# Determination of total safranal by *in situ* acid hydrolysis in supercritical fluid media: application to the quality control of commercial saffron

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A procedure allowing hydrolysis reactions to be conducted in a dynamic supercritical-$CO_2$ medium was developed with a view to quantifying total safranal (viz. free safranal present in the sample + safranal resulting from picrocrocin hydrolysis), which is the main component of the essential oil and responsible for the characteristic aroma of saffron. The results obtained were compared with the total index "safranal value", which is widely used as a quality measure of saffron products. The comparison revealed that the proposed method provides useful information not contained in the safranal value; in fact, some samples with a high "safranal index" contain low concentrations of safranal. The proposed method is very useful for quality control in commercial saffron samples.

## INTRODUCTION

Saffron, which consists of dried stigmas of *Crocus sativus* L., is a very expensive spice used mainly as a herbal medicine or a food colouring and flavouring agent in various parts of the world [1–3]. Safranal (2,6,6-trimethyl-1,3-ciclohexadien1-carboxaldehyde $C_{10}H_4O$) is formed during the handling and storage of saffron, and also by subsequent chemical or enzymatic dehydratation of the picrocrocin [5-7] (see Figure 1). It is the volatile oil responsible for the characteristic colour and aroma of saffron. Safranal can be determined by using the spectrophotometric method recommended in ISO 3632 [8], which is based on measurements of the absorbance at 330 nm of an aqueous extract of saffron [9]. However, safranal is only sparsely soluble in water and some crocins exhibit an absorbance maximum at the previous wavelength that interferes with the analysis [10,11]. In 1996, Spain adopted a SOIVRE method for the quality control of natural saffron [4]. This method determines total safranal (*viz*. free safranal present in the sample + safranal from picrocrocin hydrolysis) using a modified version of the method of C. Corradi et al. [9]. This method is labour-intensive and suffers from instability of safranal in the distillate. Iborra et al. [12] developed a selective method based on the hydrolysis of picrocrocin by β-glucosidase enzyme immobilized onto nylon-hydrazide in a continuous packed-bed reactor (PBR) for the HPLC quantification of total safranal in commercial saffron samples; despite its productivity, the method is made time-consuming and expensive by the need to use of large amounts of β-glucosidase.

The potential of supercritical fluid extraction (SFE) [13,14] as an alternative to conventional extraction procedures has been demonstrated in a wide variety of samples [16-19]. This particularly interesting when mild experimental conditions for sample extraction are

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required (e.g., when analyzing thermally unstable or highly reactive compounds) as the solvent strength can be adjusted via the pressure and temperature of the supercritical fluid. Over the past decade, a number of authors have exploited the technical advantages of supercritical fluids to develop a number of extraction and separation methods that have been beneficial to many scientific disciplines and applied to wide variety of real-world samples.

In this work, we accomplish both picrocrocin hydrolysis and the determination of "total safranal" (viz. free safranal present in the sample + safranal from picrocrocin hydrolysis), which is widely used to establish the quality of commercial saffron. To this end, we used supercritical carbon dioxide modified with formic acid in methanol as both reaction medium and extractant for safranal from saffron samples for its subsequent chromatographic determination.

