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Picrocrocin Kinetics in Aqueous Saffron Spice Extracts (*Crocus sativus* L.) upon Thermal Treatment

Ana M. Sánchez,† Manuel Carmona,† Manuel Jarén-Galán,‡ M. Isabel Mínguez Mosquera,‡ and Gonzalo L. Alonso*,†

†Cátedra de Química Agrícola, ETSI Agrónomos de Albacete, Universidad de Castilla-La Mancha, 02071 Albacete, Spain, and ‡Departamento de Biotecnología, Instituto de la Grasa CSIC, Avenida Padre García Tejero 4, 41012 Sevilla, Spain

The kinetics of picrocrocin degradation in aqueous extracts of saffron upon thermal treatment from 5 to 70 °C have been studied, together with the degradation of purified picrocrocin in water at 100 °C. The best fits to experimental data were found for a second-order kinetics model. Picrocrocin showed high stability with half-life periods ($t_{1/2}$) ranging from >3400 h at 5 °C in saffron extracts to 9 h in the experiments with purified picrocrocin at 100 °C. In saffron extracts, the evolution of the rate constant (k) with temperature showed maximum values at 35 °C, and filtration of the extracts contributed to picrocrocin stability. In the case of purified picrocrocin, the generation of safranal in the first 5 h (yield up to 7.4%) was confirmed. Spectrometric parameters used in saffron quality control ($E^{1\%}_{1cm}$ 257 nm and ΔE_{pic}) were not appropriate for documenting the evolution of picrocrocin.

KEYWORDS: Picrocrocin; saffron (*Crocus sativus* L.); kinetics; safranal; degradation

INTRODUCTION

Saffron spice, the dried stigmas of *Crocus sativus* L., is highly valued in cookery and in the food industry for the coloring properties provided by a group of glycosyl esters of crocetin ($C_{20}H_{24}O_4$, 8,8′-diapo-Ψ,Ψ′-carotenedioic acid). However, its alluring aroma and pleasant bitter taste are what mainly differentiate saffron from other natural or synthetic colorants such as safflower, turmeric, gardenia, and tartrazine. Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) is the major compound in the volatile fraction of saffron spice, whereas picrocrocin (4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) is thought to be the foremost contributor to its bitter taste. Color compounds as well as volatiles have been thoroughly researched in recent decades (*1*−*5*), but only a few studies have focused on the taste of saffron (*6*−*9*) and, in particular, on picrocrocin (*10*, *11*).

It is known that picrocrocin is converted to safranal either by a two-step enzymatic/dehydration process involving the intermediate 4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde (HTCC) or directly by thermal degradation. There is also evidence of this conversion at extreme pH (*12*−*15*).

Many applications of saffron spice involve a thermal cooking process at high temperature. Although Carmona et al. reported that thermal treatment changes the aroma profile of saffron (*16*, *17*), the effect of thermal treatment on picrocrocin and its potential impact on saffron taste are yet to be determined. Previous kinetics studies have focused on establishing the best saffron storage conditions, giving evidence that a dark, inert atmosphere, low water activity level (< 0.43), and less than ambient temperature (< 25 °C) should be maintained (*18*−*24*). They have also dealt with aqueous extracts of saffron, showing that color and crocetin ester degradations follow first-order kinetics and are sensitive upon exposure to light, thermal treatment, and acidic environment as well as the presence of additives (*25*−*27*). In comparison, only a few approximate studies have dealt with picrocrocin degradation. These studies have reported kinetics results based on measurements of $E^{1\%}_{1cm}$257 nm, the absorbance at 257 nm of a 1% saffron aqueous solution in a 1 cm path length cell, and the parameter defined by Corradi and Micheli (*28*) as $\Delta E_{pic} = E^{1‰}_{257} - E^{1‰}_{297}$, along with the difference of absorbances at 257 and 297 nm of 1‰ saffron aqueous solution in a 1 cm path length cell. These studies have shown good fits to either first- or second-order reaction models for the loss of $E^{1\%}_{1cm}$257 nm (*22*) or a second-order kinetics for ΔE_{pic} (*24*). Castellar (*29*), in the only study done with purified picrocrocin to our knowledge, gave evidence of its high stability at room temperature and 4 and −20 °C at pH 7.

The purpose of this research was to study the changes that picrocrocin undergoes in aqueous extracts of saffron spice and in water when subjected to thermal treatment. Special attention has been paid to spectral changes as well as to kinetics and thermodynamic parameters.

MATERIALS AND METHODS

Samples. Saffron spice (*C. sativus* L.) was obtained from the Protected Designation of Origin Azafrán de La Mancha with the following quality characteristics according to the ISO 3632 Technical Specification, 2003 (*30*): moisture and volatile matter content = 5.5%; coloring strength ($E^{1\%}_{1cm}$ 440 nm) = 261; $E^{1\%}_{1cm}$257 nm = 100; and $E^{1\%}_{1cm}$330 nm = 29. The initial saffron composition in picrocrocin and crocetin esters is shown in **Table 1**.

Picrocrocin was purified by preparative-scale C_{18} column chromatography according to the procedure reported by Sánchez et al. (*31*). In the

*Author to whom correspondence should be addressed (phone + 34 967 59 93 10; fax + 34 967 59 92 38; e-mail Gonzalo.Alonso@uclm.es).

