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Influence of Different Drying and Aging Conditions on Saffron Constituents

MANUEL CARMONA, AMAYA ZALACAIN, JOSÉ EMILIO PARDO, EULOGIO LÓPEZ, ANDRÉS ALVARRUIZ, AND GONZALO LUIS ALONSO*

ETSI Agrónomos, Universidad Castilla−La Mancha, 02071 Albacete, Spain

A dehydration postharvesting treatment is necessary to convert *Crocus sativus* L. stigmas into saffron spice. Three different dehydration treatments were evaluated: dehydration at room temperature; dehydration with hot air at different temperatures (70, 90, and 110 °C); and dehydration following traditional processing in Castille−La Mancha (Spain) with three different heating sources (vineshoot charcoal, gas cooker, and electric coil). The time (between 28 and 55 min) and mean temperature (between 54 and 83 °C) conditions for traditional dehydration were established for the first time. The highest coloring strength was obtained when saffron was submitted to higher temperatures and lower times. These findings may be supported by the fact that samples dehydrated at high temperature were more porous than those dehydrated at room temperature, as was observed by scanning electron microscopy (SEM) and differential scanning calorimetry (DSC). The higher the temperature during the process, the higher the proportion of *trans*-crocetin di-(β-D-gentibiosyl) ester, although *trans*-crocetin (β-D-glucosyl)-(β-D-gentibiosyl) and *trans*-crocetin di-(β-D-glucosyl) ester decrease while *cis*-crocins did not change significantly. A thermal aging process reveals that the *trans*-crocetin di-(β-D-gentibiosyl) ester increases when saffron is resubmitted to a heating treatment before it is decomposed by the extreme conditions. The picrocrocin extinction during the aging process does not imply a consistent generation of safranal.

KEYWORDS: Dehydration temperature; saffron; crocins; safranal; picrocrocin

INTRODUCTION

A dehydration postharvesting treatment is necessary to convert *Crocus sativus* L. stigmas into saffron spice. During the dehydration process, the stigmas lose ∼80% of their weight. Drying brings about the physical, biochemical, and chemical changes necessary to achieve the desired attributes of saffron. This process also plays an important role in preserving the spice. A lower moisture content, at least below the 12% value established by the International Standard ISO 3632 (*1*), maintains the quality of the product for a longer time (*2*).

The drying process differs from country to country as a result of the experience gained through trial and error and the resources that are available (*3*, *4*). There are two ways to dehydrate saffron in terms of temperature. One process is carried out at room temperature directly under sunlight or in air-ventilated conditions, as is done in India, Morocco, and Iran. In India, the stigmas are solar-dried for 3−5 days until their moisture content is reduced to 8−10% (*5*, *6*). In Morocco, the stigmas are spread on a cloth in a very thin layer and dried under the sun for several hours or in the shade for 7−10 days (*7*).

The second drying process is carried out at higher temperatures by using hot air or any other heating source. This is the process used in Spain, Greece, and Italy. In Italy, drying is carried out by spreading the stigmas in a sieve placed ∼20 cm above live oak-wood charcoal. Halfway through dehydration, the stigmas are turned to ensure uniform drying. The process is considered to be finished when saffron stigmas do not crumble and still possess a certain amount of elasticity when pressed between the fingers. The product retains between 5 and 20% of moisture (*8*).

In Greece, many efforts have been made to homogenize the practices of Greek growers in Kozani, where fresh stigmas and part of the stamens are spread on shallow layer trays (4−5 mm) of 40 × 50 cm with a silk cloth bottom. These trays are piled on frames with shelves 50 cm apart. During the first hours of the process, the room temperature is maintained at 20 °C and then increased to 35−45 °C; relative humidity should not exceed 50%. The dehydration process is finished when a moisture content of 10−11% is achieved, generally after 12 h (*4*, *9*).

The literature that explains how saffron is dehydrated in Spain, by a process called "toasting", is both scarce and inexplicit. According to Pérez (*10*), stigmas are placed on a sieve with a silk bottom that is placed over the heating source, which can be a gas cooker, live vineshoot charcoal, or, to a lesser extent, an electric coil (*11*). The process is finished when the sample has lost between 85 and 95% of its moisture, after being gently dried at 35 °C for 30 min.

