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Saffron Flavor: Compounds Involved, Biogenesis and Human Perception

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ABSTRACT

In recent years, saffron has increased in interest for both scientists and consumers, as it is the only spice able to give to food flavor, color and aroma to foods. In relation to flavor, picrocrocin is considered as mainly responsible for saffron's bitter taste, but other compounds structurally related to picrocrocin and flavonoids have been identified and could contribute to such a property. Further studies are necessary to establish picrocrocin sensory perception, as only its taste detection threshold has been established (10 mgL^{-1}). Even though it is well known that picrocrocin content is directly affected by the dehydration process of the spice, its generation pathway remains unclear. In this paper a comparison between the classical hypothesis and the alternative one is therefore presented. Among its flavor properties, picrocrocin is an excellent marker of saffron purity because till now its presence it is only reported in saffron. Also, it is especially useful in unmasking sophisticated adulterations carried out with pigments from *Gardenia jasminoides*, which contain the same carotenoid family as saffron. For both reasons, it is important to accurately determine picrocrocin content in order to gain the trust of saffron dealers and also for consumer satisfaction. This review summarizes the available methodologies for this proposal, giving emphasis to the gaps contained in the current ISO 3632 Standard, normally used in the international market.

Keywords: *Crocus sativus* L., picrocrocin, spice, crocetin esters, ISO/TS 3632

Symbols: $E_{1cm}^{1\%}$ is represented as $E_{1cm}{}^{1\%}$ throughout the text, figure legends and tables

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INTRODUCTION

In recent times, when the use of saffron in traditional medicinal recipes and beauty products has decreased, saffron is appreciated for its use in food, especially for its coloring properties. The new interest in other characteristics of this spice, such as flavor and aroma, has been enhanced as consumers become more familiar with the spice and begin to demand a higher quality of saffron. It should be noted that saffron is the only spice able to confer flavor, color and aroma in food.

The compound considered since 1930 (Lutz 1930) as mainly responsible for saffron's bitter taste is the picrocrocin, the 4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (**Fig. 1**). It is soluble in polar solvents, more soluble in water than in water-alcohol solutions (Iborra *et al.* 1992a, 1992b) and insoluble in apolar ones (Corradi and Micheli 1979a, 1979b). The structure of picrocrocin was established by Khun and Winterstein in 1934.

Picrocrocin content in saffron spice is significant, from 5% (Alonso *et al.* 2001) to 13% of dry material (Iborra *et al.* 1992b; Alonso *et al.* 2001). However, recent studies demonstrated that it could reach 18% (del Campo *et al.* 2010a) and 27% in Spanish samples (Sánchez *et al.* 2008).

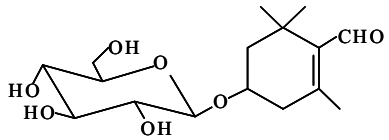

Fig. 1 Structure of picrocrocin.

Relative importance of flavor versus color properties on saffron

In international trade, saffron quality is determined by ISO 3632 standard, whose latest revision has given rise to Technical Specification ISO/TS 3632 (2003). This classifies saffron into three quality categories with regard to a large number of physical and chemical parameters such as: microscopic characteristics, presence of flower waste, mois-

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Invited Review

ture and volatile matter content, ash content, $E_{1cm}^{1\%}$ 440 nm (coloring strength), $E_{1cm}^{1\%}$ 330 nm (related to safranal content), $E_{1cm}^{1\%}$ 257 nm (related to picrocrocin content), etc. However, only coloring strength, representing the crocetin ester content, has traditionally been of importance for companies which trade with saffron. Proof is that this Standard is not capable of discriminating between qualities with regards to volatile ($E_{1cm}^{1\%}$ 330 nm) or picrocrocin ($E_{1cm}^{1\%}$ 257 nm) content. When a sample fulfills the requirements of any category regarding coloring strength (absorbance at 440 nm), it also fulfills the other spectrophotometric parameters ($E_{1cm}^{1\%}$ 330 nm, $E_{1cm}^{1\%}$ 257 nm) for the same category. This fact has been shown by del Campo *et al.* (2010a) when 435 samples were analyzed and classified by their coloring strength.

Determination of picrocrocin content

The sample mentioned above which cannot discriminate between picrocrocin or volatile content is partially due to the current methodologies included in ISO/TS 3632 (2003) for the estimation of these compounds, which are not adequate.

Determination of these two parameters is performed through the following expression:

$$E_{1cm}^{1\%}\ \lambda = \frac{D_\lambda \times 10000}{m\,(100 - H)}$$

where λ is wavelength to which is measured the maximum of absorbance, especially at 330 nm for determination of volatile content and at 257 nm for picrocrocin, D_λ is the absorbance at about 330 or 257 nm, m is the saffron mass in the working solution expressed in grams and H is the sample moisture and volatile matter content, according to ISO/TS 3632 (2003). The determination of picrocrocin through the parameter $E_{1cm}^{1\%}$ 257 nm shows a problem of selectivity since other compounds of saffron extract, primarily crocetin esters and major compounds within the extract, have also absorbed at this wavelength (**Fig. 2**) due to the glycoside bonds, causing interferences in measurement (Tarantilis *et al.* 1994; Orfanou and Tsimidou 1996; Carmona *et al.* 2006b; Sánchez *et al.* 2008, 2009).

There is another spectrophotometric parameter ΔE_{pic}, proposed by Corradi and Micheli (1979a), for measuring the picrocrocin content, which tries to avoid the interferences of the crocetin esters when it is determined by $E_{1cm}^{1\%}$ 257 nm. ΔE_{pic} is calculated as follows:

$$\Delta E_{pic} = E_{257}^{1^0/_{000}} - E_{297}^{1^0/_{000}}$$

where the first and second parameters are the maximum extinction values at λ = 257 nm and minimum of extinction measured at λ = 297 nm for saffron aqueous extract (1:10000), respectively.

When both parameters ($E_{1cm}^{1\%}$ 257 nm and ΔE_{pic}) measured spectrophotometrically are compared with picrocrocin content measured with a more effective technique by HPLC (Sujata *et al.* 1992; Tarantilis *et al.* 1995; Lozano *et al.* 1999; Alonso *et al.* 2001), ΔE_{pic} is the most suitable as demonstrated by del Campo *et al.* (2010b). The comparison of the picrocrocin concentration overestimation obtained with $E_{1cm}^{1\%}$ 257 nm and over/underestimation using ΔE_{pic} in relation to HPLC determination for different saffron origin samples is shown throughout the coloring strength range (**Fig. 3**) (del Campo *et al.* (2010b). The samples generally presented higher values of picrocrocin content using $E_{1cm}^{1\%}$ 257 nm than the results obtained using ΔE_{pic} for all coloring strength ranges for the different countries. The higher overestimation of picrocrocin content obtained using $E_{1cm}^{1\%}$ 257 nm could be justified by the interferences of crocetin esters (Tarantilis *et al.* 1994); whereas values obtained by ΔE_{pic} gave a better estimation. The results, however, are not sufficiently reliable and not sufficiently close to the true value to accept this approximation and the possibility for using it in a company's routine quality control is not feasible.

