CHARACTERIZATION AND GENETIC FINGERPRINT OF SAFFRON

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The dried stigmas of *Crocus sativus* L. are a very expensive spice known as saffron used as food flavouring and colouring agent and as traditional herbal medicine. Crocus is cultivated in India, Iran, Spain, Greece and Italy. The production process involves a big manual work and cannot be completely mechanized. In Italy from a 1000 m^2 area about 120000-150000 flowers can be obtained (4000-5000 kg) which give rise to 5-7 kg of fresh stigma, i.e. 1.0-1.3 kg of dried product.

Many papers deal with analytical aspects to set up methods for the separation and determination of the biological active components [1,2], and aroma components [3].

The purpose of this paper is the analysis of stigmas from *Crocus sativus* cultivated in Italy and Iran to characterize secondary metabolites and the quality of commercial saffron and the characterization of the biological active components of stamens and sepals in order to find a possible use of this recycling material which is the most consistent part of *Crocus sativus* flowers.

The major biologically active components of saffron are crocin analogues which are all glycosides of *trans*-crocetin, a carotenoid derivative which are responsible for colour.

Stigmas, sepals and stamens of *Crocus sativus* L. samples were analysed for their crocins, flavonols and anthocyanins content. Identification of crocins, flavonols (kaempferol and quercetin derivatives) and anthocyanins was carried out by HPLC/DAD analysis.

Other aim of this study will be the definition of a genetic fingerprint useful for the characterization of saffron germplasm.

Sample preparation. Sepals, stamen and dried stigma sample were obtained from plants harvested in 2013 from Fiesole (FI, Italy) and from three different place in Iran (Gonabad, Torbat and Ghaien).

Sepals and stamen (500 mg) were suspended in 50 mL of 70% ethanol, adjusted to pH 2.0 with formic acid, for one night. After the extraction, samples were filtered and evaporated to dryness under vacuum at room temperature, then dissolved in EtOH/H_2O (70:30) adjusted to pH 2.0 with formic acid to a final volume of 3 mL. Saffron stigmas (50 mg) were extracted with 10 mL of 70% ethanol, adjusted to pH 2.0 with formic acid for one night and then filtered to eliminate plant residues. These extracts were analysed by HPLC/DAD for the determination of saffron components.

HPLC/DAD Analysis. Analysis for flavonols, crocins and anthocyanins were carried out using a HP 1100L liquid chromatograph equipped with a DAD detector and managed by a

HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA), and were separated by using a 250 × 4.6 mm i.d. 5μm Luna C18 column (Phenomenex) operating at 25 °C. UV/Vis spectra were recorded in the 190-600 nm range and the chromatograms were acquired at 280, 330, 350 and 440 nm. The mobile phase was a two-steps linear solvent gradient system, starting from 90% H_2O (adjusted to pH 3.2 by HCOOH) up to 100% CH_3CN during a 40-min period, flow 0.8 mL min^{-1} for flavonols and crocins, while the elution method for anthocyanins was a four-steps linear solvent gradient system, starting from 95 % H_2O (adjusted to pH 2 by HCOOH up to 100% CH_3CN during a 32-min period, flow 0.8 mL min^{-1}.

Identification and Quantification of Individual Polyphenols. Quantification of individual compounds was directly performed by HPLC/DAD using a five-point regression curve ($r^2 \geq 0.998$) in the range 0-30 μg on the basis of authentic standards. In particular, crocin derivatives were determined at 440 nm using curcumin as reference compound. Flavonols were determined at 350 nm using quercitrin as reference compound and anthocyanins were determined at 540 nm using oenin as reference compound. In all cases, actual concentrations of the derivatives were calculated after applying, were possible, corrections for differences in molecular weight.

Results and Discussion

In Fig. 1 is reported a chromatogram of the Fiesole' stigmas extract, the peak identification was performed through the recording of UV spectra, and the composition of the extract is similar to that previously found [4].