* Author to whom correspondence should be addressed (fax +34 967 599238l; e-mail Gonzalo.Alonso@uclm.es).

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Dehydration treatment is accepted as being responsible for saffron organoleptic characteristics (*12*). The three attributes that saffron gives to foodstuffs are color, aroma, and taste. The compounds responsible for such attributes are crocins, a group of glycoside derivates from the carotenoid crocetin: a terpenic aldehyde known as safranal and a glycoside terpenoid, picrocrocin, respectively. Picrocrocin corresponds to 4-(β-D-glucopyranosyl)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, and by submitting this substance to hydrolysis and dehydration, it is possible to obtain safranal (*13*−*16*), which corresponds to 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (*17*−*19*).

From a chemical point of view, differences between the samples dehydrated at low and high temperatures, under sunlight or in shade, can be explained as photochemical degradation, or isomerization of the carotenoids responsible for saffron color may take place (*3*, *20*). It is more difficult to find differences between dehydration processes that are carried out at high temperatures (35−45 °C), for example, between Spain and Greece methods, as mentioned in the bibliography. Nevertheless, saffron traders can distinguish both origins by their aroma.

The aim of this paper is to characterize the time−temperature profile during the traditional dehydration process in Castille−La Mancha (Spain) in comparison with other dehydration processes. The effect of such processes is evaluated in terms of its quality determined by the Normative ISO 3632 (*1*) and the contents of the crocins picrocrocin and safranal. Variations in saffron chemical composition were studied by submitting the saffron to a thermal accelerated aging process.

MATERIALS AND METHODS

Plant Material. Harvesting and removal of the stigma from saffron flowers (*C. sativus* L.) were carried out in Motilla del Palancar (Cuenca, Spain) and in Eras and Tiriez (Albacete, Spain) according to the traditional procedures following the trade standard of the PDO "Azafrán de La Mancha" (*21*, *22*).

Saffron Dehydration Process. *Traditional Process in Castille−La Mancha.* Expert saffron growers dehydrated saffron stigmas by spreading them on a sieve, which was then placed over different heating sources. A special sieve was manufactured to record the temperature reached while the dehydration process took place. For this proposal, 11 thermocouples were placed on a traditional sieve with a silk-cloth bottom and 40 cm diameter. Taking into account its circular geometry, 8 thermocouples were placed along the sieve radius (T1−T8) and three more (T9−T11) at the same distance as sensor T5 but situated 120° apart. Another thermocouple (T12) was submerged in ice water (0 °C) and was used as a reference point. The thermocouple sensors were of "K" type, 1 m long and 1 mm in diameter. Such sensors were chosen for their lower thermal inertia and also because they are easily sown to the silk cloth with a copper wire. The data were collected in a programmed Datalogger CR10X (Campbell Scientifics) as millivolts every 360 ms. Such data were converted into centigrade degrees with a third-order equation, where the estimated maximum error was 0.35 °C in the range of 18−150 °C, resulting in a new report every 10 s. With the data generated, several graphs were created using the Excel program for Windows 2000.

To determine the time and temperature profile of the different heating sources, the assay procedure with the sieve designed was as follows:

(A) The sieve was placed over vineshoot charcoal at 20 cm height.

(B) The sieve was placed 10 cm above a metallic plate that was situated 3 cm over a gas cooker.

(C) The sieve was placed on a shelf that protected the resistance coil, at a height of 10 cm.

At Room Temperature. Saffron stigmas were dehydrated at room temperature for 7 days under dark conditions.

Hot Air Dehydration. Saffron stigmas were dehydrated at a pilot plant for food dehydration. Approximately 4.5 g of stigmas was placed on a cylindrical sample holder (9.2 cm diameter) with a perforated bottom while air at different temperatures (70, 90, and 110 °C) was passed through at 6 m s^{-1}.