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Table 1. Saffron Composition in Picrocrocin and Crocetin Esters, Spectral Characteristics, Retention Times (t_R), and Relative Retention (r)

compound[b]	mean content[a]		UV−vis λ_{max} (nm)	t_R (min)	r
	mg/L	% on a dry basis[c]			
picrocrocin	92.65 ± 11.60	19.69 ± 2.35	250	5.8	3.0
trans-5-tG	1.68 ± 0.05	0.36 ± 0.01	263, 443, 467	9.6	5.6
trans-5-nG	1.78 ± 0.05	0.38 ± 0.01	263, 422sh, 440, 467sh	10.0	5.8
trans-4-GG	73.58 ± 0.14	15.72 ± 0.03	262, 442, 465	10.3	6.0
trans-3-Gg	35.76 ± 0.19	7.64 ± 0.04	262, 441, 465	10.9	6.4
trans-2-gg	2.81 ± 0.09	0.60 ± 0.02	261, 439, 464	11.4	6.8
trans-2-G	4.59 ± 0.09	0.98 ± 0.02	259, 434, 459	12.4	7.5
trans-1-g	0.42 ± 0.05	0.09 ± 0.01	257, 434, 459	13.4	8.1
cis-4-GG	2.25 ± 0.14	0.48 ± 0.03	262, 327, 435, 458	12.0	7.2
cis-3-Gg	1.12 ± 0.05	0.24 ± 0.01	262, 325, 434, 458	12.6	7.6
total crocetin glycosides	123.98 ± 1.31	26.49 ± 0.28			

[a] Values are expressed as mean ± standard deviation of two extracts conducted in duplicate ($2 \times 2n$). [b] *trans*-5-tG, *trans*-crocetin (β-D-triglucosyl)-(β-D-gentiobiosyl) ester; *trans*-5-nG, *trans*-crocetin (β-D-neapolitanosyl)-(β-D-gentiobiosyl) ester; *trans*-4-GG, *trans*-crocetin di-(β-D-gentiobiosyl) ester; *trans*-3-Gg, *trans*-crocetin (β-D-glucosyl)-(β-D-gentiobiosyl) ester; *trans*-2-gg, trans-crocetin di-(β-D-glucosyl) ester; *trans*-2-G, *trans*-crocetin (β-D-gentiobiosyl) ester; *trans*-1-g, *trans*-crocetin (β-D-glucosyl) ester; *cis*-4-GG, *cis*-crocetin di-(β-D-gentiobiosyl) ester; *cis*-3-Gg, *cis*-crocetin (β-D-glucosyl)-(β-D-gentiobiosyl) ester. [c] mg of compound/100 mg dry weight saffron.

eluted fraction containing picrocrocin, the solvent (10% acetonitrile/water v/v) was eliminated by evaporation to dryness under reduced pressure, and the purified picrocrocin was kept at −20 °C until its utilization. The chromatographic purity of the picrocrocin obtained was 96%, calculated as the percent of the total HPLC peak area at 250 nm.

Standards and Chemicals. Gallic acid and safranal were purchased from Sigma-Aldrich (Madrid, Spain). HPLC-grade acetonitrile, cyclohexane, and phosphoric acid were from Scharlau (Barcelona, Spain). Ultrahigh-purity water was produced using a Milli-Q system from Millipore (Bedford, MA). PTFE filters (11 mm, 0.45 μm) were also purchased from Millipore, whereas C_{18} packing material for picrocrocin purification (125×10^{-8} cm pore size, 55−105 μm particle size) was supplied by Waters (Milford, MA).

Aqueous Extracts and Picrocrocin Solution. Two saffron extracts in water were prepared according to ISO 3632 Technical Specification, 2003 (*30*), but at a 500 mg L^{-1} concentration. One of the extracts was kept with its vegetal matter, whereas this was removed by filtration through filter paper in the other one. The extracts were designated nonfiltered saffron extract (NFS) and filtered saffron extract (FS), respectively. A third solution was prepared by dissolving the purified picrocrocin from saffron in water at a 50 mg L^{-1} concentration and was designated PP.

Thermal Treatment. *NFS*. Eight aliquots of an NFS (200 mL each) were transferred to 250 mL borosilicate glass bottles, hermetically screw-cap 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).

FS. Ten aliquots of approximately 20 mL were put into 50 mL Falcon tubes screw-cap sealed and kept in the dark at 5, 30, 50, and 70 °C. 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.

PP. A reflux heating at about 100 °C was applied to PP to simulate thermal treatment when cooking, in a model system. Samples were analyzed every hour for 6 h.

All experiments were conducted in duplicate.

Spectrophotometric Analysis. Changes in specific spectral characteristics of the extracts NFS, FS, and PP were monitored periodically by scanning from 190 to 700 nm using a Perkin-Elmer Lambda 25 spectrophotometer (Norwalk, CT). $E^{1\%}_{1cm}$440 nm, $E^{1\%}_{1cm}$257 nm, and $E^{1\%}_{1cm}$330 nm values, which are defined as the absorbance at 440, 257, and 330 nm, respectively, of a 1% saffron aqueous solution in a 1 cm path length cell were calculated according to ref *30* on a dry weight basis. ΔE_{pic} was also calculated in saffron extracts from the spectral data according to ref *28*. An approximate volume of 6 mL of each sample was filtered through a PTFE filter of 0.45 μm before analysis in a 4 mL cuvette. Triplicate measurements were taken for every sample at each time point.

RP-HPLC Analysis. Simultaneously with spectrophotometric analysis, picrocrocin was determined by HPLC using an Agilent 1100 HPLC chromatograph (Palo Alto, CA) equipped with a 150 mm × 4.6 mm i.d., 5 μm Phenomenex (Le Pecq Cedex, France) Luna C_{18} column thermostated at 30 °C. Gallic acid was added just before the injection to each sample up to a concentration of 10 mg L^{-1} as internal standard. Twenty microliters of the mixture was injected into the chromatograph. This addition was prompted by the continuous degradation over time of saffron components, in order to have a reference point and also ensure that differences between chromatograms were not due to the chromatographic system. The gradient system consisted 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}, and the DAD detector (Hewlett-Packard, Waldbronn, Germany) was set at 250 nm. In the saffron extracts (NFS and FS), crocetin esters were also determined by setting the detector at 440 nm. All of the samples were filtered through a PTFE filter of 0.45 μm before analysis. For each condition studied, duplicate extracts were prepared and each was analyzed 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 compound relative to that of gallic acid, obtained under identical conditions (*2*).

