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Picrocrocin Content and Quality Categories in Different (345) Worldwide Samples of Saffron (*Crocus sativus* L.)

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In this paper, 345 saffron samples were analyzed from different countries to study their picrocrocin content using different analytical techniques. The $E^{1\%}_{1\,cm}$ of 257 nm results from all samples are inflated in comparison by the high-performance liquid chromatography (HPLC) data, because of the interferences with the crocetin ester pool and especially with those with a lower trans/cis relation. A picrocrocin range update is proposed for International Organization for Standardization (ISO) 3632 normative because category III should be incremented up to 50 units, while category II should be incremented up to 60 units. More accurate data are achieved when the ΔE_{pic} measurement is carried out. Consequently, improvements to the ISO method are suggested. Fourier transform (FT)-near-infrared spectrometry analysis has also been carried out, showing excellent results from the calibration with HPLC data. This spectrophotometric technique could be used by saffron enterprises to obtain quick and more accurate data for picrocrocin determination.

KEYWORDS: Saffron; picrocrocin; crocetin esters; ISO quality standard

INTRODUCTION

Saffron, the dry stigmas of *Crocus sativus* L., is mainly used as a spice. It is valued for the color, taste, and aroma that it gives to foods and beverages (*1*). The compounds responsible for these properties are a group of carotenoids, crocetin esters, picrocrocin, and a wide group of ketones and terpenic aldehydes, with safranal as the most important compound.

Picrocrocin is supposed to be the most important substance responsible for the characteristic bitter taste of saffron (*2*). In addition to the taste, picrocrocin is also related to the aroma of saffron, because it is considered a safranal precursor generated during the dehydration of the spice (*3*).

In the international trade, saffron quality is mainly determined by specifications recommended by ISO/TS 3632-2:2003 (*4*). This specification classifies saffron into three categories, taking into account a large number of physical and chemical parameters. The most important are the presence of flower waste, moisture and volatile matter content, ash content, $E^{1\%}_{1\,cm}$ of 440 nm (coloring strength), $E^{1\%}_{1\,cm}$ of 330 nm (wavelength of safranal maximum absorbance), $E^{1\%}_{1\,cm}$ of 257 nm (wavelength of picrocrocin maximum absorbance), etc. While $E^{1\%}_{1\,cm}$ of 440 nm is directly correlated with the ability to transmit color to food and beverages, the $E^{1\%}_{1\,cm}$ of 257 nm and $E^{1\%}_{1\,cm}$ of 330 nm do not give an accurate measurement of picrocrocin and safranal, because of existing interferences. With regard to picrocrocin determination at 257 nm, the crocetin esters also absorb there (*5*−*8*). The direct consequence is that the categories established by ISO/TS 3632-2:2003 (*4*) related to $E^{1\%}_{1\,cm}$ of 257 nm (category I, minimum 70 units; category II, minimum 55 units; and category III, minimum 40 units) do not represent the real content of picrocrocin. To avoid these interferences, Corradi and Micheli (*9*) proposed the use of ΔE_{pic} parameter (*9*) for the determination of picrocrocin.

In addition, other techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography−diode array detection (HPLC−DAD) (*1*, *10*−*12*) and near-infrared (NIR) spectroscopy have also been used to quantify picrocrocin (*13*).

The aim of this work was to determine the accuracy of the picrocrocin determination using the parameter $E^{1\%}_{1\,cm}$ of 257 nm, thus determining the most important interferences in the measurement, second, to relate the real content determined by HPLC with quicker and cheaper estimations of picrocrocin carried out by ΔE_{pic} (UV−vis) and NIR spectroscopy, and finally, to define truthful levels of picrocrocin for the different categories established in the current ISO/TS 3632-2:2003 (*4*) to improve it based on the analysis of a wide number of saffron samples from different countries and submitted to different manufacturing procedures.

MATERIALS AND METHODS

Samples and Reagents. A total of 345 samples from different countries were analyzed: 50 from Italy, 52 from Spain, 107 from Greece,

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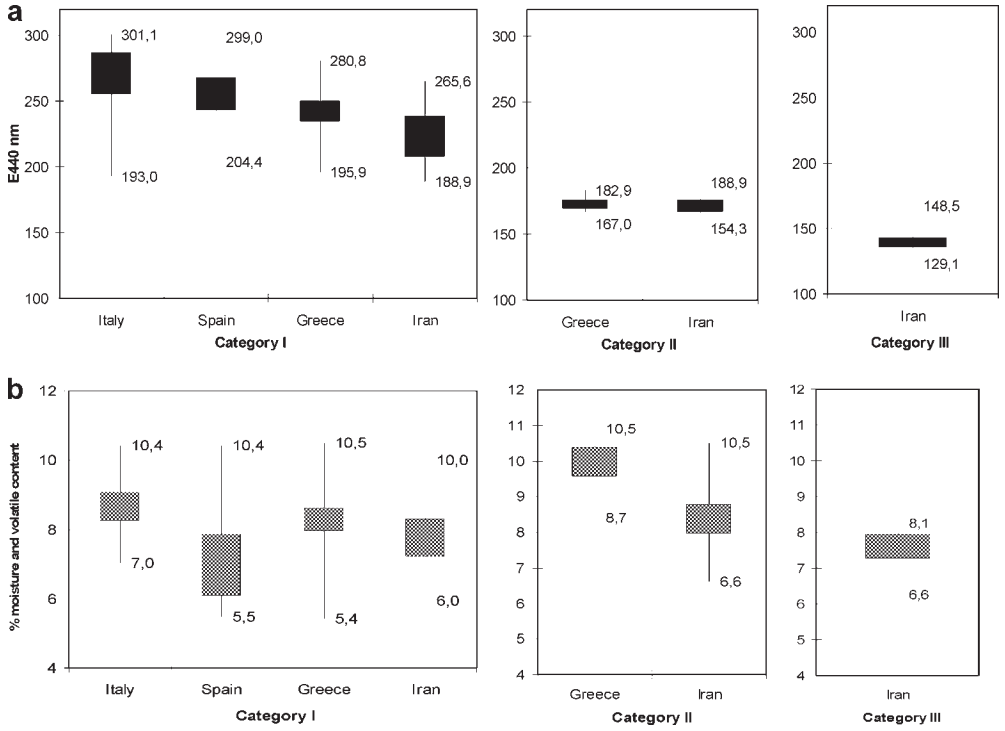

Figure 1. (a) Coloring strength distribution for the samples from different countries in the different ISO categories. (b) Moisture and volatile matter content distribution from the different countries in the different ISO categories.