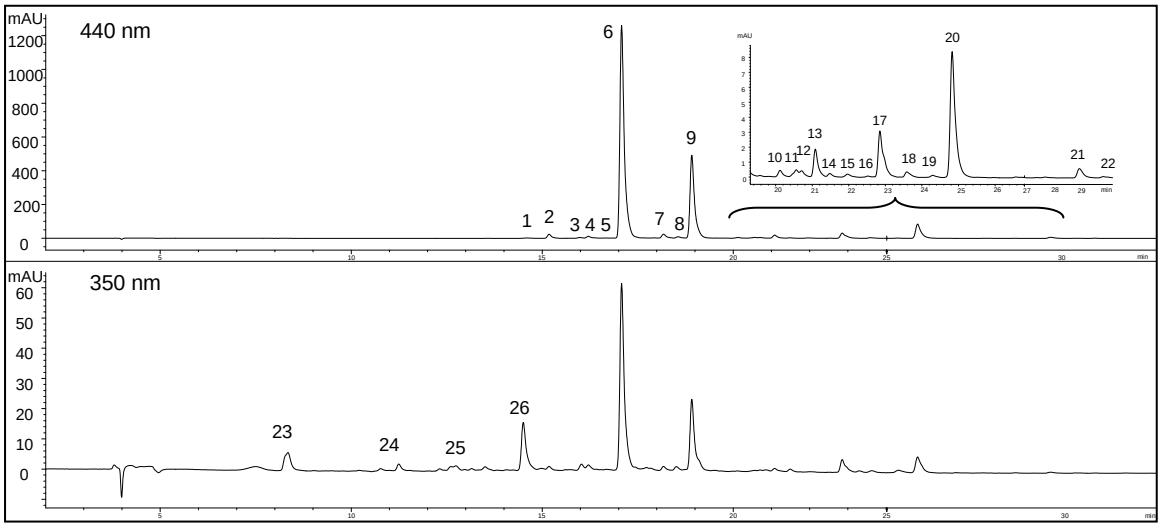

Figure 1. Chromatographic profile, acquired at 440 nm and 350 nm by HPLC-DAD, of an hydroalcoholic extract from saffron stigmas (Fiesole) at relative maximum of absorbance of crocins and flavonoids. Identified compounds: 1. Trans crocin 5, 6. trans crocin 4, 9. trans crocin 3, 12. trans crocin 2', 17. cis crocina 4, 20. trans crocina 2, 21. cis crocina 1, 2.3.4.7.8.10.11.13.14.15.16.18.19.22. crocin derivatives, 23. K-3-sophoriside -7- glucoside, 24. K derivative, 25. K -3,7,4'-triglucoside, 26. K-3-sophoroside. (k=kaempferol).

In Table 1 are reported the quantitative data of dried stigmas analysed.

COMPOUNDS	STIGMA (FI) mg/g	STIGMA GONABAD mg/g	STIGMA TORBAT mg/g	STIGMA GHAIEN mg/g
Crocins				
Trans crocin 5	1.55	1.63	1.98	1.61
crocin derivative	2.76	1.05	1.67	1.30
crocin derivative	0.50	0.66	0.51	0.65
crocin derivative	1.59	0.92	0.90	1.17
crocin derivative	0.50	0.53	0.51	0.52
trans crocin 4	372.01	168.91	238.02	197.80
crocin derivative	2.76	1.32	2.18	1.69
crocin derivative	1.00	1.19	2.44	1.30
trans crocin 3	123.88	61.25	85.36	71.56
crocin derivative	0.69	0.40	0.51	0.39
crocin derivative	0.72	1.71	1.41	1.64
trans crocin 2'	1.22	1.64	3.63	2.53
crocin derivative	2.35	0.53	0.51	0.52
crocin derivative	0.48	0.13	0.26	0.26
crocin derivative	0.50	n.d	n.d	n.d
crocin derivative	0.25	n.d	n.d	n.d
cis crocina 4	12.12	30.42	19.38	26.88
crocin derivative	0.63	0.79	0.77	0.65
crocin derivative	0.38	0.53	0.38	0.52
trans crocina 2	20.91	26.00	24.30	24.86
crocin derivative	n.d	0.40	0.38	0.39
cis crocina 1	1.50	1.58	2.22	1.73
crocin derivative	n.d	0.53	0.51	0.52
crocin derivative	0.13	0.40	0.38	0.39
TOTALE	**548.45**	**302.51**	**388.23**	**338.87**
Flavonoids				
K-3-sophoriside -7-glucoside	5.15	2.64	2.95	2.99
K derivative	1.38	1.05	0.90	1.30
K -3,7,4'-triglucoside	1.51	2.51	2.95	2.21
K-3-sophoroside	9.67	10.02	10.38	8.83
TOTALE	**17.71**	**16.22**	**17.18**	**15.33**

Table 1 Quantitative data for dried stigmas. Average value ± SD of three samples. Data are expressed as mg/g dried sample.

It should be noted that the four samples differ mainly in trans-crocin 4 and trans-crocin 3 contents, which are present in a larger amount in Fiesole' s sample. The crocins content of Fiesole's sample is quite high giving evidence to the very good quality of the sample. Among

flavonols kaempferol-3-O-sophoroside is the main compound according to our previous work [4] and the Spanish sample analysed by Carmona et al. (2007) [5].

According to Vignolini et al. 2007 [4], crocins, flavonols (kaempferol and quercetin derivatives) and anthocyanins (delphinidin derivatives) of Fiesole' sepals and stamens were been identified and quantified (table 2).

	STAMEN (FI) mg/g	SEPALS (FI) mg/g
crocins	0.84	n.d
flavonols	11.03	79.38
anthocyanins	n.d	5.46

Table 2 Quantitative data for sepals and stamen (Fiesole' sample). Average value ± SD of three samples. Data are expressed as mg/g dried sample.

From all these data the possible exploitation of alternative tissues like stamens and sepals as phytochemical resource can be point out. For each kg of stigma about 1000 kg of flowers are processed; therefore sepals and stamens are important by-product of saffron production and their use could increase the *Crocus sativus* flowers economic value.

Further investigation are in progress for the definition of a genetic fingerprint useful for the characterization of saffron germplasm.

References

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