Identification and Quantification of Picrocrocin and Crocetin Esters. Identification of picrocrocin and crocetin esters in the initial saffron extract by LC-DAD-MS was carried out as previously described (*32*). As reported (*2*, *31*), analytes in samples were identified by their t_R, in comparison to a known standard, as given in **Table 1**: picrocrocin (t_R 5.8 min), gallic acid (t_R 1.5 min), and nine crocetin esters (t_R between 9.6 and 13.4 min). Respective maxima in the UV−vis region were used as additional means of identification.

A calibration curve of the picrocrocin concentration, c (mg L^{-1}), as a function of its HPLC peak area, a, was constructed with the purified picrocrocin. It exhibited good linear regression in the range of 2−315 mg L^{-1}: $c = 0.0290a + 0.5194$, R value = 0.999 for a total of six data points.

The quantification of crocetin esters was based on the following equation according to ref *2*

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

where M_{wi} stands for the molecular weight of the crocetin ester i, $E^{1\%}_{1cm}$ 440 nm is the coloring strength, A_i is the percentage peak area of the crocetin ester i at 440 nm, and $\varepsilon_{t,c}$ is the molecular coefficient absorbance value (89000 for *trans*-crocetin esters and 63350 for *cis*-crocetin esters (*33*)).

Safranal Extraction, Separation, Identification, and Quantification in the PP solution. The PP solution was subjected to the highest thermal treatment (100 °C) to monitor the safranal content with simulated cooking in a model system.

Stir Bar Sorptive Extraction (*SBSE*). The PP solution was poured into a 10 mL volumetric flask. Safranal was extracted by introducing a

polydimethylsiloxane coated stir bar (0.5 mm film thickness, 10 mm length, Twister, Gerstel GmbH, Mülheim and der Ruhr, Germany) into the sample by stirring (700 rpm) at room temperature for 60 min. The stir bar was then removed from the sample, rinsed with distilled water, dried with a cellulose tissue, and later transferred into a thermal desorption tube for TD-GC-MS analysis.

TD-GC-MS of Safranal. Safranal was analyzed using thermal desorption equipment (Perkin-Elmer ATD-400, Norwalk, CT), coupled to a gas chromatograph with a mass selective detector (HP-6890 and HP-5973, with a NIST library; Hewlett-Packard, Palo Alto, CA). The carrier gas was helium of chromatographic purity (220 kPa). In the thermal desorption tube, the volatile compounds were desorbed from the stir bar at the following conditions: oven temperature at 290 °C; desorption time, 1 min; cold trap temperature, −30 °C; helium inlet flow, 45 mL min^{-1}. Desorbed compounds were separated in the gas chromatograph with a fused silica capillary column (BP21 stationary phase 50 m length, 0.22 mm i.d., and 0.25 μm film thickness) (SGE, Ringwood, Australia). The chromatographic program was set at 50 °C (held for 2 min), raised to 230 °C at 12 °C min^{-1}, and then held for 20 min. For mass spectrometry analysis, electron impact mode (EI) at 70 eV was used. The mass range varied from 35 to 500 units, and the detector temperature was 150 °C. The t_R and mass spectrum of safranal were compared to those of the commercial standard (t_R = 11.7 min; mass fragmentation pattern, 107 (100), 91 (86), 121 (62), 150 (47)).

Safranal Quantification by TD-GC. Three series of safranal standard solutions in 75% ethanol/water (v/v) with 0.01, 0.1, 0.25, 0.5, and 1 mg L^{-1} concentration were prepared and analyzed by TD-GC-MS after the SBSE described above. Each series had a total of 10 data points. Reproducibility at 0.01 mg L^{-1} was RSD = 2.5%, and at 1 mg L^{-1}, RSD = 5.3%. A calibration curve of the concentration (s, expressed in mg L^{-1}) was established for the series of safranal standards as a function of safranal's peak area (b) at m/z 107: $s = (9 \times 10^{-10} \pm 3 \times 10^{-11}) \times b - (0.0228 \pm 0.00091)$, R value = 0.993 for the worst fit.

Data Processing. The kinetics parameters, that is, reaction order, rate constants (k), and half-life periods ($t_{1/2}$), were obtained using the integral method (*34*). The mathematical fit was performed by assuming that the reaction was of zero, first, or second order, obtaining for each order of reaction the corresponding regression lines. Thermodynamic parameters were calculated as described previously (*2*).

The SPSS 17.0 for Windows (SPSS Inc.) statistical program was used. Significant differences in kinetics data were determined by analysis of variance (ANOVA), whereas NFS and FS data were subjected to a comparison of means with a Student t test (α = 0.05).

RESULTS AND DISCUSSION

Thermal Decomposition of Picrocrocin in Saffron Extracts. *Picrocrocin Retained*. As for crocetin esters (data not shown), the results for which were in total agreement with those previously reported (*2*), data indicated a general trend for the degradation of picrocrocin at all temperatures studied. The picrocrocin retained throughout the thermal treatment at 5, 30, 50, and 70 °C of NFS and FS is shown in **Figure 1**. In NFS the picrocrocin retained decreased at 5, 30, and 50 °C, whereas at 70 °C the values fluctuated very close to the initial level and only sporadic increases of < 10% were observed at 237 and 311 h. Nevertheless, after this time, a continuous decrease of the retained picrocrocin was also found at 70 °C. By comparison among temperatures, in NFS the results indicated that the retained picrocrocin did not decrease to a higher extent at a higher temperature, contrary to what was expected. In fact, the most prominent decrease in the picrocrocin retained was found at 30 °C instead of 70 °C. In FS, all thermal treatments caused a drop in picrocrocin and, as in NFS, from 5 to 70 °C the picrocrocin retained was lowest at 30 °C.