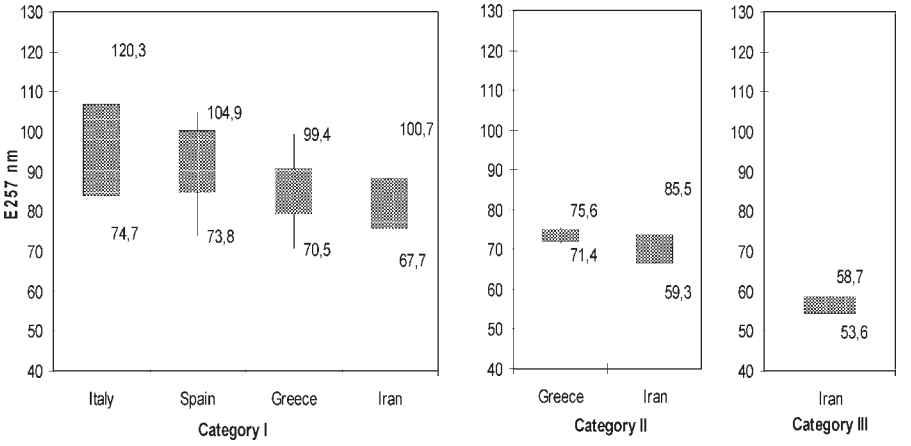

Figure 2. $E^{1\%}_{1\,cm}$ of 257 nm distribution of the samples from different countries in the ISO categories.

and 136 from Iran. All of the samples were harvested in years 2005 and 2006.

Saffron Working Solution. A total of 500 mg of ground saffron, previously passed through a sieve of 0.5 mm pore diameter, were placed in a 1 L volumetric flask, and 900 mL of distilled water were added. The solution was stirred by a magnetic bar (1000 rpm) for an hour while being kept away from light exposure. The flask was then filled to the 1 L mark, and the solution was homogenized through agitation.

Moisture and Volatile Matter Content. Determination of moisture and volatile matter content of saffron was carried out according to ISO/TS 3632-2:2003 (*4*).

UV−Vis Determinations. The spectrophotometric determinations were carried out following ISO/TS 3632-2:2003 (*4*). In addition, two different estimations of the picrocrocin content were carried out. (1) The first one was the $E^{1\%}_{1\,cm}$ of 257 nm parameter according to the formula: picrocrocin (mg/100 mg) = $E^{1\%}_{1\,cm}$ of 257 nm × M_w × 10/ε, where M_w is the molecular weight of the picrocrocin and ε is the molar absorption coefficient. (2) The second method employed used the ΔE_{pic} parameter as defined by Corradi and Micheli (*9*) according to ΔE_{pic} = $E^{1\%}_{1\,cm}$ of 257 − $E^{1\%}_{1\,cm}$ of 297.

Identification and Quantification of Crocetin Esters and Picrocrocin by HPLC. The nomenclature for the crocetin esters identified and their identification have been carried out according to Carmona et al. (*7*). The crocetin ester quantification was also estimated using the method based on the extinction coefficient and the related area calculated. Hence, the crocetin ester concentrations were calculated using the following expression: concentration (mg/100 mg) = (A × 100/A_t) × (M_w/ε) × $E^{1\%}_{1\,cm}$ of 440 nm/10, where the extinction coefficient (ε) was 89 000 and 63350 M^{-1} cm^{-1} (*1*) for *trans*- and *cis*-crocetin esters, respectively, A was the area of the crocetin ester peak in the chromatogram, A_t was the total area of the crocetin esters, $E^{1\%}_{1\,cm}$ of 440 nm was the coloring strength, and M_w was the molecular weight of the crocetin ester identified and quantified, with T-5tG, 1139 g mol^{-1}; T-5ng, 1139 g mol^{-1}; T-4GG, 977 g mol^{-1}; T-4ng, 977 g mol^{-1}; T-3Gg, 815 g mol^{-1}; T-2G, 653 g mol^{-1}; C-4GG, 977 g mol^{-1}; C-3Gg, 815 g mol^{-1}; T-2gg, 653 g mol^{-1}; T-1g, 491 g mol^{-1}; C-4ng, 977 g mol^{-1}; C-1g, 491 g mol^{-1}; and C-2G, 653 g mol^{-1}.

The identification of picrocrocin in the samples and its quantification were carried out using the standard isolated in a previous paper (*14*). Quantification was based on a six-point calibration curve of the picrocrocin standard (R^2 = 0.9998).

Table 1. Picrocrocin Content by HPLC Expressed in mg/100 mg and Its Estimation Using Different Spectrophotometric Techniques[a]

technique	HPLC (mg/100 mg)				A_{257} (mg/100 mg)			
coloring strength (ucs)	Italy	Spain	Greece	Iran	Italy	Spain	Greece	Iran
120−130				4.35 ± 0.18				11.21 ± 0.12
130−140				4.82 ± 0.61				10.35 ± 0.24
140−150				5.17 ± 0.24				10.78 ± 0.25
150−160				5.95 ± 1.28				12.25 ± 1.10
160−170			7.09 a ± 0.17	6.10 a ± 0.89			13.21 a ± 0.09	12.84 a ± 0.37
170−180			7.93 b ± 0.47	6.09 a ± 0.87			13.61 a ± 0.28	13.46 a ± 1.02
180−190			7.86 b ± 0.16	7.11 a ± 0.56			13.94 a ± 0.18	13.91 a ± 1.12
190−200	10.92 b ± 0.35		9.32 b ± 0.11	7.38 a ± 0.41	14.49 a ± 0.04		14.65 a ± 0.13	13.97 a ± 0.52
200−210	10.31 b ± 0.28	10.52 b ± 0.12	7.35 a ± 0.15	7.66 a ± 1.03	14.91 b ± 0.30	13.93 a ± 0.18	13.28 a ± 0.15	14.76 b ± 0.45
210−220			10.49 a ± 1.54	8.38 a ± 1.41			13.73 a ± 0.26	14.59 a ± 0.82
220−230	12.14 b ± 1.34		10.30 a,b ± 1.13	9.13 a ± 0.98	16.48 a ± 0.94		15.10 b ± 0.51	15.49 b ± 0.46
230−240	13.38 b ± 0.10	11.63 a,b ± 3.06	10.90 a,b ± 0.89	9.88 a ± 1.36	16.62 b ± 0.76	16.78 b ± 1.33	15.34 a ± 0.60	16.20 b ± 0.48
240−250		11.26 b ± 2.63	11.15 b ± 1.64	9.94 a ± 1.04		16.35 a ± 0.61	15.95 a ± 0.54	16.35 a ± 0.84
250−260	14.02 b ± 0.30	11.70 a,b ± 1.93	12.80 a,b ± 1.68	10.21 a ± 0.72	17.56 a,b ± 0.48	17.63 b ± 0.66	16.68 a ± 0.50	17.32 a,b ± 0.48
260−270	15.72 b ± 1.56	13.63 b ± 2.56	14.84 b ± 0.91	8.74 a ± 0.12	17.33 a ± 1.58	17.94 a ± 0.90	17.57 a ± 0.47	17.65 a ± 0.12
270−280	16.69 a ± 1.09	14.20 a ± 3.11			18.80 a ± 0.39	18.12 a ± 1.55		
280−290	17.13 a ± 1.30	15.68 a ± 2.59	14.75 a ± 0.10		19.56 a ± 0.90	18.75 a ± 0.57	18.61 a ± 0.12	
290−300	16.94 a ± 0.95	16.24 a ± 0.99			20.02 a ± 0.91	20.12 a ± 0.10		

