Improved Conditions to Extract Exogenous Yellow Water-Soluble Colorants Added to Saffron Avoiding Crocetin Esters Interferences

Amaya Zalacaín, Inmaculada Blázquez, Eva M. Díaz-Plaza, Manuel Carmona and Gonzalo L. Alonso
E.T.S.I.A., Universidad de Castilla-La Mancha,
Campus Universitario s/n, E-02071 Albacete
Spain

Keywords: adulteration, annato, hydrolysis, naphtol yellow, quinoleine yellow, sunset yellow, tartracine

Abstract

Saffron samples from 4 different production countries were extracted for 10 minutes. The extract was then submitted to different conditions of time (0, 5, 10, 15 min), temperature (RT, 60 and 90 ºC) and pH (2.0, 1.0, 0.1). Different solutions of yellow colorants (naphtol yellow, quinoleine yellow, annato, tartracine and sunset yellow) were assayed at these conditions. The degradation of crocetin esters and colorants was followed by UV-Vis spectrometry. At pH extract of 0.1 and 10 min at 90 ºC, the crocetin esters disappear and no colorants were affected.

INTRODUCTION

Saffron, dry stigmas of *Crocus sativus* L., is mainly used as a spice and colorant. The three attributes that saffron proportionate to foodstuffs are colour, aroma and taste. The most appreciated one is its colour. The international trade market by ISO 3632 normative since 1993, establish four categories depending on the colouring strength, calculated from UV-Vis measurements at 440 nm in aqueous extracts of this spice. Such measurements are related with the total crocins content. Crocins are all glycosides of the carotenoid *trans*-crocetin which is a dicarboxylic acid carotenoid. They are soluble in water, which implicates a great advantage when being used as colorant in the food industry regards to the majority of the carotenoids (Alonso et al., 1998).

Dyes are used to supplements and enhance natural colours destroyed during processing or storage, and substantially increase the appeal and acceptability of foodstuffs. However, some synthetic colorants may be toxic, especially consumed in large amounts. Therefore, safety data for every synthetic colorant food additive have been repeatedly determined and evaluated by the Food and Agricultural Organisation (FAO) and world Health Organisation (WHO). The current methodologies to detect saffron adulterations with exogenous acidic water-soluble colorants require an isolation step that involves the use of a polyamide solid-phase extraction cartridge. The high amount of crocetin esters in saffron produces much interference that cannot be fully eliminated before the colorant isolation step. Only the Manuel Suisse, among all methodologies applied to analyse saffron, proposes a previous isolation step before using the SPE cartridge. This step consists in previous extraction with TBAC and other organic solvents (Swedish National Food Administration, 1984; Manuel Suisse des denrées alimentaries, 1989).

The aim of this work was to study the optimum extraction conditions of temperature, pH and time to degrade crocetin esters into insoluble crocetin without degradation of yellow colorants in a step previous to colorant isolation.

MATERIALS AND METHODS

Powdered saffron samples from 4 different production countries (Iran, Greece, China and Spain) were studied. For eliminating the crocins interferences, first of all crocins were extracted from the saffron matrix by preparing a solution of 0.3 g in 5 ml of water. This solution was shacked for 1 minute and kept it under dark conditions and at room temperature for 10 minutes. After this, the solution was again shacked for 1 min and

then centrifuged (Selecta, Spain) at 4000 r.p.m. for 10 minutes. An UV-Vis spectrum was carried out on the supernant solution by using a Perkin-Elmer Lambda 25 (Norwalk, USA) spectrophotometer ranged between 190 and 700 nm. An acid hydrolysis was carried out to the supernant solution by adding concentrated sulphuric acid (95%) (Panreac, Spain). Different hydrolysis conditions were assayed: a) pH: 0.1, 1 and 2; b) Time: 0,5, 10 and 15 minutes and c) temperature: at room temperature, 60 and 90 ºC. Then each pH solution has been heated at the different temperatures and different times. After this process is finished, the solution was again centrifuged at 4000 r.p.m. to eliminate the crocetin.

