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Phytochemical composition of Moroccan saffron accessions by headspace solid-phase-microextraction

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Abstract

The phytochemical composition of 19 saffron samples collected under different Moroccan environments is evaluated by Headspace Solid-Phase Micro-extraction coupled with gas chromatography–mass spectrometry. The aim is to categorize Moroccan saffron volatiles and to highlight the impact of the environment on the chemical composition of the spice. This is the first research conducted on Moroccan saffron volatiles according to their geographical origin. A total of 57 volatile components have been identified. Differences in compound proportion and composition among the accessions were observed. The main chemical classes of volatiles identified were monoterpene hydrocarbons (6 to 42%), oxygenated monoterpenes (3 to 29%) and non-terpene derivatives. Only 14 compounds were found in common to all the accessions. The most important, in decreasing proportions, are safranal, 1, 8-cineole, 4-keto-isophorone, isophorone and α -pinene. 70% of the accessions analyzed contain β -isophorone, an isomere of isophorone which is a criterion of saffron high quality. Other components are identified in very few accessions, in lower amount, e.g., rose oxide (26% of accessions), allo-ocimene (13%) and piperitone (4%). A difference in the volatiles composition has been noticed consequently to accessions origin and drying mode. The cluster analysis based on Jaccard similarity and complete link method has identified five groups, at 75% of similarity. This study highlights the impact of the environment on saffron volatiles composition when the drying is done naturally, and this could be used as chemical fingerprinting for the authenticity of the product, according to its origin.

Keywords: *Crocus sativus* L., spice, SPME-GC-MS, environment, drying mode, Jaccard Index.

1. Introduction

Saffron spice has many uses based on its colour, aroma and taste. The most important are in food industry and cuisine as a dye, but it is also used for medicinal purposes. Saffron extract has been reported to show interesting anti-tumor activity^[1].

Morocco is the only country in Africa and MENA region that produces saffron spice. The use of Moroccan saffron for medicinal purposes and as a dye has a long history and has been practiced for centuries^[2]. The actual area of saffron cultivation is around 1000 ha. Its area of production is mainly in the south of Morocco, especially in the Anti-Atlas Mountains, and takes more and more importance in other area, particularly in the high Atlas Mountains. Most of the saffron drying is done naturally, mostly in shade, for 5–12 days according to climatic conditions.

Saffron quality depends on the concentration of its three major metabolites providing the unique colour, flavour and odour to the style-stigmas. During the dehydration and storage processes of saffron, the hydrolysis of picrocrocin (monoterpene glycoside) that is considered to be the main bitter principle of saffron and precursor of safranal, yields a monoterpene aldehyde that constitute one of the main components of saffron volatiles and responsible for its aroma^[3-6]. The amount of this volatile component in dried styles-stigma structures is among the most important indicator of saffron quality. Apart from safranal, other volatile constituents contribute to the final aroma of saffron^[7-11]. Several investigations have been previously carried out on saffron volatiles and more than 90 volatiles have been identified^[12, 13]. According to some authors, most of the volatiles are derived from the thermal degradation of carotenoids and hydrolysis of glycoside precursors^[14]. Many studies have been conducted on the techniques of saffron volatile extraction and identification^[15, 16]. Among them, an easy technique based on Head-Space Solid Phase Micro-extraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) has been then proposed for preconcentration

and detection of many organic compounds. This method has been proven to be a powerful technique^[17]. According to^[18], HS-SPME has a high discriminating ability for the isolation of volatile phytochemical components and yields a high number of total organic volatiles. This method has reduced decomposition of plant compounds, and decreases the loss of these constituents. It has been previously applied to food volatile analysis^[19] and since then has been used in significant applications in food aroma^[20].

Volatiles components in Moroccan saffron have never been investigated before. This research permitted the identification of volatiles of some Moroccan representative accessions collected under different environments, mainly in the principal saffron regions (Taliouin and Taznakht), as well as other regions where saffron is dried naturally and conserved at farm level. A comparison was done with some samples dried by oven. Therefore, the main aim of the present study is to provide a description of the Moroccan saffron volatile profiles and its variability among Moroccan saffron landraces.

2. Materials and methods

2.1 Plant material collection

Nineteen saffron samples, from various environments, dried naturally or by oven, were collected for analysis during 2011. Twelve samples were collected from the main saffron zone in the south of Morocco (S1-S11 and S14), one from Ourika region (Marrakech, S12), two from Errachidia region (East region: S13, S15), one from the North of Morocco (Tetuan: S16) and one from Kosovo (S17). Two samples are bought from cooperatives: one as a PDO (Protected Designation of Origin) product (S18) and one as a bulk sample (S19) bought from the main saffron market in Morocco. All saffron samples were produced during 2011. Geographical data were collected and soils were chemically and physically analyzed (Table 1).

2.2 Sample preparation

Dried styles-stigma (0.2 g) were put into a 4 ml sealed glass vial for 48 h at room temperature and then one hour in an oil bath at 30 °C to equilibrate.

2.3 Sampling of Volatile Compounds

Polydimethylsiloxane fibres (100 µm) were mounted in a SPME manual holder (Supelco, Bellefonte, PA, USA). Fibres were conditioned prior to analyses, according to the manufacturer recommendations. The fibre was maintained over the sample for 1 min. After the sampling time, the fibre was withdrawn into the needle, then transferred immediately to the injection port of GC.