technique	ΔE_{pic} (mg/100 mg)				FT-NIR (mg/100 mg)			
coloring strength (ucs)	Italy	Spain	Greece	Iran	Italy	Spain	Greece	Iran
120−130				5.83 ± 0.12				4.54 ± 0.12
130−140				5.91 ± 0.30				5.00 ± 1.28
140−150				6.36 ± 0.15				5.92 ± 0.79
150−160				6.96 ± 0.32				6.07 ± 0.12
160−170			7.52 a ± 0.10	7.39 a ± 0.27			7.42 b ± 0.14	5.46 a ± 1.72
170−180			7.95 a ± 0.28	7.67 a ± 0.84			6.92 b ± 0.40	5.39 a ± 1.15
180−190			8.07 a ± 0.18	8.65 a ± 1.28			8.41 b ± 0.10	5.94 a ± 0.98
190−200	9.14 a ± 0.50		9.02 a ± 0.13	8.76 a ± 0.47	10.21 b ± 0.66		6.37 a ± 0.10	6.48 a ± 1.60
200−210	9.92 b ± 0.18	9.80 b ± 0.18	8.74 a ± 0.15	9.21 b ± 0.23	10.56 b ± 0.40	10.06 b ± 0.12	9.06 a,b ± 0.11	6.73 a ± 1.26
210−220			9.50 a ± 0.64	9.78 a ± 0.40			11.45 a ± 2.15	7.11 b ± 2.02
220−230	11.22 ± 0.39		10.05 a ± 0.29	10.83 a ± 0.34	11.46 b ± 0.68		10.12 a,b ± 1.31	8.46 a ± 1.30
230−240	11.99 b ± 0.17	11.41 b ± 1.71	10.52 a ± 0.69	11.32 b ± 0.39	11.62 b ± 1.02	11.72 b ± 3.73	10.75 a,b ± 1.35	8.79 a ± 1.86
240−250		11.24 a ± 0.89	11.03 a ± 0.57	11.63 a ± 0.53		10.66 b ± 1.58	10.20 b ± 1.51	9.18 a ± 2.10
250−260	12.92 a ± 0.61	12.18 a ± 0.69	12.25 a ± 0.99	12.18 a ± 0.44	13.24 b ± 0.39	12.46 a,b ± 2.15	11.37 a,b ± 1.83	9.30 a ± 1.05
260−270	13.85 a ± 0.83	13.07 a ± 1.12	13.24 a ± 0.32	12.59 a ± 0.12	15.11 b ± 1.34	11.80 b ± 2.59	12.45 b ± 2.47	9.48 a ± 0.5
270−280	14.94 a ± 0.43	13.90 a ± 1.58			16.17 a ± 1.08	14.19 a ± 4.75		
280−290	15.30 a ± 0.8	14.63 a ± 1.10	14.35 a ± 0.12		16.30 b ± 1.29	13.21 b ± 3.34	12.07 a ± 0.12	
290−300	15.78 a ± 0.91	15.01 a ± 0.78			15.86 a ± 1.30	15.65 a ± 0.12		

[a] The same letters in the same row for each technique indicate non-significant differences ($p \leq 0.05$).

NIR Spectroscopy. Samples were analyzed by Perkin-Elmer Spectrum One Fourier transform (FT)-NIR equipment coupled with a near-infrared reflectance accessory (NIRA). Data processing was acquired over a wavelength range of 10 000−4000 cm^{-1}, and the resolution was set at 16 cm^{-1}. All samples were scanned in duplicate.

NIR calibration for picrocrocin was studied in relation to the HPLC results. Equations for NIR prediction were developed by Spectrum Quant+ software (Perkin-Elmer) with the principal component analysis option and two passes to eliminate outliers. The spectra were pre-processed using the standard normal variety (SNV) transformation followed by first-derivative transformation to reduced baseline variation and enhance the spectral features (*15*). Calibrations were developed using partial least-squares regression (PLS) and included the standard error of calibration (SEC), the standard error of prediction (SEP), and the coefficient of determination in calibration (R^2cal). To evaluate how well the calibration model could predict picrocrocin content of saffron, the full cross-validation included the coefficient of determination in validation (R^2).

Statistical Analysis. Statistical analysis was performed using SPSS 15.0 statistical software (SPSS, Inc., Chicago, IL). The analyses carried out in the results of picrocrocin and crocetin esters were analysis of variation (ANOVA) and Duncan test to be able to classify the samples.

RESULTS AND DISCUSSION

Selection of Samples. The choice of saffron samples, number and the country origin of samples, is selected according to the availability of samples in the international saffron market. All samples have an origin certificate.

The higher number of samples are from Iran, which is the most important saffron-producing country (>90%). Their samples have been supplied by different international saffron trade companies, which ensure that samples were obtained directly from different saffron producers and also from different harvesting years.

Although all Greek samples came from Krokos cooperative, they are obtained from different farmers. About 50% of the Spanish samples came from the D.O. Azafrán de La Mancha, and the rest were supplied by the different Spanish saffron producers located mainly in the Castilla-La Mancha region. Italian samples came from various Sardinian producers. Although Indian saffron is also highly appreciated, it is not exported; thus, no samples were obtained.