Regardless of variables such as edaphic or climate conditions, dehydration or storage procedures, which may affect the picrocrocin content and crocetin ester, the capability of $E_{1cm}^{1\%}$ 257 nm to approximate the true values of picrocrocin is surprisingly improved when the content of crocetin esters, responsible for these interferences mainly caused by *cis* configuration, increases (**Fig. 3**). According to del Campo *et al.* (2010b) *cis*-crocetin esters are presented in lower concentration when saffron coloring strength increases and

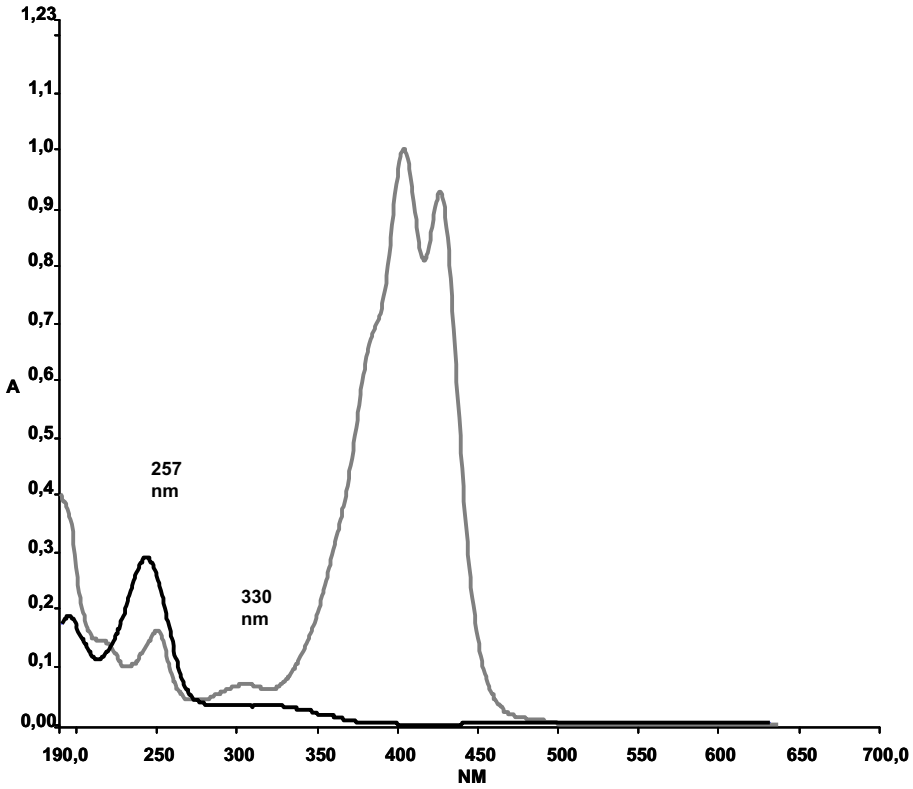

Fig. 2 UV–Vis spectra of crocetin esters and picrocrocin fractions after solid phase extraction (SPE) from a saffron aqueous extract.

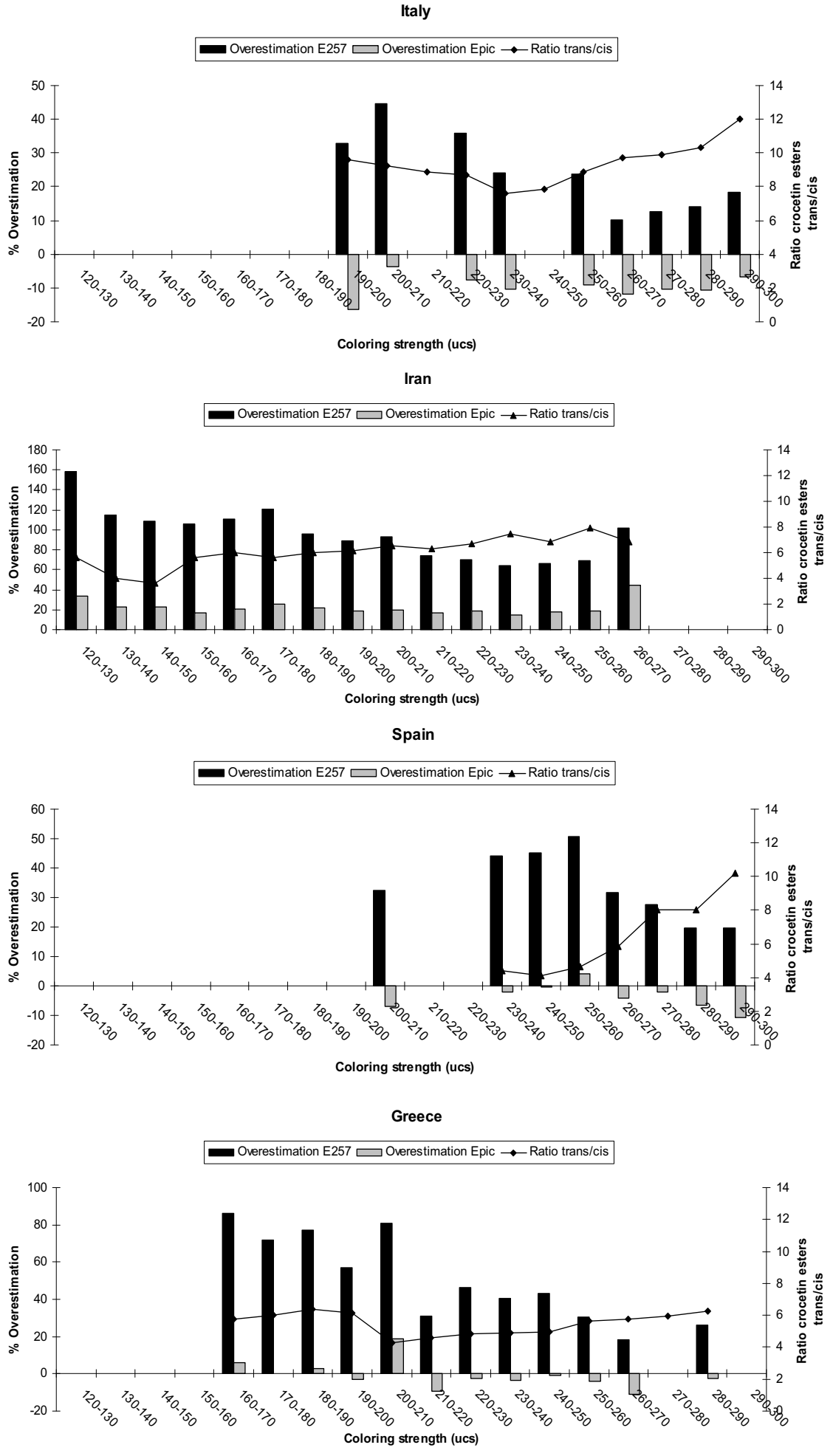

Fig. 3 Comparison of the picrocrocin content overestimation obtained with $E_{1cm}^{1\%}$ 257 nm and over/underestimation using ΔE_{pic} in each range of coloring strength and relationship with the ratio *cis*/*trans* crocetin esters for the different countries. (Adapted from del Campo *et al.* 2010b).