Moreover, from the comparison between NFS and FS it might follow that the filtration process contributed to picrocrocin stability at the three lowest temperatures shown, because the retained picrocrocin was slightly greater in the latter (FS) than in the former (NFS). For example, at 30 °C complete loss of picrocrocin was observed at about 311 h in NFS and at about 461 h in FS.

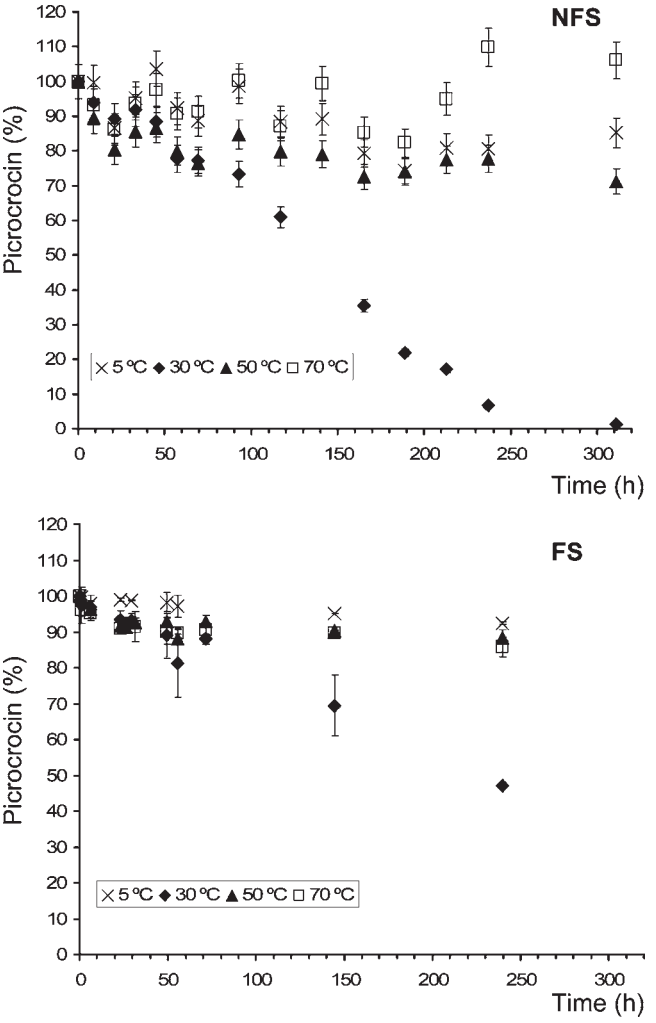

Figure 1. Percentage (mean value ± standard deviation) of picrocrocin retained throughout the thermal treatment of nonfiltered saffron aqueous extracts (NFS) and filtered saffron aqueous extracts (FS).

Kinetics Parameters. The best overall fit of the experimental data for thermal decomposition of picrocrocin in NFS and FS corresponded to a second-order kinetics model. **Table 2** shows rate constants (k) of picrocrocin in saffron extracts and its half-life periods ($t_{1/2}$) at 5, 30, 50, and 70 °C, according to this model. All rate constants of picrocrocin were negative (as was degradation) and have been expressed in **Table 2** in absolute units. In NFS the highest k value was found at 30 °C and the lowest one corresponded to 70 °C. The $t_{1/2}$ values ranged from 174 h (30 °C) to 1983 h (70 °C). With regard to the other kinetics models, the zero-order kinetics model showed better R^2 at 5 and 30 °C than the second-order kinetics model, but the deviation of experimental data from the fitted lines was higher (Table 1S of the Supporting Information).

In FS a two-stage degradation was observed at 50 and 70 °C with a faster degradation in the first hours (first stage) followed by a slower one. We designated "turning point" as the time when the regression lines of both stages intersected one another (**Tables 2**−**4**). At 50 °C the turning point was 23 h, and at 70 °C it was 21 h. The $t_{1/2}$ values ranged from 295 h (30 °C) to 3444 h (5 °C). There were small differences among R^2 values of the different reaction orders (Table 2S of the Supporting Information) and a better R^2 value was found for a zero-order model at 30 °C (Table 1S of the Supporting Information).

Quantitative differences were found between the k values of picrocrocin in NFS and FS (**Table 2**). In general, they were

Table 2. Rate Constants (k), Determination Coefficients (R^2), and Half-Life Periods ($t_{1/2}$) of Picrocrocin Degradation According to a Second-Order Kinetics, in Nonfiltered Saffron Aqueous Extracts (NFS) and Filtered Saffron Aqueous Extracts (FS) upon Thermal Treatment

extract	T (°C)	turning point[a] (h)	$(k \pm SD)^b \times 10^3$ (L mg^{-1} h^{-1})	R^{2c}	$t_{1/2}$ (h)
NFS	5		0.015 c ± 0.001	0.787 (18)	694
	30		0.079 d ± 0.002	0.932 (10)	174
	50		0.012 b ± 0.001	0.711 (16)	1388
	70		0.007 a ± 0.001	0.846 (21)	1983
FS	5		0.003 a ± 0.001	0.953 (20)	3444
	30		0.035 b ± 0.001	0.965 (10)	295
	50	23	0.039 c ± 0.001	0.997 (7)	265
			0.003 ± 0.002	0.941 (15)	3444
	70	21	0.048 d ± 0.001	0.990 (6)	218
			0.004 ± 0.001	0.924 (10)	2501

[a] Turning point is the time when the regression lines of two phases intersect with each other. [b] Values are the means of two extracts conducted in duplicate ($2 \times 2n$); SD, standard deviation. For comparison among temperatures within the same extract, different letters indicate significant differences according to Duncan's test at the 0.05% level. [c] Minimum number of experimental data points is shown in parentheses.

