Detection of Artificial Red Colorants in Saffron Using UV-Vis Spectrometry and Tristimulus Colorimetry

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Abstract

UV-Vis spectrometry, including elaboration of the 1st and 2nd derivative, was employed for the detection of red colorants in saffron extracts prepared according to the ISO 3632 –1993 standard. The sensitivity of the method was improved when saffron naturally encountered colorants, the crocins, were removed from the analytical sample. For this reason, thermal treatment of the extract at low pH was necessary. In this way even low levels of artificial red colorants (30g /kg saffron powder) were detectable. Moreover, tristimulus colorimetry was used as a screening method to control the authenticity of saffron samples prior to sample pretreatment. It seems that the chromatic parameters a* and h offer some preliminary evidence as to whereas red colorants are present in a saffron aqueous extract.

INTRODUCTION

Saffron commands a rather high value in the international spice trade that results in its frequent adulteration. One of the most common means of deception is the addition of artificial colorants. Such a practice is expected to improve the appearance of the dried stigmas or even to give rise to the coloring strength of the aqueous extract, expressed as $E^{1\%}_{440nm}$. Misleading of the consumer, or misclassification of a sample at a higher commercial quality are not the only consequences of such an illegal practice as the latter may also confront to food safety principles. The aim of the present study was to examine the potential of UV-Vis derivative spectroscopy along with color measurement in aqueous extracts of saffron for the detection of a series of red colorants. Though various spectrometric (Basker and Negbi, 1985; Sujata et al., 1992; Orfanou and Tsimidou, 1996) and chromatographic methods (Pfander and Rychener, 1982; Himeno and Sano, 1987; Sujata et al., 1992; Tarantilis et al., 1994, 1995; Alonso et al., 2001) have been developed to estimate the coloring strength or determine the natural colorants of saffron, the literature concerning detection of adulteration with synthetic ones is rather limited (Alonso et. al., 1999). In the current version of the ISO standards 3632 (1993) such a methodology is absent whereas in the revision under discussion since 2000, the application of a laborious protocol for the detection of the adulterants is proposed. The latter takes advantage of the solid phase extraction to preconcentrate the adulterant (e.g. red colorant) prior to ion pair HPLC. In an effort to enhance the quality control system of the medium size enterprises, which are responsible for the trade of saffron within the European Union, less sophisticated procedures were employed in the present study to detect the lowest attainable amounts of red colorants. The colorants examined were allura red AC (E 129), amaranth (E 123), azorubine (E 122), carminic acid (E 120), erythrosine (E 127), ponceau 4R (E 124) and red 2G (E 128).

MATERIALS AND METHODS

Twenty-two pure saffron samples were obtained from the Cooperative of Saffron Growers (Kozani, Greece). Equal amounts of each sample were used to prepare a representative saffron sample that was used in further analysis. Allura red AC, amaranth, azorubine, carminic acid, erythrosine, ponceau 4R and red 2G were purchased from Sigma-Aldrich Chemie GmbH (Steinhem, Germany). The water used was HPLC grade. The solutions were acidified with HCl 37 % (Riedel de Haën, Seelze, Germany) (for analysis). The spectrophotometer used for the analysis was Shimadzu UV 1601 (Kyoto,

Japan) equipped with the software UVPC 1601 (Personal Spectroscopy Software, v.3.9, Shimadzu). Color measurements were obtained using a MiniScan ™ XE Plus (Hunter Assoc. Inc., Reston, Virginia, USA) colorimeter with reference to D65 illuminant and 10^{o} angle of vision.

UV-Vis Spectrometric Procedure for the Detection of Red Colorants

1. Preparation of Saffron Extracts. Aqueous extracts of saffron were prepared according to the ISO 3632-2 (1993) method. Saffron powder (0.25 g) was transferred with water in a 500 mL volumetric flask. The crocins were extracted by rigorous agitation for 1 hour at ambient temperature away from direct sunlight. The working solutions were prepared by dilution of the extracts with distilled water (1:10 v/v).

2. Preparation of the Solutions of Red Colorants. A series of seven solutions for each colorant in the range of 1-10 μg/mL was obtained from stock standard solutions (200 μg/mL).

3. Preparation of Aqueous Extracts of Adulterated Saffron Samples. The admixtures were prepared by adding specific volume of each colorant solution to the saffron powder. Then extraction was as described in **1**. The working solutions were prepared by dilution of the extracts with water (2:3, v/v).

