents.²⁵

Samples. The study of thermal degradation of pigments was carried out with virgin olive oils obtained from a single industrial mill (Cooperativa Sor Àngela de la Cruz, Estepa, Seville, Spain) to avoid any effect of pedoclimatic and agricultural parameters and the industrial variables of the extraction systems in the comparative studies. To have three lots of oil with differing pigment contents, the starting material used was a mixture of two oil variety olives (Hojiblanca and Manzanilla) picked in three different months: November (sample N), December (sample D), and January (sample J). The proportion of fruits between varieties was 20:80, 80:20, and 100:0, respectively. The dates of picking correspond to high, medium, and low pigment levels (referring to the green color) and correlated inversely with the degree of fruit ripening according to the method of Walálí-Loudiyi et al.²⁷

Heat Treatment. Preliminary assays, with a commercial sample of virgin olive oil, enabled an approximate determination of the degree of conversion for the main reactions to be studied and established a range of times for an appropriate sampling at each temperature. The total time of each experiment changed depending on the assay temperature: 42 h (120 °C), 64 h (100 °C), 370 h (80 °C), and 744 h (60 °C). At least 128 aliquots (32 for each of the four assay temperatures) were separated from each oil lot (samples N, D, and J). These aliquots were put into glass tubes that were sealed in the absence of air (using nitrogen as neutral gas) and placed in thermostated ovens at the temperatures fixed for each experiment. These four temperatures were used to determine the kinetic and thermodynamic parameters (reaction order, reaction rate, and activation energies).

For each oil lot, two samples were analyzed for each time/temperature pair. The samples were removed from the thermostated ovens at fixed time intervals, depending on each experiment, to obtain a total of at least 16 duplicate samples. The samples were cooled rapidly in an ice bath and then kept at –20 °C until analysis of the pigments.

Extraction and Analysis of Xanthophyll Pigments. All procedures were performed under green lighting to avoid any photooxidation of xanthophyll compounds. Pigment extraction was performed by liquid-phase distribution. This method was developed for VOO by Mínguez-Mosquera et al.¹⁷ The technique is based on the selective separation of components between *N,N*-dimethylformamide (DMF) and hexane. The oil sample (10–15 g) was dissolved directly in 150 mL of DMF and treated with five successive 50 mL portions of hexane in a decanting funnel. The hexane phase carried over lipids and carotene fraction, whereas the DMF phase retained chlorophyll pigments and xanthophylls. This system yielded a concentrated pigment solution that was oil free and could be adequately analyzed by chromatographic techniques.

HPLC analysis of carotenoid pigments was performed according to the method described by Mínguez-Mosquera et al.²⁵ using a reverse phased column (20 cm × 0.46 cm) packed with 3 μ m C18 Spherisorb ODS2 (Teknokroma) and an elution gradient with the solvents (A) water/ion-pair reagent/methanol (1:1:8, v/v/v) and (B) acetone/methanol (1:1 v/v), at a flow rate of 1.25 mL/min. The ion-pair reagent was 0.05 M tetrabutylammonium acetate and 1 M ammonium acetate in water. The pigments were identified by cochromatography with the corresponding standard and from their spectral characteristics described in detail in previous papers.^{25,28} The online UV–vis spectra were recorded from 350 to 800 nm with the photodiode array detector. Pigments were detected at the wavelength of maximum absorption (430 nm for neoxanthin, neochrome, violaxanthin, mutatoxanthin, auroxanthin, and mutatoxanthin and 450 nm for antheraxanthin) and were quantified from the corresponding calibrated curves (amount versus integrated peak area). The calibration equations were obtained by least-squares linear regression analysis over a concentration range according to the levels of these

Table 1. Initial Content of Xanthophyll Compounds and Total Pigments in Virgin Olive Oils^a

sample ^c	group I ^b			group II			group III				total pigments ^e
	neox ^d	neoc	ratio	anther	muta	ratio	violax	luteo	auro	ratio	
N	1.00 ± 0.04	0.00 ± 0.00	ud ^f	0.84 ± 0.01	0.30 ± 0.00	2.80	0.80 ± 0.01	1.40 ± 0.01	0.55 ± 0.02	0.41	36.91 ± 0.55
D	0.73 ± 0.01	0.00 ± 0.00	ud	0.67 ± 0.01	0.20 ± 0.00	3.35	0.40 ± 0.01	0.66 ± 0.00	0.33 ± 0.07	0.40	28.23 ± 0.80
J	0.25 ± 0.00	0.00 ± 0.00	ud	0.12 ± 0.00	0.10 ± 0.00	1.20	0.15 ± 0.00	0.18 ± 0.00	0.07 ± 0.00	0.60	15.30 ± 0.26

^aData, expressed as $\mu\text{mol/kg}$, represent the mean value \pm SD for three determinations. CV \leq 3.5%. ^bEach group consists of the 5,6-epoxy xanthophyll and its corresponding 5,8-furanoxide(s). Ratio is 5,6-epoxy/5,8-furanoxide(s). ^cThe sample code corresponds to the harvest date of the olive fruits used to obtain the virgin olive oils studied: N, November; D, December; J, January. ^dneox, neoxanthin; neoc, neochrome; anther, antheraxanthin; muta, mutatoxanthin; violax, violaxanthin; luteo, luteoxanthin; auro, auroxanthin. ^eTotal chlorophyll and total carotenoid pigments. ^fud, undefined.

pigments in VOO. Injections in duplicate were made for five different volumes at each standard solution.

Kinetic Parameters. Changes in experimental data of pigment concentration, expressed in micromoles per kilogram, were used to calculate kinetic parameters by least-squares nonlinear regression analysis. The reaction order (n) and rate constant (k) were determined by trial and error using the integral method: a reaction order is initially assumed in the rate equation and then is integrated to obtain a mathematical expression that relates pigment concentration (C) with time (t). The mathematical expression that best fits the changes in the experimental data with the reaction time was selected to verify the order (assumed ad initio) and used to obtain the rate constant (k).

Thermodynamic Parameters. The effect of temperature on the rate constant was evaluated by means of the Arrhenius equation with a simple reparametrization²⁹ by using a reference temperature T_{ref}

$$k = k_{\text{ref}} \times \exp \left[\frac{-E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}} \right) \right]$$

where R is the molar gas constant ($1.98 \text{ cal mol}^{-1} \text{ K}^{-1}$), T is the absolute temperature (K), E_a is the activation energy (cal mol^{-1}), k is the specific reaction rate constant at the temperature T , and k_{ref} is the specific reaction rate constant at the reference temperature T_{ref} . The reference temperature should preferably be chosen in the middle of the studied temperature regimen.

Therefore, E_a was estimated on the basis of nonlinear regression analysis of k_i versus $1/T_{ij}$ (being $i = \text{N, D, or J}$; $j = 60, 80, 100, \text{ or } 120$ °C).

According to active complex theory, the enthalpy (ΔH^\ddagger) and entropy of activation (ΔS^\ddagger) were determined by the Eyring equation

$$\ln(k/T) = \frac{-\Delta H^\ddagger}{RT} + \frac{\Delta S^\ddagger}{R} + \ln \left(\frac{k_b}{h} \right)$$

where k is the rate constant at temperature T , k_b is the Boltzmann constant, R is the molar gas constant, and h is the Planck constant. Therefore, ΔH^\ddagger and ΔS^\ddagger were estimated on the basis of linear regression analysis of $\ln(k_i/T_{ij})$ versus $1/T_{ij}$. The Gibbs free energy was estimated according to the Gibbs equation:

$$\Delta G^\ddagger = \Delta H^\ddagger - T \Delta S^\ddagger$$

The pairs of ΔH^\ddagger and ΔS^\ddagger obtained were linearly correlated using the last equation, from which the isokinetic temperature (T_{isok}) and its corresponding Gibbs free energy (ΔG_{isok}) for the reaction could be estimated.

Calculations and Statistical Data Analysis. Data were expressed as the mean \pm SE. The data were analyzed for differences between means using one-way analysis of variance (ANOVA). The Brown and Forsythe test³⁰ was used as a post hoc comparison of statistical significance (p values < 0.05). Least-squares and nonlinear regression analysis were performed using Statistica 6.0 (StatSoft, Inc., 2001) and Statgraphics Centurion XV for Windows (Statpoint Technologies, Inc., 2005).

