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Descriptive analysis of Moroccan volatile saffron according to the storage and drying conditions

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ABSTRACT

Drying is an important step for the preparation of saffron because it determines the aroma of the spice, it differs according to the regions and producer countries, which result a variations in the quality. There are several methods of drying which the critical elements are time, drying temperature, and equipment used. The storage conditions can also influence the quality of saffron, especially its three secondary components which are: crocin, safranal, and picrocrocin. The main aim of the present study is to provide a description of volatile profiles and their variability among Moroccan saffron according to different drying and conservation methods. Twenty nine saffron samples belonging to three different Moroccan areas were analyzed using thermal desorption-gas chromatography–mass spectrometry. For not generating safranal during the isolation procedure, saffron volatiles were desorbed at 50°C during 3 min. The eight main volatile compounds, in addition to safranal, were identified and related to the dehydration procedure as well as the storage conditions. Saffron dried by the oven (40°C) and kept in the refrigerator at 8°C provided satisfactory results.

Keywords: *Crocus sativus* L; saffron volatiles; dehydration temperature; thermal desorption-Gas chromatography; storage.

INTRODUCTION

Saffron, spice derived from the flower of *Crocus sativus* L, is a plant whose its style-stigmas have been used since ancient times as a spice in cooking practices, such as colorant in the preparation of perfumes and cosmetics, as well as for medicinal purposes [1]. Nowadays, it is almost exclusively used for coloring and flavoring foods, although recent studies highlighted the interest for its medical properties; saffron and its ingredients could be considered as a promising candidate for clinical anticancer trials [2]. This sterile specie is cultivated successfully in various environments throughout the world such as Iran, India, Morocco, Greece, Spain, and Italy.

The biological and agronomic characteristics of saffron (lower activity during summer, low fertilizer requirements, and good adaptation to poor soils) make this crop an attractive alternative for the low-input agriculture able to provide good production in sustainable farming systems. It is a viable alternative crop for the marginalized areas where the low water availability severely limits the practice of other crops [3]. In Morocco, saffron is produced in the South, especially in the Anti-Atlas Mountains. The main region of its implantation is Taliouine; area characterized by difficult soil and climatic conditions where saffron cultivation has been practiced for centuries and plays an important role in the durability of production systems and the social and economic viability of populations of this region [4]. Saffron production in Morocco is carried out traditionally without the use of chemical fertilization or pesticides. The major saffron production is marketed at a national level [3].

Saffron quality depends on the concentration of its three major metabolites: crocins, picrocrocin, and safranal. Picrocrocin is considered to be the most important substance responsible for the characteristic taste of saffron [5], it's also related to the aroma of saffron, because it is considered a safranal precursor which is generated during the dehydration process of the spice [5].

Safranal is the most abundant volatile component in the style-stigmas of saffron, it's determined commercially according to ISO standards [6] by measuring the absorbance of an aqueous extract of saffron at 330 nm. However, safranal is not very soluble in water and the cis isomers of crocetin derivates are also absorbed at 330 nm. The composition of volatiles in saffron has been investigated by many researchers using techniques based on gas chromatography [7],[8]. Among them, those involving the thermal desorption and adsorption of volatiles in an adsorbent trap are the most rapid and involve the least sample manipulation. The method proposed by SANCHEZ et al [9] seemed to be the most convenient for safranal determination.

Drying process of saffron influences the physico-chemical characteristics of the product [10],[11]. The quality of saffron is also influenced by storage conditions [12]. Thus, the main aim of the present study is to provide a description of volatile profiles and its variability among Moroccan saffron landraces according to the drying, and conservation methods.

MATERIALS AND METHODS

1.1. Samples

Twenty nine samples collected during the years 2011, 2012 and 2013 were analyzed from different Moroccan regions, dried with two drying process (oven at 40°C, and natural drying) and preserved with two kinds of storage (shade, and refrigerator). The samples are pure, and bought directly from the producers.

1.2. HPLC-DAD

Fifty milligrams of sample was macerated for 1h in 100 ml of Milli-Q water. The entire process was carried out in darkness and at room temperature.

