A New Look at Saffron: Mistaken Beliefs

Manuel Carmona and Gonzalo L. Alonso
Cátedra de Química Agrícola, E.T.S. Ingenieros Agrónomos
Campus Universitario s/n, E-02071 Albacete
Spain

Abstract
   In this paper are reviewed several believes about different saffron aspects, starting with most popular ones that are complete false, and continuing with accepted hypothesis such as the role that picrocrocin plays in saffron taste and in safranal and aroma generation. As well as the implications of these findings against the accepted theories about crocin biosynthesis from zeaxanthin.

INTRODUCTION

   While the knowledge about saffron chemical content and its aroma composition are well established, several other aspects around this spice remain unknown. There are several accepted hypothesis about different aspects of saffron such as crocins biosynthetic pathway, postharvesting changes, carotenoid degradation and aroma generation that start to be doubtful. To clarify such hypothesis scientific results are necessary. But there are other most popular false believes that are easy to demonstrate that they are mistaken.

SAFFRON IS EXPENSIVE

   The saffron consumption in Spain is quite low if it is considered that in the past it has been the higher saffron country producer or that even now, it maintains an important role in the world saffron commerce. There are worldwide reasons to explain this situation and others based in particular characteristics. It is clear that saffron is a luxury commodity, even in some Asiatic cultures where it is impossible to understand food without spices. As marketing strategies, some ones present saffron as something exclusive using its historical reputation to be more expensive than gold. But it doesn't look the best way to enhance saffron consumption. Probably, it is less the people who are attracted to saffron because it is something special and restricted, than the ones that do not consider its use only because is expensive. Because it is false that saffron is expensive, at least from our European mentality with a high life level. In our country it is possible to buy 1 gram of saffron from the Protected Origin Denomination "Azafrán de La Mancha" between 3 and 6 euros depending where is bought. The price at origin of Spanish saffron is between 3 and 4 times more expensive than saffron produced in Iran, the first producer at this moment. An even taking in account this fact, nobody can say that Spanish saffron is expensive as to prepare a typical Spanish rice dish for four people, paella; only 125 mg of saffron are needed. Actually almost all kind of paella includes seafood in important quantity, so the added cost due to saffron is ridiculous.

ARTIFICIAL COLORANTS ARE MORE POWERFUL THAN NATURAL ONES

   Recent history has contributed to the belief that saffron is expensive in Spain. The Spanish apogees production coincided with the development of food colorants around the sixties. The difficult economical situation during the dictatorial period made that producers found better profits using artificial colorants for their foodstuffs and exporting their own saffron production. The historical use of tartrazine instead of saffron it has created the false believe that more amount of saffron is needed to obtain the same colour on foods. It is well known, that good quality saffron has a colouring strength between 210 and 260 units, while commercial tartrazine, 15 % rich, is between 65 and 75 units of colouring strength. Then, it is needed 3 or 4 times more of artificial colorant to get the same result. But most consumers have the idea that artificial colorants are more powerful than natural ones. The fact of the matter is that norbixin, curcumin and betanin, three very usual food pigments from plant origin, are more intense than some commonly used

synthetic colours as sunset yellow, tartrazine or amaranth. Their absorptivities at their maximum absorption wavelength are respectively 2870, 1607, 1120 for pigments and 551, 527 and 438 for synthetic dyes (Henry, 1997). Without any doubt, synthetic compounds are cheaper, more stable and it is easier to mix them looking for a special shade, but it doesn't mean necessary that they are more powerful.

AS ANTIQUE SPICE IS WELL KNOWN

But sometimes, even using good saffron, the consumer do not get the colour expected, because people do not know how to use it properly. Many people add it just at the end of the cooking without any previous grounding or extracting process. Consumer information about saffron is quite reduce and practically non-existent in relation to quality assessment. They are not aware that some companies include old saffron samples or even worst, that it is adulterated to get higher economical benefits.

The world market starts to show difficulties to absorb the increasing Iranian production. But on the paper, the worldwide production (200 tons) it does not look so high, at least if the companies makes special efforts on marketing purposes to teach consumers what is saffron and how to use it. Special emphasis may be carried out on explaining its healthy benefits of avoiding synthetic colorants. For tartrazine case, is well established the problems that it may produces, reason why its use is limited or forbidden in some European countries. Also it is important to redefine and to adapt the international quality control normative to give consumers a useful tool to distinguish between saffron qualities. Because, although the ISO/TS 3632 (2003) is not of obligatory fulfilment, the consumers should know about its existence. Once the consumer would be convinced of its importance, their exigencies could enhance the saffron quality in the market.

THE SAFFRON QUALITY IS WELL ESTABLISHED BY THE QUALITY CONTROL NORMATIVE

The first requirement that any food quality control normative has to complain is to assure product truthfulness and purity. An important contribution has been carried out with the introduction of microscopy and HPLC analysis in the recently appeared ISO/TS 3632 (2003). But there are still important gaps in the application at international level of this or any other normative. For example, the long best-before date fixed for this spice do not let to consumers to know the real quality of the saffron bought. In addition, saffron is traditionally packed in polystyrene or glass containers where it looses its properties very quickly because the compounds responsible for colour, the carotenoids, are light sensitive. A discussion at international level should be maintained to redirect consumers ideas about finding saffron in a non-transparent containers and giving more importance to the quality category labelled. Studies should be conducted to determine correct best-before date, the time that any sample maintains the category fixed during storing. Another relevant point, it is that none of the existing normative resolves the aroma and bitterness measurements. In some countries as India or Sweden, these attributes are even more important than colouring strength.

It is well known that the UV-Vis technique is not appropriated to determine the aromatic strength. The determination is carried out in an aqueous extract, but safranal and other volatile compounds are poorly water-soluble. Also at the wavelength chosen for this determination (330 nm), there are other compounds such as *cis*-crocins that are absorb as well (Tarantilis et al., 1995).

For saffron taste, something different happens, even when flavonoids and ciclohexane derivates absorb at the wavelength of 250 nm, their influence can be considered relatively small in comparison to picrocrocin. But in this case, there aren't studies that relate the absorbance at 250 nm and the taste that saffron imparts to food. From the scientific point of view, all these problems have already overcame with the use of gas chromatography-mass spectrometry for volatiles determination and the HPLC-mass spectrometry to detect and identify picrocrocin and related glycoside compounds. Techniques that are not used by saffron companies because are expensive and trained

personnel are required. An important effort has to be done by researcher in this field to adapt cheaper and easier techniques for saffron analysis.

TRADITIONAL DEHYDRATION PROCESS IN LA MANCHA TAKES PLACE AT 35º C DURING 30 MINUTES

Although there is not an economical feasible technique to measure saffron organoleptic properties, there is a complete agreement between authors about the origin of them, the dehydration process (Raina et al., 1996; Winterhalter y Straubinger, 2000; Ordoui y Tsimidou, 2004). During decades the Spanish saffron produced in La Mancha has been considered the highest quality commercial level for its aroma and taste. Saffron from others countries arise similar colouring strength but do not develop the organoleptic properties that La Mancha ones. Traditionally the explanation of trade companies has been that the special characteristics of the dehydration process used in this Spanish region give to the saffron its final attributes. But checking the bibliography data (Pérez, 1995), the divergences with other dehydration process are not so significant.