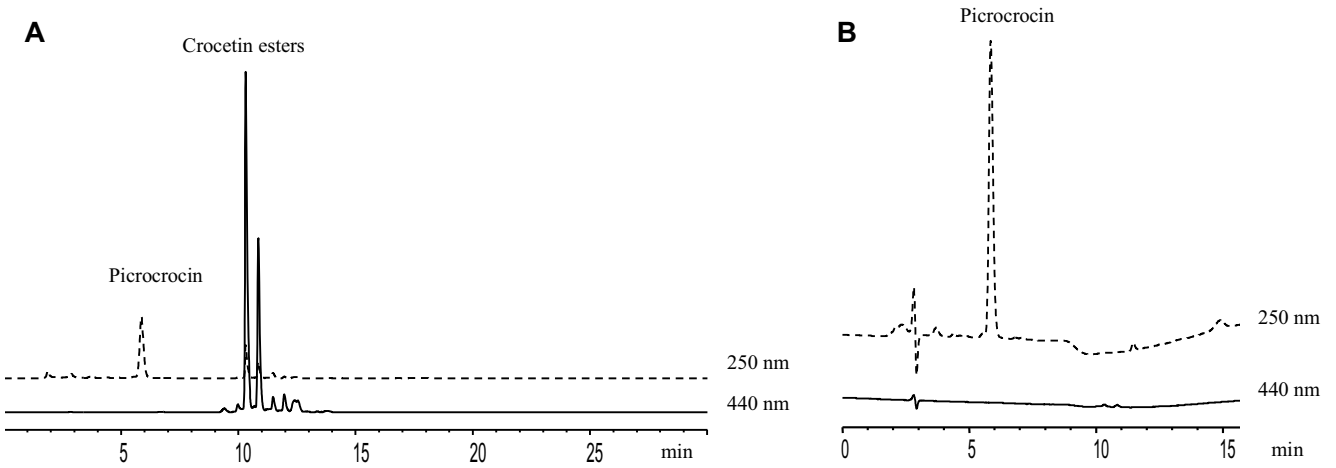

Fig. 4 Comparison of chromatograms at 250 and 440 nm of a saffron extract (A) and its corresponding picrocrocin fraction after the SPE isolation stage (B). (Adapted from Sánchez *et al.* 2009).

Table 1 Principal component statistics of NIRS calibration and validation for the $E_{1cm}^{1\%}$ 257 nm parameter measured according to ISO/TS 3632 (2003) (Adapted from Zalacain *et al.* 2005b).

Characteristic	$E_{1cm}^{1\%}$ 257 nm
Nº of analysis	221
Outliers	18
Nº independent standards	22
Nº principal components	13
SEC (Standard Error of Calibration)	6.26
SEP (Standard Error of Prediction)	6.48
R^2	0.90
Mean Value	82.27
% variance	80.37

thus the *trans/cis* ratio, observing that picrocrocin content is adjusted to real sample value.

Another technique used for determining saffron chemical composition is near-infrared (NIR) spectroscopy (Zalacain *et al.* 2005b). Principal component statistic analysis of NIRS calibration and validation for the $E_{1cm}^{1\%}$ 257 nm parameter measured according to ISO/TS 3632 (2003) were reported in **Table 1**. The number of principal components was 13, although the first two components accounted for 98% of the total variability. The low differences found between the standard error of calibration (SEC) and standard error of prediction (SEP) reveals the robustness of the equation for this parameter. For example, if the mean value of picrocrocin is 82.27, SEC value is 6.26 representing an error of about 7.6%. Although FT-NIR is a rapid technique and gives results close enough to true values, the equipment needs training and many samples are needed for a proper calibration.

But the truth is that both HPLC, and to a lesser extent NIR, require equipment that is seldom found in small or medium-size companies that process and package saffron spice. Thus, there is a real interest on the part of the companies for the development of rapid methods of routine quality control of saffron using UV–Vis spectral information. In addition, it is necessary to check if saffron is adulterated. As known, due to saffron's price, this spice was commonly adulterated with artificial colorants or *Gardenia jasminoides* extracts. The latter can increase sample coloring strength, although but picrocrocin is not present. Thus, picrocrocin is an excellent marker of saffron purity. Recently, Sánchez *et al.* (2009) has proposed a rapid method for picrocrocin routine control with a previous step of purification using a solid phase extraction (SPE) followed by UV–Vis technique. SPE is one of the most common and least expensive purification techniques and is considered as a convenient approach for sample preparation for the analysis of major and minor components of foods (Grigoriadou *et al.* 2007; Puoci *et al.* 2008). Up to the present, SPE technique had been applied to saffron extracts when detection of adulterations by artificial colorants (ISO/TS 3632, 2003; Zalacain *et al.* 2005a) was studied. The procedure for picrocrocin determination using SPE is the following: saffron aqueous extract (0.5 g L^{-1}) prepared according to the ISO/TS 3632 (2003), was centrifuged at 4000 rpm for 5 min. After having conditioned the C_{18} SPE cartridge, 1 mL of saffron extract was loaded into the SPE, washed with 10 mL water and picrocrocin was eluted with acetonitrile/water 12% (v/v) up to collecting 10 mL. The absorbance of eluted extract was measured at 250 nm in a 1 cm path length cell in the UV-Vis spectrometer. When the extract was measured by HPLC, a single peak with retention time 5.84 ± 0.03 min was detected (**Fig. 4**). The proposed method also shows a good sensitivity, the LOD is 0.30 mg L^{-1} of picrocrocin corresponding to 0.6% on a dry basis of saffron and the LOQ is 0.63 mg L^{-1} of picrocrocin that represents 1.3% on a dry basis of saffron. These values are definitely lower than 5%, minimum value for picrocrocin content (Alonso *et al.* 2001). In summary, this validated SPE gives good results for determining the content of picrocrocin in saffron spice samples from UV–Vis spectral information. The procedure is accurate, reproducible and sensitive enough for this application in samples from different countries. Furthermore, its common points with the ISO determinations in saffron, the short time necessary to carry it out and its simplicity make this procedure of particular interest for routine quality control in the industry.