20 µl of the extract filtered through a PVDF filter of 0.45 µm (Millipore) was injected into an Agilent 1100 HPLC chromatograph (Palo Alto, CA) equipped with a 150 mm*4.6 mm i.d., 5 μm Phenomenex Luna C18 column thermostated at 30 °C. The solvents were water (A) and acetonitrile (B), using the following gradient: 80% A for 5 min to 20% A in 15 min, at a flow rate of 0.8 mL/min. Dual on-line detection was carried out by a diode array spectrophotometer (DAD) set at 250, 330, and 440 nm [13]. The data reported represent the average of 4 sample replicates.

1.3. TD-GC-MS

A joined system made up of Perkin-Elmer (Norwalk, CT, USA) ATD-400 thermal desorption equipment, a model HP-6890 gas chromatograph, and a model HP- 5973 mass spectrometer provided with a NIST library (Hewlett-Packard, Palo Alto, CA) were used. A fused silica capillary column with stationary phase BP21 50 m in length, with an inside diameter of 0.22 mm, and 0.25 µm of film was employed (SGE). The carrier gas was helium of chromatographic purity (220 kPa). Fifty milligrams of sample were introduced into the desorption tube and desorbed at 3 min at 50°C.

Other conditions for the thermal desorption equipment were as follows: oven temperature of 250 °C, cold trap temperature of -30 °C, and transfer line temperature of 200 °C.

Conditions for gas chromatography were as follows: 100 °C (5 min) increased at a rate of 18 °C/min to 210 °C (15 min). In the mass spectrometer, the electron impact mode (EI) was set up at 70 eV. The mass range varied from 35 to 500 units, and the detector temperature was 150 °C [14].

All compound identification was carried out using the NIST library and by comparison with those reported previously [11]. The data reported represent the average of 2 sample replicates.

1.4. UV–Vis spectrometry

Saffron samples were analyzed according to the ISO 3632 trade standard [6]. This method allows the determination of the main characteristics of saffron related with picrocrocin, safranal, and crocins content.

According to ISO, picrocrocin, safranal and crocins are expressed as direct reading of the absorbance of 1% aqueous solution of dried saffron at 257, 330, and 440, respectively.

The analyzed saffron samples are the same samples used for HPLC analysis. Measurements of E1% of an aqueous saffron extract at 440, 330, and 250 nm, respectively, were done using a 1 cm, pathway quartz cell.

1.5. Statistical analysis

The statistical study focused on the analysis of the variability of volatile components between the 2 types of drying and 2 types of storage. The variability of different parameters is studied by the analysis of variance (ANOVA) which allows the detection of differences between the types of drying and the types of storage, and their eventual interaction. Statistical analysis is performed using the GLM procedure of the SAS software. Whenever ANOVA revealed significant differences, the multiple comparaison of means and their classification is performed using the Tukey test. All statistical analyses were performed using the Statistical Analysis System [15].

RESULTS AND DISCUSSION

1.6. Moisture and volatile matter content

The moisture and volatile matter content of 20 samples ranged from 7 to 12%, values less or equal than 12%, maximum limits established by ISO 3632 (2011), and 9 samples contains more than 13 % of moisture and volatile matter.

Coloring strength varied between 168 to 290: 24 samples belong to category I according to coloring strength and 5 to category II. $E^{\%}_{1\ cm}$ 257 nm ranged from 83 to 115 and $E^{\%}_{1cm}$ 330 nm ranged from 32 to 47.