RESULTS AND DISCUSSION

Kinetic Study. The qualitative carotenoid profile in the initial samples was typical of VOO,^{9,31} with lutein and β -carotene as major carotenoids and violaxanthin, luteoxanthin, auroxanthin, neoxanthin, antheraxanthin, mutatoxanthin, and β -cryptoxanthin as minor xanthophylls. The study of carotenoid thermal degradation in VOO has had to be separated into two sections, given the high amount of data. In the first stage of the study were reported the results for lutein, β -carotene, and β -cryptoxanthin,²⁴ and in this stage we are presenting the results concerning the 5,6-epoxide xanthophylls, neoxanthin, violaxanthin, and antheraxanthin, and their corresponding 5,8-furanoxide derivatives, neochrome, luteoxanthin, and auroxanthin, and mutatoxanthin. Table 1 shows the initial content of the pigments analyzed in this study for the high (N), medium (D), and low (J) pigmentation VOO matrices employed. The total pigment content includes chlorophylls and carotenoids as measured in this study and in previous ones.^{23,24}

Figure 1 shows the typical HPLC chromatograms for an olive oil pigment extract at three significant time points of the

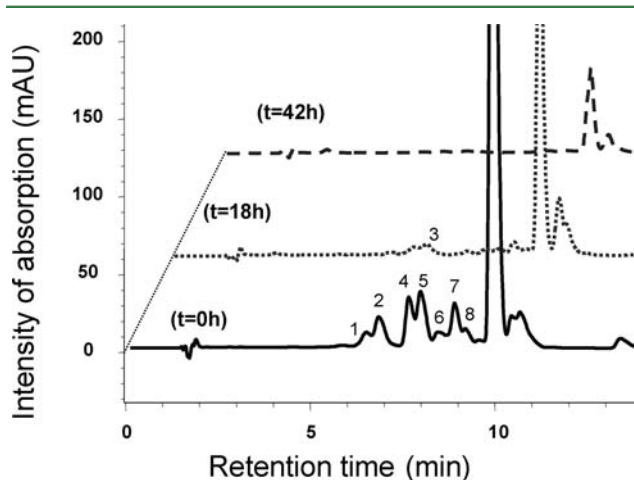


Figure 1. HPLC profile of xanthophylls from virgin olive oil (sample N), at initial sample ($t = 0$ h) and after 18 and 42 h of heating at 120 °C. Detection was by absorption at 480 nm. Peaks: 1, neoxanthin; 2, neoxanthin isomer; 3, neochrome; 4, violaxanthin; 5, luteoxanthin; 6, auroxanthin; 7, antheraxanthin; 8, mutatoxanthin.

thermal degradation process studied: initial sample ($t = 0$ h), after 18 h of heating at 120 °C, and after 42 h of heating at 120 °C. The main peak is not numbered and corresponds to lutein, the thermal degradation of which has been studied in a previous work.²⁴ In the initial sample, there were 5,6-epoxy xanthophylls including neoxanthin (peak 1), neoxanthin isomer (peak 2), violaxanthin (peak 4), and antheraxanthin (peak 7) and 5,8-

furanoid xanthophylls including luteoxanthin (peak 5), auroxanthin (peak 6), and mutatoxanthin (peak 8). Figure 2

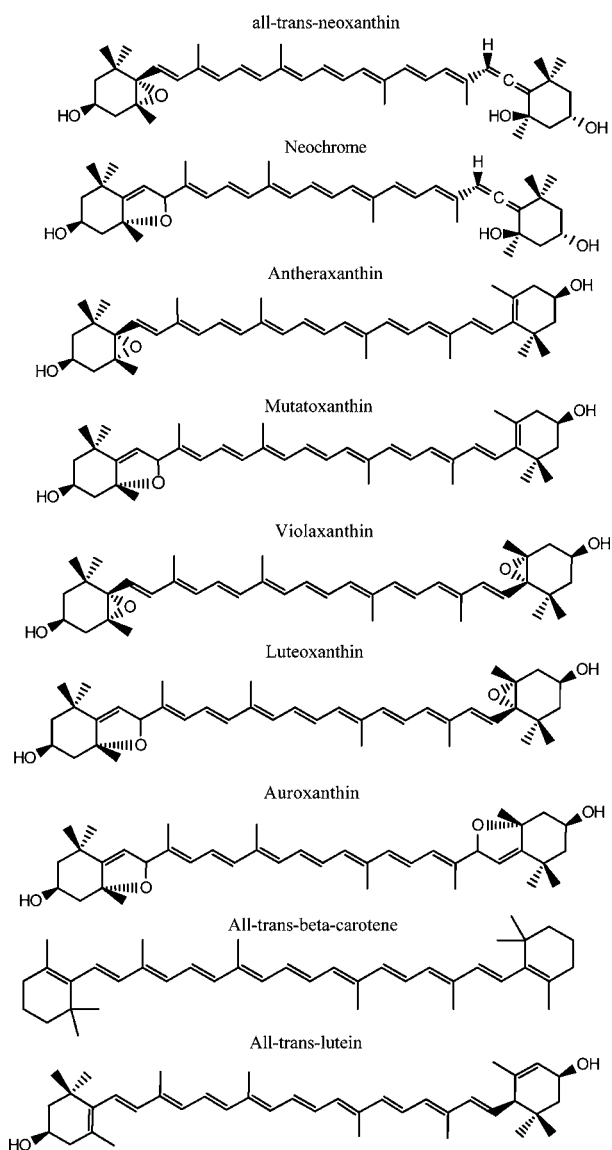


Figure 2. Structures of carotenoids.

shows the structures of the studied carotenoids. The presence of 5,8-furanoxides already in the initial sample is due to the release of intracellular acid medium during the milling of olive fruit to obtain virgin olive oil because (1) no 5,8-furanoxides have been found in olive fruits¹⁷ and (2) it is known that acid conditions might induce the isomerization of the 5,6-epoxide into a 5,8-furanoxide.³²

Three groups of xanthophylls were defined to study their evolution during heating, each group consisting of the 5,6-epoxy xanthophyll and its corresponding 5,8-furanoxide(s) (Table 1). The first group consisted of neoxanthin and neochrome (group I), the second group was made up of antheraxanthin and mutatoxanthin (group II), and the third and last group was formed by violaxanthin, luteoxanthin, and auroxanthin (group III).

In each of these groups, the initial percentages of 5,8-furanoxide xanthophylls were quite different. In group I, no 5,8-furanoxide xanthophyll was detected, in group II it represented

between 23 and 46% of the carotenoids, and in group III it exceeded 60%.

During heat treatment mentioned under Material and Methods, the concentration of 5,6-epoxy xanthophylls was gradually reduced (Figure 1) while changes in the corresponding 5,8-furanoxides were observed (Figures 3–5). Neochrome,

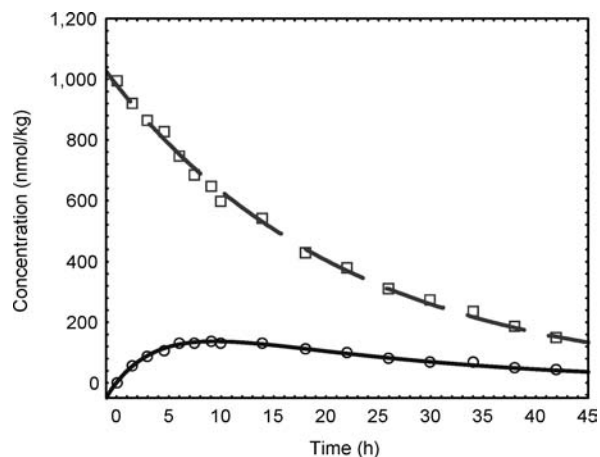


Figure 3. Evolution of concentration–time of neoxanthin (\square) and neochrome (\circ) in VOO (sample N) during 42 h at 120 °C and corresponding fits (—) to the mathematical model developed in this study (eqs 4 and 5).