Accelerated Saffron Aging Process. To simulate an accelerated aging process, a homogenized dehydrated saffron sample was placed in five Petri plates inside an oven at 50 °C. After 2 h, one sample was removed and the oven temperature was increased to 70 °C. Every 2 h, the process was repeated and the temperature was increased 20 °C from the latest step to 130 °C. Data reported represent the average of three sample replicates.

Analytical Determinations. *Coloring, Aromatic Strength, and Bitterness.* Saffron samples were analyzed following the ISO 3632 (*1*) trade standard. Measurements of $E^{1\%}$ of an aqueous saffron extract at 440, 330, and 257 nm, respectively, were carried out with a 1 cm pathway cell. Moisture was also determined according to ISO 3632 (*1*). The reported values are the average values of three replicates for each treatment sample. Significant differences in the measured properties due to the dehydration process were determined by analysis of variance (ANOVA) using the SPSS 10.0.6 for Windows (SPSS Inc.) statistical program.

Differential Scanning Calorimetry (DSC). Saffron samples were powdered and passed through a 500 μm mesh sieve. The sample was uniformly placed in aluminum pans in a DSC 6200 SII model (Seiko Instruments Inc.). Samples were heated at a rate of 10 °C/min within the range between 20 and 200 °C under synthetic air atmosphere (100 mL/min). Data collection was taken every 2 s, and interpretation of results was carried out with Station Exstar 6000 software (Seiko Instruments Inc.). Data reported represent the average of three replicates. Significant differences in the measured thermal properties due to the dehydration process were determined by ANOVA using the SPSS 10.0.6 for Windows (SPSS Inc.) statistical program.

Scanning Electron Microscopy (SEM). Plant tissue was coated with 20 nm of gold in a sputter coater model SCD 004 (Bal-Tec, Balzers, Lichtenstein). Micrographs of samples were taken in a scanning electron microscope JSM-840 (JEOL, Tokyo, Japan) configured with a 4 π energy dispersive spectrometer.

Identification and Quantitation of Crocins and Picrocrocin by LC-DAD-MS. Twenty milligrams of saffron was macerated for 1 h in 8 mL of Milli-Q water previously bubbled with helium. The whole process was carried out in darkness and at room temperature. Twenty microliters of the extract filtered through a PVDF filter of 0.45 μm (Millipore) was injected into an Agilent 1100 HPLC chromatograph (Palo Alto, CA) equipped with a 5 μm Phenomenex Luna C18 column (150 × 4.6 mm) thermostated at 30 °C. The solvents were water (acidified with formic acid, 0.25%) (A) and acetonitrile (B), using the following gradient: 80% A for 5 min to 20% C in 15 min, at a flow rate of 0.8 mL/min. Double on-line detection was carried out by a diode array spectrophotometer and a quadrupole mass spectrometer with electrospray ionization (ESI) (Agilent 1100). The probe of the mass spectrometer was connected to the UV cell outlet. The DAD detector was set at 250, 330, and 440 nm. Both the auxiliary and the sheath gases were nitrogen with a flow rate of 12 L/min. The drying gas temperature was set at 350 °C and the nebulizer pressure at 30 psi. The capillary voltage was ±2500 V and the capillary temperature 195 °C. Spectra were recorded in positive and negative ion modes between *m*/*z* 100 and 1500. Identification was carried out with Agilent Chemstation software for LC-MS. Quantification was carried out by taking into account the molecular coefficient absorbance of *trans*-crocins (89000 at 440 nm) and *cis*-crocins (63350 at 440 nm) (*20*) and expressed as the percentage of each crocin in relation to the total crocin content. Data reported represent the average of three sample replicates.

Identification and Quantitation of Safranal by Thermal Desorption−Gas Chromatography−Mass Spectrometry (TD-GC-MS). A joined system made up of thermal desorption Perkin-Elmer ATD-400 equipment (Norwalk, CT), a gas chromatograph HP-6890, and a mass spectrometer HP-5973 provided with a NIST library (Hewlett-Packard, Palo Alto, CA) was used. A fused silica capillary column with stationary phase BP21 of 50 m length, 0.22 mm of i.d., and 0.25 μm of film was used (SGE, Ringwood, Australia). The carrier gas was helium of chromatographic purity (220 kPa). Twenty milligrams of sample was introduced into the desorption tube and desorbed at 50 °C for 1 min.