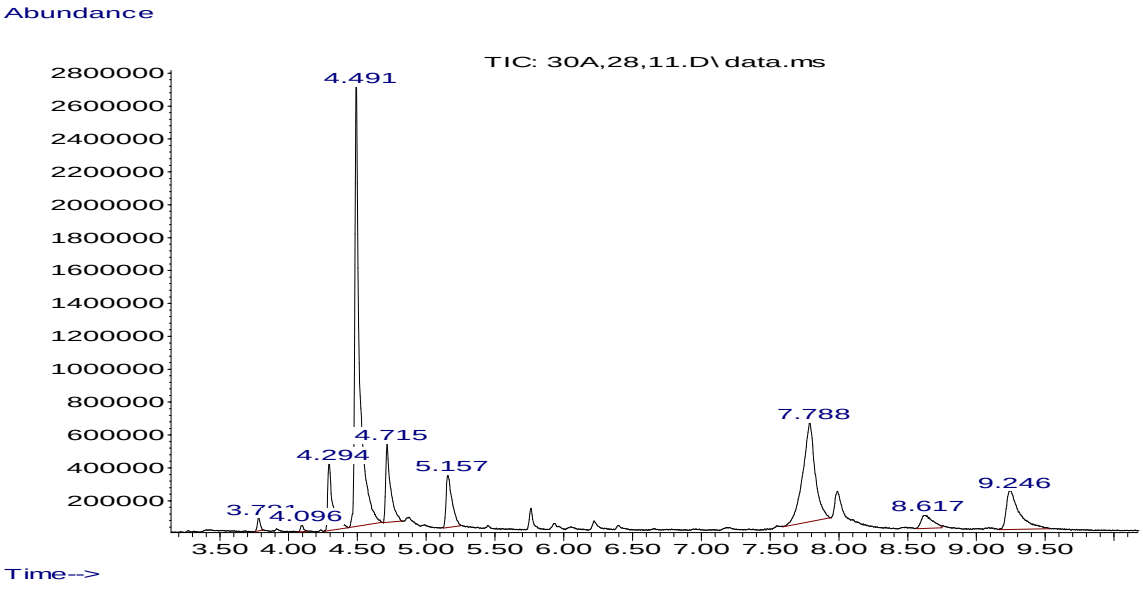

Figure 1. Chromatogram of volatile compounds obtained by ATD – GC – MS of Moroccan saffron

3.2. Chemical volatile content

Nine compounds were detected with AT-GC-MS**.** Among these compounds, safranal occupied the highest proportion, more than 50%. The amount of HTCC, in some samples, differs in comparison with the saffron profile analysis presented by other authors in the bibliography [11].

Figure1 shows a chromatogram of volatile compounds, it's representative of all samples, the time interval in this chromatogram is from 2 to 18 min approximately (the retention time of the safranal peak is 4 min 49).

Table1 gives the mass spectral data for each peak. The peak areas vary from one sample to another, but the silhouette of the chromatograms is maintained in all samples, for this reason we consider that the chromatogram under the analytical conditions described in this work can be used as an indicator of saffron authenticity.

Table 1. Mass spectral data of compounds of saffron

Retention time	Compounds	Mass spectral data [m/z (%)]
3:78	2-methylene-5,5-dimethylcyclohex-3-enal	121(100), 91(60), 107(50), 79(40), 135(20), 150 (10)
4:09	6,6-dimethylcyclohex-2-en-1-one	107(100), 125 (35), 81(33), 55(30), 140 (28)
4:29	3,5,5-trimethyl-2-Cyclohexen-1-one	82(100), 138(30), 54(10), 95(5)
4:49	2,6,6-trimethyl-1,3-Cyclohexadiene-1-carboxaldehyde	107(100), 91(80), 121(50), 79(25), 135(10)
4:71	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	68(100),96(95),152(46),109(12),41(10) 137(4)
5:15	2,2,6-trimethyl-1,4-Cyclohexanedione	139 (100), 56(95), 42 (75), 154(60), 69(52),83(24)
7:78	5-hydroxy-2,6,6-trimetyl-3-oxo-cyclohexa-1,4-dièna l	109(100), 137(95), 180(80), 152(78), 123(65), 91(45), 135(35)
8:61	4-Hydroxy-3,5,5-trimethylcyclohex-2-enone	98(100), 112(50), 70(48), 42(35)
9: 24	4-Hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC)	107(100), 135(95), 91(70), 121(68), 168(30), 150(20), 153(18)

According to the ANOVA results, the difference between the three dehydration processes (oven, sun, and shade) is significant, specially for safranal, 2,6,6-Trimethyl-2-cyclohexene-1,4-dione, and 2,2,6-trimethyl-1,4-Cyclohexanedione. According to the Tukey test, the average value of these compounds is higher when saffron is dried with the oven compared to shade or sun.

Regarding storage conditions, almost all components give high values when the saffron is stored in the refrigerator compared to shade.