Other compounds potentially involved on saffron taste

In addition to picrocrocin, other compounds that may contribute to saffron taste properties have been characterized in saffron spice although their content is much lower than picrocrocin. These compounds are structurally related to picrocrocin and flavonoids (**Fig. 5**). The identification has mainly been carried out by three research groups as shown in **Fig. 6**, with Tarantilis *et al.* (1995) proposing the first structures corresponding to 4-hydroxi-2,6,6-trimethyl-1-cyclohexen carbaldehyde 4-*O*-β-D-gentiobioside (*b*), 4-hydroxy-2,6,6-trimethyl-1-ciclohexene carboxylic 4-*O*-β-D-glucopyranoside acid (*d*) and picrocrocin (*g*). New constituents of saffron were isolated and their precursor functions with regard to their formation discussed by Straubinger *et al.* (1997, 1998a, 1998b). They identified 5-hydroxi-7,7-dimethyl-4,5,6,7-tetrahydro-3H-isobenzo-furanone 5-*O*-β-D-gentiobioside (*c*), 3′,5-dihydroxi-7,7-dimethyl perhydro-isobenzo-furanone 5-*O*-β-D-glucopyranoside (*e*) and 4-hydroxymethyl-3,5,5-trimethyl-2-ciclohexenone 4-*O*-β-D-gentiobioside (*f*). And in 2007, Carmona *et al.* (2007b) corroborated the presence of the earlier compounds such as

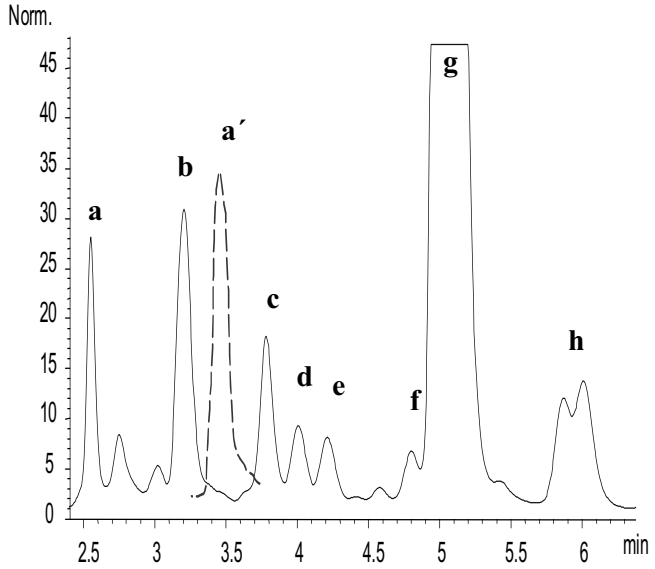

Fig. 5 Chromatogram corresponding to saffron extracts with 250 nm detection (Adapted from Carmona *et al.* 2007b).

-methyl-6-oxo-2,4 heptenate of *O*-β-D-gentiobioside (*a*) (**Fig. 5**) and extended the number of compounds tentatively identified.

After chromatographic separation it is easy to identify picrocrocin (*g*) since it is the most important substance with absorption at 250 nm, but other compounds appear with it in an aqueous saffron extract. Some of them have UV-Vis spectra almost identical to picrocrocin, as can be seen by the structure assigned to each of them and their mass spectra as reported in **Fig. 6**. The 2-methyl-6-oxo-2,4 heptenate of *O*-β-D-gentiobioside structure was assigned to peak *a* in **Fig. 5**. This compound was previously identified in saffron by the Winterhalter group (Straubinger *et al.* 1997, 1998a, 1998b). Signals at m/z 501 and 367 correspond respectively to $[M+Na]^+$ and the loss of the linear chain $[M-C_7OH_{10}]^+$ from the molecule. In chromatographic mobile phases when formic acid was added in order to promote ionisation of the substances, a considerable decrease was observed in the presence of this compound, besides the apparition of other compounds with an approximate 3.5 min retention time, shown by discontinuous line. It might be the same compound that had lost a glucose and remained in ionic form (m/z 361 $[M+2Na]^+$ in positive ion mode and at m/z 337 $[M-H+Na]^-$ in negative ion mode). Peak *b* was identified as 4-hydroxi-2,6,6-trimethyl-1-ciclohexen carbaldehyde 4-*O*-β-D-gentiobioside, where signals at m/z 515 and 339 correspond to $[M+Na]^+$ and the loss of the ring $[M-C_{10}OH_{16}+H]^+$. Peak *c* was identified as 5-hydroxy-7,7-dimethyl-4,5,6,7-tetrahydro-3H-isobenzo-furanone-5-*O*-β-D- gen -tiobioside after assigning their corresponding ions $[M+Na]^+$, $[M\text{-glucose}+2H+Na]^+$ and $[M\text{-glucose} + Na]^+$ to signals at m/z 527, 369 and 185. The signal at m/z 369 reflects the opening of lactone at the same time as one of the glucose molecules is lost. Straubinger *et al.* (1998b) found the same compound, but less glycosylated. Instead of containing the gentiobiose molecule, they only found a glucose molecule. The compound assigned to peak *d* coincides with what was shown by the Tarantilis and Winterhalter groups as 4-hydroxi-2,6,6-trimethyl-1-ciclohexene carboxylic 4-*O*-β-D-glucopyranoside acid. Fragmentation signals at m/z 369 and m/z 167 correspond to $[M+Na]^+$ and $[M\text{-glucose-}O+Na]^+$, respectively. Fragmentation signals for peak *e* were coherent with a compound of $C_{10}O_4H_{14}$ molecular form besides the glucose molecule. After thoroughly studying signals at m/z 383 and 367 corresponding to $[M-Na]^+$ and $[M-OH+Na]^+$, this peak was identified as 3´,5-dihydroxi-7,7-dimethyl perhydro-isobenzo-furanone 5-*O*-β-D-glucopyranoside, the hydrated form of the compound identified in peak c but with one less glucose. Peak *f* whose signals were at m/z 515 $[M+Na]^+$ and m/z 357 $[M-C_9OH_{12}]^+$, which assumes the freedom of the CH_3O-Gen fragment, was assigned to the 4-hydroxymethyl-3,5,5-trimethyl-2-cyclohexenone 4-*O*-β-D-gentiobioside compound. The remaining fragmentation signals (303, 232, 124) can be justified by different breakdowns of sugar. Signals from the majority peak *g* perfectly confirm that it is picrocrocin: m/z 353 corresponds to $[M+Na]^+$, while m/z 185 reflects the loss of glucose $[M\text{-glucose}+Na]^+$. Lastly, peak *h*, consisting of two shoulders, was granted the structure of two isomers, the 4-hydroxy-3,5,5-trimethyl-2-ciclohexenone 4-*O*-β-D-glucopyranoside (m/z 337 $[M+Na]^+$), compounds identified by the Straubinguer *et al.* (1998b).

On the other hand, the first identification of a flavonoid in saffron spice (by mass spectrometry) was made by Tarantilis *et al.* (1995), proposing a kaempferol structure with a disaccharide moiety (**Fig. 7A**). Straubinger *et al.* (1997) identified kaempferol 7-*O*-glucopyranosyl-3-*O*-sophoroside and kaempferol 7-*O*-sophoroside (**Fig. 7B**) by NMR and MS after counter-current preparative chromatography. Taking this determination into account, the same authors considered that the identification of a new flavonoid named kaempferol 3-*O*-gentiobioside carried out by Lozano *et al.* (1999) was not correct (Winterhalter and Straubinger 2000). Moreover, other flavonoids may be found in saffron spice, as they have already been described in other *Crocus* species (Nørbæk and Kondo 1999). The last study on the profile of flavonoids in saffron (Carmona *et al.* 2007b) showed that in aqueous extract only flavonol compounds of the flavonoid family were found. After acid hydrolysis, all the flavonoids gave kaempferol as an aglycone. They were kaempferol derivatives with three and two hexoses (**Fig. 7**). Their fragmentation patterns coincided with two standards, the -3-sophoroside-7-glucoside and the -3-sophoroside of kaempferol, respectively (Ferreres *et al.* 2004). All these data confirm the structures reported previously by Straubinger *et al.* (1997) for the main saffron flavonoids.

