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Kinetics of Individual Crocetin Ester Degradation in Aqueous Extracts of Saffron (*Crocus sativus* L.) upon Thermal Treatment in the Dark

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Kinetics of individual crocetin ester degradation in aqueous extracts of saffron upon thermal treatment in the dark has been studied. Special attention has been paid to the comparison between saffron extracts and aqueous solutions of a crocetin ester rich fraction, with a lower stability of the latter observed. The degradation reaction was the same for all crocetin esters whether they were in saffron extracts or whether they were purified, although it was affected by external factors that modified their kinetic and thermodynamic parameters, making some of them less stable than others.

KEYWORDS: Crocetin esters; saffron (*Crocus sativus* L.); thermal degradation; kinetics; thermodynamics

INTRODUCTION

Color is the most important quality characteristic of saffron spice (dried stigmas of *Crocus sativus* L.), and coloring strength values ($E_{1\,cm}^{1\%}$440 nm) of its aqueous extracts are critical for the commercial value of the spice. The yellowish red hues of the latter are due to the presence of a variety of water-soluble crocetin esters ($C_{44}H_{64}O_{24}$, 8,8′-diapo-Ψ,Ψ′-carotenedioic acid) with several glucose, gentiobiose, and neapolitanose moieties (*1*) that are also known as crocins. With appropriate flower harvest, stripping, and, above all, an adequate dehydration process, a high initial quality of spice can be achieved. However, its quality will deteriorate progressively during storage and packaging or even upon application. As evidenced by several kinetic studies in the past, optimum storage conditions of saffron should involve a dark, inert atmosphere, low water activity level (<0.43), and less than ambient temperatures (<25 °C) (*2–7*).

Due to the high water solubility of saffron pigments, most of the applications of the spice, for example, in cookery, the food and pharmaceutical industries, or even dyeing, are based on the use of aqueous extracts. The few previous studies of saffron aqueous extracts have shown that color degradation follows first-order kinetics; it is sensitive upon exposure to light, thermal treatment, and acidic environment as well as to the presence of additives (*8–10*). Vickackaite et al. studied photochemical and thermal processes that degrade saffron in methanolic solutions (*11*). Still, it should be noted that up to now most findings about crocetin ester degradation and, consequently, about color loss have been based on changes in $E_{1\,cm}^{1\%}$440 nm values. These values reflect the total result of the degradation of all the crocetin esters and are not valid for studying the changes in each one.

However, and differently from other carotenoids, such as paprika ones or carotene (*12–20*), the individual changes that each crocetin ester undergoes are not yet known. As with other spices (*21*), qualitative composition regarding trans and cis isomers is important in saffron because geometrical isomers differ in physiochemical properties (*22*), which may have an impact on quality characteristics and nutritional value (*23*) and also could discriminate saffron origins and dehydration processes (*24, 25*). Further knowledge about the kinetics of individual crocetin ester degradation in aqueous extracts of saffron would provide important information for the prevailing mechanism as well as for application in the food and pharmaceutical industries.

The aim of this work was to study overall and individual changes that crocetin esters undergo in aqueous extracts when subjected to mild thermal treatment in the dark. Special attention has been paid to kinetics in aqueous extracts prepared directly from the spice or from a crocetin ester rich fraction. The methods employed were HPLC coupled with diode array detection system and LC-MS as well as UV–vis spectrophotometry.

MATERIALS AND METHODS

Samples and Chemicals. Spanish saffron spice (*C. sativus* L.) of the Protected Designation of Origin "Azafrán de La Mancha" was collected from the 2004 harvest. HPLC-grade acetonitrile, cyclohexane, and phosphoric acid were from Scharlau (Barcelona, Spain). Gallic acid was purchased from Sigma-Aldrich (Madrid, Spain). Ultrahigh-purity water was produced using a Milli-Q system (Millipore, Bedford, MA).

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PTFE filters (11 mm, 0.45 μm) were also purchased from Millipore and C_{18} packing material (125×10^{-8} cm pore size, 55–105 μm particle size) from Waters (Milford, MA).

Saffron Aqueous Extract Preparation. Three different types of aqueous extracts were used throughout the study (500 mg L^{-1}). Two of them were prepared according to the ISO 3632 Technical Specification, 2003 (*26*), trade standard without and after removal of vegetal matter by filtration through filter paper. The extracts were designated nonfiltered saffron extract (NFS) and filtered saffron extract (FS), respectively. The third extract was prepared using as starting material a crocetin ester rich fraction (CE) that was purified by column chromatography according to a procedure described in the next paragraph.

Crocetin Ester Rich Fraction Preparation. Removal of nonpolar compounds was achieved with 30 mL of cyclohexane added to 5 g of powdered saffron for 24 h at room temperature in the dark with sporadic agitation. The organic solvent was discarded, and the residue was dried under vacuum before 60 mL of water was added and the mixture was bubbled with nitrogen. The resulting solution was magnetically stirred for 1 h at room temperature in the dark. Then, the extract was centrifuged at 4000 rpm for 10 min, and the supernatant was collected and transferred to a plastic LC column (8 cm high $\times$ 2.7 cm i.d.) filled with C_{18} packing material. Crocetin esters were eluted with 50 mL of acetonitrile/water 80% v/v after removal of flavonoids and picrocrocin with 20 mL of acetonitrile/water 2% v/v and 90 mL of acetonitrile/water 20% v/v. The solvent was evaporated under vacuum, and the crocetin ester rich fraction was kept at −20 °C until analysis.

Thermal Treatment. *NFS.* Eight aliquots of a NFS (200 mL each) were transferred to 250 mL borosilicate glass bottles, hermetically sealed, and kept in the dark at different temperatures: 5 °C (refrigerator), room temperature (20 ± 2 °C), and 30, 35, 40, 50, 60, and 70 °C (different thermostated ovens) for 91 days until total degradation of crocetin esters was attained.

FS. Ten aliquots of approximately 20 mL were put in 50 mL Falcon tubes sealed and kept in the dark at 5, 30, 50, and 70 °C for 28 days. Samples were withdrawn periodically, at intervals of 1, 2, 6, and 9 h during the first stages of the study and then after 12 or 24 h at the final stages of the experimental procedure. Each sample was used once and then discarded. All of the samples were filtered through a PTFE filter of 0.45 μm before analysis.

CE. The procedure carried out was similar to that described for FSs, and degradation was monitored for 17 days. All experiments were carried out in duplicate extracts.

Spectrophotometric Analysis. Changes in specific spectral characteristics of NFSs, FSs, and CEs at 440, 330, and 257 nm were monitored periodically by scanning from 190 to 700 nm using a Perkin-Elmer Lambda 25 spectrophotometer (Norwalk, CT). $E_{1\,cm}^{1\%}$ at 440 nm, $E_{1\,cm}^{1\%}$ at 257 nm, and $E_{1\,cm}^{1\%}$ at 330 nm values were calculated according to the method of ref *26* on a dry sample basis. Triplicate measurements for every sample at each time point were taken.

RP-HPLC of Crocetin Esters. Simultaneously to spectrophotometric analysis, gallic acid was added to the sample up to a concentration of 10 mg L^{-1}, and 20 μL of the mixture was injected into an Agilent 1100 HPLC chromatograph (Palo Alto, CA) equipped with a 150 mm $\times$ 4.6 mm i.d., 5 μm Phenomenex (Le Pecq Cedex, France) Luna C_{18} column thermostated at 30 °C. The addition of gallic acid just before the injection was used to check the correct working of the HPLC system. Crocetin esters were eluted using a gradient system consisting of a mixture of water +0.1% phosphoric acid (A) and acetonitrile (B) (20% B, 0–5 min; 20–80% B, 5–15 min and 80% B, 15–20 min). The flow rate was 0.8 mL min^{-1}. For each condition studied, duplicate extracts were prepared, and each was chromatographed twice. Apart from retention time (t_R), the relative retention (r) was calculated as the ratio of the adjusted retention time (the total elution time minus the hold-up time) of each crocetin ester relative to that of gallic acid, obtained under identical conditions (*27*).