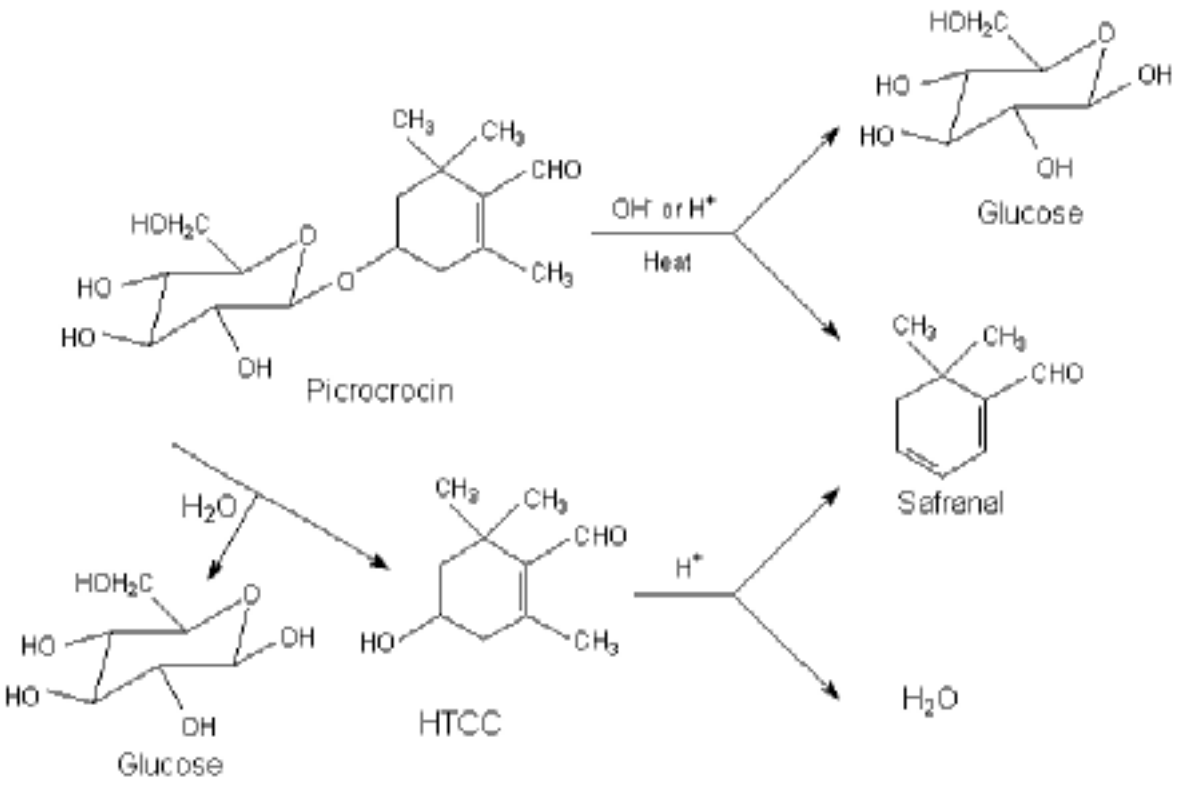


**Figure 1.** Chemical conversion of picrocrocin into HTCC and safranal.

## I. EXPERIMENTAL SECTION

The SFE system used comprised a Hewlett-Packard 7680-T extractor equipped with a quaternary HPLC pump (model 1050A) coupled in line with the extractor. The trap was filled with octadecylsilica (ODS, C18). SFE-grade $CO_2$ and N-38-grade $CO_2$ were supplied by Air liquide (France) in deep-tube cylinders. Spectrophotometric measurements were made on a Hewlett-Packard 8453 diode array spectrophotometer furnished with quartz cell of 1 cm path length. Extracts were chromatographed on Hewlett-Packard HPLC system consisting of an HPLC quaternary pump (model 1050A), an LC analytical column (model Ultrabase C18, 250 mm x 4.6 mm i.d., 5 µm particle size), a diode array detector (model 1040A) and a Rheodyne injection valve (model 1050i, 20 µl). Data was acquired and the equipment controlled by

using Agilent ChemStation software, which was run under Microsoft Windows NT on an IBM compatible PC.

***Analysis of safranal using the ISO 3632 method***

An amount of 20 mg of saffron was extracted with 200 ml of water at room temperature in the dark for 24 hours. The absorption spectrum for the aqueous extract was recorded between 200 and 700 nm, using a cell of 1 cm path length and water as reference. The "safranal value" was calculated from the expression

$$\mathbf{E = ABS_{330}\ v/100xp}$$

where $ABS_{330}$ is absorbance at 330 nm, v the volume of $H_2O$ added (ml), and p the mass of the saffron sample in g.