Coloring Strength, Moisture, and Volatile Content. Coloring strength is the parameter employed by saffron trade companies to determine its price, established on basis of the current three quality categories: category I, higher than 190 units of coloring strength (ucs); category II, from 150 to 190 ucs; and category III, between 100 and 150 ucs (*7*). From the analyzed samples, 84.35% belonged to category I according to ISO/TS 3632-2:2003 (*4*),

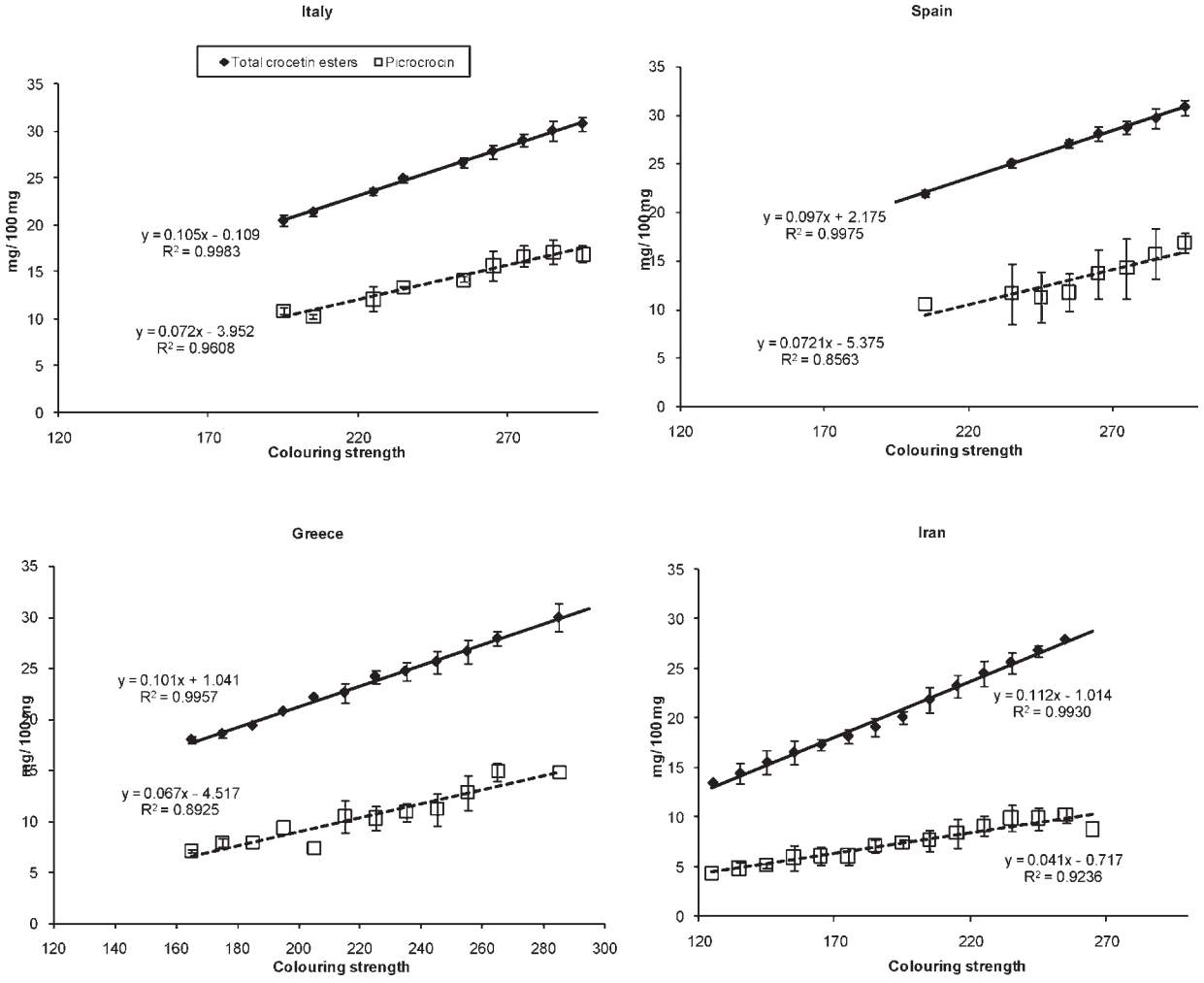

Figure 3. Relation of the total crocetin ester amount and picrocrocin concentration to the coloring strength in the samples from different countries.

with their distribution being 50 Italian, 52 Spanish, 99 Greek, and 90 Iranian samples. Within this range, sample distribution was mainly allocated in the upper echelon (**Figure 1a**). Saffron samples from Spain presented a coloring strength more uniform around the average value (253.5 ucs), with a maximum limit (299.0 ucs) close to the Italian one. Greek samples presented similar distribution, with an average value of 241.8 ucs and with 75% of the samples within a 16 ucs range (234.1−250.1 ucs). Finally, Iranian samples presented the narrowest range of coloring strength (77 ucs), and their distribution was in the lower echelon (**Figure 1a**).

Second category samples were composed of 46 samples: 8 samples from Greece and 38 samples from Iran. Category III was only configured by 8 samples from Iran. It is noteworthy that Greek samples at categories I and II can be considered the most homogeneous because they show the lowest variability. With regard to moisture and volatile matter content, all collected samples presented values lower than 12%, with the maximum limits established by ISO/TS 3632-2:2003 (*4*) for *Crocus sativus* L. to be considered as saffron spice (**Figure 1b**). The range for category I can be considered similar (between 5.4 and 10.5%) for the different countries (**Figure 1b**), except for Italian samples (minimum of 7.0%). There was no relationship between ISO quality categories and moisture and volatile matter content, because the range for category II (6.6−10.5%) was similar to the values of category I and the category III measurements were lower (6.6−8.1%) (**Figure 1b**).

Picrocrocin Determination by ISO/TS 3632-2:2003. Currently, the only standardized method to determine picrocrocin content is that proposed by ISO/TS 3632-2:2003 (*4*). This standard does not establish a concentration of picrocrocin for each defined ISO quality category; it simply establishes a minimum value of molar absortivity for the saffron solutions: 40 units for category III, 55 units for category II, and 70 units for category I, from the results obtained using UV−vis spectrophotometry.

The 345 saffron samples were analyzed according to the standard method, and results are shown in **Figure 2**. Again, Italian samples presented the widest variability (45.6 units), although the distribution was more uniform compared to the $E^{1\%}_{1\ cm}$ of 440 nm data shown in **Figure 1a**. All samples from category I, except three Iranian samples, presented a $E^{1\%}_{1\ cm}$ of 257 nm mean value over the minimum level established for this category. The reduced number of outliers related to the analyzed samples (291) suggested that 70 units is an adequate value as the minimum limit for this parameter (**Figure 2**). On the contrary, samples from category II presented a value over 60 units for this parameter, except for one Iranian sample (59.3 units) (**Figure 2**). Thus, the limit could be increased from the current 55 units to reflect the reality of the international market. Category III samples did not fall below 53 units (**Figure 2**), quite far from the 40 units established in ISO/TS 3632-2:2003 (*4*); thus, for category II, this could be increased on the normative.