Identification and Quantification of Crocetin Esters. Identification of crocetin esters by LC-DAD-MS was carried out as previously described (*1*). Respective maxima in the UV–vis region and retention times were used as additional means of identification. Due to the lack of pure standards of each crocetin ester, quantification was based on the equation

$$\%\ \text{of crocetin ester } i \text{ on dry basis} = \frac{\text{Mw}_i(E_{1\text{cm}}^{1\%}440\text{nm})A_i}{10\epsilon_{\text{t,c}}} \quad (1)$$

where Mw_i stands for the molecular weight of the crocetin ester i, $E_{1\,cm}^{1\%}440$ nm is the coloring strength, A_i is the percentage peak area of the crocetin ester i at 440 nm, and $\epsilon_{t,c}$ is the molecular coefficient absorbance value (89000 for *trans*-crocetin esters and 63350 for *cis*-crocetin esters (*22*)).

Kinetic Studies. The kinetic parameters of each reaction—reaction order, rate constants (k), and half-life periods ($t_{1/2}$)—were obtained using the integral method (*28*). This method uses a trial-and-error procedure to find reaction order. If the order assumed is correct, the appropriate plot of the concentration−time data [concentration against time (zero-order), ln concentration against time (first-order), and concentration^{-1} against time (second-order)] should be linear. The result showing the best correlation coefficient (R^2) was selected. Absolute temperature (T) dependence of the degradation rate constant was determined by the Arrhenius equation

$$\ln k = \ln A - \frac{E_a}{RT} \quad (2)$$

where R is the gas constant, E_a is the activation energy, and A is the pre-exponential factor. Therefore, E_a was estimated on the basis of linear regression analysis of ln k versus T^{-1}.

Thermodynamic Studies. According to the activated complex theory, the enthalpy and entropy of activation (ΔH^* and ΔS^*) were determined by the equation

$$\ln k = \ln \frac{k_B T}{h} - \frac{\Delta H^*}{RT} + \frac{\Delta S^*}{R} \quad (3)$$

where k is the degradation constant at temperature T, k_b is the Boltzmann constant, and h is the Planck constant. The pairs of ΔH^* and ΔS^* obtained were linearly correlated according to the equation

$$\Delta H^* = T_{\text{isok}}\Delta S^* + \Delta G_{\text{isok}} \quad (4)$$

from which the isokinetic temperature of reaction (T_{isok}) and its corresponding Gibbs free energy of the reaction (ΔG_{isok}) were calculated.

Nomenclature for Crocetin Esters. Abbreviations in nomenclature were adopted from Carmona et al. (*1*): *trans*-5-tG, *trans*-crocetin (β-D-triglucosyl)-(β-D-gentibiosyl) ester; *trans*-5-nG, *trans*-crocetin (β-D-neapolitanosyl)-(β-D-gentibiosyl) ester; *trans*-4-GG, *trans*-crocetin di-(β-D-gentibiosyl) ester; *trans*-3-Gg, *trans*-crocetin (β-D-glucosyl)-(β-D-gentibiosyl) ester; *trans*-2-G, *trans*-crocetin (β-D-gentibiosyl) ester; *trans*-2-gg, *trans*-crocetin di-(β-D-glucosyl) ester; *trans*-1-g, *trans*-crocetin (β-D-glucosyl) ester; *cis*-4-GG, *cis*-crocetin di-(β-D-gentibiosyl) ester; and *cis*-3-Gg, *cis*-crocetin (β-D-glucosyl)-(β-D-gentibiosyl) ester.

Statistics. Evaluation of the statistical significance of differences was performed using analysis of variance (ANOVA) with the aid of the SPSS 14.0 for Windows (SPSS Inc.) statistical program.

RESULTS AND DISCUSSION

Saffron Quality Characteristics and Initial Content in Crocetin Esters. Quality characteristics were evaluated according to ISO 3632 (2003) specifications (*26*). Results indicated that the sample used belonged to commercial category I: moisture and volatile matter content, 5.5%; coloring strength ($E_{1\,cm}^{1\%}440$ nm), 261; $E_{1\,cm}^{1\%}257$ nm, 100; $E_{1\,cm}^{1\%}330$ nm, 29. **Table 1** shows the individual crocetin ester composition as percentage on a dry basis. Each compound shown in this table was identified by LC-DAD-MS, and the results (data not shown) were totally in agreement with those previously reported (*1*). The five major

Table 1. Individual Crocetin Ester Composition, Spectral Characteristics in the UV–vis Region, Retention Times (t_R) and Relative Retention (r)

	compd								
	trans-5-tG	*trans*-5-nG	*trans*-4-GG	*trans*-3-Gg	*trans*-2-gg	*trans*-2-G	*cis*-4-GG	*cis*-3-Gg	*trans*-1-g
mean content[a] (g/100 g)	0.36	0.38	15.72	7.64	0.60	0.98	0.48	0.24	0.09
SD[b]	0.01	0.01	0.03	0.04	0.02	0.02	0.03	0.01	0.01
UV–vis max (nm)	263, 443, 467	263, 422sh, 440, 467sh	262, 442, 465	262, 441, 465	261, 439, 464	259, 434, 459	262, 327, 435, 458	262, 325, 434, 458	257, 434, 459
t_R (min)	9.6	9.9	10.2	10.8	11.4	12.4	12.0	12.6	13.4
r (min)	5.6	5.8	6.0	6.4	6.8	7.5	7.2	7.6	8.1

[a] Values are the means of two extracts conducted in duplicate ($2 \times 2n$) (g of compd/100 g of saffron, dry basis). [b] Standard deviation.

crocetin esters were, in a decreasing order *trans*-4-GG > *trans*-3-Gg > *trans*-2-G > *trans*-2-gg > *cis*-4-GG. It is noteworthy that the first one alone was found to represent 59% of the total crocetin ester content of the aqueous saffron extract, whereas the first three mentioned accounted for >90% of the total esters recorded. Respective maxima in the UV–vis region, t_R, and r are also shown in **Table 1**.

Changes in Coloring Strength ($E_{1\,cm}^{1\%}$440 nm) Values and Other Spectral Characteristics of Aqueous Extracts during Storage. The results of experiment with NFSs showed certain similarities between the closest temperatures. Thus, in light of these similarities, it was decided to rule out 20, 40, and 60 °C in the thermal treatment for FSs and CEs. The percentage of $E_{1\,cm}^{1\%}$440 nm values retained throughout the thermal treatment of NFSs, FSs, and CEs are displayed in **Figure 1**. Upon thermal treatment of NFSs and FSs, a greater retention of $E_{1\,cm}^{1\%}$440 nm values for the former was evidenced at all temperatures studied, except for 5 °C. A continuous extraction of crocetin esters from the remaining vegetal material in NFSs that was enhanced at higher temperatures could justify this observation. Kinetic curves at each temperature indicate a greater loss in coloring strength values of CEs, with regard to those of NFSs and FSs, when exposed to the same storage conditions. Such a finding might imply that either coexisting polar saffron constituents such as picrocrocin and flavonoids contribute to the stability of color in nonpurified aqueous extracts or that the purification process has a detrimental effect on individual crocetin esters. These results will be broached from a kinetic and thermodynamic point of view later on, in the two sections devoted to them.

The most outstanding changes observed in UV–vis spectra upon storage of aqueous extracts at different temperatures (**Figure 2**) consisted of (a) a continuous and dramatic decrease in the maximum at about 440 nm and, as a result, a progressive discoloration that was accompanied by a slight shift (**Table 2**) toward lower wavelengths; (b) an increase in absorbance at 275–315 nm followed by a decrease when time passed; (c) an increase in the absorbance around 315–380 nm at all temperatures studied but 5 °C that also disappeared over time in FSs and NFSs; and (d) a slight decrease in the maximum at about 256 nm accompanied by a slight shift of this peak toward lower wavelengths in saffron extracts but toward higher ones in CEs (**Table 2**). Obviously, not all of these changes came only from crocetin esters but also might come from other saffron components such as picrocrocin and flavonoids. Nonetheless, this study will focus mainly on crocetin esters with only brief descriptions of other components, because these components are currently under study in our laboratory.