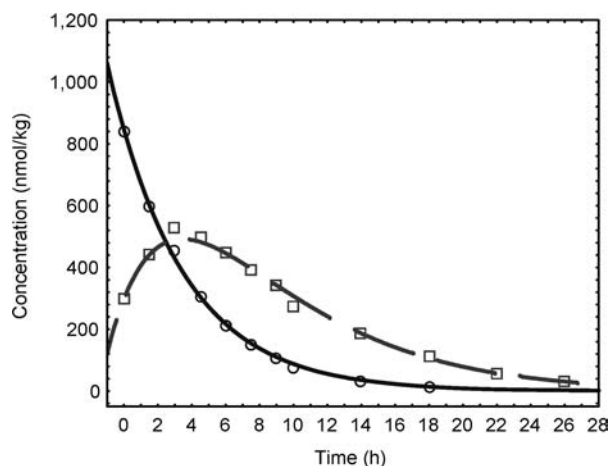


Figure 4. Evolution of concentration–time of antheraxanthin (\circ) and mutatoxanthin (\square) in VOO (sample N) during 42 h at 120 °C and corresponding fits (—) to the mathematical model developed in this study (eqs 9 and 10).

mutatoxanthin, and auroxanthin gradually increased over time, until they reached a maximum concentration (Figures 3–5). Then, they began to decline, probably oxidized to colorless compounds. In contrast, the intermediate compound luteoxanthin maintained a gradual decrease in concentration from the start of treatment.

At maximum concentration, the highest percentage of 5,8-furanoxides compared to epoxides was found in group III (luteoxanthin + auroxanthin), reaching values of up to 95%, followed by group II (mutatoxanthin), which ranged from 50 to 60%, and finally group I (neochrome), which in no case exceeded 40%. For each group, the time required to achieve this maximum percentage of 5,8-furanoxides increased with decreasing temperature, and in all cases the highest time values

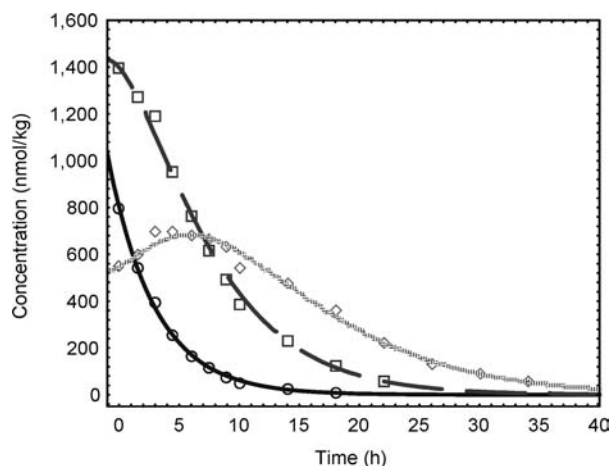


Figure 5. Evolution of concentration–time of violaxanthin (○), luteoxanthin (□), and auroxanthin (◇) in VOO (sample N) during 42 h at 120 °C and corresponding fits (—) to the mathematical model developed in this study (eqs 15–17).

corresponded to neochrome for all temperatures and matrices studied.

These results lead us to suggest the percentages of 5,8-furanoxide xanthophylls as chemical markers of heat treatment in a VOO. To support this claim, we will examine the experiment conducted at 120 °C in greater detail. Table 2

Table 2. Ratios between Isomers 5,6-Epoxy and 5,8-Furanoxide by Groups^a

time (h)	neox/neoc	violax/luteo + auro	anther/muta
0	ud ^b	0.41	2.83
1.5	16.92	0.29	1.35
3	9.57	0.21	0.86
4.5	7.63	0.15	0.62
6	5.86	0.11	0.47
7.5	5.22	0.09	0.38
9	4.78	0.07	0.31
10	4.57	0.06	0.27
14	4.11	0.03	0.18
18	3.90	0.02	0.13
22	3.82	0.00	0.00
26	3.74	0.00	0.00
30	3.98	0.00	
34	3.49	0.00	
38	3.52		
42	3.61		

^aneox, neoxanthin; neoc, neochrome; anther, antheraxanthin; muta, mutatoxanthin; violax, violaxanthin; luteo, luteoxanthin; auro, auroxanthin. ^bud, undefined.

shows the changes in the ratio of 5,6-epoxides to 5,8-furanoxides for the different groups of xanthophylls experienced during the heat treatment. This ratio decreased significantly during heat treatment and showed differences between groups. In the violaxanthin group (III), this relationship began at values <1 in the initial sample and decreased to 0 (100% of 5,8-furanoxides) after 22 h of heat treatment at 120 °C. In the antheraxanthin group (II), the initial sample started with values >1 but progressively decreased, reaching 0 after 22 h at 120 °C. In the neoxanthin group (I) the relationship started at undefined values due to the 5,8-furanoxide not being detected

in the initial sample and decreased significantly during heat treatment, but in no case was <3. These results marked a difference compared to other groups of xanthophylls. Even a short time of heat treatment at 120 °C (e.g., 1.5 h) was sufficient to decrease the initial 5,6-epoxide/5,8-furanoxide ratio in all groups, but this decrease was significant mathematically only for group I (neoxanthin). For the other groups of xanthophylls, no significant differences were observed for this ratio after 1.5 h of heat treatment because the values corresponding to the initial sample (Table 1) showed a wide range of variation between different VOO matrices. Therefore, the ratio neoxanthin/neochrome (or the percentage of neochrome) offers the best possibility to be used as a chemical marker of thermal treatment in VOO.

Similar losses of 5,6-epoxy xanthophylls after heat treatment have been described in other foods. Thermal effects were clearly observed on violaxanthin and antheraxanthin after pasteurization³³ and microwave heating³⁴ of orange juice. High losses of violaxanthin were also noted after cooking of pumpkin puree³⁵ and green vegetables,³⁶ being more prone to degradation than β -carotene. There are also a few papers in which the isomerization of the epoxide function in position 5,6 into a furanoxide function in position 5,8 is reported as a common reaction for the xanthophylls during thermal processing,^{13,37–39} but this is the first work in which kinetic study is performed on this subject.

Zepka and Mercadante⁴⁰ studied the degradation compounds of carotenoids formed during heating of a simulated cashew apple juice. They also reported that the loss of total carotenoids was not compensated by the other isomers formed, indicating that isomerization and oxidation to both colored and noncolored compounds were the main reactions occurring during heating of carotenoids in aqueous-based and juice systems.

On the basis of the observed changes in the xanthophylls mentioned above, the kinetic models indicated in Figure 6 were

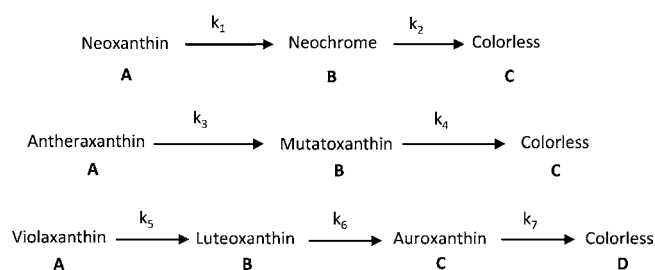


Figure 6. Kinetic mechanisms for thermal degradation pathway of neoxanthin (A), antheraxanthin (B), and violaxanthin (C) in VOO.

proposed. All kinetic models proposed involve consecutive reactions. The first reactions determine the formation of the 5,8-furanoxides, and the final reactions determine the destruction of the chromophores resulting in the formation of noncolored compounds (nc).

Group I. In accordance with the mechanism proposed (Figure 6), neoxanthin (5,6-epoxide) leads to neochrome (5,8-furanoxide) and the last reaction leads to noncolored products.

The corresponding kinetic equations are expressed as follows:

$$V_{\text{neoxanthin}} = -\frac{d[A]}{dt} = k_1[A]^n \quad (1)$$

Table 3. Rate Constants (k) and Determination Coefficients (R^2) Estimated for the Kinetic Mechanism of the Thermal Degradation of Neoxanthin, Antheraxanthin, and Violaxanthin in VOO