Table 1. Saffron Analytical Determinations Following the ISO 3632 (1993) Normative and Data Measured during Different Dehydration Processes[a]

	heating source						
				hot air			
parameter	vine-shoot	gas cooker	electric coil	70 °C	90 °C	110 °C	room T^a
Analytical Determinations							
coloring strength	263 e	252 d	250 c	225 b	226 b	227 b	210 a
bitterness	29 c	28 b	26 a	28 b	27 b	27 b	30 c
aromatic strength	104 c	101 c	95 b	93 b	93 b	92 b	83 a
moisture (%)	7.7 d	7.4 c	7.1 c	7.3 c	5.6 b	4.4 a	7.4 c
Dehydration Process							
dehydration time (min)	28	32	55	4	3	2	10080
T^a max (°C)	110.4	121.1	75.3	70.0	90.0	110.0	22.5
mean T^a (°C)	75.7	82.8	54.1	70.0	90.0	110.0	20.4

[a] Different letters between columns indicate significant differences at the 0.05% level.

Other conditions for the thermal desorption equipment were as follows: oven temperature, 250 °C; cold trap temperature, −30 °C; transfer line temperature, 200 °C. Conditions for gas chromatography were 100 °C (5 min), ramped at 18 °C/min to 210 °C (15 min). In the mass spectrometry analysis, the electron impact mode (EI) at 70 eV was used. The mass range varied from 35 to 500 u, and the detector temperature was 150 °C. Data reported represent the average of three sample replicates.

RESULTS AND DISCUSSION

Time−Temperature Profile. Some preliminary trials were carried out with a digital thermometer to determine the approximate temperature reached on the sieve surface when the traditional dehydration process was carried out in Castille−La Mancha (Spain). The maximum temperature reached, ∼100 °C, revealed values much higher than the ones mentioned in the bibliography (35 °C). The contradictory data obtained made it necessary to measure the temperature of the process more accurately. A sieve with 12 thermocouples was used for this purpose while using different heating sources.

In the case of using vineshoot charcoal, the dehydration process started quickly, reaching a temperature of 80 °C after a few minutes. Saffron stigmas were turned in the sieve to avoid burning after 12 min. Stigmas reached temperatures >80 °C until the charcoal died out, the entire process lasting ∼28 min (**Table 1**). While using a gas cooker, the temperature increment was more gradual; between 6 and 7 min was necessary to reach 80 °C, although temperatures of 100 °C were easily surpassed. The stigmas were turned in the sieve after 15 min. The process was kept at temperatures >80 °C until the process ended, in ∼32 min (**Table 1**). The dehydration process using an electric coil as heating source took place at lower temperatures, ranging from 50 to 60 °C, and therefore the process took longer, 55 min. The sieve position was constantly moved to achieve a more uniform dehydration process.

Saffron Quality Characteristics by ISO 3632. All dehydrated saffron samples were analyzed following the ISO 3632 (*1*) Normative, which is used to certify saffron quality on the international market. Results are shown in **Table 1**. In the traditional process followed in Castille−La Mancha, the moisture of the samples ranged from 7 to 8%, thus achieving values of <12%, which is the maximum allowed by the International Normative. Parameters such as coloring strength, bitterness, or aromatic strength fell into the best ISO category, category I, for all samples. The high temperatures reached during dehydration (**Table 1**) explain the common belief of saffron producers that toasted saffron does not get moldy, nor is it attacked by insects, as is sometimes observed when saffron is dehydrated at room temperature (*6*). The temperature reached is high enough to eliminate an important proportion of microorganisms.