4. Elimination of Crocins. The solutions of saffron mixtures with (or without) colorants were acidified to pH 0.1 and heated at 90^{o}C for 30 min. The solutions were filtered through a Gooch filter no 4 and then the spectrum of the filtrate was obtained in the UV-Vis region.

5. UV-Vis Spectra Recording. The UV-Vis spectra of all solutions were recorded in the region 200-600 nm. Measurements in triplicate were taken for each solution. The spectra were stored and the derivatives were calculated using the software facilities.

Evaluation of the Chromatic Parameters a*, b*, C* and h

Two ceramic plates, black and white, were used for the calibration of the colorimeter. The working solutions were the same as for the spectrometric analysis. A black plastic ring was placed into a glass container (d=6.5 cm), which was then filled with 20 ml of each solution and sealed with a white ceramic plate. A mean value of nine readings was taken as the overall value for each case. The CIEL*a*b* coordinates a* (red-green component) and b*(yellow-blue component) along with the parameters C*(chroma) and h (hue angle) were recorded and used to evaluate the apparent color of the solutions.

RESULTS AND DISCUSSION

One of the expected difficulties in ensuring the authenticity of natural products is the variability in the chemical composition of the authentic products. In the case of saffron natural variability could be attributed to the genetic material, the origin of the sample as well as to the processing conditions. Therefore, in order to exploit the potential of spectrometry to detect adulteration with red colorants, it was considered necessary to analyze first a considerable number of authentic samples from the same region (Kozani area), processed by different producers to establish the characteristics of the UV-Vis spectrum of aqueous saffron extracts. The spectrum of the extracts for the 22 samples and the 1^{st} and 2^{nd} spectrum derivative revealed several characteristics (Figure 1, Tables 1 & 2) that were repeatable in all cases, indicating, thus, a typical UV-Vis profile for Greek authentic saffron samples.

Taking into account preliminary results presented by Alonso et al. (1999) and the levels of adulteration reported therein, we decided to work at a much lower level of adulteration. For the selected adulteration levels of 30g colorant/kg saffron powder no significant difference in the values of $E^{1\%}_{440nm}$ between an authentic saffron sample and an adulterated one was found (Table 3). Moreover, UV-Vis zero-order spectra of the aqueous extracts of adulterated samples did not differ from that of an authentic one

(Figure 2). The only exception was the case of carminic acid. The addition of this natural colorant in saffron caused a slight abnormality to the saffron spectrum in the region 270-330 nm. The zero crossing points at 1st spectrum derivative of the adulterated samples were slightly different from those of authentic saffron only in the UV region (Figure 3a). However, the presence of red colorants in saffron samples could be suspected after the examination of the 2nd spectrum derivative. For most adulterated samples, a new absorbance maximum in the region above 530 nm, along with a clearly different UV profile from the typical one of saffron, were both indicative of adulteration with red artificial colorants (Figure 3b).

The presence of synthetic food colorants in saffron could be stated only after a proper chemical treatment of the adulterated samples in order to eliminate interference caused by crocins, the natural colorants present in saffron. Working solutions of authentic saffron, adulterated sample and pure colorant were subjected to extreme conditions of pH (0.1) and temperature (90^{o} C) for 30 min. The concentration of the colorant solution was the same as in the mixture with saffron. The spectra of the chemically treated solutions gave evidence for the adulteration of samples due to absorbance at the 450-550 nm region (Figure 4). The repeatability of the method chosen for the elimination of crocins was tested in different authentic saffron samples showing in each case, a very low residual absorbance at 440 nm (<0.03 abs) (Figure 5). In the case of erythrosine, the detection level was much higher (>6% w/w) due to the low stability of this substance under the harsh conditions of crocins' elimination.

In parallel to the above approach it was also examined whether tristimulus colorimetry, widely used in the food quality control, could be useful as at least a screening method in the control of saffron authenticity. The chromatic parameters a*, b*, C* and h were recorded for the extracts of authentic saffron (Table 4), pure colorants (Table 5) and saffron mixtures with colorants (Table 6). An obvious increase in the values of a* (or decrease in h) was observed in the case of the adulterated samples indicating the presence of the red colorants. Tristimulus colorimetry by means of the CIEL*a*b* and CIEL*C*h systems of trichromatic theory has already been used to indicate the different origin (Alonso et al., 2003) or different process of the spice (dried stigmas or powder) (Mitsopoulou, 2000; Pardo et al., 2002). However, evaluation of the apparent color of saffron aqueous extracts has not been reported. The presence of red artificial colorants in the spice, even at very low concentration, brings about noticeable changes in the values of a* and h, while the yellow component (b*) as well as the overall chroma (C*) are slightly influenced.