It remains to establish what their contribution to the saffron taste could be, as it is well known that flavonoids have many functions in the biochemistry, physiology and ecology of plants, as well as in both human and animal nutrition (Forkmann and Martens 2001).

Biogenesis of compounds responsible for flavor and bioconversion

The classical biogenesis pathway for the generation of picrocrocin and subsequent volatile compounds is shown in **Fig. 8**. Zeaxanthin breaks on both ends to generate crocetin-dialdehyde plus two molecules of picrocrocin which is later converted into safranal, while crocetindialdehyde is oxidized to give rise to crocetin and later on crocetin esters by the action of a glycosyltransferase (Côté *et al*. 2000).

This hypothesis was supported by Buchecker and Eugster (1973) who confirmed that the configuration of the carbon supporting the hydroxyl group in picrocrocin and in the zeaxanthin, the R configuration, is the same (**Fig. 9**). Also, Himeno and Sano (1987), followed by Lozano *et al.* (1999), proposed that during the dehydration process, 4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde (HTCC) could be the intermediate compound for the synthesis of safranal from picrocrocin either because of the temperature reached or the action of glycosidases (**Fig. 10**).

There is some evidence that the three types of compounds: crocetin esters, picrocrocin (and its related compounds) and volatiles could be interrelated in a different manner, as crocetin esters can generate safranal and other volatile compounds as much as picrocrocin and their analogues (Carmona *et al.* 2007a). This evidence was shown when the effects of dehydration processes on the different compounds in saffron spice were studied.

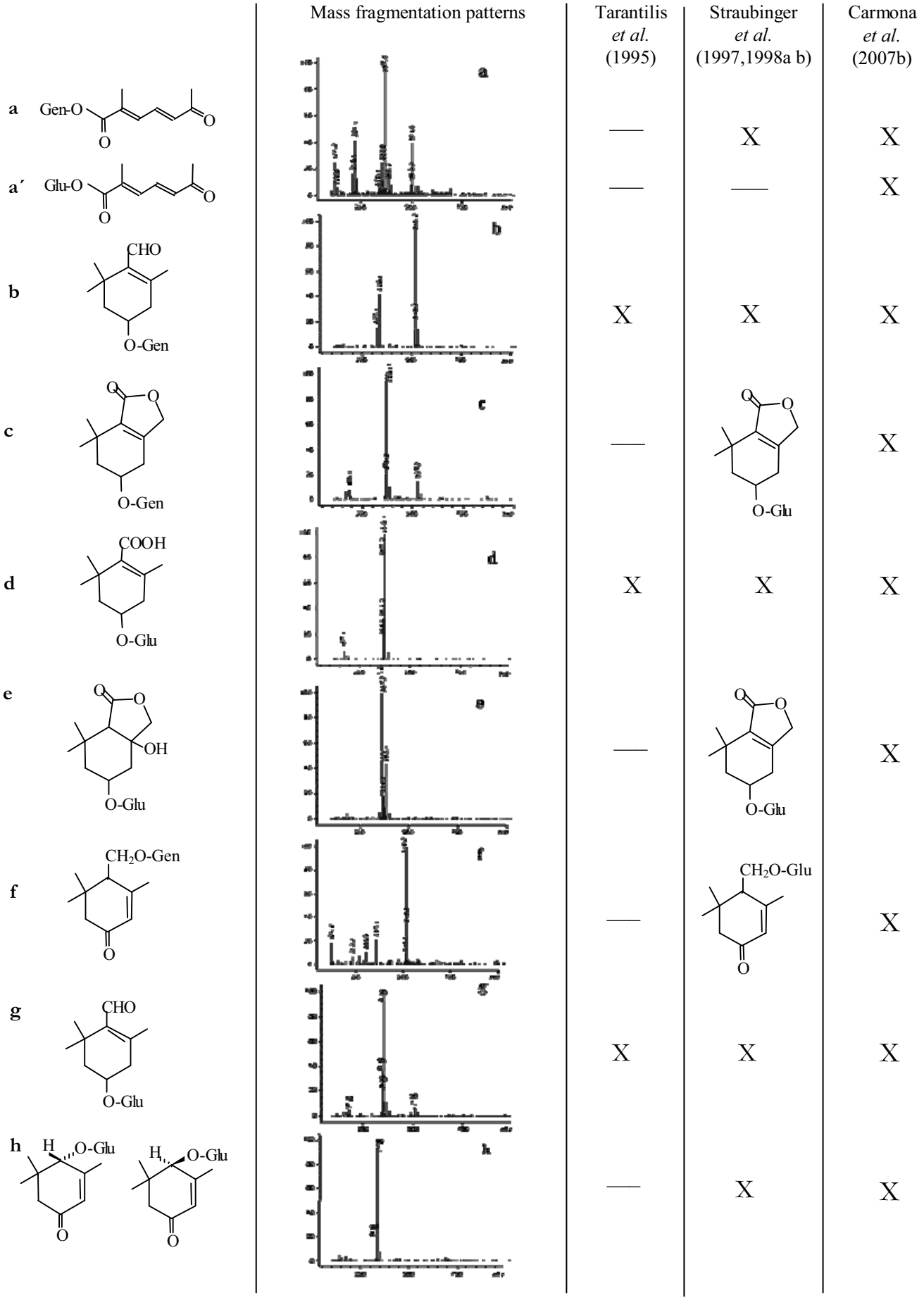

Fig. 6 Structures and mass fragmentation patterns proposed for the compounds in Fig. 5 detected by HPLC-DAD at 250 nm in aqueous saffron extract. In columns at the right, results are compared to those previously identified by other authors: Tarantilis *et al.* (1995), Straubinger *et al.* (1997, 1998a, 1998b) and Carmona *et al.* (2007b). Note: X means that these authors had already proposed its existence. When the compound is not the same but a similar one, its structure is given.