***Determination of safranal by SFE-HPLC***

$CO_2$ was aspirated from a cylinder furnished with a dip-tube at a constant flow rate of 1 ml $min^{-1}$ (liquid), using a double-piston pump, and passed through the extraction vessel. The sample (0.25-0.1 g), homogenized with 0.5 g of diatomaceous earth, was placed in a 7-ml stainless steel extraction thimble to which 100 µl of formic acid was added. The extraction cell was accommodated in the extraction chamber and allowed to equilibrate at the preset temperature before extraction. Once the target pressure (221 bar) and temperature (120 ºC) were reached, the $CO_2$, modified with 5% (v/v) methanol, bypassed the extraction cell and the sample was hydrolysed in the static mode for 15 min, after which the $CO_2$ was passed though the sample and the dynamic period (15 min) started. After $CO_2$ depressurization, the analyte was deposited in an ODS trap at 35 ºC. In a subsequent step, a methanol stream was pumped at 1 ml $min^{-1}$ through the nozzle and trap by means of syringe pump, the analyte being collected in two 2 ml vials, the methanol extract (4 ml) was filled to 5 ml with methanol in a calibrated flask and injected into the HPLC system for analysis. The nozzle temperature during the extraction and rinsing steps was 45 and 35 ºC, respectively. Gradient elution was used to separate picrocrocin and safranal with appropriate solvents, namely A (methanol) and B (1% aqueous acetic acid, v/v) at room temperature. The gradient program used was follows: initial 0-1 min, A-B (40:60, v/v); 1-6 min, linear range to A-B (55:45, v/v); 6-23, linear range to A-B (75:25, v/v); holding for 7 min; and restoring the initial conditions after 30 min. The flow-rate was kept constant at 1.0 ml $min^{-1}$ throughout.

## II. RESULTS AND DISCUSSION

The feasibility hydrolysis of picrocrocin in supercritical $CO_2$ was studied in a real sample matrix (viz. the La Mancha saffron sample). We aimed to maximize the recovery of total safranal from real samples. For this purpose, an amount of 0.025 g of saffron sample was homogenized with 0.5 g of diatomaceous earth for insertion into the extraction cell. The SFE procedure was developed under the previously established optimum conditions for safranal. Triplicate extractions were performed, and the extracts collected in vials that were filled to mark in a 5 ml calibrated flask. The extracts were then analysed by HPLC method without further treatment. Quantification was based on a standardized safranal solution. Figure 2 shows the chromatograms obtained for the methanol SFE extracts of the saffron sample. The

extracts were monitored at 250 and 310 nm. Inspection of the chromatogram revealed the presence of a high peak for picrocrocin presumably due to its incomplete hydrolysis to safranal (chromatogram A). However, mixing the methanol with some formic or acetic acid (1%) sufficed to maximize the peak of safranal and minimize that for picrocrocin (chromatogram B). The static extraction time was then increased to 30 min with the aim of improving safranal production. No improvement was thus obtained; however. In order to ensure complete hydrolysis of picrocrocin, 100 µl of formic acid was added to the sample in the thimble (chromatogram C). We choose this acid because it is more volatile than acetic acid.

We constructed analytical graphs from triplicate measurements at each point that were linear over the range 50-1500 µg $ml^{-1}$. A typical equation, A = (212.6±191.1)+(31.89±0.32) [C], with a regression coefficient of 0.9995, was thus obtained. The precision of the method, as the relative standard deviation for 10 replicate measurements each, was 2% for 100 µg $ml^{-1}$ safranal. The limit of detection, defined as the concentration of safranal giving a signal equivalent to three times the standard deviation of the blank plus the net blank intensity, was 38.4 µg $ml^{-1}$.

The proposed method was applied to the analysis of natural saffron samples. Table 1 shows the results obtained for nine different types of natural saffron samples. As can be seen, relative proportion of total safranal differed among samples. Thus samples 5 and 8 contained much more safranal than the others. The total safranal concentration also differed among samples. We determined the "safranal value" in accordance with *ISO 3632.* As can be seen in Table 1, the "safranal value" for sample 1 was up to two times lower than those for all other samples. Also samples 2 and 6 had a similar safranal number but a rather different concentration of total safranal. The safranal value of sample 5 was higher than that sample 8 even though its safranal concentration was lower. Therefore, the proposed method gives a more real information about the saffron quality than the ISO method based on the "safranal value". In fact, as results reported in Table 1 show, total safranal concentration is not directly correlated with safranal values.