When the data of $E^{1\%}_{1\ cm}$ of 257 nm are converted into milligrams of picrocrocin/100 mg of saffron (A_{257}) (**Table 1**) and compared to

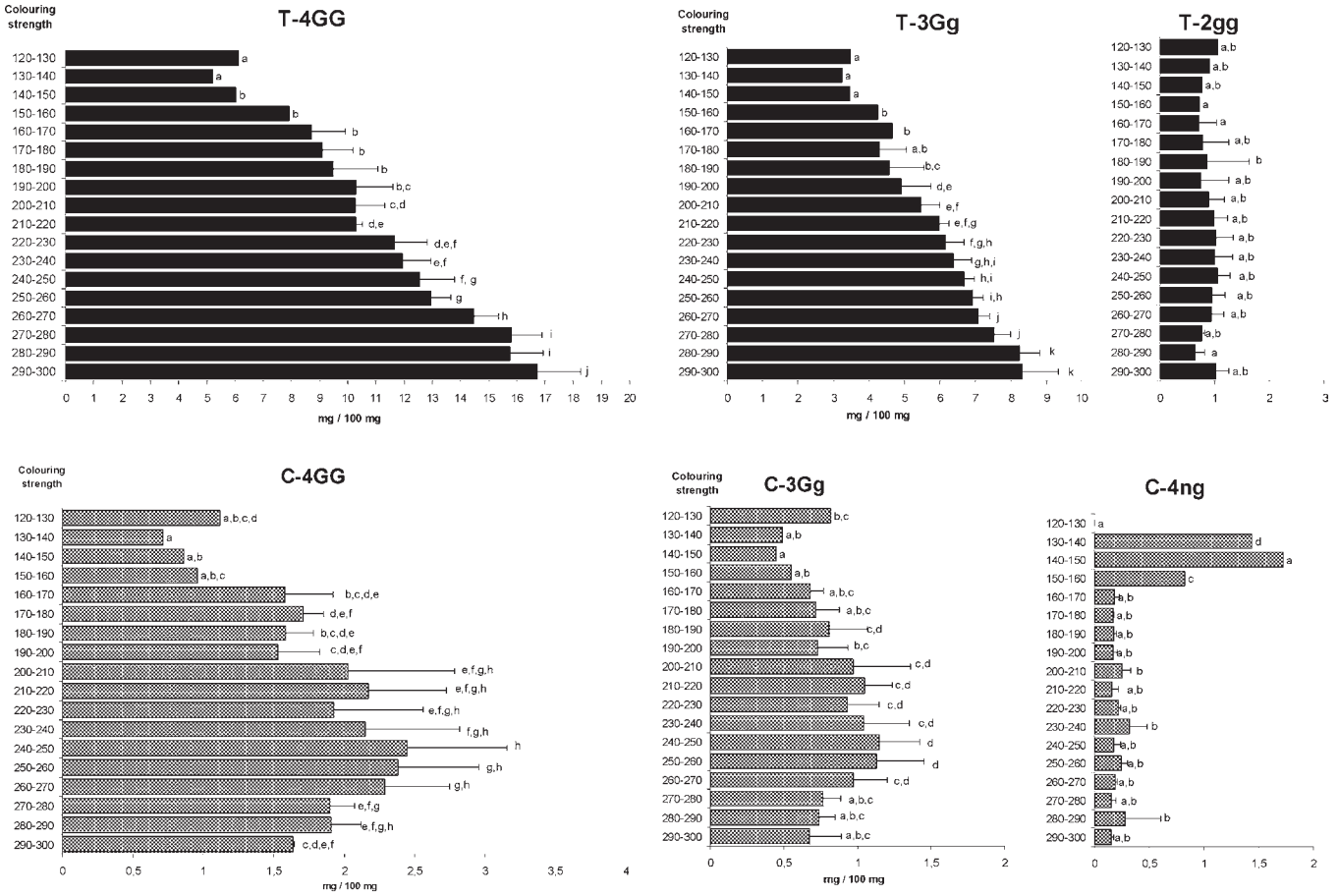

Figure 4. Evolution of the concentration of the six main crocetin esters according to the coloring strength. In each compound, the same letter in the columns indicates non-significant differences among the samples ($p \leq 0.05$).

the picrocrocin quantification by HPLC, an overestimation of the picrocrocin content calculated from the absorbance at 257 nm through the $E_{1\,\mathrm{cm}}^{1\%}$ of 257 nm parameter is observed. According to other authors (*5*), this overestimation could be justified by the interferences of crocetin esters.

However, the overestimation did not show a good relationship with the HPLC data. For UV−vis determination, statistical differences within the same coloring strength section at a level of 0.05 only appeared for different countries, but it does not coincide with the same data obtained with A_{257} (**Table 1**).

Study of the Relationship between Crocetin Esters and Picrocrocin. The crocetin ester quantification was carried out to be able to conclude with the potential interference of them in the determination of picrocrocin content in each country (**Figure 3**). With regard to crocetin esters, similar performances were found for the four countries, with good linear behavior with slopes around 0.1, confirming that coloring strength is a good reference parameter directly related to chemical composition.

Picrocrocin also followed a linear relation, although not so precise, with a similar increase rate for Italy, Spain, and Greece with a slope around 0.07. Iranian samples again showed a different relation because they maintained a linear progression, although the rate was almost half the value of the other countries. Looking for a relation between the total crocetin ester content and picrocrocin for the different countries, these presented a good linear relation, being higher than 0.86 in all of the countries. When the coloring strength increased from 160 to 270 ucs, the overestimation decreased, approximately from 6 to 3 units, especially Greek samples (UV−vis from 13.21 to 17.57 and HPLC from 7.09 to 14.84 mg/100 mg) (**Table 1**). Picrocrocin can therefore play an important role in the saffron quality control, the importance of which has not been considered up until now. Great numbers of adulterated saffron batches have appeared with gardenia fruit powder because it contains the same crocetin esters but no picrocrocin. Consequently, picrocrocin can be the biomarker key to avoid fraud. The use of the different dehydration procedures may modify picrocrocin content (*16*). Finally, the picrocrocin increment is lower than the crocetin ester increment; therefore, their potential interference would also increase with the coloring strength. However, this hypothesis was the opposite of what we found.

From the identification and quantification of each crocetin ester, we were looking for further information about their interference. **Figure 4** shows the behavior of these six main crocetin esters (T-4GG, T-3Gg, T-2gg, C-4GG, C-3Gg, and C-4ng). The sum of the amount of the six compounds represents more than 92% of the total crocetin ester content.