Table 3. Rate Constants (k), Determination Coefficients (R^2), and Half-Life Periods ($t_{1/2}$) of $E^{1\%}_{1cm}$ at 257 nm Evolution According to a Second-Order Kinetics, in Nonfiltered Saffron Aqueous Extracts (NFS) and Filtered Saffron Aqueous Extracts (FS) upon Thermal Treatment

extract	T (°C)	turning point[a] (h)	$(k \pm SD)^b \times 10^3$ (AU^{-1} h^{-1})	R^{2c}	$t_{1/2}$ (h)
NFS	5		0.002 c ± 0.001	0.894 (21)	5854
	30		0.006 d ± 0.001	0.968 (19)	1171
	50	45	−0.024 b ± 0.001	0.368 (5)	
			0.001 ± 0.001	0.923 (17)	16727
	70	21	−0.047 a ± 0.002	0.665 (3)	
			0.002 ± 0.001	0.976 (19)	5854
FS	5		0.006 b ± 0.001	0.924 (15)	1829
	30		0.007 b ± 0.001	0.991 (16)	1547
	50		0.003 a ± 0.001	0.817 (20)	3353
	70	32	0.030 c ± 0.001	0.771 (12)	335
			0.003 ± 0.001	0.849 (11)	3353

[a] Turning point is the time when the regression lines of two phases intersect with each other. [b] Values are the means of two extracts conducted in duplicate ($2 \times 2n$); SD, standard deviation; AU, absorbance units. For comparison among temperatures within the same extract, different letters indicate significant differences according to Duncan's test at the 0.05% level. [c] Minimum number of experimental data points is shown in parentheses.

Table 4. Rate Constants (k), Determination Coefficients (R^2), and Half-Life Periods ($t_{1/2}$) of ΔE_{pic} Degradation According to a Second-Order Kinetics, in Non filtered Saffron Aqueous Extracts (NFS) and Filtered Saffron Aqueous Extracts (FS) upon Thermal Treatment

extract	T (°C)	turning point[a] (h)	$(k \pm SD)^b \times 10^3$ (AU^{-1} h^{-1})	R^{2c}	$t_{1/2}$ (h)
NFS	5		0.002 a ± 0.001	0.944 (21)	7611
	30		0.016 b ± 0.001	0.919 (21)	951
	50	57	0.074 c ± 0.003	0.943 (6)	206
			0.001 ± 0.001	0.664 (16)	21745
	70	21	0.208 d ± 0.008	0.980 (3)	73
			0.005 ± 0.001	0.969 (19)	3044
FS	5		0.010 a ± 0.001	0.976 (15)	1326
	30	56	0.107 b ± 0.004	0.965 (12)	118
			0.008 ± 0.001	0.914 (7)	1575
	50		0.228 c ± 0.009	0.981 (12)	55
	70		0.261 d ± 0.010	0.939 (12)	48

[a] Turning point is the time when the regression lines of two phases intersect with each other. [b] Values are the means of two extracts conducted in duplicate ($2 \times 2n$); SD, standard deviation; AU, absorbance units. For comparison among temperatures within the same extract, different letters indicate significant differences according to Duncan's test at the 0.05% level. [c] Minimum number of experimental data points is shown in parentheses.

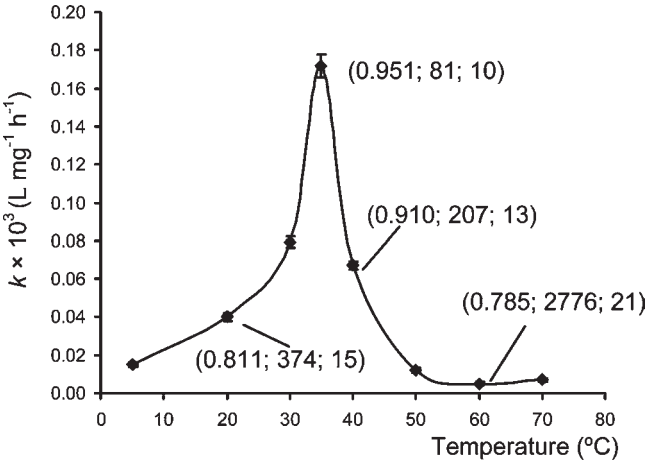

Figure 2. Rate constants (k) of picrocrocin degradation according to a second-order kinetics in nonfiltered saffron aqueous extracts (NFS) upon thermal treatment. At 20, 35, 40, and 60 °C the determination coefficients, half-life periods, and minimum number of experimental data points are presented in parentheses (R^2; $t_{1/2}$; n).

significantly lower in FS except for the first stage of the degradation at 50 and 70 °C.

With regard to the dependence of the k values of picrocrocin with temperature, in NFS these k values increased when temperature increased from 5 to 30 °C. Contrary to what was expected, increments of temperature up to 50 and 70 °C resulted in a decrement in the reaction rate, and the highest temperature corresponded to the lowest k value. In FS, by comparing the k values at all studied temperatures and focusing attention on the first stage at 50 and 70 °C, an increase was noted with temperature. To follow in detail the evolution of k with temperature and to determine the temperature at which k was maximum, we also studied the kinetics of picrocrocin in NFS at 20, 35, 40, and 60 °C (**Figure 2**). The degradation rates showed the maximum k values at 35 °C. This behavior could be explained by a catalytic activity (for example, enzymatic) where the optimum is reached at 35 °C, although further research should be done. Moreover, saffron is subject to dehydration at temperatures up to 80 °C (*16*), indicating a need to consider occurrence of more than one reaction simultaneously.