reaction ^a	sample ^b	120 °C			100 °C			80 °C			60 °C		
		$k^c \times 10^3$ (h ⁻¹)	SE	R^2	$k^c \times 10^3$ (h ⁻¹)	SE	R^2	$k^c \times 10^3$ (h ⁻¹)	SE	R^2	$k^c \times 10^3$ (h ⁻¹)	SE	R^2
D neoxanthin k_1	N	44.91a	1.21	0.99	10.99a	0.14	1.00	3.66a	0.29	0.95	0.74a	0.03	0.97
	D	48.74b	1.05	1.00	17.48b	0.70	1.00	4.47b	0.16	0.99	0.97b	0.02	0.99
	J	60.59c	1.32	0.99	15.46c	0.49	0.98	9.17c	0.30	0.99	0.99b	0.03	0.98
D neochrome k_2	N	213.47d	2.48	0.99	51.18d	0.79	0.99	10.40d	0.38	0.94	1.31c	0.06	0.99
	D	228.23e	3.20	0.99	81.93e	1.30	0.97	13.53e	0.37	0.96	1.68d	0.05	0.99
	J	182.67f	2.87	0.99	63.51f	1.20	0.96	27.04f	0.53	0.99	1.38e	0.11	0.93
D antheraxanthin k_3	N	228.23g,e	3.50	1.00	141.74g	5.30	0.97	10.79g,d	0.27	1.00	6.86e	0.13	1.00
	D	182.69h,f	1.57	1.00	96.99h	1.34	1.00	8.91h,c	0.24	1.00	5.87f	0.07	1.00
	J	152.48i	2.28	1.00	77.99i	1.15	1.00	15.05i	0.39	1.00	4.91g	0.27	0.97
D mutatoxanthin k_4	N	178.92j	2.98	1.00	111.68j	3.11	0.97	8.61j,c,h	0.21	0.95	4.55h,g	0.05	0.99
	D	144.82k	1.46	1.00	69.15k	1.06	0.99	7.78k	0.19	0.94	4.70i,g	0.05	0.99
	J	131.86 l	1.23	0.99	51.64l,d	0.20	1.00	14.94l,i	0.26	0.99	3.10j	0.06	0.96
D violaxanthin k_5	N	257.95m	3.65	1.00	170.17m	4.86	0.99	109.7m	1.47	1.00	54.06k	0.50	1.00
	D	289.35n	2.67	1.00	204.92n	6.43	0.99	93.67n	1.61	1.00	63.80l	0.92	1.00
	J	267.38o	1.56	1.00	165.10o	0.64	1.00	90.27n,o	1.74	1.00	54.36k	0.32	1.00
D luteoxanthin k_6	N	191.42p	2.91	1.00	150.27p,g	3.52	0.98	89.92o	0.60	1.00	35.09m	0.11	1.00
	D	226.70q,e,g	1.23	1.00	187.17q	5.29	0.98	73.86p	1.24	1.00	40.34n	0.18	1.00
	J	195.52p	0.91	1.00	140.76r,g	0.93	1.00	70.58q	0.85	1.00	43.98o	0.28	1.00
D auroxanthin k_7	N	145.85r	1.43	0.98	119.36s	1.40	0.98	68.60r,p,q	6.37	1.00	21.46p	0.04	1.00
	D	155.69s,i	1.41	0.99	142.22t,g,q	2.56	0.96	53.82s	0.60	0.99	23.82q	0.06	1.00
	J	135.91t	0.33	1.00	110.37u,j	0.62	0.99	51.68s	1.55	1.00	17.04r	0.05	1.00

^aReactions according to the kinetic mechanism shown in Figure 6; D, degradation. ^bSample code as in Table 1. ^cValues are obtained from a minimum of 16 experimental data points analyzed in duplicate; SE, standard error. At each temperature, different letters between rows indicate significant differences ($p \leq 0.05$).

$$V_{\text{neochrome}} = \frac{d[B]}{dt} = k_1[A]^n - k_2[B]^n \quad (2)$$

$$V_{\text{colorless}} = \frac{d[C]}{dt} = k_2[B]^n \quad (3)$$

[A] is the concentration of neoxanthin, [B] the concentration of neochrome, and [C] the concentration of noncolored products (nc). k_1 and k_2 are rate constants for the various reactions, and n is the reaction order.

From the balance of materials of all species, the concentration of colorless compounds over time is derived by the following equation:

$$[A]_0 + [B]_0 + [C]_0 = [A] + [B] + [C]$$

$[A]_0$ is the initial concentration of neoxanthin, $[B]_0$ the initial concentration of neochrome, and $[C]_0$ the initial concentration of nc. Concentrations $[A]$ – $[C]$ are those described for eqs 1–3.

Solving the kinetic mechanism, assuming an order of 1 ($n = 1$) and that all reactions are irreversible, we get

$$[A] = [A]_0 e^{-k_1 t} \quad (4)$$

$$[B] = \frac{k_1[A]_0}{k_2 - k_1} [e^{-k_1 t} - e^{-k_2 t}] + [B]_0 e^{-k_2 t} \quad (5)$$

Group II. The kinetic mechanism of group II is similar to that of group I. Thus, antheraxanthin (5,6-epoxide) leads to mutatoxanthin (5,8-furanoxide), and this leads to noncolored products (Figure 6).

The corresponding kinetic equations are expressed as

$$V_{\text{antheraxanthin}} = -\frac{d[A]}{dt} = k_3[A]^n \quad (6)$$

$$V_{\text{mutatoxanthin}} = \frac{d[B]}{dt} = k_3[A]^n - k_4[B]^n \quad (7)$$

$$V_{\text{colorless}} = \frac{d[C]}{dt} = k_4[B]^n \quad (8)$$

[A] is the concentration of antheraxanthin, [B] the concentration of mutatoxanthin, and [C] the concentration of nc. k_3 and k_4 are rate constants for the different reactions, and n is the reaction order.

Using the material balance of all species, the next equation allows us to obtain the concentration of colorless products over time:

$$[A]_0 + [B]_0 + [C]_0 = [A] + [B] + [C]$$

$[A]_0$ is the initial concentration of antheraxanthin, $[B]_0$ the initial concentration of mutatoxanthin, and $[C]_0$ the initial

Table 4. Thermodynamic Parameters^a for the Thermodegradation Reaction of Xanthophyll Compounds in Virgin Olive Oil

reaction ^b	sample ^c	ΔS^\ddagger [(cal/mol·K)]	SE ^d	ΔH^\ddagger (kcal/mol)	SE	E_a (kcal/mol)	SE	ΔG^\ddagger_{298} (kcal/mol)	SE
neoxanthin	N	-38.74	1.07	16.75	0.39	17.79	0.12	28.30	0.39
neoxanthin	D	-39.18	0.71	16.41	0.26	17.10	0.12*	28.09	0.26
neoxanthin	J	-39.59	3.69	16.11	1.33	17.84	0.21	27.91	0.33
neochrome	N	-23.84	0.93	21.30	0.34	22.16	0.06*	28.40	0.34
neochrome	D	-24.40	2.16	20.91	0.78	21.63	0.32	28.18	0.78
neochrome	J	-27.75	5.47	19.69	1.98	21.56	0.38	27.96	1.98
antheraxanthin	N	-36.31	5.50	16.29	1.99	15.51	0.62	27.11	1.99
antheraxanthin	D	-38.24	4.99	15.76	1.80	15.14	0.42	27.16	1.80
antheraxanthin	J	-40.71	2.02	14.88	0.73	15.08	0.35	27.01	0.73
mutatoxanthin	N	-35.02	5.18	16.96	1.87	16.25	0.66	27.39	1.87
mutatoxanthin	D	-39.54	4.27	15.46	1.54	14.99	0.31*	27.24	1.54
mutatoxanthin	J	-39.21	1.08	15.60	0.39	16.49	0.17	27.29	0.39
violaxanthin	N	-62.04	0.31	6.17	0.11	6.84	0.07	24.66	0.11
violaxanthin	D	-62.16	0.54	6.02	0.19	6.73	0.15	24.54	0.19
violaxanthin	J	-61.74	0.37	6.28	0.13	6.93	0.06	24.68	0.13
luteoxanthin	N	-60.59	1.24	6.89	0.45	7.66	0.37	24.95	0.45
luteoxanthin	D	-59.79	1.54	7.05	0.56	7.89	0.46	24.87	0.56
luteoxanthin	J	-62.94	0.76*	6.02	0.27*	6.59	0.18*	24.77	0.27
auroxanthin	N	-58.48	1.79	7.89	0.65	8.80	0.53	25.31	0.65
auroxanthin	D	-58.76	2.44	7.68	0.88	8.67	0.71	25.19	0.88
auroxanthin	J	-57.03	2.20	8.49	0.79	9.43	0.59	25.48	0.79

^a ΔS^\ddagger , activation entropy; ΔH^\ddagger , activation enthalpy; E_a , activation energy, ΔG^\ddagger , Gibbs free energy. ^bReactions according to the kinetic mechanism shown in Figure 6. ^cSample code as in Table 1. ^dSE, standard error. * indicates significant differences for a parameter between different samples ($p \leq 0.05$).

concentration of nc. Concentrations $[A]$ – $[C]$ are those described for eqs 6–8.