The mentioned changes were more marked as temperature increased. In FSs, the absorption band between 400 and 500 nm regions disappeared after the extracts were kept for 23 h at 70 and 50 °C, whereas it was not until 56 h at 30 °C and 672 h at 5 °C when this region was insignificant. The same happened in NFSs at 21, 45, 2184, and 816 h at 70, 50, 30, and 5 °C, respectively, whereas in CEs this happened at 6 h at the three first temperatures and at 50 h at 5 °C.

Even so, experimentation showed some differences in the spectra of NFSs and FSs recorded for the highest temperature with regard to those obtained with the same extracts kept at lower ones for a longer time (**Figure 3**). When we compared the spectra of two samples from the same saffron extract kept at different temperatures, with similar absorbance at about 440 nm, a broader visible band with a slight shift toward lower wavelengths was observed at the highest temperature. The increment of absorbance in 275–380 nm was also higher, whereas the decrement at 256 nm was lower. The lack of

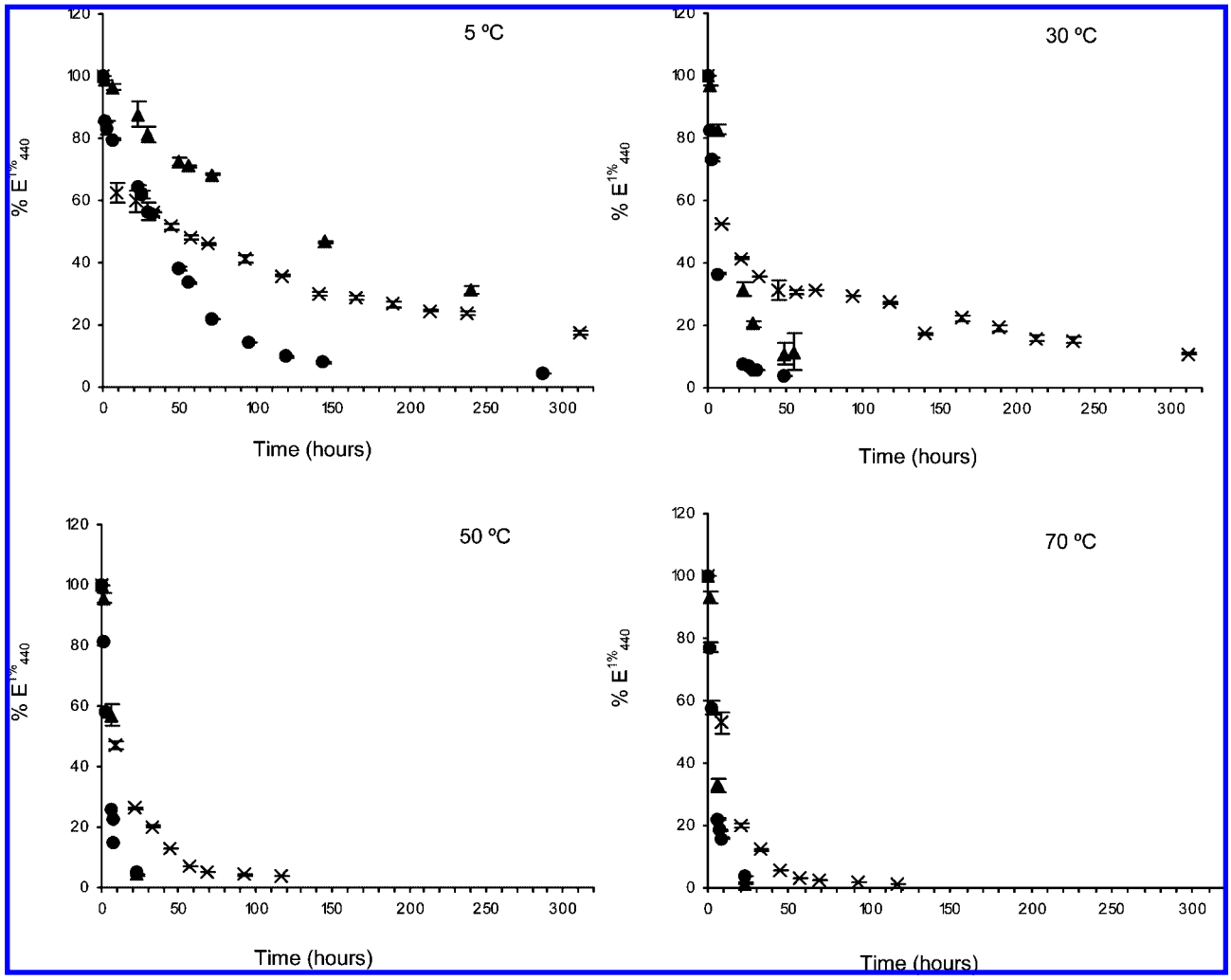

Figure 1. Percentage [mean value of two extracts conducted in triplicate ($3 \times 2n$) $\pm$ standard deviation] of $E^{1\%}_{1\,cm}440$ nm retained throughout the thermal treatment of crocetin ester rich fraction solutions: CE (●), nonfiltered saffron aqueous extracts, NFS (×), and filtered saffron aqueous extracts, FS (▲).

parallelism between this behavior and that of CEs, in which case both spectra were overlapped, was noteworthy. Unlike the main maximum in the visible region, the maximum at 254, 256, and 263 nm of NFS, FS, and CE, respectively, was more stable and showed a slight decrease in absorbance. This decrease was more noticeable at lower temperatures after a longer time elapsed. Therefore, this maximum in time became the only peak in the spectra of saffron extracts.

Changes in HPLC Profile during Storage. To match the previous information with changes shown individually by crocetin esters, their chromatographic profiles were carefully examined. The results from HPLC analyses showed that the decrease in absorbance of the band between 400 and 500 nm corresponded to the decrease in the area of total crocetin esters, until reaching almost total disappearance for all temperatures studied. Also, as has been noted for UV–vis changes, higher temperatures accelerated degradation.

No significant appearance of new compounds was detected at 440 nm, indicating that degradation products had no absorbance at this wavelength or that they could not be detected by our chromatographic method. Moreover, small differences (<10% of their remaining values at each time) between the evolution of $E^{1\%}_{1\,cm}440$ nm and the percentage of the total crocetin ester content were found.

To justify the hypsochromic effect at approximately 440 nm along with the varying width of the visible band, the relative content of each crocetin ester as well as that of trans and cis isomers was examined. Taking into account that the total crocetin ester content was influenced by both time and temperature, the results were normalized by expressing each crocetin ester content as a percentage of the whole. Also, the change observed in the proportion of *trans*- and *cis*-crocetin esters could explain the hypsochromic shift shown. However, moments with maximum shifts were not in line with moments with maximum proportions of *cis*-crocetin esters. Thus, our research focused on the sum of crocetin esters with the lowest maximum wavelength (**Table 1**). The results were in better agreement with the only exceptions of NFS at 5 and 50 °C, where maximum proportions of these crocetin esters were reached earlier than maximum hypsochromic shifts (**Figure 4**). On the basis of the above findings, the hypsochromic effect was generated by a change in the proportion of crocetin esters with maximum wavelength lower than 440 nm, which included cis isomers, rather than by an isomerization of *trans*-crocetin esters to *cis*-crocetin esters. The different width of the band between 400 and 500 nm seemed to be caused by the different relative content of each crocetin ester.