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Tables

Table 1. Zero crossing points at the 1st spectrum derivative of Greek saffron aqueous extracts

$\lambda_{zero\ absorbance}$(nm)	222[a] ± 1.69[b]	258 ± 0.38	293 ± 0.61	329 ± 0.35	348 ± 0.50	443 ± 0.31

[a] Mean value (*n*=22) of 6 replication for each extract, [b] SD

Table 2. Wavelengths of maximum absorbance in the 2nd spectrum derivative (inversed) of Greek saffron aqueous extracts

λ_{max}(nm)	211[a] ± 0.64[b]	227 ± 0.48	263 ± 0.18	297 ± 0.97	326 ± 0.27	415 ± 0.36	441 ± 0.64	472 ± 0.27

[a] Mean value (*n*=22) of 6 replication for each extract, [b] SD

Table 3. Mean values of colouring strength of aqueous extracts of saffron mixtures with red colorants (3% w/w).

Mixtures of saffron with:	$E^{1\%}_{440nm}$	$E^{1\%}_{440nm}$ of representative Greek saffron sample
Allura red AC	173 ± 3 (***n=4)***	
Amaranth	165 ± 3 (***n=6)***	
Azorubine	166 ± 4 (***n=6)***	
Erythrosine	161 ± 3 (***n=5)***	166 ± 1
Carminic acid	166 ± 3 (***n=6)***	
Ponceau 4R	171 ± 3 (***n=6)***	
Red 2G	171 ± 2 (***n=5)***	

[a] *n*=6

Table 4. Frequency distribution of the chromatic parameters of 22 Greek saffron aqueous extracts.

a*		b*		C*		h	
R	f %	R	f %	R	f %	R	f %
2.47-5.44	27.27	114.09-117.92	31.82	114.32-118.11	31.82	86.00-87.39	68.18
5.44-8.41	72.73	117.92-121.76	68.18	118.11-121.90	68.18	87.39-88.78	31.82

R: range, F: frequency

Table 5. Tristimulus values recorded for the solutions of pure colorants (6 ppm)

Colorant	a*	b*	C*	h
Allura red AC	29.18 ± 0.59	4.95 ± 0.14	29.59 ± 0.60	9.63 ± 0.09
Amaranth	29.41 ± 0.03	-4.23 ± 0.02	29.71 ± 0.03	351.882 ± 0.02
Azorubine	20.84 ± 0.17	-1.88 ± 0.06	20.93 ± 0.18	354.86 ± 0.02
Erythrosine	3.92 ± 0.08	1.31 ± 0.05	4.13 ± 0.09	18.52 ± 0.48
Carminic acid	7.60 ± 0.03	3.74 ± 0.15	8.47 ± 0.04	26.21 ± 1.00
Ponceau 4R	21.68 ± 0.36	3.56 ± 0.08	21.97 ± 0.36	9.33 ± 0.12
Red 2G	29.66 ± 0.46	-1.77 ± 0.02	29.71 ± 0.46	356.59 ± 0.02

Table 6. Tristimulus values for the extracts of saffron mixtures with red colorants.

Added colorant (6 ppm)	a*	b*	C*	h
Allura Red AC	25.04 ± 0.51	110.73 ± 1.40	113.53 ± 1.27	77.26 ± 0.39
Amaranth	24.74 ± 0.53	107.18 ± 0.18	110.00 ± 0.06	77.01 ± 0.29
Azorubine	21.21 ± 0.31	106.60 ± 0.84	108.69 ± 0.77	78.74 ± 0.24
Erythrosine	10.47 ± 0.04	117.62 ± 0.36	118.09 ± 0.36	84.91 ± 0.02
Carminic acid	15.36 ± 0.85	110.91 ± 0.39	111.97 ± 0.50	82.12 ± 0.40
Ponceau 4R	14.64 ± 1.06	108.91 ± 1.93	109.89 ± 1.77	82.34 ± 0.68
Red 2G	22.22 ± 0.71	113.98 ± 0.85	116.21 ± 0.96	78.78 ± 0.27