Solving the kinetic mechanism, assuming an order of 1 ($n = 1$) and that all reactions are irreversible, we get

$$[A] = [A]_0 e^{-k_3 t} \quad (9)$$

$$[B] = \frac{k_3[A]_0}{k_4 - k_3} [e^{-k_3 t} - e^{-k_4 t}] + [B]_0 e^{-k_4 t} \quad (10)$$

Group III. Violaxanthin differs from neoxanthin and antheraxanthin in its structure due to its two 5,6-epoxy groups. Therefore, the transformation of one of these epoxy groups into a 5,8-furanoid group leads to luteoxanthin. If the second epoxy group is transformed into a 5,8-furanoid group, then this leads to auroxanthin. Accordingly, the proposed model (Figure 6) presents an additional consecutive reaction kinetic model from groups I and II described above. This further complicates the model and, consequently, its mathematical resolution.

The corresponding kinetic equations are expressed as follows:

$$V_{\text{violaxanthin}} = -\frac{d[A]}{dt} = k_5[A]^n \quad (11)$$

$$V_{\text{luteoxanthin}} = \frac{d[B]}{dt} = k_5[A]^n - k_6[B]^n \quad (12)$$

$$V_{\text{auroxanthin}} = \frac{d[C]}{dt} = k_6[B]^n - k_7[C]^n \quad (13)$$

$$V_{\text{colorless}} = \frac{d[D]}{dt} = k_7[C]^n \quad (14)$$

$[A]$ is the concentration of violaxanthin, $[B]$ the concentration of luteoxanthin, $[C]$ the concentration of auroxanthin, and $[D]$ the concentration of nc. k_5 , k_6 , and k_7 are rate constants for the different reactions, and n is the reaction order.

The next equation allows us to obtain the concentration of colorless products over time:

$$[A]_0 + [B]_0 + [C]_0 + [D]_0 = [A] + [B] + [C] + [D]$$

$[A]_0$ is the initial concentration of violaxanthin, $[B]_0$ the initial concentration of luteoxanthin, $[C]_0$ the initial concentration of auroxanthin, and $[D]_0$ the initial concentration of nc. Concentrations $[A]$ – $[D]$ are those described for eqs 11–14.

Resolving the kinetic mechanism, assuming an order of 1 ($n=1$) and that all reactions are irreversible, we get

$$[A] = [A]_0 e^{-k_5 t} \quad (15)$$

$$[B] = \frac{k_5[A]_0}{k_6 - k_5} [e^{-k_5 t} - e^{-k_6 t}] + [B]_0 e^{-k_6 t} \quad (16)$$

$$\begin{aligned}
 [C] = & k_5 k_6 [A]_0 \left[\frac{e^{-k_5 t}}{(k_7 - k_5)(k_6 - k_5)} \right. \\
 & \left. + \frac{e^{-k_6 t}}{(k_7 - k_6)(k_6 - k_5)} + \frac{e^{-k_7 t}}{(k_7 - k_5)(k_7 - k_6)} \right] \\
 & + \frac{k_6 [B]_0}{(k_7 - k_6)} [e^{-k_6 t} - e^{-k_7 t}] + [C]_0 e^{-k_7 t} \quad (17)
 \end{aligned}$$

In accordance with the proposed kinetic eqs 4, 5, 9, 10, and 15–17, and by nonlinear regression analysis of the experimental data, the rate constants for each of the proposed reactions in the mechanisms were estimated. For treatment of the high-pigmentation matrix (sample N) at 120 °C, Figures 3–5 show the concentration changes found and the regression estimated. Table 3 shows the values for the estimated rate constants, together with the standard error and determination coefficient (R^2) for each reaction studied. The determination coefficients obtained showed a good fit of the experimental data to the equations proposed and demonstrate that the first-order mechanism is appropriate for describing the thermal degradation of neoxanthin, antheraxanthin, and violaxanthin in VOO.

Studies describing the kinetics of carotenoid degradation in fruit- and vegetable-based products are rather limited, although this information would be very useful and industrially relevant for predicting changes in bioactive compounds during the processing and shelf life of these foods.¹⁸ In those studies, analysis of kinetic data also suggested a first-order model to describe the degradation of carotenoids in green table olives,⁴¹ paprika oleoresins,¹⁹ citrus juice,¹³ carrot puree,¹⁸ and oils enriched with β -carotene and lutein.²¹

In general, all kinetic constants doubled or tripled for each 20 °C increase in temperature, demonstrating a marked effect of temperature in reaction rates, similar to other carotenoids in VOO.²⁴ However, this effect was lower than that found in the thermal degradation of chlorophyll compounds in VOO.²³

The rate constant estimated for neoxanthin isomerization was significantly lower than that of antheraxanthin and violaxanthin (Table 3), in all temperatures and matrices, suggesting that neoxanthin has a relatively greater heat resistance. This result partly agrees with Fratianni et al.,³⁴ who found that violaxanthin was the most unstable compound followed by antheraxanthin.

The chemical structures of the carotenoids significantly affect thermal stability. In group I, the ratio of rate constants between neoxanthin and neochrome was <1 in all cases (average = 0.4 ± 0.1 of four temperatures and samples studied), indicating that the 5,8-isomer degradation into nc products is the preferred reaction. This explains why the maximum concentration of 5,8-furanoxide (neochrome) does not exceed that of its predecessor 5,6-epoxide (neoxanthin) at any point in the heat treatment.

In contrast, in the other two xanthophyll groups, the 5,8-furanoxide formation reaction was always preferred. In group II, the rate constant of mutatoxanthin formation (k_3) was always higher than its degradation to colorless products (k_4). Similarly, in group III, the formation rate constants 5,8-furanoxides (luteoxanthin from violaxanthin (k_5) and auroxanthin from luteoxanthin (k_6)) were always higher than the rate constant of the final degradation reaction of auroxanthin to colorless products (k_7).

Thermodynamic Study. The Arrhenius model and transition state theory were used to determine the influence of temperature on the reaction rates. Table 4 displays the values

estimated for the thermodynamic parameters (entropy, enthalpy, activation energy, and Gibbs free energy), with their respective standard errors for each matrix and reaction analyzed.

To study the effect of matrix type on the reaction mechanism, we compared the thermodynamic parameters estimated in the three types of VOO. In general, no significant differences were found in the parameters ΔS^\ddagger and ΔH^\ddagger characterizing the reactions of isomerization and degradation of xanthophylls (t test $P \leq 0.05$) (Table 4). These results enable all of the matrices to be considered a single-reaction medium. An exception is the isomerization reaction of luteoxanthin to auroxanthin, for which significant differences in the corresponding thermodynamic parameters (E_a , ΔS^\ddagger , ΔH^\ddagger) were found in matrix J, suggesting a slight effect of the matrix in this case. Also, differences in the activation energy were found in matrix D, N and D for the degradation of neoxanthin, neochrome, and mutatoxanthin, respectively.

With respect to the estimated values for activation energy of isomerization reactions, higher values were found in xanthophylls with a single epoxide group (neochrome and mutatoxanthin) than in those with two epoxy groups (luteoxanthin and auroxanthin), in all matrices studied. Mathematically, this can be interpreted as follows: a temperature increase produces a greater increase in the rate constant for 5,6-monoepoxy compounds degradation; that is, a smaller temperature change is needed to form 5,8-monofuranoxo compounds more rapidly.

In all cases, values for the $T\Delta S^\ddagger$ term were negative (due to the negative values of entropy); however, enthalpy values (ΔH^\ddagger) were positive, as were the Gibbs free energy values (ΔG^\ddagger), making the reactions nonspontaneous.

Isokinetic Ratio. The isokinetic ratio was studied along the same lines as previous studies^{23,24} to determine whether there were changes in the reaction mechanisms (first case) or whether some specific step in the mechanism had greater importance under our different experimental conditions (VOO matrices with high, medium, and low pigmentation) (second case).

The isokinetic effect (or isoequilibrium) is defined as the intersection point between the straight Arrhenius (or van't Hoff) lines that show the thermodynamics of a series of similar reactions or reactions in various media.⁴² This cutoff point is the isokinetic temperature at which reactions take place at identical rates. Specifically, the experiments study the same reaction taking place in various oily matrices. Thus, we are in agreement with the second case: a greater importance of a particular step in the mechanism.

To study the existence of an isokinetic ratio among oily matrices, the Arrhenius straight lines obtained for each of the three oily matrices studied were represented together. The study was repeated for each of the reactions including the mechanism for thermal degradation of neoxanthin, antheraxanthin, and violaxanthin. No isokinetic ratio was found for any of them.