Making it easier, there are two modus operandi to carry out saffron dehydration in terms of temperature. One method consists of spreading the stigma over large areas and drying them at room temperature in the sunlight or in air-ventilated conditions. This is the method employed in Iran, India and Morocco (Sampathu et al., 1984; Nauriyal et al., 1997; Hassnain, 1998; Ait-Oubahou and El-Otmani, 1999). The other drying process is carried out at higher temperatures using any kind of heating source, and is the procedure used in Spain, Greece and Italy. In Italy, the drying process is carried out by spreading the stigmas in a sieve placed about 20 cm above live oak-wood charcoal (Tammaro, 1999). In Greece, the fresh stigmas and stamens are spread out on a thin layer trays (4-5 mm) of 40 x 50 cm with a silk cloth bottom. Such trays are piled on frames with shelves 25-30 cm apart. During the first hours of the process, the room temperature is maintained at 20 ºC and then increased to 30-35 ºC. The dehydration process is finished when a moisture content of 10-11% is achieved, generally after 12 hours (Goliaris, 1999).

The bibliography that explains how saffron is dehydrated in Spain, by the process called "toasted" is both scarce and inexplicit. According to Pérez (1995), the process is finished when the sample has lost between 85 and 95 % of its moisture, after being gently dried at 35 ºC. The process consists in spreading a thin layer of stigmas (2 cm) on a sieve with a silk bottom. The sieve is placed above the heating source. In the Castilla-La Mancha region, the heating source most commonly used is a gas cooker, followed by live vineshoot charcoal, and in a lesser extent an electric coil (Alonso et al., 1998). After being heated for 10 to 15 minutes, the stigmas are turned over and the sieve is again placed above the heating source.

From the data obtained, it is difficult to find reasons to justify the different organoleptic properties between the different saffron production countries. It is easy to postulate differences between the samples dehydrated in sunlight or in shade, as photochemical degradation or isomerization of the carotenoids responsible for saffron colour (Speranza et al., 1984; Basker, 1999) may take place. It is more difficult to find differences between similar dehydration processes, for example between Spain and Greece where the process takes place around 35-40 ºC, as mentioned in the bibliography.

Looking for those differences with other dehydration process, a suitable mechanism to measure the temperature and time consuming during La Mancha traditional dehydration process was tested (Carmona et al., 2004a) and unexpected results were obtained. When different heating sources as vine-shoot charcoal or gas cooker heating were used, saffron reached temperatures higher than 100º C. These temperatures let to finish the process in a short time, around 30 minutes. In the same work it was demonstrated that it is so important the temperature achieved as the time consumed by the modus operandi. It looks crucial the temperature reached during the first minutes as saffron colouring strength could be affected, probably due to the significant changes in the structural properties of the material during water removal. In the early stages of the dehydration process, the cellular tissues are elastic enough to shrink into the space left by

the evaporated moisture and volatile compounds. As the process proceeds, structural changes in the cellular tissues result in a more rigid network (Krokida and Maroulis, 2000). But if the process takes place quickly, the intracellular interstices are not filled and the vegetable material results in a more porous material affecting the quality of the final product (Achanta and Okos, 2000). The methodology used nowadays to determine saffron quality in terms of colouring strength is based on a water suspension for 1 hour (ISO/TS 3632, 2003), time that is not enough to exhaust the colour pigments in the vegetable material. More porous saffron may have its pigments more readily soluble in water, resulting in a better colouring strength. Anyway in these trials the samples with the best colouring strength were those ones dehydrated at higher temperature and less time, the same results than the obtained in 1994 by Morimoto and co-workers.

OPTIMUM TEMPERATURE TO OBTAIN BEST SAFFRON IS BETWEEN 35 AND 45º C

After knowing the temperature reached during traditional "toasted" it is necessary to reconsider another accepted hypothesis. Several authors propose that picrocrocin, responsible of saffron bitterness, is converted to safranal during dehydratation process (Figure 1) (Himeno y Sano, 1987; Raina et al., 1996; Winterhalter y Straubinger, 2000). The high temperature during Mancha procedure, could explain the important aromatic content. But it cannot justify that picrocrocin content of the saffron dehydrated in that way is also important. It is supposed that so high temperatures should produce the hydrolysis of picrocrocin to generate safranal. To check this possibility, sensorial analysis trials were developed (Pardo et al., 2002). A homogeneous batch of fresh stigmas were dehydrated following three different dehydratation process: at room temperature, as traditionally in Castilla-La Mancha and using hot air at different temperatures. The samples generated were submitted to the analysis of 102 consumers and 15 expert judges and also to ISO 3632 (1993). The results obtained are summarized in Table 1.

Statistically the samples with higher colouring strength were those ones dehydrated by the traditional process. For aroma assays, it wasn't observed a process influence, the most aromatic samples were those dehydrated at higher temperatures. In bitterness case, the differences where observed between the samples dehydrated at room temperature and at high temperature. Curiously more temperature meant more bitterness, just the opposite of proposed by theory.

The use of sensorial analysis let to define the quality control concept in another sense. Normally the quality is related to the corresponding normative but employing sensorial analysis it can be used a more simple conception. If saffron is valorised by its colouring strength, aroma and taste, it will be better saffron that one with these attributes present in more amount. With this point of view the best saffron was the one dehydrated at higher temperature. The results were unambiguous, although the studies were conducted by judges from only one country that could be used to consume determined kind of saffron. Larger studies should be done with this idea to know which type of saffron demand every market. Because even when there are some sensorial analysis studies (Narasimhan et al., 1992; Raina et al., 1996), there is not any designed to analyse the same sample by judges from different countries that would let to know the most valuated characteristics for each one. Only trade companies know the profile request for each market but it should be established in detail applying scientific tools.

The companies use to commerce with Spanish saffron also remark the stability of the colouring strength of this saffron in comparison with others. It is an important parameter, as it was already mentioned, the long best-before date of this spice makes important the colour disappearing rate. The trade companies also think that the particular dehydratation process has something to do with it. A possible explanation could be the arguments offered by Kato (2000) when they studied the light stability of the different crocins present in *Gardenia jasminoides*, which are the same than in *Crocus sativus*. They found as more stable those ones that contained gentibiose in one or both extremes (Figure 2). More sensible were shown the crocins that contains only glucose with a similar

376

behaviour than crocetin. When it was studied the evolution of trans-crocin 4 (digentibiosilcrocetin ester) in relation with the increasing temperature during dehydration process, traditional one and using hot air, surprisingly the results were independent of the kind of process, in all cases trans-crocin 4 content was increased with the temperature (Figure 3) (Carmona et al., 2004b). This could explain the mayor resistance of Spanish saffron to the degradation during storage, upper amount of crocin esterified by gentibiose moieties it would mean superior stability.

In summary, the temperature that can be reached to get the maximum saffron organoleptic properties is much higher than the proposed until this moment. Something confirmed by the historical reputation of La Mancha saffron now that it is know the temperatures used in this production area. In Mancha saffron as well as in saffron dehydrated with hot air a high aromatic strength do not means a bitterness reduction.