Figures

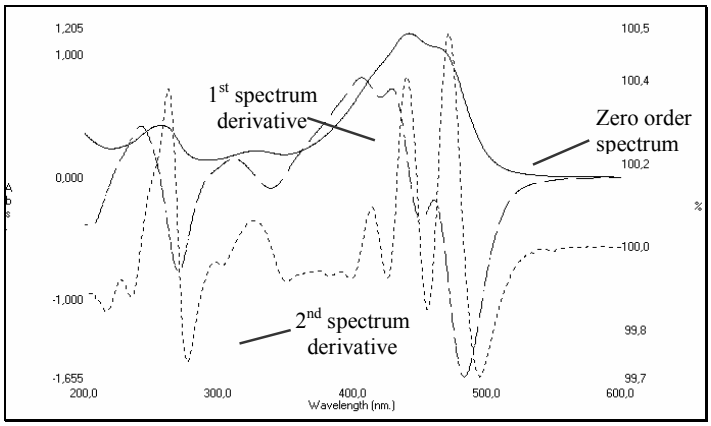


Fig. 1. Typical UV-Vis spectrum of aqueous extracts of saffron and its 1st and 2nd derivatives.

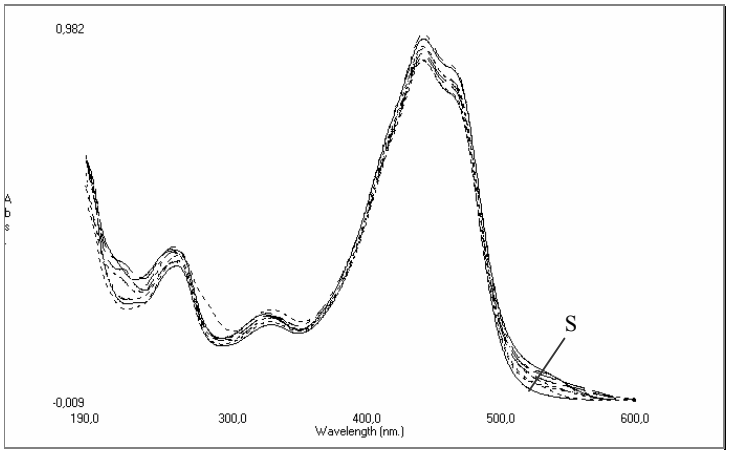
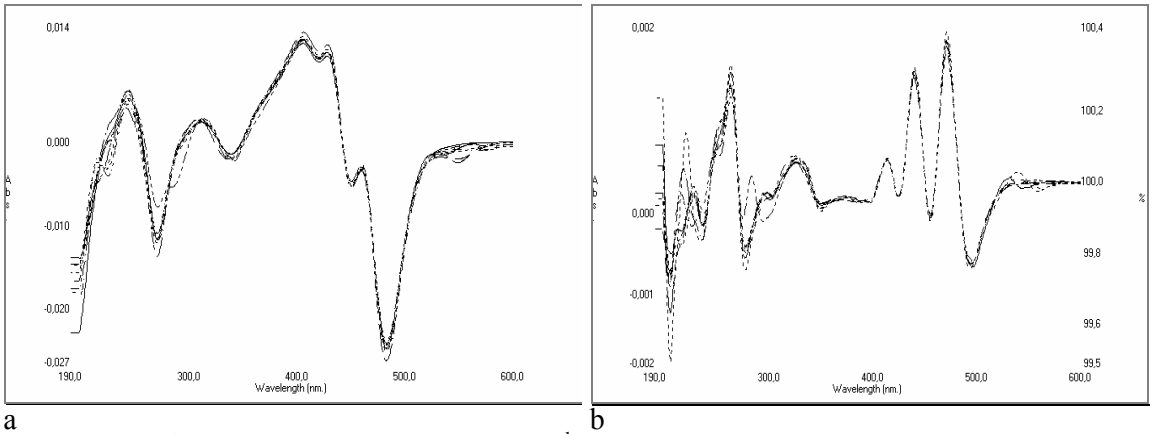


Fig. 2. UV-Vis spectra of aqueous extracts of authentic saffron (S) and of its mixtures with red artificial colorants (3% w/w).



a b

Fig. 3. a). 1st spectrum derivative and b). 2nd spectrum derivative of aqueous extracts of saffron and its mixtures with red artificial colorants (3% w/w).