The next step consists of concentrating the colorants on a polyamide solid-phase cartridge (125 mg, Machery-Nagel, Germany) and it is necessary to adjust the pH solution to 2. The cartridge was conditioned with 10 mL of water. The extract at pH 2 was passed thought the cartridge that was dried under gradual suction on a Supelco Visiprep-12 vacuum manifold at a pressure of 15 mm Hg for 10 min. The column was washed again to eliminate the rest of crocins with 45 mL of methanol, 45 mL of acetone and 45 mL of methanol; all solvents are of chromatography grade. Finally, the colorants are eluted with 3 mL of methanol: ammonium (95:5). The obtained extract is dried under vacuum and re-diluted with 300 μL of water that has been analysed spectrophotometrically (190-700 nm).

The same process was carried on the following yellow colorants: annato, naphtol yellow, quinoleine yellow, sunset yellow and tartracine. All of them standards supplied by Sigma-Aldrich Química (Spain).

RESULTS AND DISCUSSION

An Spanish normative, UNE 34013 (1965), quantified the crocin content in saffron in terms of crocetin precipitate by submitting crocins to an acid hydrolysis. This hydrolysis reaction was also studied by several authors (Sampathu et al., 1984; Solinas and Cichelli, 1988), where crocins decomposed into crocetin and glucose by this mentioned process. For this reason, several conditions were studied as it is shown in Materials and Methods section. Once the pH solution was adjust to 0.1, then time and temperature parameters were studied. When hydrolysis took place at room temperature, different reaction times were assayed and as it can be observed there was no significant differences between spectra, meaning that crocins precipitation did not take place. If higher temperatures were assayed, for example at 60 ºC (Figure 2), after 10 and 15 minutes approximately 90% of the crocetin ester content has been eliminated. If the solution is heated at 90 ºC (Figure 3), most crocetin ester content (99%) has been eliminated when hydrolysis time was set at 10 and 15 minutes.

Once the hydrolysis conditions were fixed for eliminating crocetin ester matrix, the behaviour of yellow colorants is also assayed. As it is shown in Figure 4, colorants do not modified its spectra when analysed at so extreme conditions. But as a polyamide cartridge is needed for concentrating the colorants, a sample pH needs to be set at 2 to retain the colorants in the mentioned matrix. No differences were observed when this final step is inserted in the method (Figure 4).

As conclusions, the advantage of this hydrolysis process is that all crocetin esters interferences when analyse the presence of colorants in the saffron extract are eliminated. At this hydrolysis conditions, solution pH 0.1, heating time of 10 min at 90 ºC, did not affect the behaviour of the yellow colorants analysed.

ACKNOWLEDGEMENTS

We would like to thank the Craft consortium G6ST-CT-2002-50257, with special mentioned to the companies Verdú-Saffron-Spain (Spain), C.E.A.E. (Spain), Aromatic AB (Sweden), Krokos Kozani Cooperative (Greece) and Unicam (Spain).

Literature Cited

Alonso, G.L., Salinas, M.R. and Sáez, J.R. 1998. Crocin as coloring in the food industry.

Recent Res. Develop. Agric. Food Chem. 2: 141-154.
ISO 3632. 1993. Saffron (*Crocus sativus* L.). Part 1 (Specification) and Part 2 (Test methods).The International Organization for Standarization, Genève, Swittzerland.
Manuel Suisse des denrées alimentaries.1989. Colorants pour denrées alimentaires. Chapter 42a.
Sampathu, S.R., Shivashankar, S. and Lewis, Y.S. 1984. Saffron (*Crocus sativus* Linn.). Cultivation, processing, chemistry and standardization. CRC Crit. Rev. Food Sci. Nut. 20: 123-157.
Solinas, M. and Cichelli, A. 1988. Analisi HPLC dei compostti responsabili del colore dell´aroma dello zafferano. Industrie Alimentari, XXVII, 634-640.
Swedish national Food Administration, Adulteration of saffron, Krog G. (Eds), 1984.
UNE 34013h1 and h2. 1965. Azafrán. Métodos de ensayo