PICROCROCIN IS RESPONSIBLE OF SAFFRON BITTERNESS

Everyone agrees that picrocrocin is responsible for saffron bitterness, but is not clear the relation between picrocrocin disappearing and aroma production. Since the thirties´ when picrocrocin was isolated for first time and taste it as bitter, everybody defend that picrocrocin is responsible of saffron bitterness in each paper or review without any other argument. It is a perfect proposal, three main compounds for the three organoleptic properties: crocin for colour, safranal for aroma and picrocrocin for taste. In case of picrocrocin probably is true for the important presence of this compound in comparison with others. But nobody has supported this idea with scientific results. In fact this compound is not the only one present in saffron with bitter taste. In Figure 4a it can be seen the first minutes of a typical saffron HPLC chromatogram, where the main peak corresponds to picrocrocin, identified by its UV-Vis (Figure 4b) and it mass fragmentation pattern (Figure 4c).

Using column chromatography is possible to fractionate the extract getting an enriched fraction where no picrocrocin is found (Figure 5a). This fraction also taste bitter. There are two groups of compounds presents in such extract. The most important ones are those with UV-Vis spectra similar to the Figure 5b, corresponding to flavonoids (Figure 5c) (Straubinger et al., 1997). These compounds are included in a very extended family of natural product, where one of its usual properties is the bitterness. Also it appears some of the compounds originated by carotenoid degradation (Figure 6) (Straubinger et al. 1997, 1998a,b).

Until the moment that using sensorial analysis is established the real contribution of each compound or group of compounds to the saffron bitterness it should be avoided the sentence "picrocrocin is responsible of saffron bitterness". It should be changed by other supported by research results, "picrocrocin is bitter".

PICROCROCIN IS THE SAFRANAL STOCK

Coming back again over the supposed hypothesis of picrocrocin conversion into safranal, the study of compound evolution during storage could be interesting to clarify this point. It is well know that saffron aroma change during ageing from floral notes to pungent character, where the safranal contribution to the aroma is more important. It was thought that was due to the picrocrocin conversion to safranal during this period. To study this hypothesis an accelerated ageing process was simulated using increasing thermal treatment. A homogenous batch of dehydrated stigmas was divided in 5 portions that were introduced in an oven at 50º C. After two hours the first sample was removed from the oven and the temperature was elevated 20º C more. Two hours after the temperature was stable at 70º C, was extracted the second sample. The procedure was repeated several times until was generated a sample that was submitted two hours at 130º C and other two hours at each lower temperature. The results are shown in Figure 7. The picrocrocin evolution was decreasing as expected but surprisingly did not produce a safranal increment. It is necessary to remark that picrocrocin content is according to some authors more than 60 times safranal content (Lozano et al., 2000). For this reason the unexpected

results are remarkable. Only from certain temperature safranal generation was produced but without relation to picrocrocin disappearing. This performance cannot be explained by the accumulation as HTCC of the hydrolysed picrocrocin because also decrease its content. The conclusion obtained was that picrocrocin is not the safranal stock.

Even when the research results are clear, it is difficult to propose a hypothesis that goes against everything published during more than 60 years on this field. Although, nobody has presented definitely results in that sense over the spice. It is well described that from picrocrocin isolated and submitted to heat or enzymatic treatment it is obtained safranal. The doubtful question it is if this really happens on the stigmas whether they are fresh or when are already dehydrated. This is not the first time that controversial results about this point have been obtained. Other authors have found similar data but they gave it different explanation trying to justify the unique reasonable hypothesis at that moment. Himeno and Sano in 1987 studied the evolution of HTCC, safranal and crocin during the ripening stages of the stigmas. They found that only at final stage it was possible to detect safranal and HTCC. They justified the safranal appearance for the significant amount of picrocrocin that disappeared. The proportion with the previous picrocrocin content at other stages was important, but in absolute value the μg of picrocrocin that disappeared at this moment were more or less the same amount of crocin disappeared (Figure 8a). There was not relation between the 67 μg of picrocrocin disappeared, and the production of HTCC (0.60 μg) and safranal (0.049 μg). If the ratio over the fresh weight that each compound represents is taking into account, the results shown in Figure 8b are obtained. Now, the decreasing behaviour observed in Figure 8a, looks as a logical evolution during ripening after arising to the higher levels for crocin and picrocrocin around the stage V or VI. The negative evolution of the crocin accumulated is more pronounced than picrocrocin. Then, the conclusion from these results could be that safranal is produced from crocins as well, or from other compounds different from picrocrocin.

The same conclusion can be extracted from Loskutov et al. (2000) paper where safranal evolution was studied on a saffron extract. They demonstrated that safranal content increase as straight line when the solvent was ethanol/water but it was constant when the solvent was acetonitrile. The explanation offered by these authors was that acetonitrile extract safranal selectively and not picrocrocin. In this way, the conversion of picrocrocin into safranal during storage of the solution was impossible. On the other hand, this conversion takes place in an aqueous extract by means of some enzymatic activity. Both hypothesis look reasonable but it is more difficult to explain how is possible that safranal content increases between the 2 and 6 hours of analysis, when after 4 hours picrocrocin was undetectable (Figure 9). Also the low slope line give the idea that the conversion rate was also slow. But how this can be possible, if using the same argument as before, picrocrocin content is more than 60 times higher than safranal (Lozano et al., 2000). Its complete disappearance between 0 and 4 hours would have produced an enormous safranal production. Sometimes are justified some research results due to an unknown enzymatic activity, when it is impossible to find any other justification. But in this case, it doesn't look the most appropriate hypothesis. First of all, the saffron used by this authors was Spanish origin and knowing that saffron produce in Spain is submitted to temperatures higher than 100° C, it is difficult to propose any endogenous enzymatic activity after this process. Secondly, the environmental conditions (ethanol: water 80:20) chosen, are not the best as it is not the best medium for any enzymatic activity neither a microorganism growth that may bring an exogenous activity in such short period time. Really, it looks that a chemical degradation was taking place independently if picrocrocin is present or not. It suggests that safranal and other aromatic compounds may be produced in a different way, as it has been believed until now.

To confirm this idea it is also possible to check the paper of Lozano et al. (2000), which has already been referenced. These authors studied the optimal extraction conditions for using supercritical carbon dioxide to determine safranal and HTCC. Studying the temperature conditions they founded different behaviour for HTCC and safranal. While for HTCC, the temperature increment produces a slightly enhancement of

378

the extraction yield, for safranal a Tª over 90º C produced an inflexion point that could be related with a safranal generation. This results were confirmed when the influence of the density of supercritical carbon dioxide was studied. For the best extraction setting (0.4 g/ml), safranal content represented a 167 % of total content previously determined on alcoholic extraction by HPLC. The only possible explanation, according to the authors, was that new safranal was chemically synthesised from its saffron precursors, HTCC or picrocrocin. But the total HTCC content of the supercritical extract plus the residue was practically unchanged in relation to the original saffron sample, and no picrocrocin decrement could be detected. Once again, it was impossible to relay directly and without doubts that the disappearance of picrocrocin in the spice was the origin of safranal production.