It has been reported (*24*) that the proportion of *trans*-4-GG in saffron spice (dry stigmas), as well as its absolute amount, were higher after 2 h at 50 °C and after 4 h at 70 °C when compared with a nonaged sample. It was concluded that saffron could increase its *trans*-4-GG content when it was resubmitted

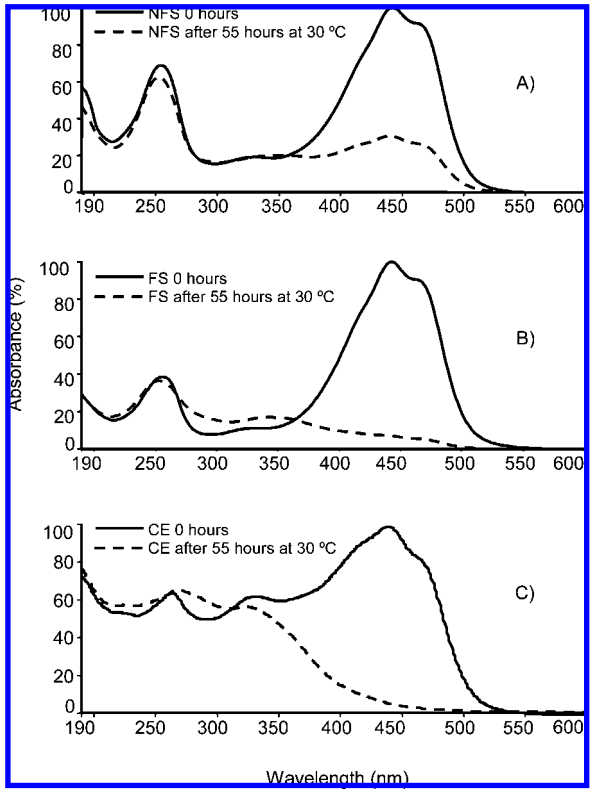

Figure 2. UV−vis spectra of (**A**) nonfiltered saffron aqueous extracts (NFS), (**B**) filtered saffron aqueous extracts (FS), and (**C**) crocetin ester-rich fraction solutions (CE) at the initial moment (—) and after 55 h (- - -) at 30 °C.

to a heating treatment. However, this appears not to happen in saffron crocetin ester rich fraction solutions, because increments in neither the percentage nor the absolute content of the above compound were observed. The same happened for all crocetin esters quantified in CEs. On the contrary, higher absolute contents than the initial ones in NFS for *cis*-3-Gg at 5 and 30 °C after 9 h and for *trans*-2-gg at 70 °C after 9 h have been observed. In FS, within the first 6 h of the experiments, the same trend was found for *trans*-5-nG at 5, 30, and 70 °C; for *trans*-3-Gg at 5 °C; for *trans*-2-gg at 5, 30, and 50 °C; for *cis*-4-GG, *cis*-3-Gg, and *trans*-1-g at 5, 30, 50, and 70 °C; and for *trans*-2-G at 50 and 70 °C. Concerning their relative proportion, increases were shown in all crocetin esters except *trans*-4-GG, which gave rise to situations in which *trans*-3-Gg surpassed *trans*-4-GG. By comparison of these two major carotenoids, *trans*-4-GG and *trans*-3-Gg, it was shown that when the latter increased, the former decreased and vice versa in saffron extracts, at all temperatures but 30 °C in NFS, at which the percentage of *trans*-3-Gg fluctuated while the percentage of *trans*-4-GG decreased, and at 70 °C in FS, at which both decreased. In CE both remained stable or decreased at the same time.

Despite the different rates of degradation, these findings would support the generation of the other crocetin esters from *trans*-4-GG by the detachment or attachment of glucose moieties, as previously reported (*11*) in methanolic solutions along with the cis−trans isomerization promoted by light.

The main changes shown by the HPLC results at 250 nm were a slight decrease in the picrocrocin peak area, the disappearance of signals corresponding to crocetin esters, and the appearance of a peak with a $t_R = 8.7$ min ($r = 5.0$ min) and $\lambda_{max} = 286$ nm after 23 h at 70 °C. On the contrary, a peak with $t_R = 11.2$ min ($r = 6.7$ min) and with a spectrum similar

Table 2. Maxima Shifts of UV–vis Spectra Observed in Saffron Aqueous Extracts and Crocetin Ester Rich Fraction Solutions upon Thermal Treatment

T (°C)	starting material[a]	λ peak at initial time (nm)	max shift[b] (nm)	time of max shift (h)
70	NFS	443	−3.0 ± 0.3	9
		254	−3.0 ± 0.4	2184
	FS	443	−3.0 ± 0.3	6
		256	−4.0 ± 0.5	672
	CE	439	−4.0 ± 0.2	2
		263	+3.0 ± 0.4	6
50	NFS	443	−6.0 ± 0.3	33
		254	−2.0 ± 0.1	93
	FS	443	−1.0 ± 0.4	6
		256	−3.0 ± 0.5	672
	CE	439	−4.0 ± 0.3	2
		263	+4.0 ± 0.2	387
30	NFS	443	−14.0 ± 0.4	984
		254	−9.0 ± 0.3	2184
	FS	443	−3.0 ± 0.6	30
		256	−3.0 ± 0.5	50
	CE	439	−2.0 ± 0.2	2
		263	+6.0 ± 0.4	385
5	NFS	443	−13.0 ± 0.3	575
		254	−2.0 ± 0.2	2184
	FS	443	−1.0 ± 0.3	240
		256	−3.0 ± 0.5	672
	CE	439	−4.0 ± 0.4	32
		263	+8.0 ± 0.3	385

[a] Nonfiltered saffron aqueous extracts (NFS), filtered saffron aqueous extracts (FS), and crocetin ester rich fraction solutions (CE). [b] Values are the means of two extracts conducted in triplicate ($3 \times 2n$). Shifts toward lower wavelengths are indicated by (−), whereas shifts toward higher wavelengths are indicated by (+).

to that of picrocrocin slightly increased at this temperature and at 50 °C, but showed a more prominent increase at 30 and 5 °C at longer times. On the basis of its UV–vis spectrum and retention time in comparison to the bibliography data (*29, 30*), this peak could possibly be 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC).

To justify the increase in absorbance observed between 275 and 380 nm, special attention was paid to specific moments. For example, in FSs at 70 °C, this change, unnoticed after 1 h, was observed after 6 h (**Figure 5**). Thus, the increase in absorbance at 330 nm occurred between 1 and 6 h. Comparison of the chromatograms at 330 nm for those times showed that several peaks with t_R between 3 and 4 min (r between 1.2 and 1.9 min) and around 7 min ($r = 3.9$ min), for which λ_{max} was between 325 and 385, appeared and could have contributed to the mentioned increase. Among them, the most noticeable peak eluted at $t_R = 4.2$ min ($r = 2.0$ min), and its spectrum had two maxima at 241 and 335 nm, the latter being higher. A baseline increase in the region of the chromatogram where crocetin esters were eluted was also observed. However, neither the *cis*-4-GG nor the *cis*-3-Gg area showed a higher level after 6 h, even though the previous measurement observed increases of 31 and 6% in each. The effect of these increases in the UV–vis spectrum might become apparent in delay with regard to the HPLC signal. Consequently, the increase in absorbance around 330 nm could be attributed to the isomerization of crocetin esters, together with the appearance of some degradation products. After 23 h at 70 °C, the spectrum showed slightly higher absorbance between 275 and 315 nm, but it decreased at higher wavelengths. This decrease was in consonance with the decrease in the peaks with retention times around 4 min and with the decreases in

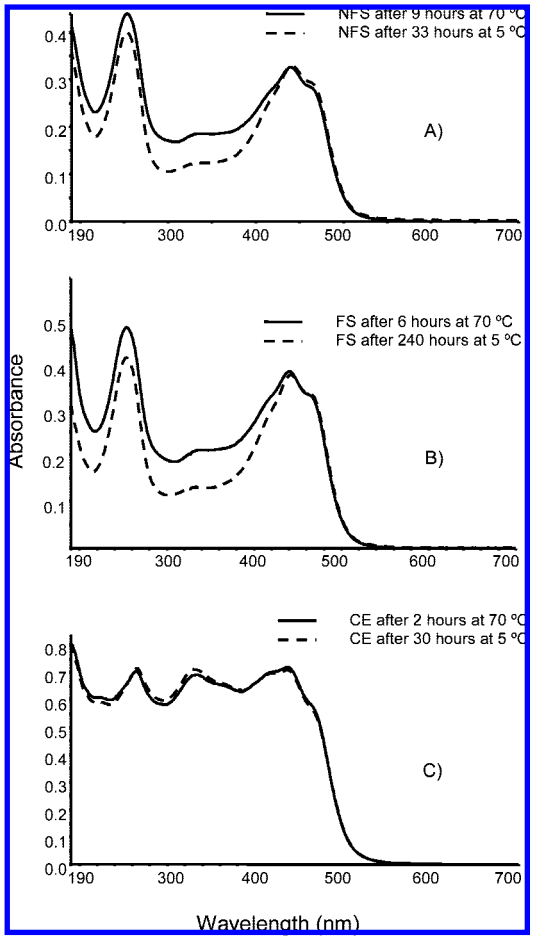

Figure 3. Comparison of UV−vis spectra of (**A**) a nonfiltered saffron extract (NFS), (**B**) a filtered saffron extract (FS), and (**C**) a crocetin ester rich fraction solution (CE) when their coloring strengths were the same but subjected to different temperatures.

picrocrocin and in crocetin esters shown in the chromatograms of the extracts after 23 h.

