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Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions

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ABSTRACT

The primary goal of this study was to propose saffron as a sustainable substitute crop with high added value in some Moroccan agricultural areas with low and erratic rainfalls, for their socio-economical development. The quality of the saffron spice has to be evaluated prior to recommendation for commercial production. For this purpose, saffron was grown in experimental plots for the first time in eleven different experimental zones with a disparity of altitudes, soils and climates. High-performance liquid chromatography (HPLC) was used to quantify the most important saffron components crocins, picrocrocin, and safranal which are respectively responsible for its colour, taste and odour. The respective average values, in % dry matter, across all sites altogether are 29.01 ± 5.6; 14.04 ± 7.1 and 0.22 ± 0.11. The statistical analysis shows that crocins are stable under each specific environment tested ($p > 5\%$) for 3 years of study. Meanwhile, there was a large variability in safranal content for the same period ($p < 0.05$). This suggests that post-harvest processing of saffron produced under different environments may need to be improved. Analysis of environmental impact on saffron quality showed that just the altitude affects crocins ($R^2 = 0.84$, $p < 0.05$).

1. Introduction

The flowers of saffron (*Crocus sativus* L.), a plant from the family Iridaceae, possess red-orange tripartite stigmas. This triploid sterile monocot species is not known to grow in the wild, but has been cultivated for its stigma for a long time. It is highly valued as a culinary spice for its flavouring and colouring properties (Rios et al., 1996), and is the subject of ongoing scientific research for its potential medicinal properties. Interest in the impact of saffron carotenoids on human health is growing due to their high antioxidant capacity (Abdullaev, 2002; Pham et al., 2000; Verma and Bordia, 1998).

Saffron is a perennial crop well adapted to arid and semi-arid lands which produce stigmas annually. It is also adaptable to temperate and sub-tropical climates, and can be grown on soils varying from sandy to well-drained clay loams. It blooms in autumn and spends a long period of dormancy (aestivation) in the summer. It is said to be native to the Mediterranean environment that is characterized by cool to cold winters, with autumn–winter–spring rainfall, and warm dry summers with very little rainfall. The Mediterranean environment is recognized worldwide as the best region to produce saffron, with regards to its quality, which is attributed to many factors. However, in Europe, especially in the Mediterranean basin, saffron production faces a crisis. The production of saffron has decreased due to the rise in labour costs which has made production unprofitable in spite of its high market price. The result is a significant decrease in areas for growing saffron during the last few decades in some traditional saffron producing regions in Europe (Negbi, 1999). This situation is advantageous to Morocco where this crop requires mostly family labour and, while it is the only country in Africa and in the Middle East that grows saffron.

The use of Moroccan saffron for medicinal purposes has a long history and has been practiced for centuries (Migration et Développement, 2006); however, its area of production is limited to a small region in the south, especially in the Anti-Atlas Mountains, on about 600 ha. The main region of its implantation lies in the Taliouine zone (Altitude 1200–1630 m, latitude 30°26′N and a longitude of 8°25′W), a remote area in the Southwest of Morocco (Ait Oubahou and El Otmani, 1999) with cold winters and hot summers. The temperature varies between −2 °C during winter and 45 °C during summer. Rainfall ranges annually from 100 to 300 mm. The average yield varies from 2 to 6 kg/ha during its 5–6 years of soil occupation, with a maximum reached between

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the third to the fifth year. Higher yields are achieved on some well-maintained plots (10–12 kg/ha). The annual production is about 3 tons. Saffron production in Morocco is carried out traditionally without the use of mineral fertilization or pesticides. The major saffron production is marketed at a national level.

Saffron quality depends on the concentration of its three major metabolites providing the unique colour and flavour to the stigmas. Picrocrocin ($C_{16}H_{26}O_7$) is considered to be the main bitter principle of saffron. It is a monoterpene glycoside precursor of safranal ($C_{10}H_{14}O$), the major volatile oil responsible for the aroma. β-Glucosidase action on picrocrocin liberates the aglycone, 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC, $C_{10}H_{16}O_2$)) which is transformed to safranal by dehydration during the drying process of the plant material (Lozano et al., 2000; Winterhalter and Straubinger, 2000). The UV absorption maximum for picrocrocin is 254 nm (Alonso et al., 1999). The aroma of saffron comes from an essential oil, which is primarily composed of the terpene aldehyde, safranal, being the most abundant volatile component in the stigmas of saffron (>60% of essential oil) (Roedel and Petrzika, 1991; Tarantilis and Polissiou, 1997). The absorbance maximum for safranal is 330 nm. The dye substances collectively referred to as the crocins, come from the water-soluble glycosidic *cis*- and *trans*-carotenoid crocin, glucosyl esters of crocetin. Crocins dissolve easily in water to provide an orange-red solution. This is the reason for its application as a food colorant. The absorbance maxima of crocins are at about 440 nm in distilled water (ISO/TS 3632, 2003).