But the implications of this proposal go further. If the way to produce safranal it is not the accepted one until this moment, also the hypothesis to produce picrocrocin and crocins from zeaxanthin (Figure 10) it starts to be suspicious.

ZEAXANTHIN IS THE CROCINS STARTING MATERIAL

The firmest support to the classic theory comes from a recent discovery of an enzyme called *CsZCD* (*C. sativus* zeaxanthin cleavage dioxygenase) (Bouvier y col., 2003). Such enzyme expressed in *Escherichia coli* and later purified, it has been able to convert zeaxanthin into crocetindialdehyde. This paper shows empirical evidences on the possible crocins biosynthesis pathway, although there are some points that need to be clarified in detail to accept its final conclusions. For example, the authors did no detect any zeaxanthin, the proposed precursor in *C. sativus* stigmas. There is no doubt that its presence may support such theory as it happen with other carotenoids. Recently the same research group found a group of enzymes responsible of bixin production, a C_{24} compound, by breaking the double bonds C(5,6) and C(5´,6´) in licopene (Bouvier et al., 2003b). Until that moment the hypothesis was accepted based on the presence of complementary compounds (Britton, 1998). Small amounts of licopene and C_{32} compounds that have one of the two end groups, supported this hypothesis (Mercadante et al., 1997). It is truth that the absence of zeaxanthin is not an obstacle, as it may have been quantitatively transformed by *CsZCD* action. This may be carried out easily by analysing saffron stigmas while its formation, as Himeno and Sano (1997) did, and that zeaxanthin disappearance is related to crocetindial together with the presence of the enzyme in the tissue.

The authors also submitted saffron flower to a stress dehydration process similar to the one that take place the second day after flowering. The perianth was removed from the other floral parts and left on a filter paper till half weight loss. In such conditions, the enzyme expression was increased considerably and it is here when the contradiction it appears. If during the flowering the presence of zeaxanthin was undetectable, then it has no sense that after such stress conditions the plant increases the gene expression to produce larger amount of enzyme if there is no substrate to work with.

But where the proposal seems to be weaker, is on relation to the enzyme specificity.

The authors affirmed that the enzyme was specific of zeaxanthin as "apparently" it was not possible to use violaxanthin as substrate in an experiment followed by thin layer chromatography. With the actual knowledge about carotenoids biosynthesis and related enzymes, it is necessary to be more precise. It is not enough to isolate C_{10} and C_{20} compounds that seem to be complementary and to find an enzyme capable of breaking a C_{40} compound. To assure that *CsZCD* is specific, several trials with many other substrates need to be carried out. The idea that the same enzyme in the carotenoid biosynthesis pathway can recognise and use as a substrate any carotenoid with the same partial structure is begin to be accepted (Britton, 1998). There are several examples in this direction. Rose flowers contain mostly C_{27}-alcohols but also C_{13} ethers, ketones and alcohols. The enzyme systems responsible for the catabolism of the apparent carotenoids are unknown. But it has been postulated that the cleavage reactions are assisted by a

carotene-9,10:9´,19´-dioxygenase that is not specific for any particular end group (Eugster and Märki-Fisher, 1991). Another interesting case, is about the cyanobacterium *Microcystis* sp., which liberates large amounts of β-cyclocitral by a caroteneoxygenase action (Jüttner and Höflacher, 1985). Such enzyme with an activity very similar to the one proposed for *CsZCD*, catalyses the specific cleavage of the C(7,8) and C(7´,8´) double bonds in β-carotene as well as in zeaxanthin, producing in the last case 3-hydroxy-β-cyclocitral. This enzyme has not have the proposed specificity for *CsZCD*. Everything said before does not hide the importance of such enzyme discovery, but it may be that the progenitor on which the enzyme acts in vivo is not zeaxanthin. It is necessary to characterize better this enzymatic activity to support the classic hypothesis of crocins biosynthesis.

When works of this research group about the dioxygenases of *Crocus sativus* and *Bixa orellana* are compared, something wrong appears, although it is not possible to say what without refutation experiments. It is difficult to understand why they follow the activities of the cloned enzymes by TLC, when in the same work they use HPLC-DAD and mass spectrometry. The TLC technique does not give useful information just the opposite, it offer contradictory results. In the first paper when zeaxanthin was transformed by *CsZCD* it appeared an aldehyde having a R_f value of 0,73 (retention factor). Zeaxanthin had a lower R_f, approximately 0,56 (calculated for us from the TLC plates presented in the paper). In the second article, when lycopene was converted by *BoLCD* (*Bixa orellana* lycopene cleavage dioxygenase) it appeared an aldehyde having a R_f value of 0,75. But in this case zeaxanthin had higher R_f than the aldehyde produced, approximately 0,93 (calculated for us from the TLC plates presented in the paper). The R_f is characteristic per each compound on each chromatographic system (mobile and stationary phase). As they used the same system in both papers something is wrong. There are two possible explanations, the compound use as zeaxanthin reference is not zeaxanthin in any of the two works, or against the author proposal, and the *BoLCD* enzyme transforms quantitatively zeaxanthin to produce a different compound with higher R_f. In this case other arguments would match as both dioxygenase with high relationship (97%) will be able to catalyse zeaxanthin transformation to give different final products. Then, also the idea of the specificity of this enzyme would disappear, if *BoLCD* can be involved in the conversion of zeaxanthin and lycopene, maybe *CsZCD* could convert lycopene as well.

While all this questions are not clarified by the authors, it is not acceptable any of their conclusion. With the deep work developed by this research group, probably one of pathways proposed for crocetindialdehyde or bixin aldehyde biosynthesis is true, but not both of them. It can said that it remains unknown the biosynthetic pathway of crocins in saffron.

CONCLUSION
While the knowledge about chemical composition of saffron is well established, others aspects as crocins biosynthetic pathway, postharvesting changes, carotenoid degradation and aroma generation are poor known. There are enough research results to suspect that several of the hypothesis accepted to explain these saffron aspect are mistaken.

Literature Cited
Achanta, S. and Okos, M.R. 2000. Quality changes during drying of food polymers. In: Mujumdar, A.S. (ed.). Drying Technology In Agriculture and Food Sciences. Science Pub. Inc., Plymouth, pp 133-148.
Alonso, G.L., Salinas, M.R., Sánchez-Fernández, M.A. and Garijo, J. 1998. Técnicas culturales, métodos de deshidratación y formas de conservación en la producción del azafrán en España [Culture techniques, dehydration methods and storage conditions in the production of saffron in Spain]. Agrícola Vergel 169: 357-370.
Ati-Oubahou, A. and El-Otmani, M. 1999. Saffron cultivation in Morocco. In: Negbi, M.

(ed.). Saffron. *Crocus sativus* L. Medicinal and Aromatic Plants. Industrial Profiles, Harwood Acad. Pub. Amsterdam, pp 87-94.

Basker, D. 1999. Saffron technology. In: Negbi, M. (ed.). Saffron. *Crocus sativus* L. Medicinal and Aromatic Plants. Industrial Profiles, Harwood Acad. Pub. Amsterdam, pp 95-101.