According to Raina (Raina et al., 1996) [16], too low temperature, 30 °C, causes a very long drying time (27-53 h) which provoke the biodegradation of crocin and does not allow the transformation of picrocrocin to safranal, the intermediate of this reaction remains majority and distorts the smell of saffron. A brief drying, 2 to 4 hours at 60 °C, results a thermal degradation of pigments. The optimal temperature is between 35 and 45 °C for a drying time of 5-6 h. Only the electric oven and solar drying are convenable for obtaining a high quality of saffron. Pardo's results (Pardo et al., 2002) [16] confirm that in low-temperature drying (in the shade), saffron loses its color, aroma, and flavor.

Nevertheless, values may change depending on the amount placed on each tray and the total amount to dry. In this study, saffron was dried at 40 ° C with a drying time which varies from 30 to 60 minutes. To ensure the humidity, it is necessary to weigh the fresh product from extraction of flowers and submit it to the hot air of the oven until the loss of 4/5 of initial weight.

Using electric Oven reduces drying time and danger of contamination by dust and insect excrement, and therefore limits the risk and the loss of aroma and the specific color of saffron.

The graph reported in Figure 2 represents the comparison of safranal identified by LC-DAD and absorbance at 330 nm of aqueous solution of saffron using the UV–Vis according to ISO 3632 (2011). There is no correlation between safranal identified by LC- DAD and the absorbance at 330 nm since there are other substances present in saffron absorbing at 330 nm, such as cis-crocetin esters isomers.

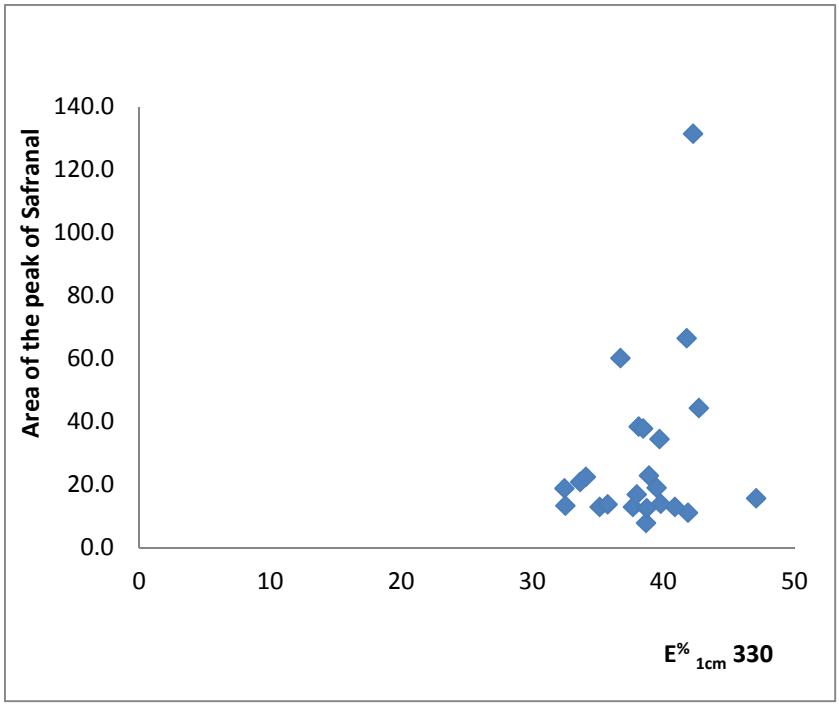

Figure 2. Comparison between the absorbance of saffron at 330 nm according to ISO 3632 (2003) and the area of the peak of safranal identified by LC-DAD analysis

CONCLUSION

The style-stigmas of *Crocus sativus* lose 80% of their weight during the dehydration process to preserve saffron for a long time, its moisture content must be reduced to the 12% established by ISO/TS 3632 Specification, this step is important to obtain a high quality of saffron, but poor conservation of spice can significantly affect the coloring, the aroma and the taste properties of the spice. Saffron is very hygroscopic and must be stored in a dry place because with high moisture it loses its aroma, and becomes black. In this study, we obtained a satisfactory results of saffron dried by the oven at 40°C and kept in the refrigerator at 8°C. It is important to follow the progress of dehydration so that the stigmas are not too dry and therefore brittle, because the humidity can vary from one batch to another.

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