It was noted that samples dehydrated following the traditional process had higher coloring strength than the ones dehydrated by using hot air procedures. The highest coloring strength was achieved by the sample dehydrated over vineshoot charcoal. Surprisingly, it was not the sample dried at the highest temperature. When samples dehydrated traditionally were analyzed carefully, no correlation was found between the parameters of coloring strength and temperature. Instead, a linear equation was achieved when the time variable was also taken into account ($y = -0.0003x + 299.83$; $R^2 = 0.9997$; the *X*-axis is the product between dehydration temperature and time, and the *Y*-axis is the coloring strength). This confirms that the time required for the process is as important as the temperature reached. Hence, if saffron with the highest coloring strength is desired, it is better to submit it to a higher temperature and lower times than to lower temperature and longer processing time.

The dehydration rate may therefore also be responsible for higher coloring strength, probably due to the significant changes in the structural properties of the material when water is removed. In the early stages of the dehydration process, the cellular tissues are elastic enough to shrink into the space left by the evaporated moisture, creating the final rigid network as the process proceeds (*23*). However, if the process takes place rapidly, the intracellular interstices are not filled and the vegetable material results in a more porous material, affecting the quality of the final product (*24*). Using SEM, a sample dried at quicker rates was seen to have more intracellular interstices, resulting in lower vegetable tissue cohesion, in contrast with what was observed with the same sample dried at a lower rate (**Figure 1**). This supports the idea that higher dehydration temperatures affect the porosity of the sample, at least in the sample surface. DSC was the technique chosen to evaluate the internal porosity of the samples, as it can offer an idea of how water is retained inside the vegetable material as the temperature increases (*25*). The typical DSC graph for a saffron sample showed an endothermic peak due to moisture and volatile matter evaporation. Samples that were not significantly different in terms of their moisture content, such as samples dehydrated at room temperature and the ones dehydrated traditionally (gas cooker and electric coil) (**Table 1**), showed differences in the endotherm temperature value and the enthalpy required for this process (**Table 2**). It was more difficult to eliminate the moisture and volatile contents in saffron dehydrated at room temperature because of its lower porosity. Similar behavior was observed when hot air dehydrated samples were studied. In this case, the lower temperature and enthalpy values of the endothermic peak, in comparison with traditional toasted, can be explained by the shorter time employed in dehydration (**Table 1**). These results confirm the importance of temperature and also the processing rate to determine final saffron characteristics, both physical and chemical.

Effect of Dehydration Process on Saffron Chemical Composition. Once the effect of the dehydration process was determined on the coloring strength, the main parameter considered in international commerce, interest was focused on clarifying its relationship to crocin content. These compounds were analyzed by LC-DAD-MS, obtaining chromatograms such as the one shown in **Figure 2**. The fingerprint was almost identical to that obtained by Tarantilis (*26*), although due to

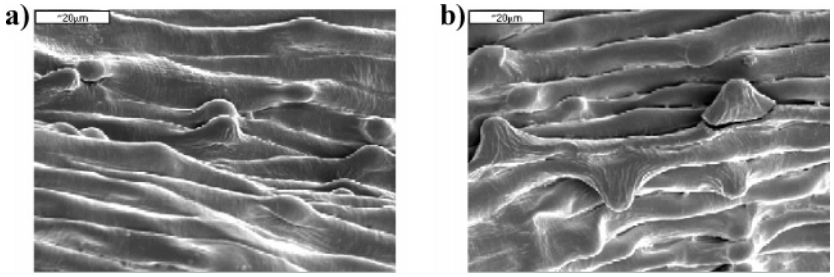
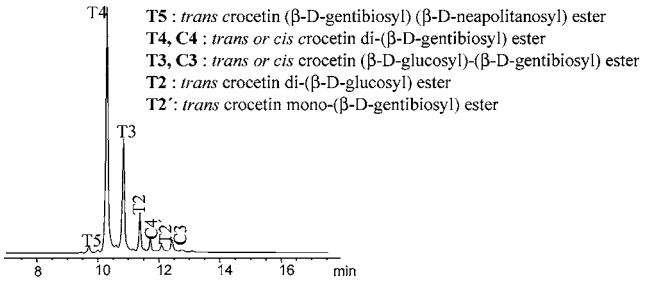


Figure 1. SEM photographs of the same saffron (**a**) dehydrated at room temperature and (**b**) dehydrated at high temperatures.