Bouvier, F., Suire, C., Muttener, J. and Camara, B. 2003a. Oxidative remodeling of chromoplasr carotenoids: Identification of the carotenoid dioxygenase CsCCD and CSZCD genes involved in crocus secondary metabolite biogenesis. Plant Cell 15: 47-62.

Bouvier, F., Dogbo, O., and Camara, B. 2003b. Biosynthesis of the food and cosmetic plant pigment Bixin (Annato). Science 300: 2089-2091.

Britton, G. 1998. Overview of carotenoids biosynthesis. In: Britton, G., Liaaen-Jensen, S., and Pfander, H. (eds). Carotenoids. Vol. 3. Biosynthesis and Metabolism. Birkhäuser Verlag, Switzerland, pp 13-147.

Carmona, M., Zalacain, A., Pardo, J.E., López, E., Alvarruiz, A. and Alonso, G.L. (2004a). Time-temperature effect on dehydration of saffron stigmas. Send for publication.

Carmona, M., Zalacain, A., Salinas, M.R. and Alonso, G.L. (2004b). Changes on saffron chemical composition during the dehydration and accelerated aging process. Send for publication.

Eugster, C.H. and Märki-Fischer, E. 1991. Angew. Chem. Int. Ed. Engl. 30: 654-672.

Goliaris, A.H., 1999. Saffron cultivation in Greece. In: Negbi, M. (ed.). Saffron. *Crocus sativus* L. Medicinal and Aromatic Plants. Industrial Profiles, Harwood Acad. Pub. Amsterdam, pp 73-87.

Hassnain, F.M. 1998. Saffron cultivation in Kashmir. Surinder Singh Sethi, Rima Publishing House, New Delhi, India.

Henry, B.S. 1997. Natural food colours. In: Hendry, G.A.F. and Houghton, J.D. (eds.), Natural Food Colorants. Blackie Acad., London, pp 40-79.

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

Jütner, F. and Höflatcher, B. 1985. Evidence of β-carotene 7,8 (7´,8´) oxygenase (β-ciclocitral, crocetindial generating) in *Mycrocystis*. Arch. Microbiol. 141: 337-343.

Kato, Y. (2000). Gardenia Yellow. In: Lauro, G. and Francis, J.K. (eds.). Natural Food Colorants. Marcel Dekker, New York, pp 87-96.

Krokida, M. and Maroulis, Z. 2000. Quality changes during drying of food materials. In: Mujumdar, A.S. (ed.). Drying Technology in Agriculture and Food Sciences. Science Pub Inc., Plymouth, pp 61-106.

Loskutov, A.V., Beninger, C.W., Hosfield, G.L. and Sink, K.C. 2000. Development of an improved procedure for extraction and quantification of safranal in stigmas of *Crocus sativus* L. using high performance liquid chromatography. Food Chem. 69: 87-95.

Lozano, P., Delgado, D., Gomez, D., Rubio, M. and Iborra, J.L. 2000. A non-destructive meted to determine the safranal content of saffron (*Crocus sativus* L.) by supercritical carbon dioxide extraction combined with high performance liquid chromatography and gas chromatography. J. Biochem. Bioph. Meth. 5: 367-378.

Mercadante, A.Z., Steck, A. and Pfander, H. 1997. Isolation and identification of new apocarotenoids from annato (*Bixa orellana*) seeds. J. Agric. Food Chem. 45: 1050-1054.

Morimoto, S., Umezaki, Y., Shouama, Y., Saito, H., Nishi, K. and Irino, N. 1994. Post-harvest degradation of carotenoids glucose esters in saffron. Planta Med. 60: 438-440.

Nauriyal, J.P., Gupta, R. and George, C.K. 1997. Saffron in India. Arecaunt Spices Bull. 8: 59-72.

Narasimhan, S., Chand, N. and Rajalakshmi D. 1992. Saffron quality evaluation by sensory profile and gas chromatography. J. Food Quality 15:303-314.

Ordoudi, E. and Tsimidou, M., 2004. Saffron quality: Effect of agricultural practices, processing and storage. In: Dris, R. (ed.). Harvest and Quality Evaluation of Food

Crop. Kluwe Acad. Pub., Dordrecht, The Netherlands (in press).

Pardo, J.E., Zalacain, A., Carmona, M., López, E., Alvarruiz, A. and Alonso, G.L. 2002. Influence of the type of dehydration process on the sensory properties of saffron spice. Ital. J. Food Sci. 14: 413-422.

Pérez, M. 1995. El azafrán. Historia, cultivo, comercio, gastronomía.[Saffron. History, cultivation, commerce, gastronomy]. Mundi-Prensa, Madrid, Spain.

Raina, B.L., Agarwal, S.G., Bhatia, A.K. and Gaur, G.S. 1996. Changes in pigments and volatiles of saffron (*Crocus sativus* L.) during processing and storage. J. Sci. Food Agric. 71: 27-32.

Sampathu, S.R., Shivashankar, S. and Lewis, Y.S. 1984. Saffron (*Crocus sativus* Linn.). Cultivation, processing, chemistry and standardization. CRC Critical Rev. Food Sci. Nut. 20: 123-157.

Speranza, G., Dadá, G., Manitto, P., Monti. D. and Gramatica, P. 1984. 13-cis crocin: a new crocinoid of saffron. Gazzeta Chim. Ital. 114: 189-192.

Straubinger, M., Jezussek, M., Waibel R. and Winterhalter, P. 1997. Novel glycosidic constituents from saffron. J. Agric. Food Chem. 45:1678-1681.

Straubinger, M., Bau, B., Eckestein, S., Fink, M. and Winterhalter, P. 1998. Identification of novel glycosidic aroma precursors in saffron (Crocus sativus L.). J. Agric. Food Chem. 46: 3238-3242.

Straubinger, M., Bau, B., Eckestein, S., Jezussek, M. and Winterhalter, P. 1998. Isolation of new saffron constituents using counter-current chromatography. In: Schreier, P., Herderich, M., Humpf, H.U. and Schwab, W. (eds.). Natural Product Analysis. Vieweg: Braunschweig/Wiesbaden, Germany, pp 27-34.

Tammaro, F. 1999. Saffron (*Crocus sativus* L.) in Italy. In: Negbi, M. (ed.). Saffron. *Crocus sativus* L. Medicinal and Aromatic Plants. Industrial Profiles, Harwood Acad. Pub. Amsterdam, pp 53-61.

Tarantilis, P.A., Tsoupras, G. and Polissiou, M.G. 1995. 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 699: 107-118.

Winterhalter, P. and Straubinger, RM. 2000. Saffron. Renewed interest in an ancient spice. Food Rev. Int.16: 39-59.

Tables

Table 1. Colouring strength, aroma and bitterness of the same sample differently dehydrated.