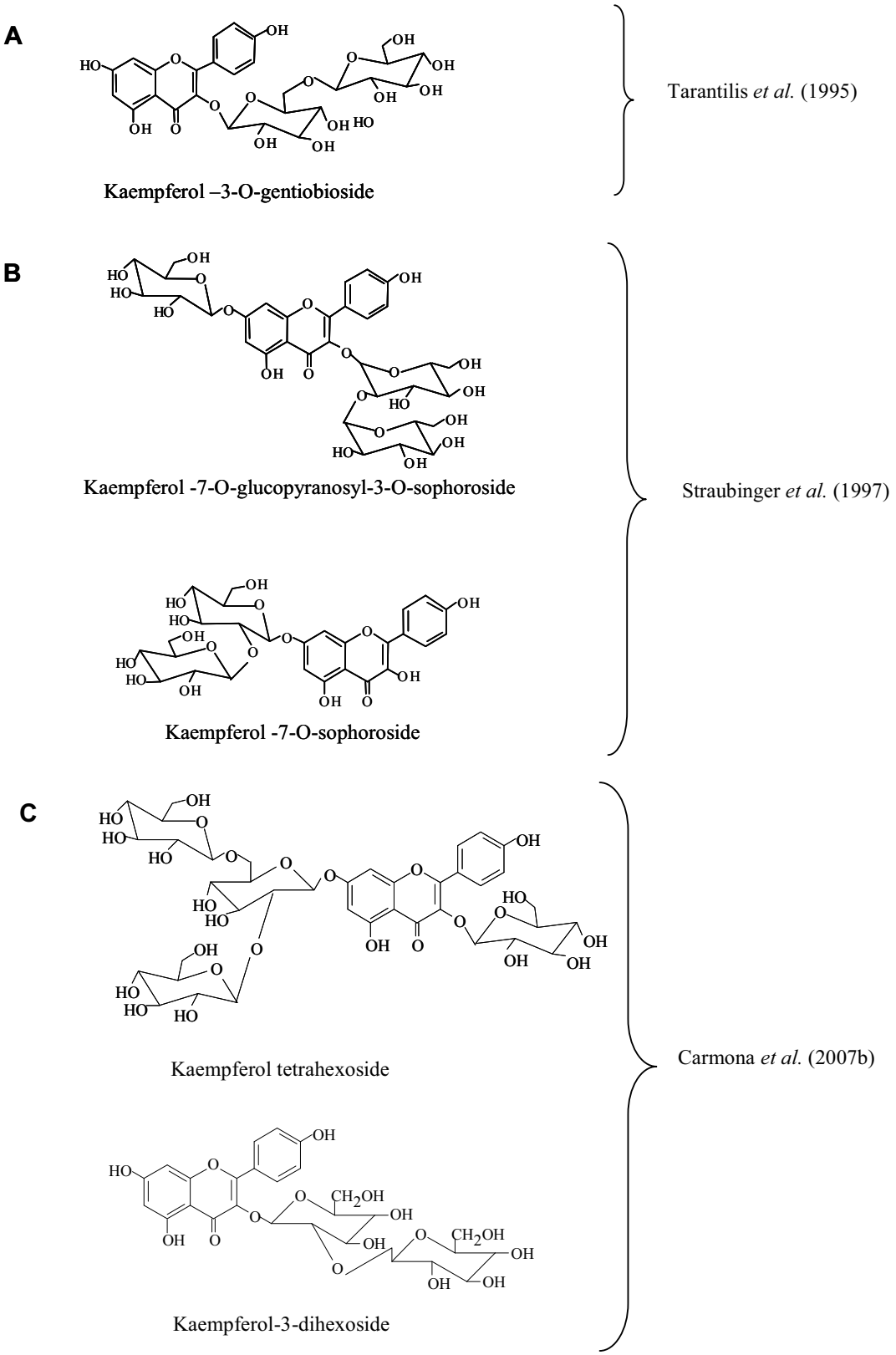

Fig. 7 Structure of the flavonoids identified in saffron spice.

1. Effect of post-harvesting treatments on picrocrocin

While it is known that the dehydration procedure is responsible for saffron sensorial properties: color, flavor and aroma (Pardo *et al.* 2002; Carmona *et al.* 2006a, 2006c), the best conditions to carry out this postharvest treatment remain unclear. Changes in the compounds responsible for these characteristics have been studied when dehydration processes are performed at low (Nauriyal *et al.* 1997; Hassnais 1998; Ati-Oubahou and El-Otomani 1999), high (Carmona *et al.* 2005; Gregory *et al.* 2005) and at mild temperatures (Tammaro 1999; Ordoudi and Tsimidou 2004; del Campo *et al.* 2010a).

In this last paper, the effect of different mild conditions applied to saffron samples with the same origin was investigated. The results showed that picrocrocin content is higher when the higher temperature is applied within the range assayed (between 18 and 55°C). According to Alonso *et al.* 1993 when similar temperatures were used (25-40°C), the same behavior was observed for picrocrocin. The increment

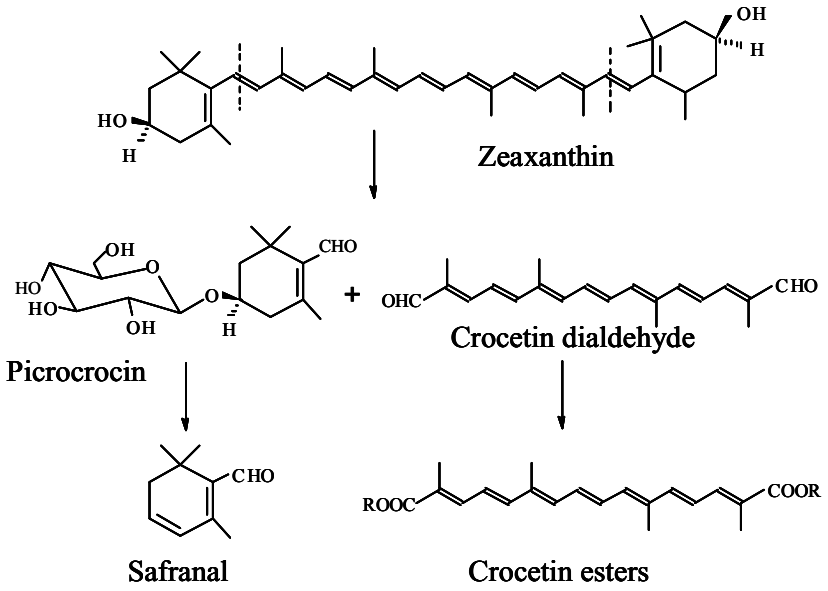

Fig. 8 Classical biogenesis pathway for the generation of picrocrocin and volatile compounds in saffron. (Adapted from Carmona *et al.* 2006d).

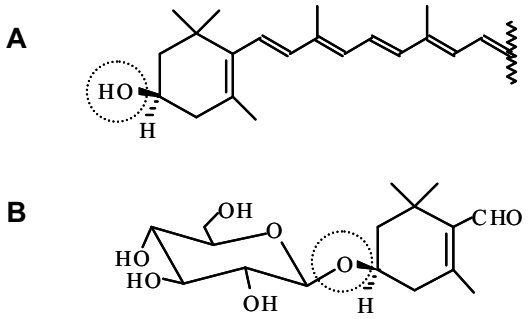

Fig. 9 Diagram representing the configuration of the carbon that supports the hydroxyl group in zeaxanthin (A) and picrocrocin (B).