T-4GG and T-3Gg content increased throughout the entire range of coloring strength. However, T-4GG showed statistical differences within categories II and III established by ISO/TS 3632-2:2003 (*4*), i.e., 120−150 and 160−190 ucs at a 0.05 level. Because T-4GG is the major compound with around 48% of the total crocetin ester content, it also has a high contribution to the saffron coloring strength; therefore, the categories established by ISO/TS 3632-2:2003 (*4*) are partially justified.

T-2gg content only presented light variations. Two groups showed significance differences, but the samples in the level 150−160 and 280−290 ucs presented the lowest values. Meanwhile, samples belonging to the 180−190 ucs level have the highest value of this crocetin ester. However, the rest of the

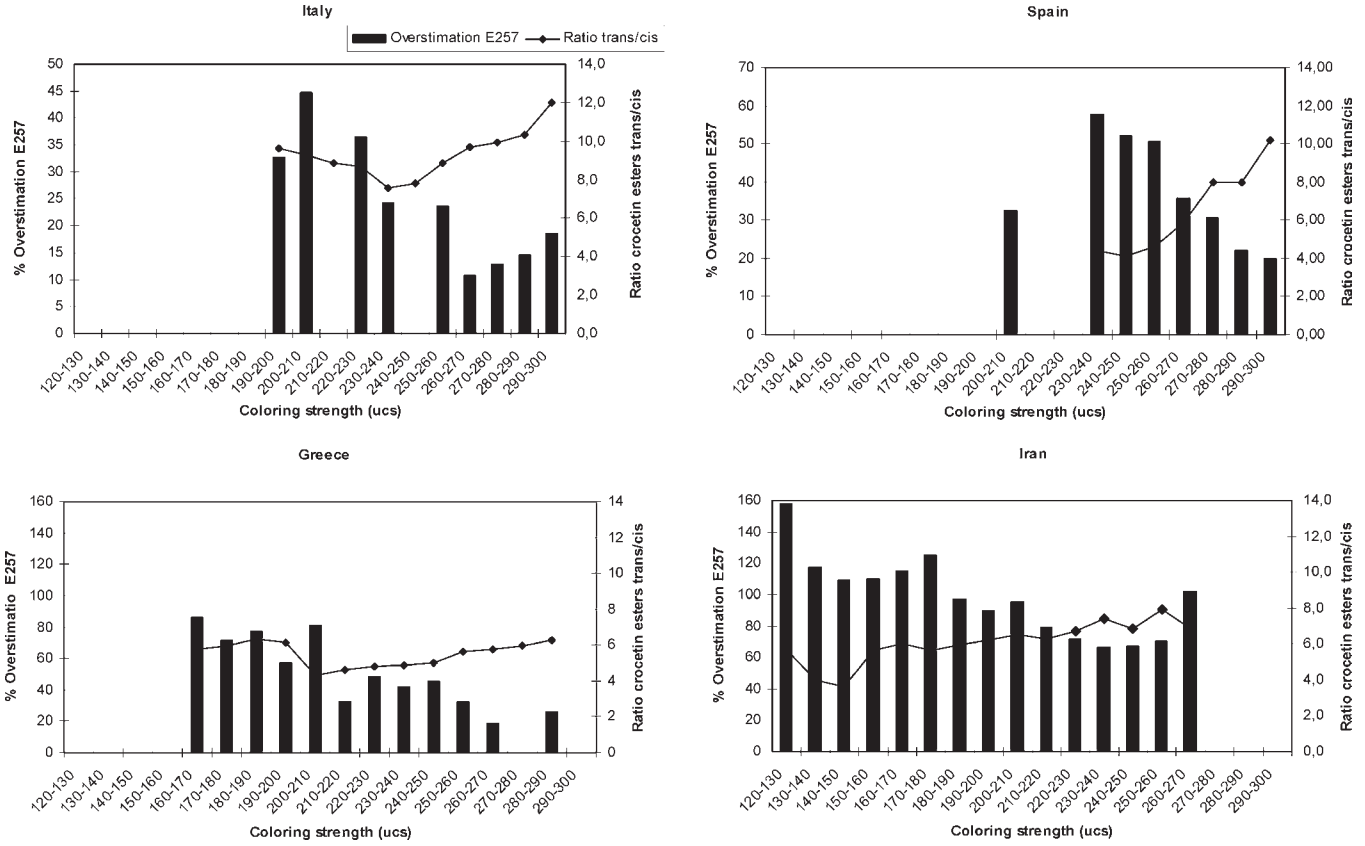

Figure 5. Overestimation of the $E^{1\%}_{1\,cm}$ of 257 nm refers to the picrocrocin amount obtained by HPLC in each range of coloring strength in samples from different countries. Evolution of the ratio *cis*/*trans*-crocetin esters with the coloring strength from the different countries.

Table 2. Descriptive Statistics of the Picrocrocin Calibration Content in Each Country (mg/L) Using NIR Spectroscopy

	mean value	SD[a]	maximum	minimum	CV (%)[b]	SEC[c]	SEP[d]	intercept	slope	R^2
Italy (N = 50)	14.77	2.41	18	9.72	16.32	0.84	0.91	0.38	0.90	0.90
Spain (N = 52)	11.90	3.10	18.30	4.13	26.05	1.08	1.16	1.48	0.97	0.97
Greece (N = 107)	10.52	1.90	14.79	5.89	18.09	1.01	1.03	2.20	0.79	0.79
Iran (N = 136)	7.22	1.97	14.67	2.61	27.29	1.14	1.18	1.60	0.78	0.77

[a] SD = standard deviation. [b] CV = (SD/mean) × 100. [c] SEC = standard error of calibration. [d] SEP = standard error of prediction.

samples had intermediate values in both groups with no significant differences.

C-4GG content increased up to the 240−250 level and then decreased slowly, although this increment was not significant until the last section 290−300 ucs. C-3Gg content showed similar behavior, because it increased to a maximum between 230 and 260 ucs before it decreased. On the contrary, C-4ng content presented the highest contents with low coloring strength, but its content decreased in the following levels, presenting a similar concentration.

This different evolution could justify the higher overestimation of the picrocrocin using $E^{1\%}_{1\,cm}$ of 257 nm in samples with a higher content of *cis*-crocetin esters. To check this hypothesis, the degree of overestimation for each country related to the existing ratio *trans*/*cis*-crocetin esters was studied (**Figure 5**). The overestimation ranges were from 10.24 to 44.64% in Italian samples, from 19.58 to 50.65% in Spanish samples, from 18.37 to 86.23% in Greek samples, and finally, from 63.81 to 157.80% in samples from Iran. The results for Iranian samples were surprisingly higher in relation to the rest of the countries.