Thermodynamic Parameters. Due to the observed variation of picrocrocin k values with temperature, the activation energy (E_a) was calculated. The Arrhenius equation parameters were the following: (a) for NFS, E_a = 12.5 kJ mol^{-1}; ln A = 16.2 (with A in L mg^{-1} h^{-1}); R^2 = 0.993; and (b) for FS, E_a = 38.3 kJ mol^{-1}; ln A = 3.7 (with A in L mg^{-1} h^{-1}); R^2 = 0.992. As previously described in crocetin esters (*2*), these results showed higher E_a values in FS than in NFS. The interpretation is that changes in temperature modify the reaction rate constant more when the extract is filtered than when it remains unfiltered.

The variations of activation enthalpy and entropy (ΔH^* and ΔS^*) in NFS were 15.1 kJ mol^{-1} and 408.3 J mol^{-1} K^{-1}, respectively. Moreover, in FS these results were ΔH^* = 35.5 kJ mol^{-1} and ΔS^* = 243.0 J mol^{-1} K^{-1}.

Changes in $E^{1\%}_{1cm}$257 nm and ΔE_{pic} versus Picrocrocin. $E^{1\%}_{1cm}$ 257 nm and ΔE_{pic} are parameters that have been used in the

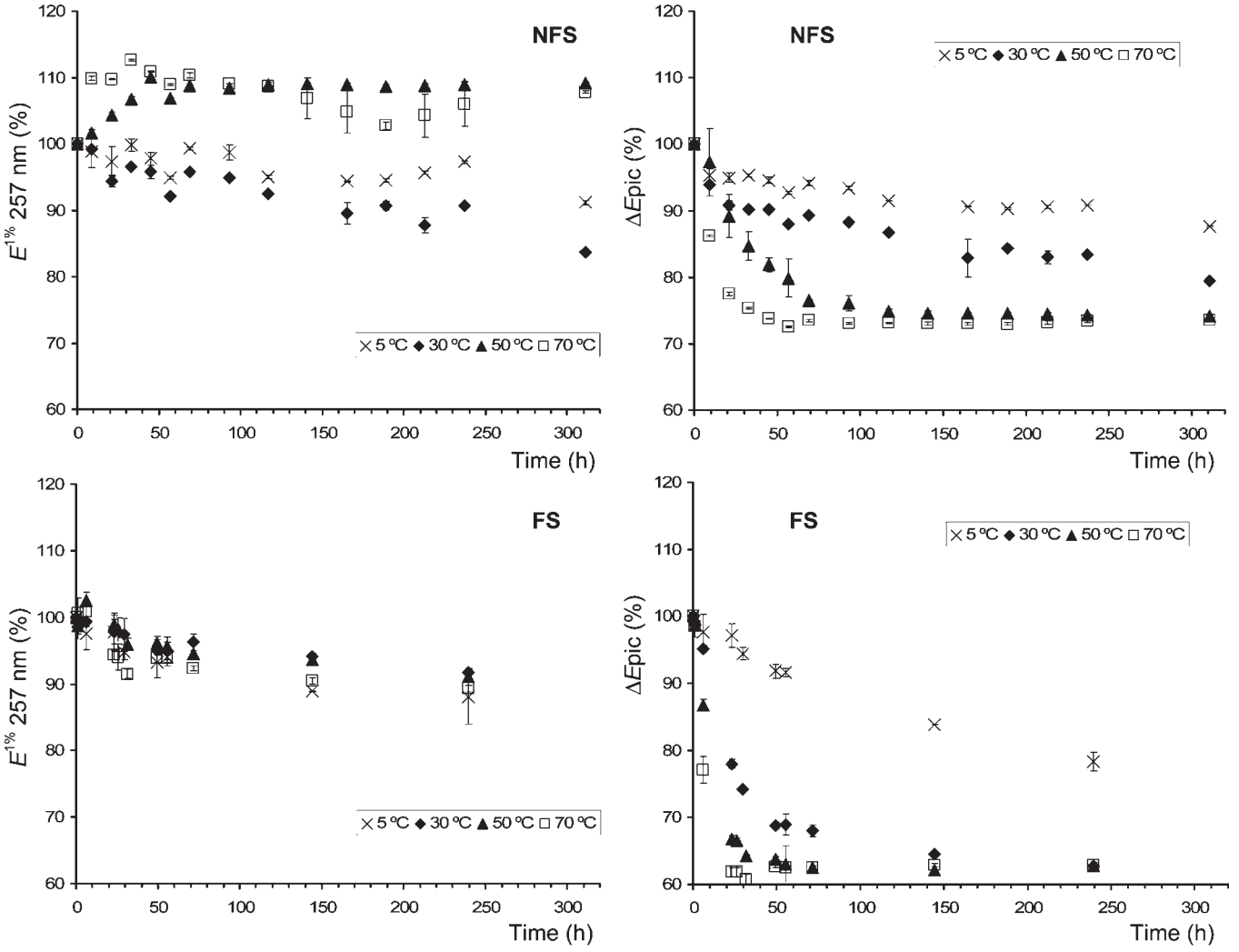

Figure 3. Percentage (mean value ± standard deviation) of $E^{1\%}_{1cm}$257 nm and ΔE_{pic} retained throughout the thermal treatment of nonfiltered saffron aqueous extracts (NFS) and filtered saffron aqueous extracts (FS).

standards and studies of saffron quality as an approximation of picrocrocin content. Despite the low selectivity of these parameters (*35*), it was interesting to follow their evolution to determine their ability to depict picrocrocin changes in saffron and connect the results obtained herein with previous studies (*24*, *25*).