Figure 7 shows the example of the violaxanthin isomerization reaction. We could not conclude that there was an isokinetic ratio as the Arrhenius straight lines for the three samples (N, D, and J) did not present any common cutoff points. These straight lines are almost parallel, but are also very close to one another (all points lie within the same interval of confidence). Consequently, all points can be explained by a single Arrhenius line, so that the reaction mechanism is not affected at any stage

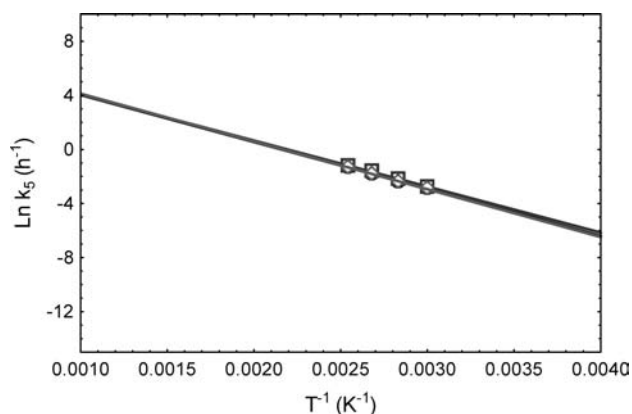


Figure 7. Arrhenius plot for 5,6-epoxide/5,8-furanoxide isomerization of violaxanthin in VOO oil samples studied (N, ○; D, □; J, ◇) (confidence intervals, 95%).

by different pigment contents in the oily matrix. This same result was observed for the other reactions studied.

They are, therefore, isenthalpic and isoentropic straight lines. This observation is consistent with the thermodynamic parameters (Table 4), which do not show significant differences (*t* test $P \leq 0.05$) between the various oily matrices. Thus, there is no isokinetic ratio, and one can conclude that the type of oily matrix does not affect the isomerization reaction mechanisms of neoxanthin, antheraxanthin, violaxanthin, and luteoxanthin and the degradation reactions of nc from neochrome, mutatoxanthin, and auroxanthin during any of its steps. Consequently, the thermodynamic parameters characterized here can be extrapolated to any type of VOO matrix.

The isokinetic effect can also be considered in a series of similar reactions, as in the case of the degradation of neochrome, mutatoxanthin, and auroxanthin to form colorless products and in the case of isomerization of neoxanthin, antheraxanthin, and violaxanthin to 5,8-furanoids. The average values of the rate constants obtained in the three VOO matrices studied were used to obtain the Arrhenius straight lines (Figures 8 and 9).

In the first case (Figure 8), the confidence intervals of Arrhenius straight lines for mutatoxanthin and neochrome overlap (100% of data between confidence limits), whereas the overlap is lower with the confidence intervals of auroxanthin

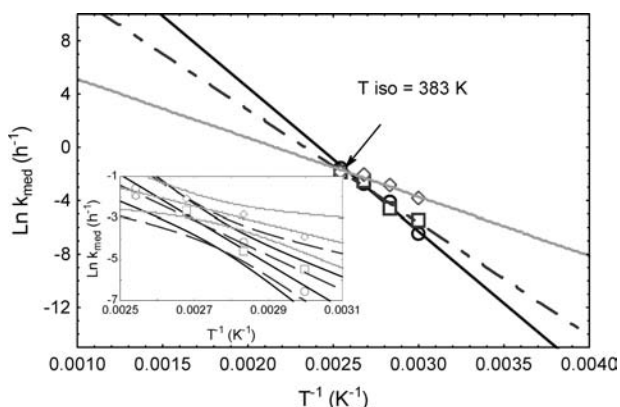


Figure 8. Arrhenius plot for a series of similar reactions: neochrome (○), mutatoxanthin (□), and auroxanthin (◇) degradation to colorless in VOO (average values from the three samples (N, D, J); confidence intervals, 95%).

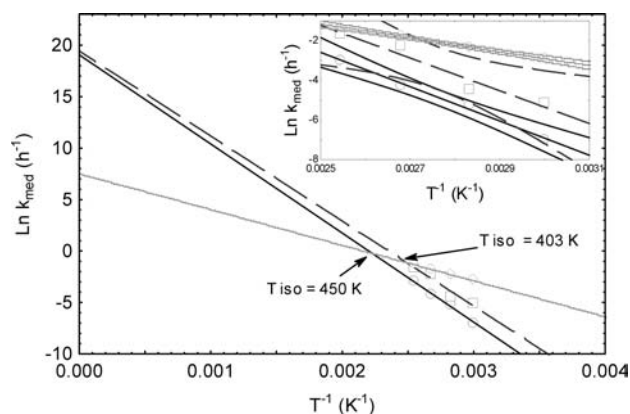


Figure 9. Arrhenius plot for a series of similar reactions: neoxanthin (○), antheraxanthin (□), and violaxanthin (◇) 5,6-epoxide/5,8-furanoxide isomerization reaction in VOO (average values from the three samples (N, D, J); confidence intervals, 95%).

straight lines (50% of data within confidence limits). This results in two straight lines that are cut at an isokinetic temperature of 383 K (± 15) and indicate the same isomerization mechanism, but affected by the temperature change in one or another of its steps. Thus, at temperatures below the isokinetic temperature, the formation of colorless products from auroxanthin is the most rapid, followed by mutatoxanthin and neochrome, respectively. At temperatures above isokinetic, the formation of nc from neochrome is the most rapid, followed by mutatoxanthin and auroxanthin, respectively.

In the second case (Figure 9), the three lines were considered to be independent because the level of overlap was less than in the previous case (100% of violaxanthin and antheraxanthin data were found only within their respective confidence limits). Thus, the lines intersect in pairs, leading to three isokinetic temperatures. One of these, the intersection of neoxanthin and antheraxanthin, takes place at high temperature (>1000 K), well above the boiling point of olive oil. The other two isokinetic temperatures are below the boiling point of olive oil, 450 K (≈ 177 °C) for the intersection of violaxanthin and neoxanthin and 403 K (≈ 130 °C) for violaxanthin and antheraxanthin. The isomerization mechanism of these pigments is the same, but some of the mechanism steps are influenced by temperature (the influence of temperature is similar for neoxanthin and antheraxanthin, and very different from violaxanthin). Therefore, above the isokinetic temperature, isomerizations are faster in xanthophylls with an epoxide group (antheraxanthin and neoxanthin), whereas at lower temperatures the isomerization of violaxanthin with two epoxide groups is preferred.

Compensation Effect. A kinetically compensated system requires that the various thermodynamic parameters obtained for the same reaction in different environments define an isokinetic line. This theoretical line includes all of the various kinetic and thermodynamic coordinates of a single reaction, with the isokinetic temperature (T_{iso}) being the line slope and the increase in Gibbs free energy of all reactions at the T_{iso} being the intercept, according to

$$\Delta H^\ddagger = T_{iso} \Delta S^\ddagger + \Delta G^\ddagger$$

There are some papers describing degradation reactions of carotenoids in different reaction media and reporting the existence of an isokinetic line defined by thermodynamic

parameters and its application in stability prediction studies.^{43,44}

Liu and Guo⁴⁵ demonstrated that the compensation effect and the isokinetic effect are not necessarily synonymous as had been previously thought and that the existence of one does not imply the existence of the other. Errors are inevitable in experiments, and the data used are therefore estimators of the corresponding variables. Consequently, it is possible that the real values are not correlated, although their estimators are. This would be the case for the so-called false compensation effect. Krug et al.⁴⁶ proposed that the straight line in the plane ΔH versus ΔS was only a manifestation of the statistical pattern of the compensation and that this hypothesis can be ruled out if the estimation of the line slope is sufficiently different from the harmonic temperature (T_{hm}), defined as

$$T_{\text{hm}} = \frac{n}{\sum_{i=1}^n \frac{1}{T_i}}$$

Liu and Guo⁴⁵ proposed a method for distinguishing the real compensation effects from the false ones, based on the graphical representation of experimental values of enthalpies and entropies with their error bars in the ΔH^\ddagger versus ΔS^\ddagger plane.