Saffron Samples	Colouring Strength [1]	Aroma [2]	Bitterness [3]
Room Temperature	220.46 [a]	26 [a]	18 [a]
Toasted	247.96 [c]	59 [c]	50 [b]
Hot air 70 ºC	225.51 [b]	43 [b]	58 [b]
Hot air 90 ºC	226.44 [b]	36 [b]	47 [b]
Hot air 110 ºC	227.41 [b]	61 [c]	52 [b]

(1) Colouring strength measured by ISO 3632 (1993)

(2)(3) Aroma and bitterness measured by sensorial analysis (Pardo et al., 2002)

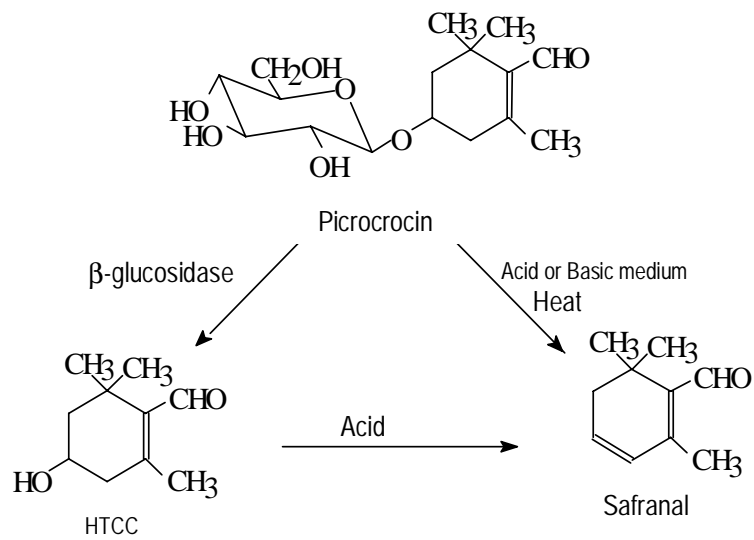Different letters between rows indicate significant differences at 0.05% level.

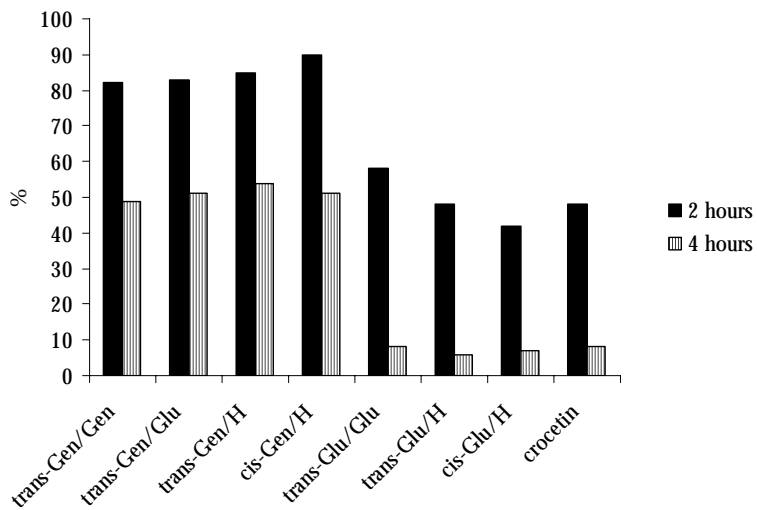Fig. 1. Mechanism of safranal generation from picrocrocin proposed by several authors.

Fig. 2. Residual content (%) of different gardenia crocetin derivates after submitting them to a light degradation process (Kato, 2000).
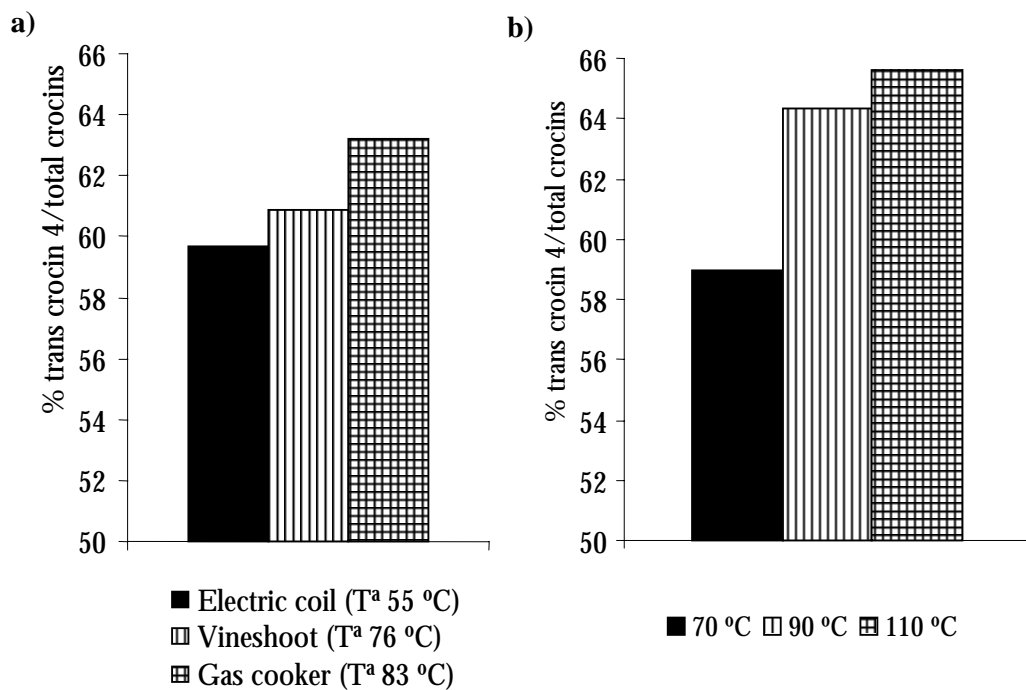
a)

■ Electric coil (Tª 55 ºC)
⬚ Vineshoot (Tª 76 ºC)
⊞ Gas cooker (Tª 83 ºC)

b)

■ 70 ºC ⬚ 90 ºC ⊞ 110 ºC

Fig. 3. Content of trans-crocin 4 depending on the mean temperature of the dehydration process. a) Using different traditional heating sources in Castilla-La Mancha. B) Using hot air.
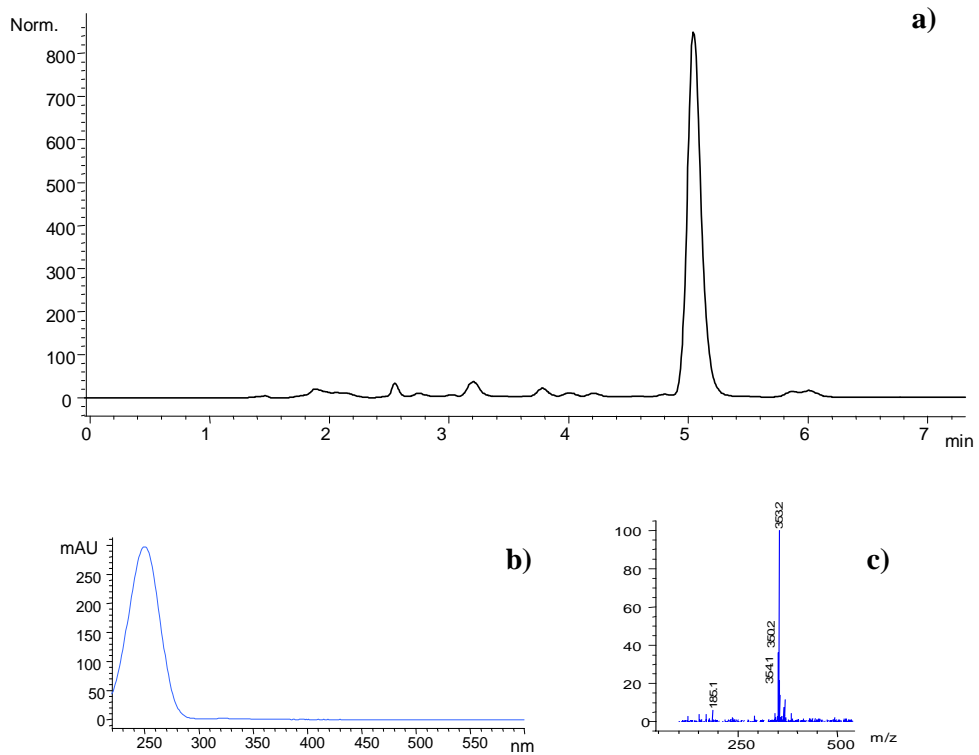
Fig. 4. a) First eluted minutes of a saffron aqueous extract chromatogram with DAD detection set at 257 nm. b) UV-Vis spectra of the major peak in a) which corresponds to picrocrocin. c) Mass fragmentation pattern of the major peak in a) which corresponds to picrocrocin