## CONCLUSIONS

Based on the results obtained in this work, the proposed supercritical fluid extraction method is a straightforward, effective choice for the determination of total safranal and hence for assessing saffron quality. One important advantage of this method is to give a information closer to the real quality of saffron samples. Future works could be carried out dealing with picrocrocin hydrolysis, enzimatically performed, in dynamic supercritical-$CO_2$ media.

## Acknowledgements

Spain's Ministerio de Ciencia y Tecnología (MCyT) is gratefully acknowledged for funding this work with Grant CTQ2004-01220.

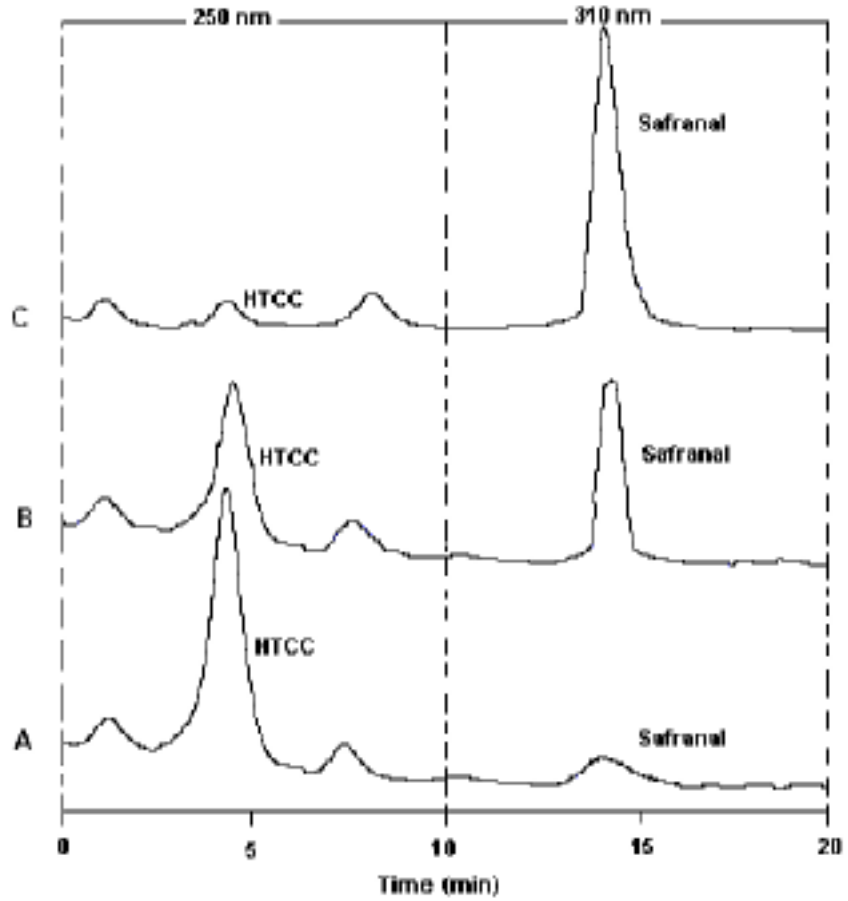


**Figure 2.** HPLC chromatograms for the supercritical carbon dioxide extract from La Mancha saffron samples as recorded at two different wavelengths.

**Table 1**. Analysis of natural saffron samples

| | **ISO 3632 method** | **Proposed method** |
|---|---|---|
| Sample | Safranal value (‰) | Total safranal concentration (g $kg^{-1}$) |
| Sample 1 | 33.15 | 11.7 ± 1.7 |
| Sample 2 | 54.11 | 39.1 ± 0.4 |
| Sample 3 | 56.81 | 47.7 ± 1.4 |
| Sample 4 | 56.53 | 41.0 ± 2.7 |
| Sample 5 | 62.79 | 68.3 ± 1.8 |
| Sample 6 | 55.75 | 61.6 ± 1.7 |
| Sample 7 | 58.00 | 56.4 ± 1.7 |
| Sample 8 | 60.26 | 74.9 ± 1.6 |
| Sample 9 | 58.17 | 58.5 ± 1.6 |

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