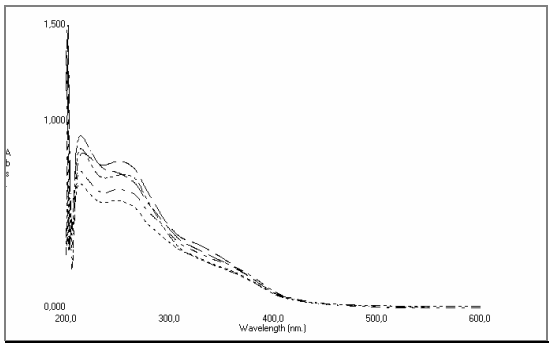


Fig. 5. Repeatability of crocin elimination method (pH 0.1, 90°°C, 30 min).

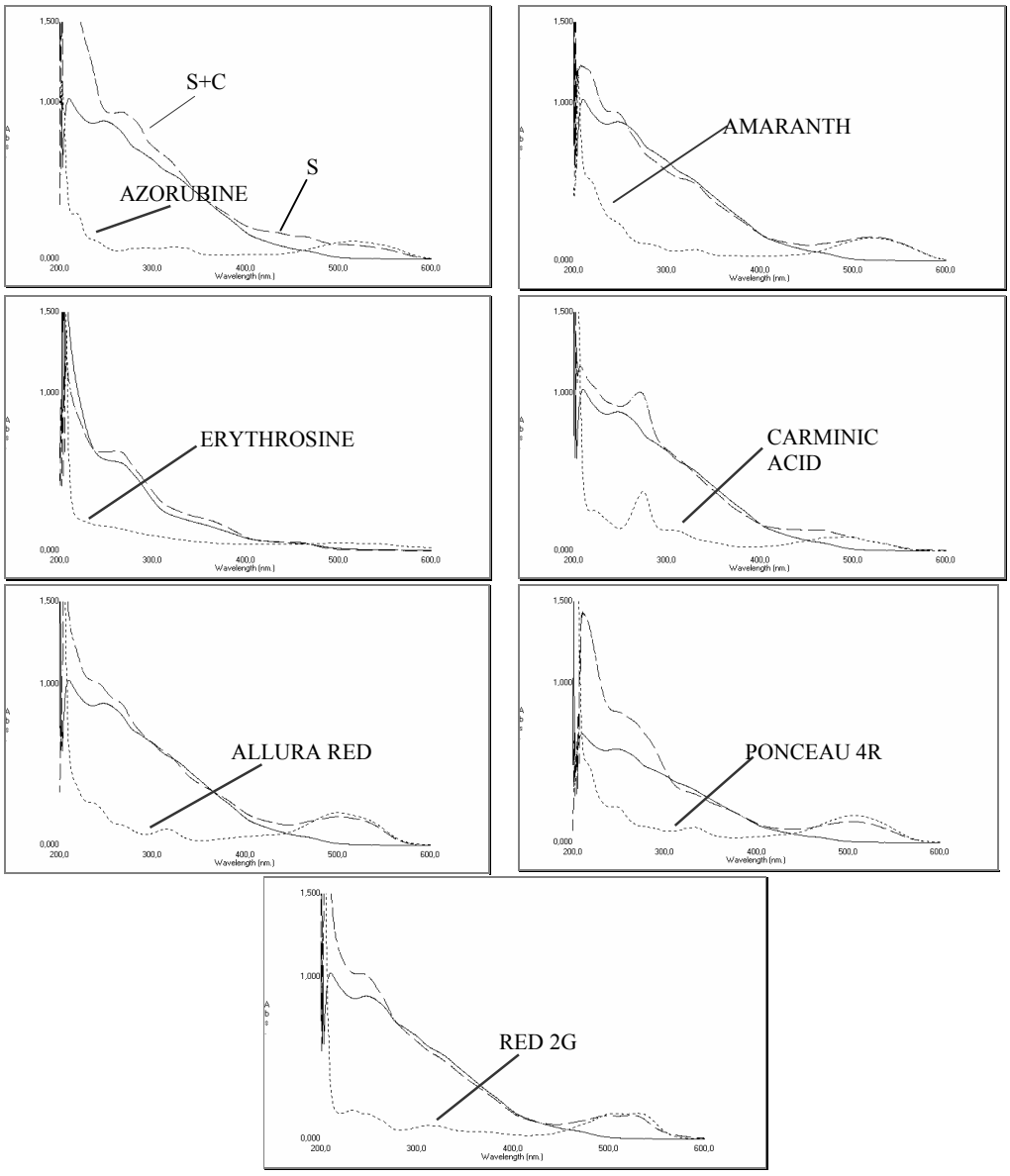


Fig. 4. UV-Vis spectra of chemically treated solutions of authentic saffron (S), of mixtures of saffron with colorants (S+C) and of pure red colorant.