a)

b)

c)

Kaempferol 7-O-glucopiranosil-3-O-sophoroside

Kaempferol 7-O-sophoroside
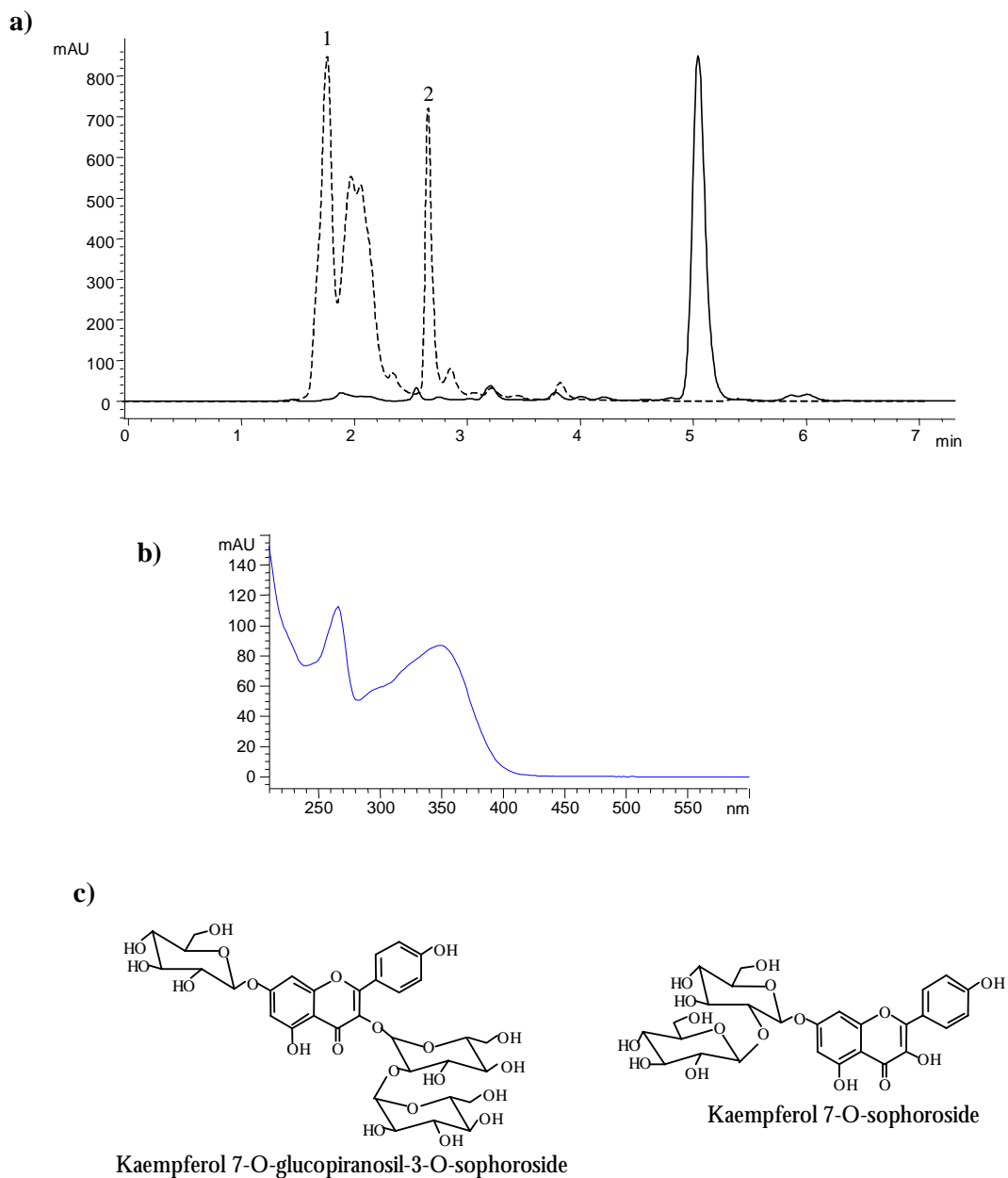
Fig. 5. a) Overlaid HPLC chromatograms of an aqueous saffron extract (—) and a fraction obtained by column chromatography (----) from the same extract. b) UV-Vis spectra of peaks 1 and 2 (from a) which correspond to flavonoid compounds. c) Flavonoid structures isolated and identified in saffron extracts by Straubinger et a. 1997.
b) Flavonoid UV-Vis spectra
c) Sevaral flavonids structures
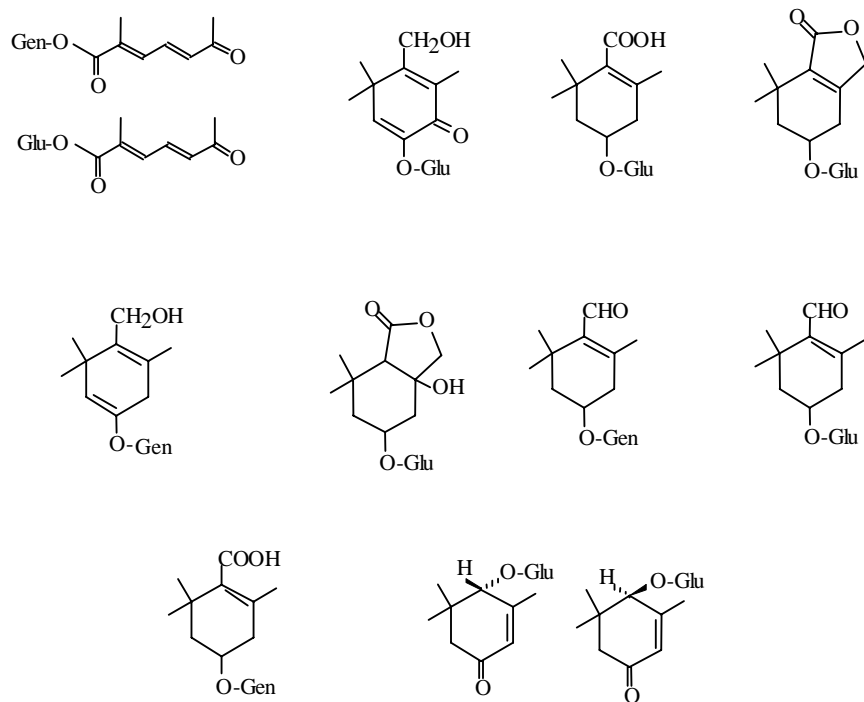
Fig. 6. Compounds generated by carotenoid degradation identified by Winterhalter group (Straubinguer et al., 1997, 1998).
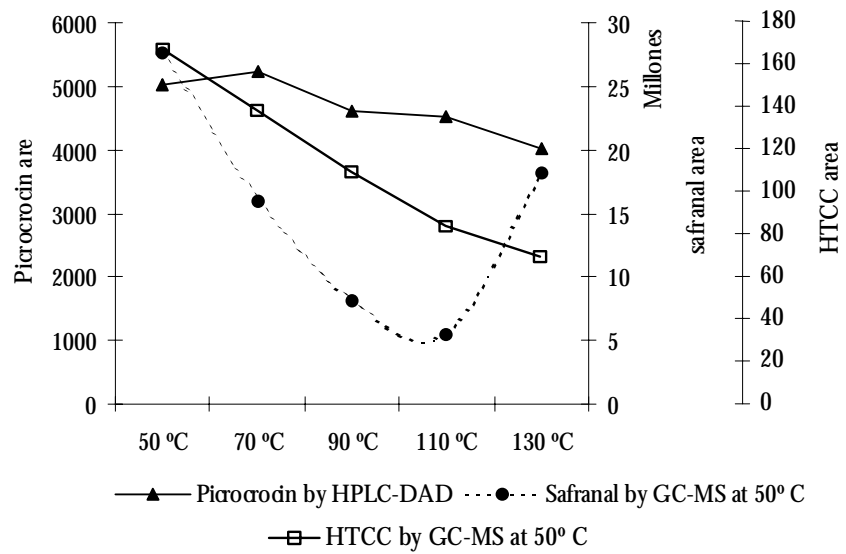
Fig. 7. Evolution of picrocrocin, HTCC and safranal content in a Spanish saffron
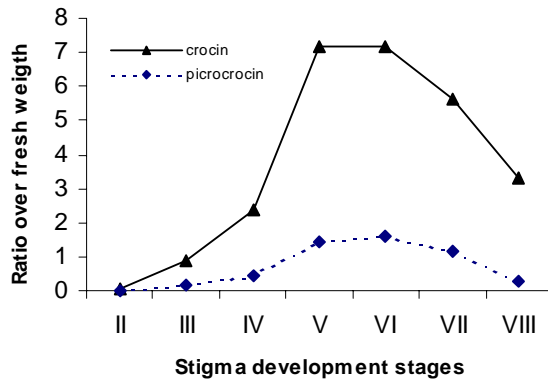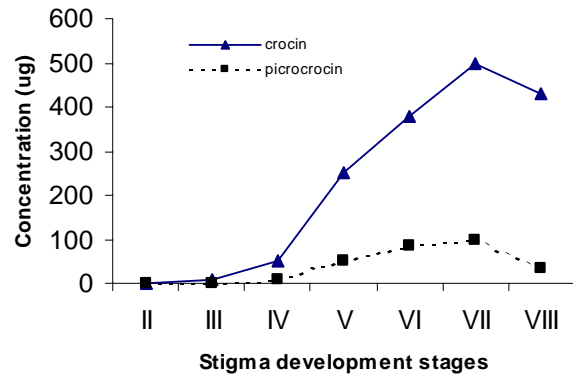sample submitted to an accelerated aging by thermal treatment.