Table 2. Temperature and Energy of the Endothermic Peak Observed in the DSC Graph for Saffron Samples[a]

sample	T^a_{min} endotherm (°C)	Δ enthalphy (mJ/mg)
room T^a	89.3 e	25.03 d
traditional dehydration process		
electric coil	84.6 d	23.87 c
vineshoot	83.9 d	22.31 c
gas cooker	81.9 d	20.77 c
hot air dehydration process		
70 °C	77.3 c	18.72 b
90 °C	74.4 b	17.58 b
110 °C	72.9 a	15.93 a

[a] Different letters between rows indicate significant differences at the 0.05% level.



	Mass fragmentation pattern (positive ion mode)	Mass fragmentation pattern (negative ion mode)	UV λ_{max}
T5	1161(38), 797 (100), 592 (50)	1137 (20), 651 (26), 415 (100)	420, 440
T4	999(100), 675(10), 513 (25)	975(78), 651 (54), 533 (100)	420, 460
T3	837 (100), 675 (7), 329(15)	813(20), 651(100), 489(15)	440,468
T2	675(100), 513(85), 386(84)	651 (100), 487(41), 287(25)	435,461
C4	999 (100), 511(30), 386(11)	975 (75), 651(100), 533(30)	330,435,462
T2′	675(100), 329 (27)	651 (100), 563(30), 475 (9)	440,461
C3	837(100), 675 (10), 543(85)	837 (20), 651 (100), 565(23)	330,435,460

Figure 2. Saffron fingerprint chromatogram at 440 nm and the mass fragmentation pattern in the positive and negative ion modes.

the different chromatographic conditions, *trans*-crocin 2 and *trans*-crocin 2′ eluted earlier (**Figure 2**). The mass fragmentation pattern in the negative and positive ion modes was used for crocin identification: *trans*-crocetin (*β*-D-gentibiosyl) (*β*-D-neapolitanosyl) ester *(trans*-crocin 5), *trans*-crocetin di-(*β*-D-gentibiosyl) ester *(trans*-crocin 4), *trans*-crocetin (*β*-D-glucosyl)-(*β*-D-gentibiosyl) *(trans*-crocin 3), *trans*-crocetin di-(*β*-D-glucosyl) ester *(trans*-crocin 2), and *trans*-crocetin mono-(*β*-D-gentibiosyl) ester *(trans*-crocin 2′), as well as the *cis* isomers crocins 4 and 3. These coincide with the Tarantilis paper (*26*) except for *trans*-crocin 5. The mass fragmentation pattern of *trans*-crocin 5 (*m*/*z* 1161, 797, and 592) corresponding to $[M + Na]^+$, $[M - \text{gentibiose}]^+$, and $[M + 2Na]^+/2$, respectively, was in agreement with the structure proposed by Pfister (*27*), neapolitanose as trisaccharide moiety, instead of the one proposed by Tarantilis (*26*), three glucose moieties with (1→6) linkage. In the last case, the loss of gentibiose or two glucose from both ends will show a prominent ion of *m*/*z* 814. The *m*/*z* signal 797 was due to the losses of a glucose moiety at position 6 of either of the two ends (162 u) together with the glucose at position 2 (179 u) from the trisaccharide neapolitanose. The structural assignations done are in complete agreement with other previous papers (*27*−*30*), which makes it reasonable to consider that the compounds were properly identified, even though there are no commercial standards available. As the presence of *trans*-crocin 5 and 2′ was insignificant, their quantitation was not carried out.