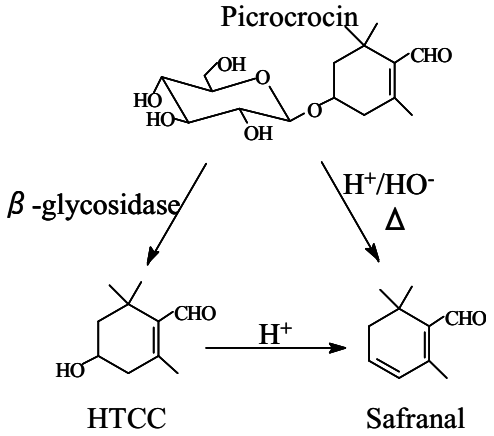

Fig. 10 Diagram of the chemical or enzymatic formation of safranal from picrocrocin. (Based on Himeno and Sano 1987).

of picrocrocin content with the dehydration temperature has been previously described for higher temperatures used in the Spanish dehydration process (Carmona *et al.* 2005), revealing that picrocrocin content increases up to 70°C and then decreases. It is worthy to mention that this outcome is in disagreement with the accepted theory that during the dehydration procedure, picrocrocin, the supposed precursor of safranal and other volatiles, is transformed in order to generate these compounds, thus reducing its content.

There is some evidence that high temperature procedures would promote the production of compounds responsible for flavor and aroma. Results from different authors employing drying conditions of 80°C for 30 min in an oven (Loskutov *et al.* 2000), presumably without air circulation, 110°C for 2 min (Pardo *et al.* 2002; Carmona *et al.* 2006b) and 70°C for 30 min with a strong air flow (Loskutov *et al.* 2000) support the idea that during the dehydration stage, important physical and chemical changes take place that are not explained. The new hypothesis proposed by Carmona *et al.* (2006d) for the generation of the compounds responsible for flavor and aroma of saffron is presented in **Fig. 11**. Aroma formation would take place from crocetin esters by the action of a carotenase. In this way it is easy to explain why odorous C9 and C10 compounds are found in one plant and not in gardenia. The presence of an enzyme could be responsible for the difference. This hypothesis is compatible with existing knowledge on the formation of six-member rings in carotenoid linear ends. Besides being simple, it complies with the principle of metabolic economy, a single route that can progress forward when given additional enzymes. This hypothesis is coherent with the disappearance of crocetin esters found by Himeno and Sano during anthesis (1987) and with the other relevant results found. During stigma development, crocetin esters and picrocrocin would maintain a more or less constant proportion (Himeno and Sano 1987) since HTCC, found in very small amounts at this stage, would be glycosylated to picrocrocin. This mechanism, customarily used by plants to accumulate metabolites in large quantities to avoid degradation or cell damage, would prevent their oxidation. At this moment the safranal content is negligible (**Fig. 12A**).

At the time of anthesis and during the long dehydration procedures at low temperature, specific or unspecific glycosydases would act on picrocrocin (Lozano *et al.* 1999), changing it again into HTCC. Its concentration would increase greatly, not only through the transformation of picrocrocin, but also because carotenase would actively work on crocetin esters (**Fig. 12B**). Raina *et al.* (1996) emphasized that temperatures lower than 35-45°C required too long a drying period, resulting in excessive enzymatic degradation of crocetin esters.

When non enzymatic cleavage is possible for the low water activity after dehydration or due to the high temperature reached during the dehydration procedure, the crocetin esters, more labile compounds than picrocrocin, could convert directly into HTCC and safranal without passing through picrocrocin generation (**Fig. 12C**), permitting at the same time an increment in picrocrocin content (Loskutov *et al.* 2000; Pardo *et al.* 2002).

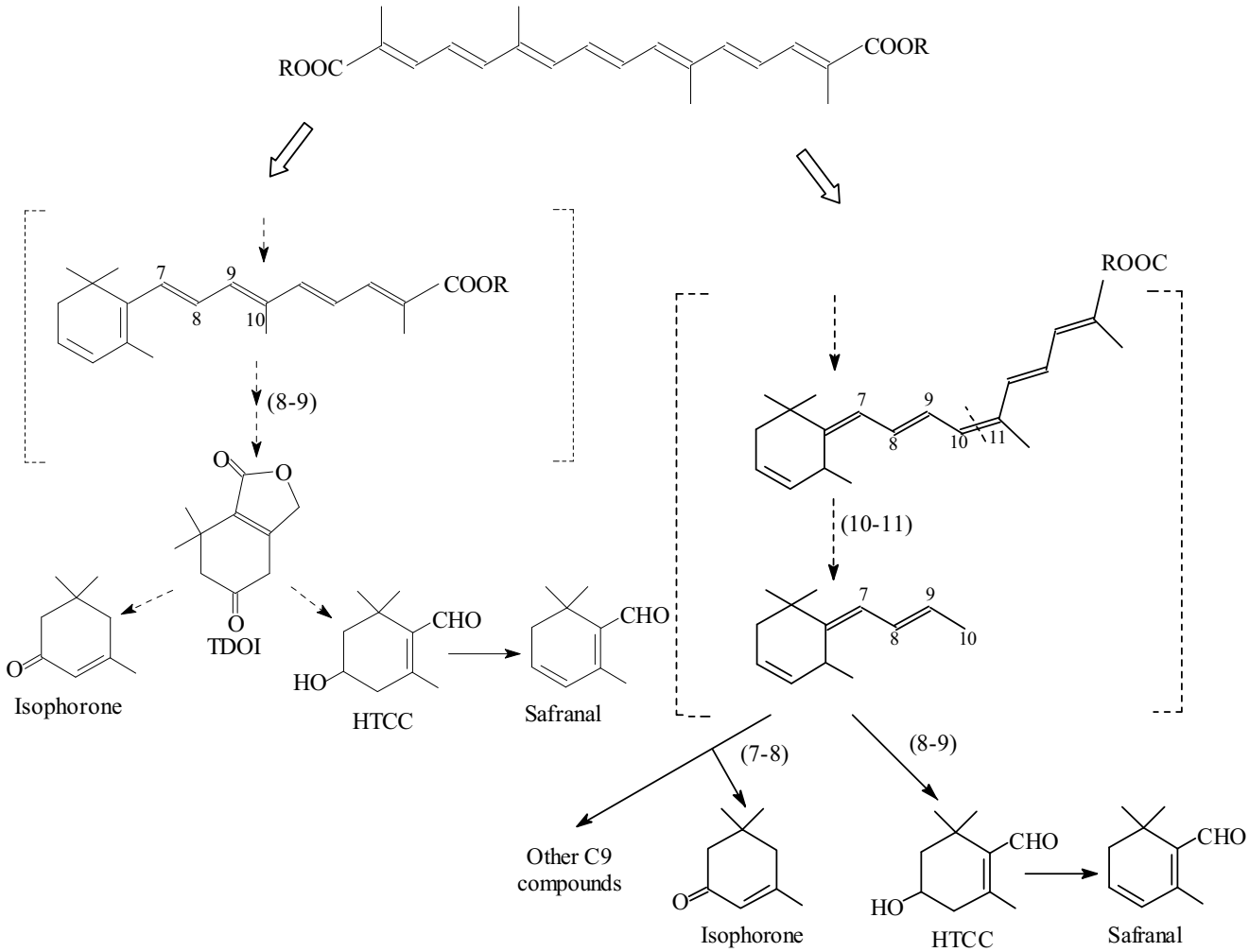

Fig. 11 New hypothesis for aroma formation from *Crocus sativus* L. crocetin esters. (Adapted from Carmona *et al.* 2006d).