Therefore, the changes in the UV–vis spectrum from 315 to 380 nm could be due to such compounds present in the first minutes of chromatograms.

The same changes in the spectra and chromatograms were observed in FS at 50 °C, the only difference being that after 23 h, the absorbance between 275 and 360 nm was higher than at 6 h and also the area of the peaks with t_R around 4 min was higher. At 30 °C, these changes happened later in time (between 6 and 23 h) and remained longer (the increase in the absorbance was still noticeable after 240 h).

Kinetic Parameters. Table 3 shows rate constants (k) and half-life periods ($t_{1/2}$) of coloring strength loss ($E^{1\%}_{1\,cm}$440 nm), according to a first-order kinetic model, in saffron aqueous extracts and crocetin ester rich fraction solutions at 5, 30, 50, and 70 °C. Prior to discussing these results, it is necessary to point out that all rate constants were negative (as was degradation), with increases or decreases being expressed in absolute value.

The differences found in the k, depending on the initial material used, except for 5 and 30 °C when NFSs and FSs were compared, indicated that they could be considered as different reaction media in which the rest of the components modulate the degradation as stated for paprika carotenoids (*16, 31*). These k values showed a prominent increase with temperature, especially in FSs, for which k at 5 °C was approximately 10, 30, and 40 times lower than those at 30, 50, and 70 °C,

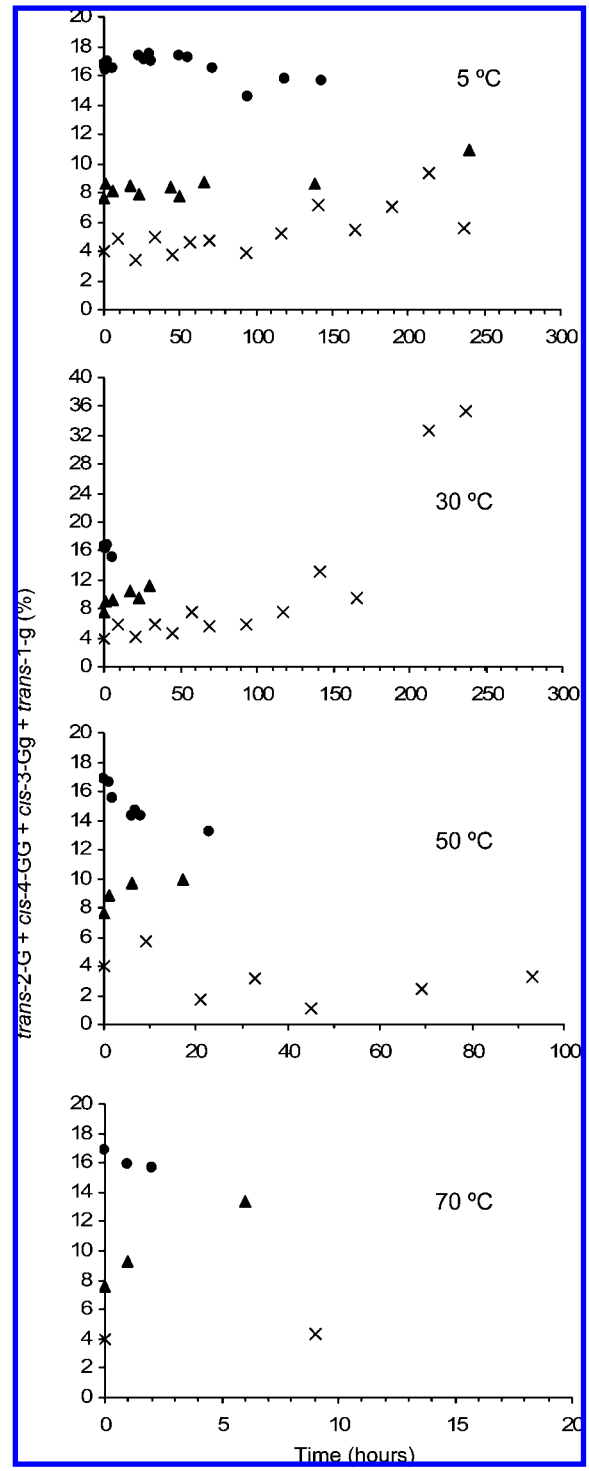

Figure 4. Sum of *trans*-2-G, *cis*-4-GG, *cis*-3-Gg, and *trans*-1-g, as percent of the total crocetin esters content, in crocetin ester rich fraction solutions (CE, ●), nonfiltered saffron aqueous extracts (NFS, ×), and filtered saffron aqueous extracts (FS, ▲) upon thermal treatment.

respectively. The only exceptions were CEs at 50 and 70 °C, which had very close k. These results illustrate the great effect of temperature on coloring strength stability and also confirmed the results previously reported (*9*). Degradation rate constants for CE were always higher than those for saffron extracts, giving evidence, once more, of their higher lability. However, only at 50 and 70 °C were k values of FSs significantly higher than those of NFSs. Half-life periods of the $E^{1\%}_{1\,cm}$440 nm degradation ranged from 151 h for FSs at the lowest temperature (5 °C) to 3 h for CEs at 50 and 70 °C. With regard to each crocetin ester,

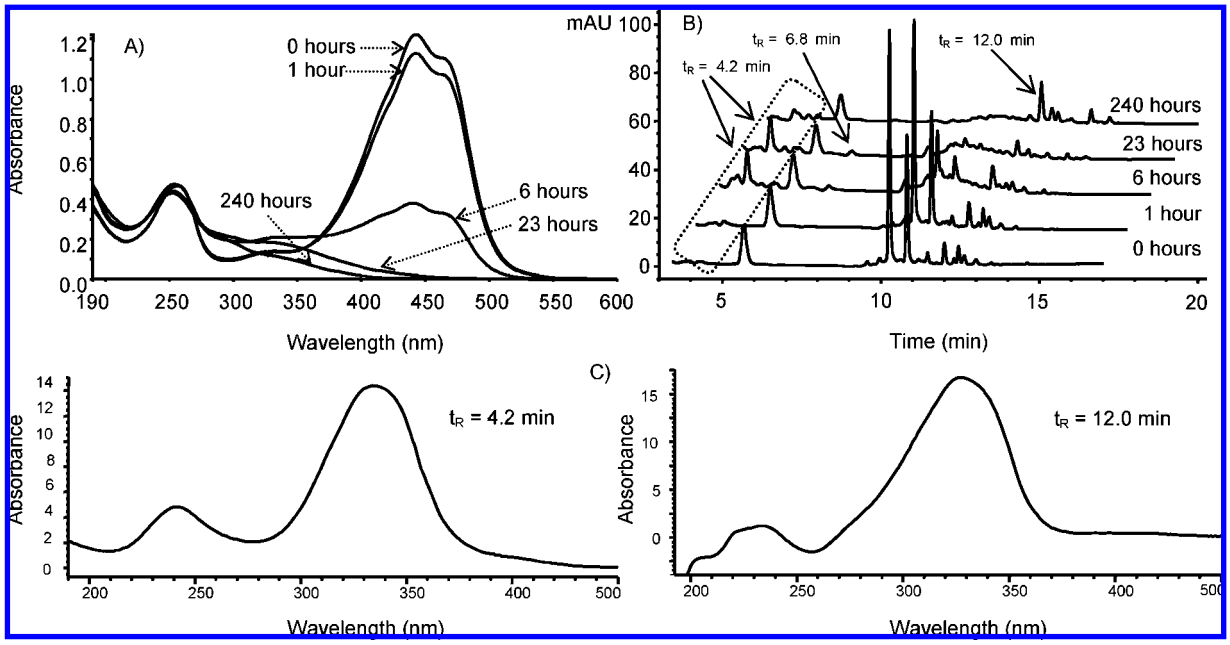

Figure 5. Evolution of filtered saffron aqueous extracts (FS) kept at 70 °C: (**A**) UV−vis spectra; (**B**) HPLC chromatograms at 330 nm; (**C**) UV−vis spectra of peaks with t_R values of 4.2 and 12.0 min.