Table 3. Identification and Quantitation of Crocins in Saffron Samples Submitted to Different Dehydration Processes and to a Thermal Accelerated Aging Process[a]

	trans-crocin 4	*trans*-crocin 3	*trans*-crocin 2	*cis*-crocin 4	*cis*-crocin 3
	Dehydration Process[b] (%)				
room temp	49.1 a	29.7 b	10.4 d	8.3 b	2.5 a
resistance coil	58.6 b	31.7 c	3.4 c	3.9 a	2.3 a
vineshoot	59.6 b	31.1 c	2.4 b	4.6 a	2.2 a
gas cooker	61.9 c	29.1 b	1.8 a	4.3 a	2.2 a
hot air					
70 °C	57.7 b	29.9 b	5.4 c	4.6 a	2.4 a
90 °C	63.2 c	29.5 b	1.4 a	4.3 a	1.5 a
110 °C	64.3 c	27.4 a	1.3 a	5.1 a	2.0 a
	Thermal Accelerated Aging Process[b] (%)				
TD[c]	52.5 d	25.4 b	6.1 a	11.5 a	4.4 a
TD + 50 °C	55.3 e	26.1 c	3.9 a	10.8 a	3.9 a
TD + 70 °C	53.9 e	25.9 c	4.5 a	11.4 a	4.4 a
TD + 90 °C	51.5 c	25.5 b	5.8 a	11.8 a	5.3 a
TD + 110 °C	47.1 b	22.4 b	7.5 b	15.3 b	7.7 b
TD + 130 °C	34.8 a	17.1 a	16.1 c	22.4 c	9.6 c

[a] Different letters between rows indicate significant differences at the 0.05% level. [b] Expressed as the percentage of each crocin in relation to the total content [c] Traditional dehydration.

The total crocin content, when saffron samples were dehydrated by hot air, increased linearly with temperature following the equation total crocin area = $976.8\ T^a_{\text{hot air}} + 27165$; $R^2 = 0.99$. Similar results were observed when traditional dehydration processes were carried out: total crocin area = -0.0175 (time × temperature) + 31220; $R^2 = 0.93$. In this case, the slope of the equation is negative because the variable time × temperature was higher when the temperature values were lower.

Knowing that the total crocin content increased with temperature, the results were normalized by expressing each of them as a percentage of the total crocin content (**Table 3**). The proportions of *trans*-crocins 4, 3, and 2 were similar to the ones found by Morimoto et al. (*31*). The higher the temperature of the process (gas cooker, hot air at 90 and 110 °C), the higher the proportion of *trans*-crocin 4, which is the most abundant carotenoid. However, *trans*-crocins 3 and 2 decreased (**Table 3**). These results are more in accordance with the crocin biosynthesis hypothesis proposed in *Gardenia jasminoides* (*28*), where *trans*-crocins 2 and 2′ and *cis*-crocin 2 are direct

intermediate precursors of crocin 4, than with the other proposal in saffron, which postulates that *trans*-crocin 4 would be biosynthesized first and the other crocins would be obtained by the loss of a glucose or gentibiose moiety (*31*). Anyway, crocin behavior observed cannot be justified by a selective degradation of *trans*-crocins 2 and 3. Crocins are considered to be thermal-resistant compounds, and the different crocetin glycosylation degree does not affect their heat resistance (*32*), as occurs when light irradiation is applied to crocins, where crocins containing gentibiose are more stable than the ones with a glucose extreme (*33*).

As results presented in this paper reject the Morimoto et al. (*31*) hypothesis and also make it difficult to justify the pathway proposed by Ichi et al. (*28*), a new hypothesis to explain the increment of total crocin and *trans*-crocin 4 composition ratio with temperature is proposed. Carotenoids are known to be embedded in the cell matrix, probably in the chromoplast (*34*), but up to now the structure of the mature chromoplast which houses red saffron pigments is not clear (*35*). The chromoplast structure, which exists in various forms, determines the expression and the quality of color, as well as the ease with which carotenoids are liberated (*36*). In saffron, a high amount of secondary metabolites, ~20%, are located in such chromoplasts, so the high temperature employed during dehydration could facilitate crocin liberation, as shown in this paper, while allowing the most effective breakage to be carried out with the compound with highest glycosylation and degree of symmetry, *trans*-crocin 4.