Figures

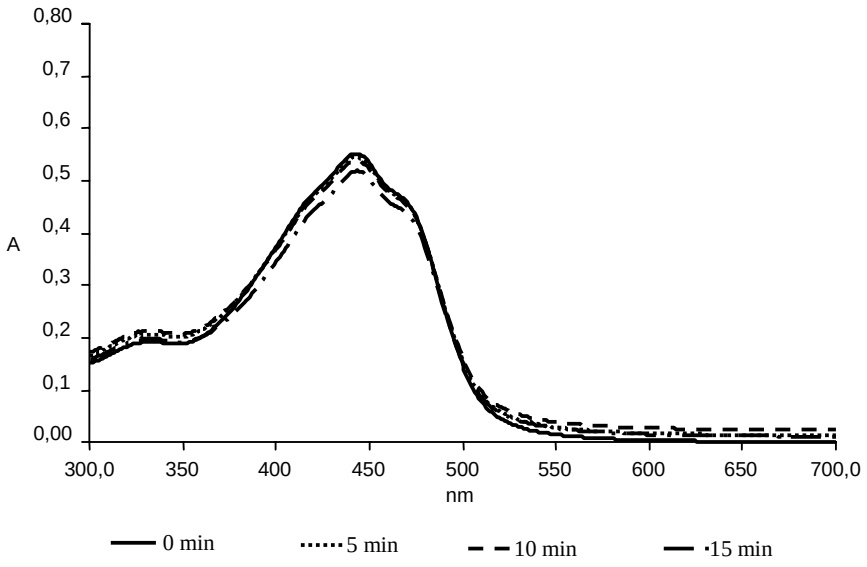

Fig. 1. Effect of heating time on the saffron extract spectra when hydrolysis take place at room temperature and sample pH is at 0.1.

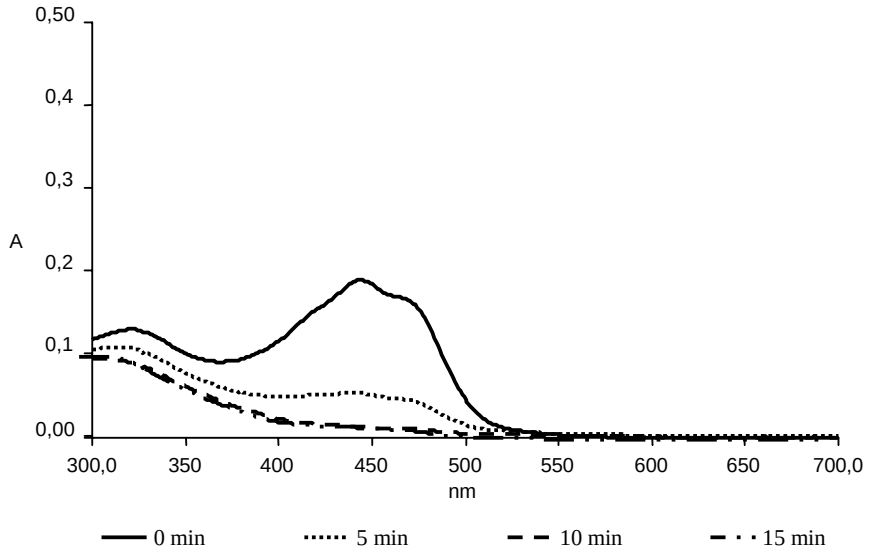

Fig. 2. Effect of heating time on the saffron extract spectra when hydrolysis take place at 60 ºC and sample pH is at 0.1.