Table 3. Rate Constants (k), Determination Coefficients (R^2), and Half-Life Periods ($t_{1/2}$) of Coloring Strength Loss ($E^{1\%}_{1cm}$440 nm) in Saffron Aqueous Extracts and Crocetin Ester Rich Fraction Solutions upon Thermal Treatment

T (°C)	starting material[a]	($k \pm$ SD)[b] $\times 10^3$ (h^{-1})	R^2(m)[c]	$t_{1/2}$ (h)
5	NFS	4.7 a ± 0.4	0.920 (45)	147
	FS	4.6 a ± 0.5	0.998 (16)	151
	CE	19.1 b ± 1.0	0.991 (26)	36
30	NFS	40.6 a ± 0.9	0.883 (9)	17
	FS	43.7 a ± 5.5	0.955 (11)	16
	CE	103.4 b ± 5.2	0.987 (12)	7
50	NFS	62.9 a ± 2.4	0.976 (9)	11
	FS	140.0 b ± 4.2	0.994 (9)	5
	CE	224.7 c ± 11.2	0.992 (12)	3
70	NFS	76.5 a ± 0.4	0.996 (9)	9
	FS	184.2 b ± 8.8	0.999 (9)	4
	CE	234.0 c ± 11.4	0.996 (12)	3

[a] Nonfiltered saffron aqueous extracts (NFS), filtered saffron aqueous extracts (FS), crocetin ester rich fraction solutions (CE). [b] Values are the means of two extracts conducted in triplicate (3 × 2n), SD = standard deviation. [c] Minimum number of experimental data points. At each temperature, different letters between rows indicate significant differences at the 0.05% level.

in most cases studied their degradation adjusted to a first-order kinetics model (**Table 4**), but it was not always possible to apply such a model to the degradation because either their areas were stable or they increased slightly in the first hours and then decreased. The k of each crocetin ester was also clearly dependent on temperature and increased along with temperature. Only *cis*-4-GG had similar k values for the higher temperatures (50 and 70 °C) in the three starting solutions. Besides, low differences were found between k of CE at 50 and 70 °C.

In the first thermal treatment for NFSs, the crocetin esters that had the lowest k and therefore degraded more slowly were the *trans*-3-Gg at 5 °C ($t_{1/2}$ = 198 h), whereas at 30 and 50 °C they were the *trans*-2-gg ($t_{1/2}$ = 301 and 59 h, respectively) and at 70 °C it was the *cis*-4-GG ($t_{1/2}$ = 14 h). On the contrary, crocetin esters with the highest degradation rates were *trans*-5-tG at 5 °C ($t_{1/2}$ = 99 h) and *trans*-4-GG at other temperatures ($t_{1/2}$ = 53 h at 30 °C, 8 h at 50 °C, 6 h at 70 °C). In the second thermal treatment for FSs, the most stable crocetin esters were *trans*-2-G ($t_{1/2}$ = 165 h) and *trans*-5-tG ($t_{1/2}$ = 136 h) at 5 °C, whereas at 30 and 50 °C they were the *trans*-5-nG ($t_{1/2}$ = 32 and 9 h, respectively) and at 70 °C it was the *cis*-4-GG ($t_{1/2}$ = 7 h). On the other hand, crocetin esters with the highest degradation rates were *trans*-4-GG ($t_{1/2}$ = 104 h), *trans*-3-Gg ($t_{1/2}$ = 106 h), and *cis*-4-GG ($t_{1/2}$ = 123 h) at 5 °C; *trans*-4-GG at 30 °C ($t_{1/2}$ = 12 h); *trans*-2-G at 50 °C ($t_{1/2}$ = 4 h); and *trans*-2-gg and *trans*-4-GG at 70 °C ($t_{1/2}$ = 3 h). Finally, in the third thermal treatment for CEs, the *trans*-5-nG was the most stable crocetin ester ($t_{1/2}$ = 60 h), whereas *trans*-2-G and *cis*-3-Gg were the most labile ones at all temperatures ($t_{1/2}$ = 23 h at 5 °C, 3 h at 30 °C, 2 h at 50 and 70 °C), although at 70 °C there were no significant differences with the *trans*-4-GG.

No evidence of any relationship between the number or type of glycoside moiety and lability of crocetin esters was found. Furthermore, at almost all temperatures, the k values of the two mayor crocetin esters (*trans*-4-GG and *trans*-3-Gg) did not present significant differences. However, *trans*-3-Gg at 5, 50, and 70 °C in NFSs and at 30 °C in FSs degraded at a lower rate, whereas it was less stable than *trans*-4-GG at 5 °C in CEs. Analogously, *cis*-4-GG and *cis*-3-Gg had equal k values at 30 °C but *cis*-4-GG was slightly more stable than *cis*-3-Gg at 50 °C in FSs. The crocetin ester *cis*-4-GG showed equal or lower k values compared to the major *trans*-crocetin esters (*trans*-4-GG, *trans*-3-Gg, and *trans*-2-G) except for NFS at 50 °C, at which *trans*-3-Gg had a higher stability, and for FS at 5 °C, at which the same happened with *trans*-2-G. The crocetin ester *cis*-4-GG was less stable than *trans*-5-nG in all cases but in NFS and FS at 70 °C. Half-life periods of crocetin esters ranged from 198 h at 5 °C to 2 h at 70 °C. The results also indicated that each crocetin ester was affected in a different way by the temperature increase. For example, in FSs, an increment of temperature from 5 to 30 °C resulted in k values of crocetin ester degradation being multiplied by a factor ranging from 7 to 11 approximately. From 30 to 50 °C, this factor varied between 3 and 4, whereas an increase in temperature from 50 to 70 °C multiplied k by a factor of 1−2. Especially important

Table 4. Degradation Rate Constant (k), Determination Coefficient (R^2), and Half-Life Period ($t_{1/2}$) of Each Crocetin Ester in Saffron Aqueous Extracts and Crocetin Ester Rich Fraction Solutions upon Thermal Treatment