Independent of variables such as edaphic or climate conditions, dehydration or storage procedures may have effects on the picrocrocin content and crocetin ester. There is a similar evolution of the prediction capability of $E^{1\%}_{1\,cm}$ of 257 nm depending upon the trans/cis ratio, because it is reduced as the coloring strength and the trans/cis ratio increase. The highest values of the trans/cis ratio were found in samples from Italy (12.0%), where the overestimation was lower (19%). Spain showed higher overestimation of $E^{1\%}_{1\,cm}$ of 257 nm (58%) with a lower relation of the trans/cis value (4.4%). Finally, Greek samples presented higher values of overestimation, especially in intermediate ranges of coloring strength and lower trans/cis ratios. In the case of Iran, a reduction in the overestimation was also observed when the coloring strength increased, despite the fact that additional interferences from other compounds could influence it.

Picrocrocin Determination Using Other Spectrophotometric Methodologies. Other spectrophotometric techniques used the parameter ΔE_{pic} (*9*). The results obtained using ΔE_{pic} were more accurate than those obtained with $E^{1\%}_{1\,cm}$ of 257 nm (**Table 1**) and can be proposed to ISO.

FT-NIR is another technique that is apt for use in the enterprises because results are obtained easily and quickly and can successfully be applied to saffron (*13*). The good performance of NIR parameters can also be seen in **Table 2**, where the PLS calibration statistics are shown. SEC and SEP values showed that

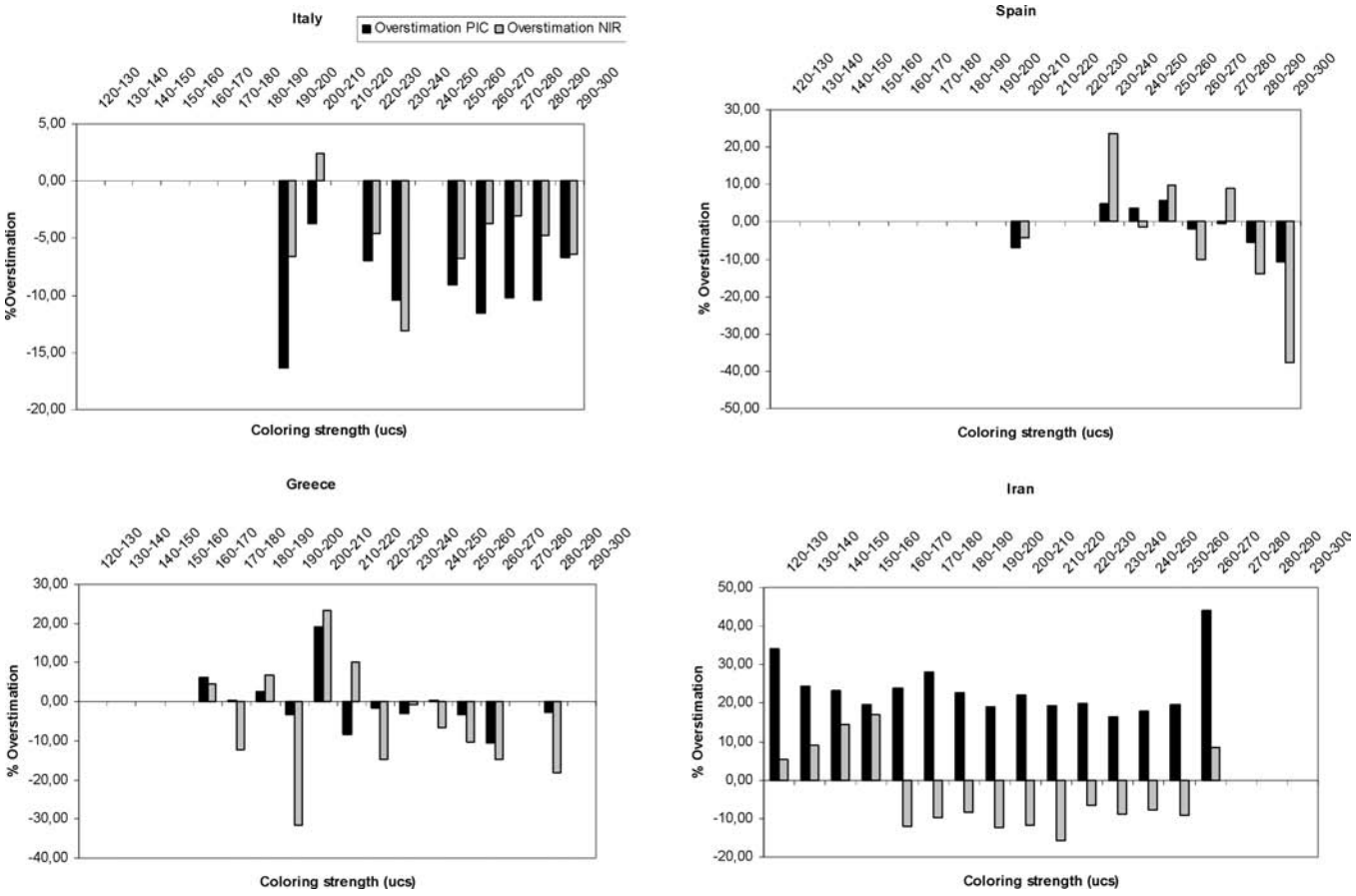

Figure 6. Comparison of the picrocrocin concentration over/underestimation obtained with ΔE_{pic} and overestimation using FT-NIR in each range of coloring strength for the different countries.

Table 3. Categories According to the Current ISO Standard and the Proposed Limits for Picrocrocin Content of Saffron by HPLC and Its Estimation by UV−Vis Parameters

	categories according to the current ISO 3632 standard		proposed limits for the UV−vis technique		
ranges of coloring strength	coloring strength $E^{1\%}_{1\ cm}$ of 440 nm	picrocrocin	limits for $E^{1\%}_{1\ cm}$ of 257 nm parameter	limits for picrocrocin estimation using ΔE_{pic} parameter	proposed limits for real picrocrocin content by HPLC
100−110					
110−120					
120−130	category III	category III	category III	≥4.2 mg/100 mg of saffron	≥2 mg/100 mg of saffron
130−140	100−150 units	≥40 units	≥50 units		
140−150					
150−160					
160−170	category II	category II	category II	≥6 mg/100 mg of saffron	≥4.3 mg/100 mg of saffron
170−180	150−190 units	≥55 units	≥60 units		
180−190					
190−200					
200−210					
210−220					
220−230					
230−240					
240−250	category I	category I	category I	≥8.5 mg/100 mg of saffron	≥7.5 mg/100 mg of saffron
250−260	190−300 units	≥70 units	≥70 units		
260−270					
270−280					
280−290					
290−300					

NIR spectroscopy provides a suitable prediction of the picrocrocin content in each country, indicating that the PLS models based on NIR spectra explain more than 77% of the variation in the data. The calibration curves of the picrocrocin content obtained between NIR results and HPLC for each country were reported in **Table 2**.