To apply this study to our experimental data, the linear regressions ΔH^\ddagger versus ΔS^\ddagger were estimated for each of the reactions. Table 5 shows the values obtained for the line slope

Table 5. Isokinetic Temperature (T_{isok}) and Determination Coefficients (R^2) Estimated by Leffer's Compensation Law ($\Delta H^\ddagger_i = \Delta H^\ddagger_0 + \beta \Delta S^\ddagger$) for the Thermal Degradation Reactions of Xanthophylls in Virgin Olive Oil

reaction ^a	β^c	SE ^d	R^2
D, neoxanthin	751.8	14.4*	0.99
D, neochrome	394.1	40.6	0.99
D, violaxanthin	591.1	248.9	0.89
D, luteoxanthin	338.2	35.0*	0.99
D, auroxanthin	448.9	44.1*	0.99
D, antheraxanthin	322.8	23.9*	0.99
D, mutatoxanthin	327.9	74.9	0.99
group of reactions ^b			
neox, violax, and anther degradation reactions	424.1	16.1*	0.99
neoc, auro, and muta degradation reactions	385.7	3.7*	1.00

^aReactions according to the kinetic mechanism shown in Figure 6; D, degradation. ^bGroup of reactions: neox, neoxanthin; neoc, neochrome; anther, antheraxanthin; muta, mutatoxanthin; violax, violaxanthin; luteo, luteoxanthin; auro, auroxanthin. ^c $\beta = T_{\text{isok}}$. ^dSE, standard error. * indicates significant differences ($p \leq 0.05$) with the mean harmonic temperature (T_{hm}) = 362 K.

(T_{iso}) and the corresponding determination coefficients (R^2). An isokinetic line was obtained in all cases ($R^2 > 0.95$), except for violaxanthin isomerization. However, by comparing the estimated isokinetic temperature and the T_{hm} under study conditions (362 K), we deduced that the compensation effect could be true only for the degradation of neoxanthin, luteoxanthin, auroxanthin, and antheraxanthin, for a series of similar reactions that involved the isomerization of neoxanthin, violaxanthin, and antheraxanthin, and for the degradation of neochrome, auroxanthin, and mutatoxanthin to colorless products. Finally, applying the error bar method proposed by

Liu and Guo⁴⁵ showed that there is no true compensation effect in these cases, except for the group of reactions of neochrome, auroxanthin, and mutatoxanthin to colorless products (Figure 10B).

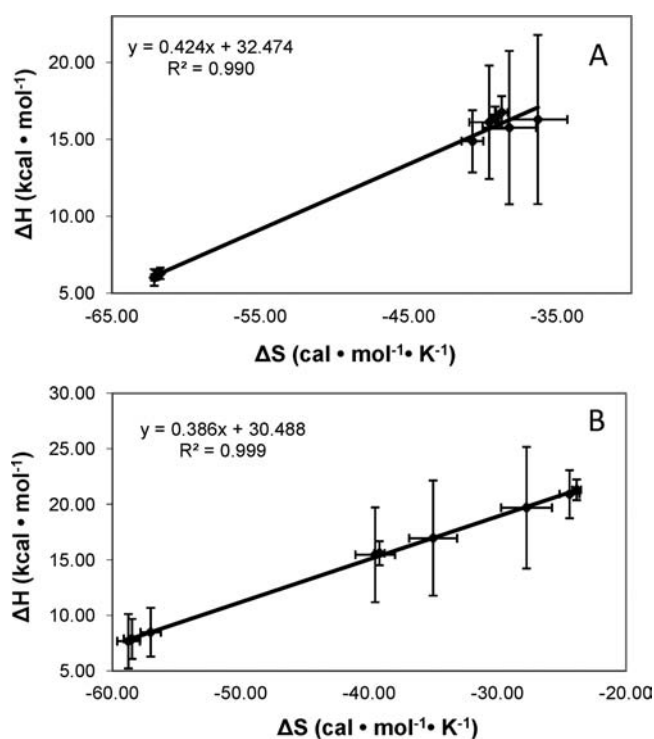


Figure 10. Graphic representation of ΔH^\ddagger versus ΔS^\ddagger by error bars method.⁴⁵ (A) false compensation effect for the group of 5,6-epoxide/5,8-furanoxide isomerization reactions of neoxanthin, violaxanthin, and antheraxanthin; (B) true compensation effect for the group of degradation reactions of neochrome, auroxanthin, and mutatoxanthin to noncolored products.

The degradation of 5,6-epoxy xanthophylls in VOO during heat treatment followed first-order kinetics. The analysis of the 5,8-furanoxide compounds (reaction intermediates) that appear during the thermodegradation of neoxanthin, antheraxanthin, and violaxanthin to colorless products has established that the degradation process is not simple and takes place in several consecutive elemental steps. The marked effect of temperature on the reaction mechanism was revealed. The thermal stability varied among carotenoids and was greater for neoxanthin but was significantly affected by changes in their chemical structure. A true kinetic compensation effect exists only for the case of similar reactions in the degradation of neochrome, mutatoxanthin, and auroxanthin to colorless products.

No significant effect of the oily medium on the reaction mechanisms of any of these xanthophylls has been found from the isokinetic study, which compared kinetic and thermodynamic parameters determined in the three VOO matrices of different pigment contents (high, medium, and low). The thermodynamic parameters characterized in this study could therefore be applied to any type of VOO matrix yielding a mathematical model developed from activation energies, which predict xanthophyll degradation and 5,8-furanoxide formation if the time–temperature profile of the processing method is known. Reaction conditions similar to those used in the soft deodorization of VOO (1.5 h at 120 °C) are sufficient to increase the percentage of 5,8-furanoxides, decreasing the

natural 5,6-epoxide/5,8-furanoxide ratio. This criterion was significant for neoxanthin/neochrome ratio and could be proposed as a chemical marker of heat treatment in VOO.

AUTHOR INFORMATION

Corresponding Author

*Phone: 34-954691054. Fax: 34-954691262. E-mail: gandul@cica.es.

Funding

This work was supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT-EU, Spanish and European Government) and FEDER (CE) in the Project AGL-2007-66139-C02-01 and by the Junta de Andalucía (AGR 148-2009-10).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Sergio Alcañiz-García for his technical assistance.