		5 °C			30 °C			50 °C			70 °C		
compd	starting material[a]	($k \pm$ SD)[b] $\times 10^3$(h^{-1})	R^{2c} (m)	$t_{1/2}$ (h)	($k \pm$ SD) $\times 10^3$ (h^{-1})	R^2 (m)	$t_{1/2}$ (h)	($k \pm$ SD) $\times 10^3$ (h^{-1})	R^2 (m)	$t_{1/2}$ (h)	($k \pm$ SD) $\times 10^3$ (h^{-1})	R^2(m)	$t_{1/2}$ (h)
trans-5-tG	NFS	7.0 e ± 0.3	0.968 (11)	99	*			*			*		
	FS	5.1 bc ± 0.4	0.987 (13)	136	45.5 d ± 5.1	0.997 (12)	15	132.8 gh ± 5.9	0.995 (5)	5	196.0 f ± 13.1	0.994 (5)	4
	CE	16.8 g ± 0.8	0.982 (27)	41	100.8 f ± 5.0	0.988 (16)	7	194.2 l ± 9.7	0.992 (12)	4	222.4 gh ± 11.1	0.991 (12)	3
trans-5-nG	NFS	5.6 cd ± 1.0	0.944 (13)	124	9.7 ab ± 1.7	0.955 (11)	71	32.6 c ± 2.2	0.980 (7)	21	74.1 b ± 12.0	0.979 (4)	9
	FS	*			21.9 c ± 3.4	0.985 (12)	32	75.0 e ± 3.4	0.988 (9)	9	136.1 d ± 17.4	0.952 (5)	5
	CE	11.6 f ± 0.6	0.996 (19)	60	65.8 e ± 3.3	0.982 (18)	11	148.4 i ± 7.4	0.982 (12)	5	156.0 e ± 7.8	0.998 (14)	4
trans-4-GG	NFS	5.6 cd ± 0.5	0.982 (20)	124	13.0 b ± 5.1	0.981 (16)	53	92.4 f ± 4.6	0.989 (7)	8	112.2 c ± 5.6	0.992 (6)	6
	FS	6.7 de ± 0.5	0.985 (13)	104	58.3 e ± 6.6	0.997 (12)	12	165.4 jk ± 8.1	0.994 (7)	4	229.3 ghi ± 13.3	0.994 (5)	3
	CE	25.5 j ± 1.3	0.990 (29)	27	153.9 i ± 7.7	0.991 (14)	5	281.5 o ± 14.1	0.995 (12)	2	300.4 kl ± 15.0	0.996 (12)	2
trans-3-Gg	NFS	3.5 a ± 0.2	0.972 (18)	198	5.4 ab ± 0.3	0.930 (17)	128	15.8 b ± 0.8	0.896 (15)	44	78.1 b ± 0.8	0.991 (7)	9
	FS	6.6 de ± 1.7	0.961 (13)	106	43.8 d ± 5.0	0.992 (12)	16	169.3 k ± 8.1	0.995 (7)	4	222.9 gh ± 2.1	0.999 (5)	3
	CE	27.4 k ± 1.4	0.983 (27)	25	159.7 i ± 8.0	0.987 (14)	4	278.1 o ± 13.9	0.996 (12)	2	292.8 k ± 2.9	0.996 (12)	2
trans-2-gg	NFS	*			2.3 a ± 0.2	0.911 (19)	301	11.7 ab ± 0.6	0.965 (15)	59	67.8 b ± 3.4	0.982 (5)	10
	FS	*			*			152.7 i ± 5.2	0.991 (7)	5	243.7 ij ± 7.8	0.993 (5)	3
	CE	16.1 g ± 0.8	0.979 (27)	43	116.9 g ± 5.8	0.986 (10)	6	240.9 n ± 12.0	0.997 (12)	3	256.3 j ± 10.3	0.994 (12)	3
trans-2-G[d]	FS	4.2 ab ± 0.1	0.979 (13)	165	45.2 d ± 10.7	0.989 (12)	15	182.6 l ± 10.9	0.980 (7)	4	215.6 g ± 7.1	0.997 (5)	3
	CE	29.6 l ± 1.5	0.976 (27)	23	211.3 j ± 10.6	0.994 (12)	3	331.5 p ± 16.6	0.995 (12)	2	315.4 l ± 15.8	0.993 (12)	2
cis-4-GG	NFS	*			*			53.8 d ± 2.7	0.972 (6)	13	51.3 a ± 2.6	0.961 (5)	14
	FS	5.7 cd ± 0.2	0.979 (13)	123	37.5 d ± 7.6	0.983 (12)	19	103.5 f ± 6.0	0.987 (7)	7	106.6 c ± 18.3	0.963 (5)	7
	CE	22.3 i ± 1.1	0.983 (27)	31	133.5 h ± 6.7	0.990 (10)	5	241.7 n ± 12.1	0.995 (12)	3	235.5 hi ± 11.8	0.996 (12)	3
cis-3-Gg[e]	FS	*			37.9 d ± 8.4	0.983 (12)	18	121.1 g ± 8.9	0.973 (5)	6	*		

[a] Nonfiltered saffron aqueous extracts (NFS), filtered saffron aqueous extracts (FS), crocetin ester rich fraction solutions (CE). [b] Values are the means of two extracts conducted in duplicate ($2 \times 2n$), SD = standard deviation. [c] Minimum number of experimental data points. [d] Separation of the peak was not possible in NFS, and it was coeluted with *cis*-3-Gg in CE. [e] It was coeluted with *trans*-2-G in CE, and it did not follow a first-order kinetics in NFS. *, first-order kinetics was not followed. Different letters between rows indicate significant differences at the 0.05% level.

Table 5. Arrhenius Equation Parameters, Activation Energy (E_a), and Pre-exponential Factor of Each Crocetin Ester Degradation and Coloring Strength Loss ($E^{1\%}_{1\,cm}$440 nm) in Saffron Aqueous Extracts and Crocetin Ester Rich Fraction Solutions

	starting material[a]	$E_a \pm$ SD[b] (kJ mol^{-1})	ln $A \pm$ SD (A, h^{-1})	R^2
trans-5-tG	NFS	*	*	*
	FS	45.6 jk ± 1.2	14.7	0.958
	CE	32.3 cd ± 0.7	10.1	0.912
trans-5-nG	NFS	32.4 i ± 0.7	8.7	0.871
	FS	39.8 gh ± 0.8	12.0	0.998
	CE	33.0 de ± 0.8	10.1	0.913
trans-4-GG	NFS	41.2 hi ± 0.8	12.6	0.946
	FS	44.3 j ± 0.9	14.4	0.951
	CE	30.9 bc ± 1.1	10.0	0.890
trans-3-Gg	NFS	39.2 g ± 1.1	10.7	0.846
	FS	45.4 jk ± 2.7	14.7	0.962
	CE	29.6 b ± 1.2	9.5	0.882
trans-2-gg	NFS	55.8 m ± 1.2	16.6	0.982
	FS	*	*	*
	CE	34.8 e ± 1.3	11.2	0.895
trans-2-G[c]	NFS	*	*	*
	FS	50.3 l ± 0.9	16.5	0.950
	CE	29.5 b ± 0.8	9.6	0.826
cis-4-GG	NFS	21.9 a ± 1.3	5.2	0.981
	FS	37.4 f ± 1.3	11.3	0.926
	CE	29.7 b ± 1.3	9.4	0.867
$E^{1\%}_{1\,cm}$ 440 nm	NFS	34.1 e ± 0.3	9.8	0.873
	FS	46.5 k ± 0.4	15.0	0.961
	CE	31.8 cd ± 0.4	10.0	0.911

[a] Nonfiltered saffron aqueous extracts (NFS), filtered saffron aqueous extracts (FS), crocetin ester rich fraction solutions (CE). [b] Values are the means of two extracts conducted in duplicate ($2 \times 2n$) ± standard deviation (SD) for crocetin esters and the means of two extracts conducted in triplicate ($3 \times 2n$) ± SD for $E^{1\%}_{1\,cm}$440 nm. [c] Separation of the peak was not possible in NFS and was coeluted with *cis*-3-Gg in CE. *, there were not enough data to calculate the activation energy. Different letters between rows indicate significant differences at the 0.05% level.

was the change in the degradation rate of crocetin esters when temperature was increased from lower temperatures, justifying the necessity to study certain thermodynamic parameters such as the activation energy (E_a).

Now that all degradation rate constants have been discussed, we point out that k values of coloring strength were situated among the k values corresponding to crocetin esters, as well as its E_a (described below). In general, the coloring strength (**Tables 3** and **4**) was more stable (lower k) than the two major crocetin esters with the following exceptions: no significant differences were found with *trans*-3-Gg at 30 °C in FS and at 5 and 70 °C in NFS; at 50 °C in NFS, *trans*-3-Gg had lower k than $E^{1\%}_{1\,cm}$440 nm.