If the results obtained by ΔE_{pic} are compared to the results obtained by FT-NIR already calibrated with HPLC data, the samples generally presented higher values of picrocrocin content using FT-NIR than the results obtained using ΔE_{pic} for all coloring strength ranges. In **Figure 6**, it is shown that the underestimation was higher in Italian samples using the first technique.

The results obtained in samples with lower coloring strength presented higher underestimation than the results obtained from the samples with higher coloring strength using the parameter ΔE_{pic} and the results from FT-NIR. Unexpected outcomes were obtained in Iranian samples, because they presented an overestimation using ΔE_{pic} higher than the underestimation obtained with FT-NIR. In conclusion, the FT-NIR achieved more accurate results than those obtained using ΔE_{pic} in Iranian samples. Because the Iranian samples represent 90% of the total commercial saffron samples, it could be said that FT-NIR spectroscopy could be a good technique to determine picrocrocin content in saffron.

Determination of New Quality Categories. The results shown in this paper indicate that the ISO standard should be updated to adapt more accurately to the current categories in relation to picrocrocin content. During the latest update of ISO 3632 (year 2003), it was impossible to reach an agreement between all countries involved, mainly because of the absence of robust scientific data. Attending to the results obtained in this paper, the proposal is shown in **Table 3**. In relation to the actual measurements ($E_1^{1\%}{}_{cm}$ of 257 nm), a minimum level (50) for category III should be proposed. Although our samples did not present a coloring strength lower than 120 units, an increase minimum level for category II up to 60 should be proposed, while no changes in value are needed for category I (**Table 3**).

If picrocrocin could be expressed as ΔE_{pic} (expressed in mg/100 mg), the quality categories should be as reported in **Table 3**.

Finally, if HPLC determination could be included in the ISO normative, because its benefits as a precise analytical technique are already known, then the limits proposed are around 1 mg/100 mg of saffron for category I and almost 2 mg/100 mg of saffron for the remaining categories (**Table 3**), as a result of the overestimation of the picrocrocin content using the spectrophotometric technique in samples with a high content of *cis*-crocetin esters.

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LITERATURE CITED

(1) Alonso, G. L; Salinas, M. R.; Garijo, J.; Sánchez-Fernández, M. A. Composition of crocetin esters and picrocrocin from Spanish saffron (*Crocus sativus* L.). *J. Food Qual.* **2001**, *24*, 219–233.

(2) Corradi, C.; Micheli, G. Determinazione spettrofotometrica del potere colorante, amaricante ed odoroso dello zafferano. *Boll. Chim. Farm.* **1979**, *118*, 553–562.

(3) Himeno, H.; Sano, K. Synthesis of crocin, picrocrocin and safranal by saffron stigma-like structures proliferated in vitro. *Agric. Biol. Chem.* **1987**, *51*, 2395–2400.

(4) International Organization for Standarization (ISO). ISO 3632-1/2. Technical Specification. Saffron (*Crocus sativus* L.). Part 1 (Specification) and Part 2 (Test Methods). ISO, Geneva, Switzerland, **2003**.

(5) Tarantilis, P. A.; Polissiou, M. G.; Manfait, M. Separation of picrocrocin, *cis*−*trans*-crocins and safranal of saffron using high-performance liquid chromatography with photodiode-array detection. *J. Chromatogr., A* **1994**, *664*, 55–61.

(6) 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.

(7) Carmona, M.; Zalacain, A.; Sánchez, A. M.; Novella, J. L.; Alonso, G. L. Crocetin esters, picrocrocin and its related compounds present in *Crocus sativus* stigmas and *Gardenia jasminoides* fruits. Tentative identification of seven new compounds by LC−ESI−MS. *J. Agric. Food Chem.* **2006**, *54*, 973–979.

(8) Orfanou, O.; Tsimidou, M. Evaluation of the colouring strength of saffron spice by UV−vis spectrometry. *Food Chem.* **1996**, *51* (3), 463–469.

(9) Corradi, C.; Micheli, G. Carateristiche generali dello zafferano. *Boll. Chim. Farm.* **1979**, *118*, 537–552.

(10) Lozano, P.; Castellar, M. R.; Simancas, M. J.; Iborra, J. L. Quantitative high-performance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L.) products. *J. Chromatogr., A* **1999**, *830*, 477–483.

(11) Sujata, V.; Ravinshankar, G. A.; Venkataraman, L. V. Methods for the analysis of the saffron metabolites crocin, crocetins, picrocrocin and safranal for the determination of the quality of the spice using thin-layer chromatography, high-performance liquid chomatography and gas chromatography. *J. Chromatogr., A* **1992**, *624*, 459–462.

(12) Tarantilis, P. A.; Tsoupras, G.; Polissiou, M. G. Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography−UV−visible photodiode-array detection−mass spectrometry. *J. Chromatogr., A* **1995**, *699*, 107–118.

(13) Zalacain, A.; Ordoudi, S. A.; Díaz-Plaza, E. M.; Carmona, M.; Blazquez, I.; Tsimidou, M. Z.; Alonso, G. L. Near-infrared spectroscopy in saffron quality control: Determination of chemical composition and geographical origin. *J. Agric. Food Chem.* **2005**, *53*, 9337–9341.

(14) Sánchez, A. M.; Carmona, M.; Zalacain, A.; Carot, J. M.; Jabaloges, J. M.; Alonso, G. L. Rapid determination of crocetin esters and picrocrocin from saffron spice (*Crocus sativus* L.) using UV−visible spectrophotometry for quality control. *J. Agric. Food Chem.* **2008**, *56*, 3167–3175.

(15) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.* **1989**, *43*, 772–777.

(16) Carmona, M.; Zalacain, A.; Pardo, J. E.; Alvarruiz, A; Alonso, G. L. Influence of different drying and aging conditions on saffron constituents. *J. Agric. Food. Chem.* **2005**, *53*, 3974–3979.

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