Fig. 8. Graphs generated from the results obtained by Himeno and Sano (1987) when crocin, picrocrocin, HTCC and safranal content was studied during stigma development. a) Results obtained by representing concentration (µg) of crocin and picrocrocin against stigma development stage. b) Ratio obtained by representing concentration (µg) of crocin and picrocrocin against fresh stigma weight.
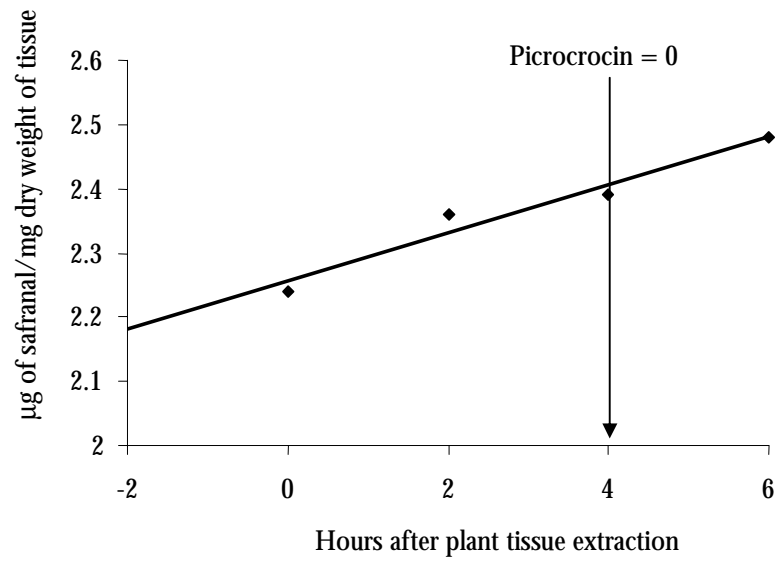
Fig. 9. Evolution of safranal content in a saffron ethanol/water extract (adapted from
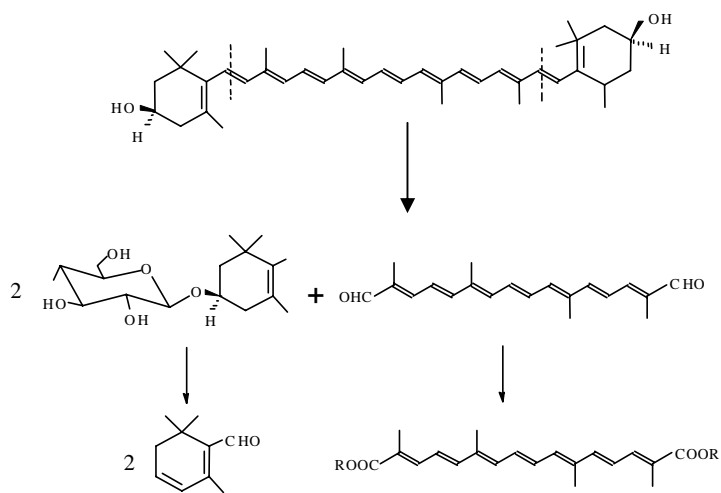   Loskutov et al., 2000).

Fig. 10. Accepted hypothesis for crocins, picrocrocin and safranal biosynthesis by oxidative cleavage at both extremes of zeaxanthin.