REFERENCES

- (1) Krinsky, N. I. The biological properties of carotenoids. *Pure Appl. Chem.* **1994**, *66*, 1003–1010.
- (2) Goodwin, T. W. *The Biochemistry of the Carotenoids, Vol. I, Plants*, 2nd ed.; Chapman and Hall: New York, 1980.
- (3) Meléndez-Martínez, A. J.; Vicario, I. M.; Heredia, F. J. Carotenoid pigments: structural and physicochemical considerations. *Arch. Latinoam. Nutr.* **2007**, *57*, 109–117.
- (4) Britton, G.; Liaaen-Jensen, S.; Pfander, H. *Carotenoids. Vol. 5: Nutrition and Health*; Birkhäuser Verlag: Basel, Switzerland, 2009.
- (5) Castenmiller, J. J.; West, C. E. Bioavailability and bioconversion of carotenoids. *Annu. Rev. Nutr.* **1998**, *18*, 19–38.
- (6) Granado-Lorencio, F.; Olmedilla-Alonso, B.; Herrero-Barbudo, M. C.; Blanco-Navarro, I.; Pérez-Sacristán, B.; Blázquez-García, S. In vitro bioaccessibility of carotenoids and tocopherols from fruits and vegetables. *Food Chem.* **2007**, *102*, 641–648.
- (7) Bermúdez, B.; Pacheco, Y.; López, S.; Abia, R.; Muriana, J. G. Digestion and absorption of olive oil. *Grasas Aceites* **2004**, *55*, 1–10.
- (8) International Olive Oil Council (IOC). Trade Standard Applying to Olive Oils and Olive-Pomace Oils, 2009; Madrid: IOC/T.15/NC n° 3/Rev. 4; available online at <http://www.internationaloliveoil.org/downloads/NORMAEN1.pdf>.
- (9) Gandul-Rojas, B.; Mínguez-Mosquera, M. I. Chlorophyll and carotenoid composition in virgin olive oils from various spanish olive varieties. *J. Sci. Food Agric.* **1996**, *72*, 31–39.
- (10) Rao, A. V.; Rao, L. G. Carotenoids and human health. *Pharmacol. Res.* **2007**, *55*, 207–216.
- (11) Rodríguez-Amaya, D. B. *A Guide to Carotenoid Analysis in Foods*; International Life Sciences Institute (ILSI) Press: Washington, DC, 1999.
- (12) Oliver, J.; Palou, A. Chromatographic determination of carotenoids in foods. *J. Chromatogr., A* **2000**, *881*, 543–555.
- (13) Dhuique-Mayer, C.; Tbatou, M.; Carail, M.; Caris-Veyrat, C.; Dornier, M.; Amiot, M. J. Thermal degradation of antioxidant micronutrients in Citrus juice: kinetics and newly formed compounds. *J. Agric. Food Chem.* **2007**, *55*, 4209–4216.
- (14) Meléndez-Martínez, A. J.; Vicario, I. M.; Heredia, F. J. Geometrical isomers of violaxanthin in orange juice. *Food Chem.* **2007**, *104*, 169–175.
- (15) Lee, H. S.; Coates, G. A. Effect of thermal pasteurization on Valencia orange juice color and pigments. *Lebensm. Wiss. Technol.* **2003**, *36*, 153–156.
- (16) Gallardo-Guerrero, L.; Roca, M.; Mínguez-Mosquera, M. I. Distribution of chlorophylls and carotenoids in ripening olives and between oil and alperujo when processed using a two-phase extraction system. *J. Am. Oil Chem. Soc.* **2002**, *79*, 105–109.
- (17) Mínguez-Mosquera, M. I.; Gandul-Rojas, B.; Garrido-Fernández, J.; Gallardo-Guerrero, L. Pigments present in virgin olive oil. *J. Am. Oil Chem. Soc.* **1990**, *67*, 192–196.
- (18) Lemmens, L.; De Vleeschouwer, K.; Moelants, K. R. N.; Colle, I. J. P.; Van Loey, A. M.; Hendrickx, M. E. β -Carotene isomerization kinetics during thermal treatments of carrot puree. *J. Agric. Food Chem.* **2010**, *58*, 6816–6824.
- (19) Pérez-Gálvez, A.; Jarén-Galán, M.; Mínguez-Mosquera, M. I. Effect of high-temperature degradative processes on ketocarotenoids present in paprika oleoresins. *J. Agric. Food Chem.* **2000**, *48*, 2966–2971.
- (20) Henry, L. K.; Catignani, G.; Schwartz, S. Oxidative degradation kinetics of lycopene, lutein, and 9-cis and all-trans β -carotene. *J. Am. Oil Chem. Soc.* **1998**, *75*, 823–829.
- (21) Achir, N.; Randrianatoandro, V. A.; Bohuon, P.; Laffargue, A.; Avallone, S. Kinetic study of β -carotene and lutein degradation in oils during heat treatment. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 349–361.
- (22) Mancebo-Campos, V.; Fregapane, G.; Salvador, M. D. Kinetic study for the development of an accelerated oxidative stability test to estimate virgin olive oil potential shelf life. *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 969–976.
- (23) Aparicio-Ruiz, R.; Mínguez-Mosquera, M. I.; Gandul-Rojas, B. Thermal degradation kinetics of chlorophyll pigments in virgin olive oils. 1. Compounds of series a. *J. Agric. Food Chem.* **2010**, *58*, 6200–6208.
- (24) Aparicio-Ruiz, R.; Mínguez-Mosquera, M. I.; Gandul-Rojas, B. Thermal degradation kinetics of lutein, β -carotene and β -cryptoxanthin in virgin olive oils. *J. Food Compos. Anal.* **2011**, *24*, 811–820.
- (25) Mínguez-Mosquera, M. I.; Gandul-Rojas, B.; Gallardo-Guerrero, L. Rapid method of quantification of chlorophylls and carotenoids in virgin olive oil by HPLC. *J. Agric. Food Chem.* **1992**, *40*, 60–63.
- (26) Khachik, F.; Beecher, G. R.; Whittaker, N. F. Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J. Agric. Food Chem.* **1986**, *34*, 603–616.
- (27) Walali-Loudiyi, D.; Chimitah, M.; Loussert, R.; Mahhou, A.; Boulouha, B. Morphologic and physiologic characters of olive tree clones from Picholine Marroqui variety. *Olivae* **1984**, *3*, 26–31.
- (28) Hornero-Méndez, D.; Gandul-Rojas, B.; Mínguez-Mosquera, M. I. Routine and sensitive SPE-HPLC method for quantitative determination of pheophytin a and pypropheophytin a in olive oils. *Food Res. Int.* **2005**, *38*, 1067–1072.
- (29) Van Boekel, M. A. J. S. Kinetic modeling of food quality: a critical review. *Compr. Rev. Food Sci. Food Saf.* **2008**, *7*, 144–158.
- (30) Brown, M. B.; Forsythe, A. B. Robust tests for the equality of variances. *J. Am. Stat. Assoc.* **1974**, *69*, 364–367.
- (31) Gandul-Rojas, B.; Roca, M.; Mínguez-Mosquera, M. I. Use of chlorophyll and carotenoid pigment composition to determine authenticity of virgin olive oil. *J. Am. Oil Chem. Soc.* **2000**, *77*, 853–858.
- (32) Davies, B. H. Carotenoids. In *Chemistry and biochemistry of Plant Pigments*, 2nd ed.; Goodwin, T. W., Ed.; Academic Press: London, U.K., 1976; Vol. 2, pp 38–165.
- (33) Lee, H. S.; Coates, G. A. Effect of thermal pasteurization on Valencia orange juice color and pigments. *Lebensm. Wiss. Technol.* **2003**, *36*, 153–156.
- (34) Fratianni, A.; Cinquanta, L.; Panfilì, G. Degradation of carotenoids in orange juice during microwave heating. *Food Sci. Technol.* **2010**, *43*, 867–871.
- (35) Provesi, J. G.; Dias, C. O.; Amante, E. R. Changes in carotenoids during processing and storage of pumpkin puree. *Food Chem.* **2011**, *128*, 195–202.
- (36) De Sá, M. C.; Rodríguez-Amaya, D. B. Optimization of HPLC quantification of carotenoids in cooked green vegetables – comparison of analytical and calculated data. *J. Food Compos. Anal.* **2004**, *17*, 37–51.
- (37) Suzuki, Y.; Shioi, Y. Identification of chlorophylls and carotenoids in major teas by high-performance liquid chromatography

with photodiode array detection. *J. Agric. Food Chem.* **2003**, *51*, 5307–5314.

(38) Mezdari, T.; Perez-Galvez, A.; Hornero-Mendez, D. Carotenoid pigments in acerola fruits (*Malpighia emarginata* DC.) and derived products. *Eur. Food Res. Technol.* **2005**, *220*, 63–69.

(39) Cano, M. P.; Marin, M. A. Pigment composition and color of frozen and canned kiwi fruit slices. *J. Agric. Food Chem.* **1992**, *40*, 2141–2146.

(40) Zepka, L. Q.; Mercadante, A. Z. Degradation compounds of carotenoids formed during heating of a simulated cashew apple juice. *Food Chem.* **2009**, *117*, 28–34.

(41) Mínguez-Mosquera, M. I.; Gandul-Rojas, B. Mechanism and kinetic of the degradation of carotenoids during the processing of green table olives. *J. Agric. Food Chem.* **1994**, *42*, 1551–1554.

(42) Karpinski, Z.; Larsson, R. On the isokinetic effect of neopentane hydrogenolysis over metal catalysts. *J. Catal.* **1997**, *168*, 532–537.

(43) Jarén-Galán, M.; Pérez-Gálvez, A.; Mínguez-Mosquera, M. I. Prediction of decoloration in paprika oleoresins. Application to studies of stability in thermodynamically compensated systems. *J. Agric. Food Chem.* **1999**, *47*, 945–951.

(44) Sánchez, A. M.; Carmona, M.; Ordoudi, S. A.; Tsimidou, M. Z.; Alonso, G. L. Kinetics of individual crocetin ester degradation in aqueous extracts of saffron (*Crocus sativus* L.) upon thermal treatment in the dark. *J. Agric. Food Chem.* **2008**, *56*, 1627–1637.

(45) Liu, L.; Guo, Q. X. Isokinetic relationship, isoequilibrium relationship, and enthalpy-entropy compensation. *Chem. Rev.* **2001**, *101*, 673–695.

(46) Krug, R. R.; Hunter, W. G.; Grieger, R. A. Enthalpy-entropy compensation: 1. Some fundamental statistical problems associated with the analysis of Van't Hoff and Arrhenius data. 2. Separation of the chemical from the statistical effect. *J. Phys. Chem.* **1976**, *80*, 2335–2351.