Thermodynamic Parameters. Thermodynamic results are presented in **Tables 5** and **6**. Significant differences were found among crocetin ester E_a (**Table 5**), with the highest E_a in line with crocetin esters that showed the most important differences when k values for 5 °C were compared to those for 70 °C. For instance, in FSs, *trans*-2-G showed the highest E_a (the mentioned increase was >50 times) followed by *trans*-5-tG, *trans*-3-Gg, and *trans*-4-GG (their k increased by >34 times). On the other hand, *cis*-4-GG showed the lowest E_a (k increment of 20 times approximately). In addition, the order in E_a for NFSs was *trans*-2-gg > *trans*-4-GG > *trans*-3-Gg > *trans*-5-nG > *cis*-4-GG, whereas that for CEs was *trans*-2-gg > *trans*-5-nG > *trans*-5-tG > *trans*-4-GG > *cis*-4-GG > *trans*-3-Gg > *trans*-2-G. It was also found that the changes in k with temperature for the loss of coloring strength, and as a consequence the E_a, were always in the range of the corresponding values for crocetin esters.

As has been observed for degradation constant rates, there were no important differences between the E_a values of *trans*-4-GG and *trans*-3-Gg. The activation energy for the loss of coloring strength, 46.5 ± 0.4 kJ/mol (11.1 ± 0.1 kcal/mol), was higher when compared with that obtained by Tsimidou and Tsatsaroni (*9*) for temperatures ranging from 4 to 62 °C and pH 7 (7.2 ± 0.1 kcal/mol) but lower than that reported by Alonso et al. (*8*) for temperatures ranging from 0 to 35 °C (124.11 kJ/mol). The E_a was always higher for FSs, followed by NFSs and CEs, except for *cis*-4-GG, for which the E_a values of CEs were higher than those of the NFSs. Mathematically, the interpretation is that changes in temperature modify the reaction rate constant more when the extract is filtered than when they remain unfiltered or when crocetin esters are purified from the rest of saffron components. With these values, according to the activated complex theory, the energy requirement for each crocetin ester in FSs to become an activated complex is well above that of crocetin esters in NFSs and CEs. However, once energy is supplied to the reaction system (in this case an aqueous medium), it is the other parameter of the Arrhenius equation, A (**Table 5**), that determines the degree to which the reaction proceeds. The result was that for the majority of crocetin esters, the number of molecules able to form the activated complex was higher in FSs than it was in NFSs and CEs. Thus, for these reaction conditions, there was a degradative effect on the FSs.

Table 6. Increase of Activation Entalpy (ΔH^*), Entropy (ΔS^*), Gibbs Free Energy (ΔG_{isok}), and Isokinetic Temperature (T_{isok}) of Each Crocetin Ester Degradation and Coloring Strength Loss ($E^{1\%}_{1\,cm}$440 nm) in Saffron Aqueous Extracts and Crocetin Ester Rich Fraction Solutions

	NFS[a]			FS[a]			CE[a]					
	ΔH^* (kJ/mol)	ΔS^* (J/mol K)	R^2	ΔH^* (kJ/mol)	ΔS^* (J/mol K)	R^2	ΔH^* (kJ/mol)	ΔS^* (J/mol K)	R^2	ΔG_{isok} (kJ/mol)	T_{isok} (K)	R^2
trans-5-tG	36.7	221.5	1.000	43.0	199.6	0.952	29.7	237.3	0.896	112.9	348	0.984
trans-5-nG	31.4	245.1	0.926	37.0	222.1	0.970	30.4	237.9	0.899	97.1	273	0.817
trans-4-GG	37.7	221.1	0.910	41.7	201.8	0.944	28.3	238.6	0.870	115.7	361	0.938
trans-3-Gg	34.2	239.5	0.859	42.4	200.3	0.958	27.1	242.5	0.860	102.2	297	0.834
trans-2-gg	59.3	164.6	0.984	18.8	271.2	1.000	32.2	228.2	0.878	121.6	383	0.994
cis-4-GG	15.0	292.8	0.745	35.0	227.4	0.907	27.2	243.8	0.843	100.3	292	0.976
trans-2-G[b]	–	–	–	47.6	184.1	0.933	27.0	241.6	0.797	–	–	–
cis-3-Gg	–	–	–	44.7	192.8	1.000	–	–	–	–	–	–
trans-1-g	–	–	–	–	–	–	33.0	221.9	0.769	–	–	–
$E^{1\%}_{1\,cm}$ 440 nm	31.2	241.8	0.860	43.9	197.0	0.940	29.2	238.1	0.895	105.4	313	0.960

[a] Nonfiltered saffron aqueous extracts (NFS), filtered saffron aqueous extracts (FS), crocetin ester rich fraction solutions (CE). [b] Separation of the peak was not possible in NFS and was coeluted with *cis*-3-Gg in CE. –, no available data.

To elucidate whether there were formal, kinetic, and thermodynamic differences among the situations studied (NFS, FS, and CE), the results obtained were interpreted as a kinetically compensated system. According to the isokinetic theory, a single reaction can have different kinetic and thermodynamic parameters depending on the reaction conditions, although in all cases the reaction is the same (*14, 16, 32–34*). A kinetically compensated system requires that the different thermodynamic parameters obtained for the same reaction in different environments define an isokinetic line. This theoretical line includes all of the different kinetic and thermodynamic coordinates of a single reaction, having as slope the isokinetic temperature (at which the rate constant of the reaction is unique and independent of the medium) and as ordinate at the origin the increase in Gibbs free energy of all the reactions at the isokinetic temperature (eq 4). **Table 6** shows the variation of activation enthalpy, entropy, and Gibbs free energy for the degradation of each crocetin ester and loss of coloring strength. These values have been expressed as absolute values because, as rate constants must have positive values for the thermodynamic study, the sign of the calculated parameters has only a mathematical meaning. It was found that in most of crocetin esters studied, an isokinetic line was obtained ($R^2 > 0.9$). However, *trans*-5-nG and *trans*-3-Gg presented a lower determination coefficient (0.817 and 0.834). The variation of Gibbs free energy varied between 97.1 kJ/mol for *trans*-5-nG and 121.6 kJ/mol for *trans*-2-gg. The isokinetic temperatures ranged from 273 to 383 K, including the range of temperatures under study (278–343 K) and also the values previously reported for β-carotene and capsanthin co-oxidation by lipoxygenase (*16*) and carotenoid pigments in paprika oleoresins (*31*). The fact that all situations defined the same isokinetic line showed that the reaction was the same and that the environment which surrounded the reaction was responsible for the displacement of the thermodynamic parameters along the same isokinetic line. In this case, the theoretical single reaction is represented by each colored crocetin ester that becomes colorless, possibly due to the loss of conjugation in its molecule. The other functional groups of the crocetin ester (not included in the chromophore) can be considered as external factors that can modify the amount of energy required for loss of conjugation. The medium in which the reaction occurs or the environmental conditions are also external factors that do not modify the pattern of the reaction, but change its speed and temperature dependence. Also, the nature of the compounds that promote the loss of conjugation is an external factor affecting the reaction quantitatively but not qualitatively.

In conclusion, the degradation reaction was the same for all crocetin esters whether they were in saffron extracts or whether they were purified, but it was affected by external factors that modified their kinetic and thermodynamic parameters, hence making some of them more labile than others. In general, an overall loss, more marked with increasing temperature, of all crocetin esters was found and a lower stability of the purified crocetin esters was observed. Throughout degradation, the proportion of the different crocetin esters showed changes that contributed to hypsochromic effects in the UV–vis spectra of aqueous extracts. The parameters for the loss of coloring strength were always between the maximum and minimum values for individual crocetin esters, so it could be considered as a global result of their degradation. Some punctual increases in percentage of cis isomers were observed that could be responsible for changes at about 330 nm in the UV–vis spectra, together with the appearance of some degradation products.

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Supporting Information Available: Degradation rate constant (k), determination coefficient (R^2), and half-life period ($t_{1/2}$) of each crocetin ester in nonfiltered saffron aqueous extracts at room temperature, 35, 40, and 60 °C. This material is available free of charge via the Internet at http://pubs.acs.org.

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