$E^{1\%}_{1cm}$257 nm and ΔE_{pic} Values Retained. **Figure 3** shows the evolution of $E^{1\%}_{1cm}$257 nm and ΔE_{pic} values retained throughout different thermal treatments of NFS and FS as a percentage of their respective initial levels. With regard to $E^{1\%}_{1cm}$257 nm values, high stability was generally observed with decreases of < 20% at about 300 h at all temperatures studied. This observation was in agreement with the previously reported data for saffron aqueous extracts and in contrast to the pronounced decrease in $E^{1\%}_{1cm}$ 440 nm values due to the degradation of crocetin esters (*2*, *22*, *29*). In NFS, results for 5 and 30 °C presented a slight decrease over time that was less prominent for the first temperature, whereas the results for 50 and 70 °C showed an increase in $E^{1\%}_{1cm}$257 nm values in the first hours, which was faster at 70 °C, and then remained constant at a value of about 110%. On the other hand, the evolution of $E^{1\%}_{1cm}$257 nm values in FS was the same at all temperatures studied, with only slight differences among thermal treatments being found in some moments. Similar to the behavior at the two lowest temperatures in NFS, a progressive decrease in the $E^{1\%}_{1cm}$ 257 nm retained was observed in FS. Comparison of the results for both NFS and FS showed a greater retention of $E^{1\%}_{1cm}$257 nm values for NFS at all temperatures studied, except for 30 °C, in which case significant differences were not observed. It seemed that a filtration process reduced the stability to a certain extent in the $E^{1\%}_{1cm}$257 nm value. This finding was consistent with the reported effect of filtration on the loss of coloring strength (*2*), which was attributed to possible extraction of crocetin esters from the remaining vegetal material in NFS. Although similarities were found, especially in FS, the results demonstrated that $E^{1\%}_{1cm}$257 nm values do not reflect the evolution of picrocrocin (**Figure 1**).

The same occurred with the parameter ΔE_{pic}, which evolved differently from picrocrocin, and its changes were more marked as temperature increased (**Figure 3**). These results seemed to corroborate the effect of other components apart from picrocrocin in $E^{1\%}_{1cm}$257 nm and ΔE_{pic}. The simultaneous changes happening in crocetin esters throughout the thermal treatment and the possible presence of degradation products with absorbance at 257 nm could explain these discrepancies.

Kinetics Parameters. The k values and $t_{1/2}$ of $E^{1\%}_{1cm}$257 nm at 5, 30, 50, and 70 °C according to a second-order kinetics are shown in **Table 3** and those of ΔE_{pic} loss in **Table 4**. This was the best model fitting the experimental data. Results for zero- and first-order reaction models are reported in the Supporting Information (Tables 2S and 3S).

In the case of $E^{1\%}_{1cm}$257 nm (**Table 3**), in NFS at 50 and 70 °C two-stage degradations were observed with increments in

$E^{1\%}_{1cm}$257 nm values during the first stage. At 50 °C the turning point was at 45 h, whereas at 70 °C it was at 21 h. R^2 values lower than 0.7 were found in these first stages. The k values for both degradation and increments of $E^{1\%}_{1cm}$257 nm were higher as the temperature rose. Compared with other kinetics models, in NFS better R^2 values were found at 30 °C, where the highest R^2 corresponded to a first-order reaction model, and at 50 °C until 45 h, where the best R^2 value was found for a zero-order model (Table 2S of the Supporting Information). In FS, a two-stage degradation at 70 °C was also observed, but increments of $E^{1\%}_{1cm}$257 nm were not found (**Table 4**). At this temperature, the degradation was faster during the first stage and the highest k value of all studied temperatures was found. On the contrary, the lowest k value corresponded to 50 °C followed by the k values at 5 and 30 °C. As for $t_{1/2}$ values of $E^{1\%}_{1cm}$257 nm, higher values (from 1171 to 16727 h) were found in NFS when compared to FS (from 335 to 3353 h) except for the 30 °C temperature. With regard to the other kinetics models for FS, a better R^2 value was only found for a zero-order model in the first stage at 70 °C, although it was lower than 0.8 (Table 3S of the Supporting Information). When the k values of $E^{1\%}_{1cm}$257 nm in NFS and FS were compared to those for the picrocrocin (**Table 2**), it was found that the former were lower than the latter in NFS, whereas in FS there was not a fixed trend; that is, depending on temperature, the highest k value was found in NFS or in FS.

In the case of ΔE_{pic}, the second-order kinetics model was also the best model fitting the experimental data (**Table 4**). Also, the best R^2 values were found for a second-order kinetics model, with the following exceptions: in NFS at 30 °C and in the second stage at 70 °C, where the highest R^2 values were found for a first-order kinetics model; and in FS in the second stage at 30 °C, where the highest R^2 value was found for the zero-order kinetics model (Table 3S of the Supporting Information). In NFS at 50 and 70 °C, ΔE_{pic} showed a faster degradation in the first hours (first stage) followed by a slower one (**Table 4**). The turning point was lower at the highest temperature and was in agreement with the moment of almost total disappearance of the crocetin esters in the saffron extract. In FS, these two-stage kinetics were observed only at 30 °C. Contrary to picrocrocin, higher k values of ΔE_{pic} were found after the saffron extract had been filtered, as previously reported for crocetin ester degradation at 50 and 70 °C (*2*). In NFS, the $t_{1/2}$ values of ΔE_{pic} were higher than those of picrocrocin (**Table 2**) and the other way around in FS.