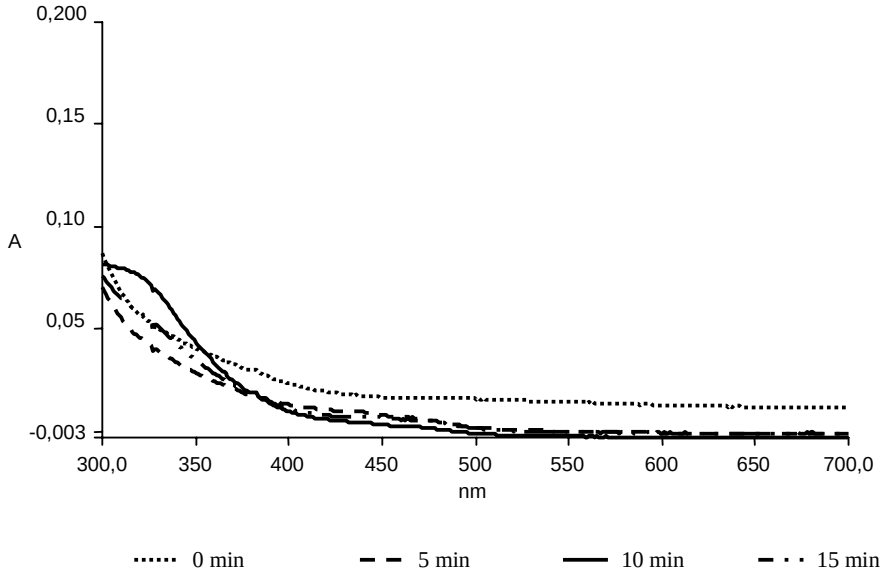

Fig. 3. Effect of heating time on the saffron extract spectra when hydrolysis take place at 90 ºC and sample pH is at 0.1.

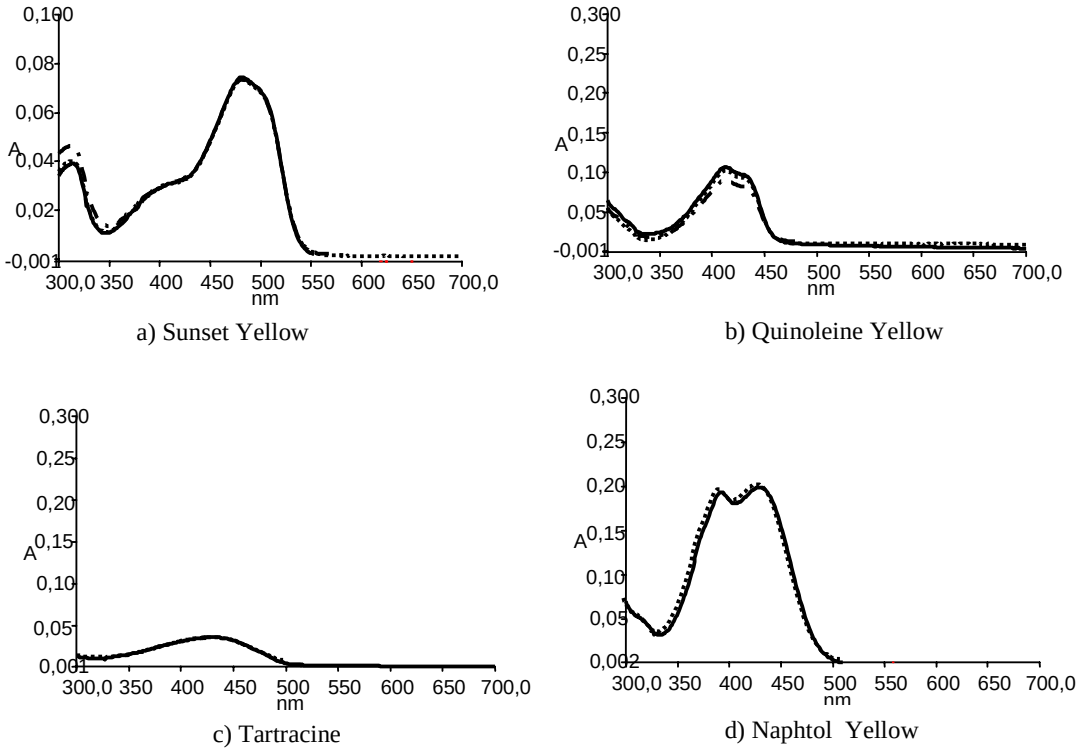

Fig. 4. Effect of the pH change from 0.1 to 2 on yellow colorant spectra.