Many methods of saffron component analysis have been described (Tarantilis et al., 1995). The chemical composition of saffron samples from many countries indicates that the values reported are strongly dependent on the methods employed for drying, extraction and analysis (Kanakis et al., 2004; Lozano et al., 2000; Zareena et al., 2001). The method of saffron quality characterization currently recommended by the International Standardization Organization is UV–vis spectrophotometry (ISO/TS 3632, 2003). Unfortunately, the method is non-specific and unable to adequately separate between genuine and adulterated saffron, and thus unable to provide a quality category on the international market (Lozano et al., 1999; Zougagh et al., 2005a). Various analytical methods have been developed including thin-layer chromatography (TLC) (Sampathu et al., 1984); reverse-phase high-performance liquid chromatography (RP-HPLC), coupled with a UV–vis detector or, more often, a photodiode array detector (PDA) (Caballero-Ortega et al., 2007), with mass spectrometry (Tarantilis et al., 1995) and gas chromatography (GC), with a mass spectrometer (MS) detector for the volatiles (Narasimhan et al., 1992; Tarantilis and Polissiou, 1997). Others methods such as near infrared spectroscopy (NIR) (Zalacain et al., 2005), nonaqueous capillary electrophoresis (CE) (Zougagh et al., 2005b) and proton nuclear magnetic resonance (^{1}H NMR) (Assimiadis et al., 1998; Tarantilis and Polissiou, 2004) have been developed with some success.

The method used in this work for the quality determination of saffron is high-performance liquid chromatography (HPLC) with PDA detector. This method is the most efficient analytical technique for the analysis of sensitive compounds in complex extracts of natural products (Alonso et al., 2001).

This research is the first to be conducted on Moroccan saffron for quality analysis under different environments for a possible extension of the regions where this valuable crop can be grown. The main goal is to support a recommendation of saffron as a substitute crops for the socio-economic development of some deprived rural areas. Saffron can be used as an alternative crop for the diversification of agricultural production as a way to improve the quality of farm life by its relatively high profit, especially for women farmers who are most often utilized in the picking of flowers and subsequent stigma sorting. It could also ensure the sustainable use and conservation of arid area since it is a perennial culture which is adapted to erratic environments. The objective of the work is to determine the range of variation in the main saffron compounds as influenced by environment and to determine the region that yields the highest saffron quality based on crocins level.

2. Materials and methods

2.1. Corms collection

Prior survey work on saffron cultural techniques, yields and problems identification in the main saffron growing zone in the south region of Morocco was done in September of 2005. Corms were collected in fields and growers' reserves. In addition, further information on cultural techniques used in the region was recorded from local specialists and through many interviews with farmers so as to conduct the experiments in the same manner as done by farmers.

2.2. Stigma collection

The experiments were conducted on *Crocus* stigmas grown under diverse environments during 2005, 2006 and 2007. The flowers on each experimental plot were picked by hand at approximately the same time of day (from 6 to 8 am). Methods for removal of the stigma from flowers and drying conditions were kept identical to the methods used by farmers in the main saffron Moroccan regions. Stigmas were brought indoors where they were separated by hand shortly after collecting in the field, and were dried, in shade, for 8–10 days. Afterward, stigmas were weighed for yield determinations and analyzed for quality determination.

Commercial Moroccan Saffron from "Taliouin" (Anti-Atlas), the main saffron growing zone in Morocco and "Le Safranier d'Ourika"

Table 1
Characteristics of the trials sites in comparison with control site (Taliouin).

Sites number	Site name	Altitude (m)	Clay (%)	Sand (%)	OM (%)	P (ppm)	K (ppm)	Soil pH (solvent:water)
S1	Marrakech	1300	13	53	4.1	211.1	522.2	7.8
S2	Rabat	75	19	72	3.5	106.5	466.7	7.9
S3	Merchouch	398	35	12	2.2	70.2	524.2	7.6
S4	Koudia	200	16	55	1.9	78.6	575.7	7.5
S5	Larache	47	11	86	2.8	28.1	89.2	6.8
S6	Ouezzen	614	25	50	3.7	24.1	276.0	7.8
S7	Chaouen	600	30	53	2.6	159.8	1087.3	7.0
S8	Oulmes	1135	25	44	4.5	1.2	178.4	6.2
S9	Meknes	714	35	26	5.7	438.2	1157.0	7.7
S10	Settat	397	15	32	3.7	24.12	276.0	7.8
S11	Taounate	509	40	14	1.7	34.8	331.8	7.9
C1 (control 1)	Taliouin	1630	23	22	1.3	17.0	412.5	8.3

OM: organic matter; P: phosphorus; K: potassium.