The only study on aqueous extracts of saffron from the literature, with k and $t_{1/2}$ values of a parameter related to picrocrocin (*24*), reported $t_{1/2}$ values of ΔE_{pic} for a second-order kinetics model slightly lower at 20 °C (391−480 h) than that found in this study in NFS (1268 h) and of the same magnitude at 40 °C (223−535 versus 507 h).

Thermal Decomposition of Purified Picrocrocin in Water and Safranal Production. The next step was to study the effect of thermal treatment on picrocrocin after purification (PP). **Figure 4** shows the evolution of the percentage of picrocrocin retained throughout the thermal treatment at 100 °C and that of the absorbance at 250 nm. A progressive fall to just 66% at 6 h was found in the retained picrocrocin, providing evidence of the high stability of picrocrocin not only at low temperatures, as reported by Castellar et al. (*29*), but also at high temperatures.

The k value of PP at about 100 °C according to a second-order kinetics was $(1.99 \pm 0.09) \times 10^3$ L mg^{-1} h^{-1}; R^2 was 0.930 for a minimum of six experimental data points, and $t_{1/2}$ was 9 h, thus demonstrating a high resistance of picrocrocin to thermal treatment. Similar R^2 and $t_{1/2}$ values were found for zero- and first-order kinetics models (Table 4S of the Supporting Information). The perception threshold of picrocrocin has recently been published (*11*). Taking into account that picrocrocin is responsible for the bitter taste of saffron, its resistance at high temperatures would mean only a slight loss of this taste when the spice is submitted to a thermal cooking process. With regard to the absorbance at 250 nm that was retained, significant differences with the picrocrocin retained were not found until 3 h of thermal treatment. The formation of degradation products could explain such later differences.

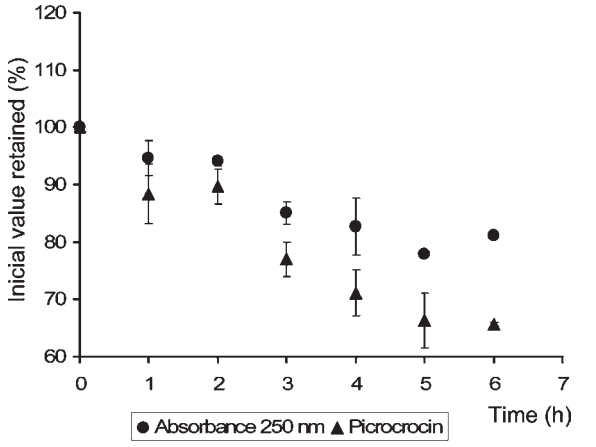

Figure 4. Percentage (mean value ± standard deviation) of picrocrocin and absorbance at 250 nm retained throughout the thermal treatment at 100 °C of a purified picrocrocin fraction (PP).

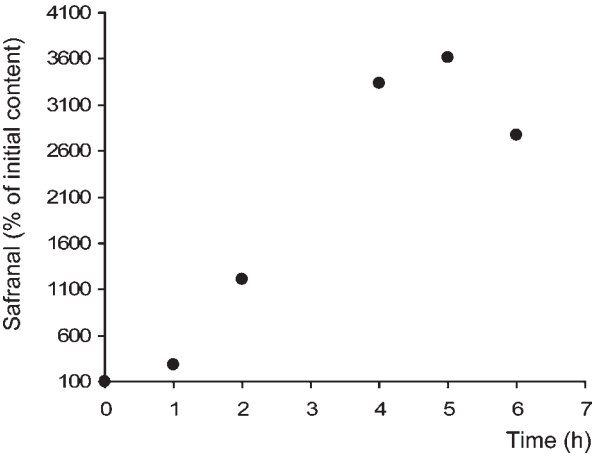

Figure 5. Evolution of safranal throughout the thermal treatment of purified picrocrocin (PP) at 100 °C, expressed as a percentage of its initial concentration.

Degradation Products: Safranal. According to past studies on picrocrocin (*12*−*15*), its loss could generate HTCC and safranal. In fact, slight increases of a peak with t_R = 11.2 min (r = 6.7 min), which could be HTCC based on its UV−vis spectrum and t_R in comparison to the bibliography data (*15*, *29*), were observed in the chromatograms at all temperatures studied. However, our main research focused on safranal, the major compound in the volatile fraction of saffron, when picrocrocin (PP) was submitted to a thermal treatment at about 100 °C and after SBSE and TD-GC-MS. The SBSE offers the advantages of low sample manipulation and great capacity of capturing volatiles at trace levels, and was assayed in saffron matrixes by Carmona et al. (*5*), who determined safranal, among other volatile compounds.

Figure 5 shows the results of safranal evolution throughout the thermal treatment, confirming the generation of this volatile compound in the first 5 h. At this moment, the yield of safranal

from purified picrocrocin was 7.4%. After this time, safranal decreased.

In conclusion, picrocrocin underwent slow degradation in saffron extracts at all temperatures studied. This degradation followed a second-order kinetics model and was affected by filtration. Filtration of the extracts contributed to picrocrocin stability. The evolution of k with temperature showed maximum k values at 35 °C. The kinetics of $E^{1\%}_{1cm}257$ nm and ΔE_{pic} did not depict the kinetics of picrocrocin degradation, hence demonstrating the need for a more selective measure of picrocrocin. In the case of purified picrocrocin in water submitted to 100 °C, high stability was also found and the generation of safranal in the first 5 h was confirmed.

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Supporting Information Available: Addtional tables and figure. This material is available free of charge via the Internet at http://pubs.acs.org.

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