Effect of Thermal Accelerated Aging Process on Saffron Chemical Composition. To confirm the temperature effect on saffron chemical composition, a sample that had been already dehydrated by the traditional process was submitted to an accelerated aging process. The proportion of *trans*-crocin 4 (**Table 3**) as well as its absolute amount (data not shown) was higher at 50 and 70 °C when compared with a nonaged sample. Again, an interesting result, saffron that has been already dehydrated can increase its *trans*-crocin 4 content when it is resubmitted to a heating treatment. However, this result comes out in relation with the biosynthetic origin of *trans*-crocin 4. Until this moment it was accepted that crocins are formed by the oxidative degradation of zeaxanthin. Recently, a relevant research group was not able to find zeaxanthin after anthesis during their studies on the characterization of the enzyme responsible for the formation of *trans*-crocin 4 from zeaxanthin (*37*). With this in mind, it is difficult to explain how *trans*-crocin 4 increases with temperature during dehydration, but even more difficult to understand was its increment after heating of the sample once it was already dehydrated.

When treated at 90 °C (TD + 90 °C, **Table 3**), the content of *trans*-crocins 4 and 3 decreased to transform into *trans*-crocin 2 and *cis*-crocin 3 and 2. In this case the isomerization process was clear over 110 °C (TD + 110 °C, **Table 3**), although it was necessary to consider the extreme conditions in which the sample had been submitted: 8 h in the oven with increasing temperatures with the last 2 h at 110 °C (TD + 110 °C, **Table 3**).

When picrocrocin was analyzed after the corresponding aging process, the same behavior as for crocins was observed: an increment up to 70 °C (TD + 70 °C, **Table 3**) and then a decrease. This appears to be a behavior related to what Himeno and Sano (*14*) found: a relationship of crocin/picrocrocin between 4.4 and 5.6 during the seven steps previous to the anthesis, similar to the one found by us during the aging process (**Figure 3**). The picrocrocin decrement is in accordance with

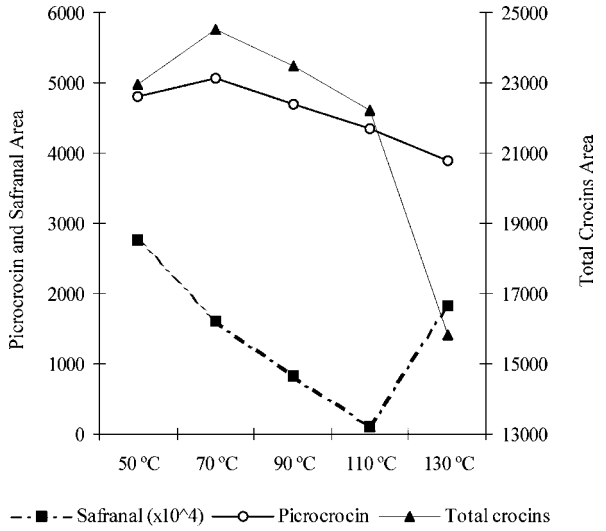


Figure 3. Total crocin, picrocrocin, and safranal contents in samples submitted to an accelerated aging process.

the theory postulated by several authors that the dehydration temperature is responsible for safranal generation from picrocrocin (*14*, *38*, *39*). Contrary to the bibliography, there was not a relationship between the increment and later decrement of picrocrocin with safranal generation. Safranal content decreased in the same way as picrocrocin did up to 110 °C (TD + 110 °C, **Table 3**), when it started to be generated. These results contradict the mentioned bibliography. If during the dehydration process the high temperatures are responsible for the conversion of picrocrocin into safranal, it is difficult to understand why it cannot happen in the same way when the dehydrated sample has been submitted to the same range of temperatures, 50−90 °C. It is well documented that isolated picrocrocin is transformed into safranal by chemical, thermal, or enzymatic treatment (*15*). These experiments support the accepted transformation hypothesis during the dehydration process. If this is true and does take place in the spice, it is at least doubtful that picrocrocin is the safranal reservoir once saffron has been dehydrated. Its decrement is not followed by an increment in the safranal content. Only a high-temperature treatment causes safranal generation.

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