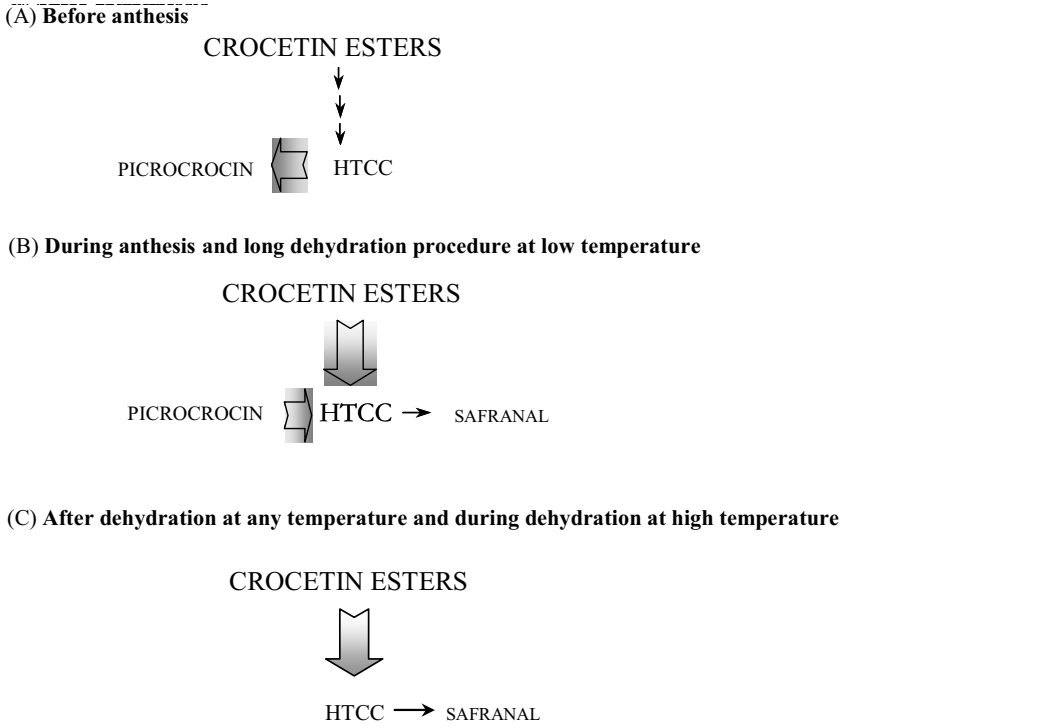

Fig. 12 Current hypothesis proposed for picrocrocin generation and conversion at different saffron stages. (Adapted from Carmona *et al.* 2006d).

Sensory perception

Only a few studies deal with saffron taste (Sarma *et al.* 1991; Narashimhan *et al.* 1992; Raina *et al.* 1996; Pardo *et al.* 2002) and, in particular, with picrocrocin and bitter taste. Sarma *et al.* (1991) made the first report on the sensory analysis of saffron produced through tissue cultures of *Crocus sativus*. In this study, stigma-like structures were produced in tissue cultures (TC stigmas) from the ovary explants of *C. sativus* on MS medium. Crocin and picrocrocin were found to be 6 to 11 times lower in TC stigmas than in the natural stigmas. The saffron obtained from tissue cultures was subjected to sensory analysis and compared with the data obtained from chemical analysis. The sensory data indicated that the saffron pigments produced in tissue cultures were one tenth those of natural stigmas. Sensory profile tests showed that the tissue culture saffron was low in floral, spicy and fatty characteristics as compared to saffron obtained from flowers.

Narasimhan *et al.* (1992) reported the assessment of the recognition threshold concentration of flavor components, the intensity of flavor attributes and the establishment of the dose-response relationships over a wide range of stimulus concentration. In this paper, the mean scores of four different saffron samples were provided. Samples A (the select grade) and B (lower grade) came from Kashmir, having a flavor threshold of 1.25 and 1.67 mg 100 mL^{-1}, respectively. Sample C, a commercial saffron, had a threshold of 2.50 mg 100 mL^{-1}, whereas for sample D, a saffron grown *in vitro*, it was 20 mg 100 mL^{-1}. Thus, the threshold of saffron flavor proved an important indicator of its strength (concentration), as well as the maturity of the sample or the part of the plant.

In addition, Raina *et al.* (1996) studied the effect of post-harvest processing on the flavor of saffron, and observed that a prolonged storage time affected the pigments and flavor concentration to a great extent, but proper storage (with 5% moisture) and packaging in a polystyrene box wrapped with MXXT cellophane reduced saffron deterioration and increased the shelf-life of the product.

In the same field are the sensory analyses carried out by Pardo *et al.* (2002), who developed affective (102 consumer judges) and discriminatory (15 expert judges) tests to determine the effect on the human perception of saffron samples dehydrated in different ways. The results (**Table 2**) showed that, as regards color, consumer judges preferred the samples dehydrated with hot-air (DHA) that were brightest and showed significant difference between toasted and dehydrated samples at room temperature (DRT). With respect to the sensory aroma parameters, consumer judges preferred DHA and toasted samples. As for the flavor parameters, significant differences between the samples were not found in the affective judges, whereas the expert judges found DHA and toasted samples more bitter than DRT ones. In all cases, saffron dehydrated at room temperature was the worst rated as it had less color, flavor and aroma.

Recently, Sánchez *et al.* (2009) carried out a study on sensory analysis of picrocrocin, previously isolated and purified (96%), to evaluate saffron's bitterness. In this study, the taste detection threshold of picrocrocin in aqueous extract was determined according to ISO 4120 (2004). The picrocrocin taste detection threshold was set at 10 mg L^{-1}. Although further research on saffron bitterness is necessary, the taste detection threshold of picrocrocin adds crucial information to take advantage of saffron's taste potential and optimize its usage in food.

Table 2 Sum of the scores given for the sensory parameters evaluated by a discriminatory test (Adapted from Pardo *et al.* 2002).

Color		Aroma	Bitterness
Red tone	Brightness		
15a	17a	26a	18a
31a	28a	59c	50b
60b	57b	43b	58b
59b	56b	36b	47b
60b	67c	61c	52b

DRT: dehydrated at room temperature; DHA: dehydrated with hot-air.
Values followed by different letters within a column are significantly different at $p<0.05$ (Duncan´s test).

CONCLUSION

Knowledge of picrocrocin is gradually becoming more important in the international trade of this spice. On one hand, consumers begin to demand a more broadly-focused saffron quality which is not based solely on color. This consumer demand has led to more accurate methods to determine picrocrocin content in an easy and reliable way. These methods have established that picrocrocin content is directly related to the handling of the spice, both during the dehydration process and storage time. From these studies, it was learned that it is much more resistant to heat treatment and degradation over time than at first thought. For this reason, it is quite possible that picrocrocin is not the only or the major precursor of saffron volatile generation, as was thought until now.

Picrocrocin is an excellent marker of saffron purity against its sophisticated adulteration when *Gardenia jasminoides* is used to increase its color, since picrocrocin is present only in the saffron spice.

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