2.4 Chromatographic analysis of the volatile components

The compounds collected from the headspace above saffron samples were analysed by a gas chromatograph (GC) connected to a mass spectrometer (MS). GC-MS analyses were performed with a Varian CP3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25; coating thickness, 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follow: injector and transfer line temperature, 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C min; carrier gas, helium at 1 ml/min; splitless injection.

2.5 Volatiles Identification

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial^[21, 22] and home-made library mass spectra built up from pure substances and components of known oils and MS literature data^[23, 24, 25, 26, 27]. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

2.6 Data Analysis

Statistical analyses were based on Jaccard similarity index and complete link method was used for comparing the similarity and diversity over samples for discrimination based on their volatile composition.

3. Results and Discussion

3.1 Sample Volatile identification

Globally, 57 volatile compounds have been identified (Table 2). Quantitative and qualitative differences have been found among samples. However, only qualitative difference, based on presence and absence of molecules, is analysed in this paper.

Fourteen volatiles are common to all accessions (Figure 1), with safranal (2,6,6-trimethyl-1,3-cyclo-hexadien-1-carboxaldehyde) as the main constituent (9-57%), followed by monoterpene hydrocarbons (6-42%) (Table 2). According to Maggi and co-workers^[28], safranal is the major saffron volatile component, and constitutes about 60% of volatile fraction. The differences in safranal and other main volatile compounds in saffron depend mainly on the conditions of processing, storage and of volatile isolation and analysis.

Some components are found in most samples in lower amounts, such as hexanal (0-1.1%), heptanal (0-0.5%), fenchone (0-0.9%), α -thujene (0-0.5%), menthone (0-0.5%), linalool (0-0.4%), verbenone (0-0.4%) (Table 2). Others are found at traces level in many accessions, such *cis*- α -ambrinol, (*E*)-geranyl acetone and (*E*)- β -ionone. Additional components are found in lower amount but in very few accessions, e.g., rose oxide (26% of accessions), allocimene (13%), piperitone (4%) (Table 2).

Seven major compounds are dominant in all samples, specifically safranal (9-57%), 1,8-cineole (3-27%), 4-keto-isophorone (3-22%), isophorone (2-20%), α -pinene (1-15%), 2,6,6-trimethyl,1,4-cyclohexadien-1-carboxaldehyde (safranal-isomer) (1-3%) and β -isophorone (0.6-6%) (Figure 1). Besides, some minor components are present in all samples such as 2(5*H*)-furanone, camphene, myrcene, α -phellandrene, δ -3-carene, α -terpinene, *p*-cymene and 4-methylene isophorone. According to a study conducted on saffron volatiles, β -isophorone, an isomer of isophorone, is considered one of the quality marker compounds for category I, and thus saffron containing this compound is of the highest quality^[29]. 70% of samples analyzed in this study contain β -isophorone (Table 2).

3.2 Distribution of volatile components according to their environment and drying methods

The matrix shown in table 3 is based on the Jaccard's similarity coefficient and indicates a high level of variation among the accessions from different sites based on volatiles

components. The similarity coefficient ranged from 0.48 to 0.91, with S15 (Errachidia) and S18 (PDO) having the highest chemical composition similarity (0.92). On the other side, S3 (Taliouin) together with S15 (Errachidia), possessed the least similarity coefficient (0.48) (Figure 2). Five most typical groups at 75% of similarities were then identified (Table 4). Statistical analysis shows that the composition of each sample is mainly due to both the drying method and the environment. Table 4 shows different sites assembled based on their volatile components similarity. It can be noticed that the G5 group includes samples that originated from different environment (Marrakech, Errachidia, Kosovo and an AOP product), which are all artificially dried, by oven (Table 1). This may suggest that the drying method is a dominant variant in saffron aroma expression and the environment impact is not noticeable when the drying is made by oven. So according to this result the saffron volatiles can't be a tool for discrimination of authenticity of saffron origin, when the drying is done artificially. All the other groups contain samples naturally dried. G2 include one identified sample (S4) and one unknown sample sold in the market (S19) in the saffron main region of Morocco. G1, G3 and G4, each include samples from the neighboring regions, having similar drying climatic

conditions. Cluster analysis was performed to obtain groups having similar common qualitative traits (Figure 2) based on volatiles. Five groups with some similarities were then identified. In the dendrogram (Figure 2), we see that the samples S11, S13 and S16 are completely separate from all the others which indicate that the distributions of volatiles in those samples are significantly different from the distribution in the remaining samples and between them. They originated from 3 different environments (Table 1) and have been dried under natural conditions. We notice also that S1 and S3 showed some similarity at 65% but are separate from the other samples. Those two samples were dried naturally, but with sun exposition and not in shade.

As we noticed in all samples originated from different environments, the volatiles composition varied according to the environment and the drying method. According to [30], storage can also be considered as responsible for the variation in aroma components in saffron. This study shows clearly that when samples are dried by oven, the environment impact is not noticeable. But when it is performed naturally, a difference between samples aroma from different environment is obvious.

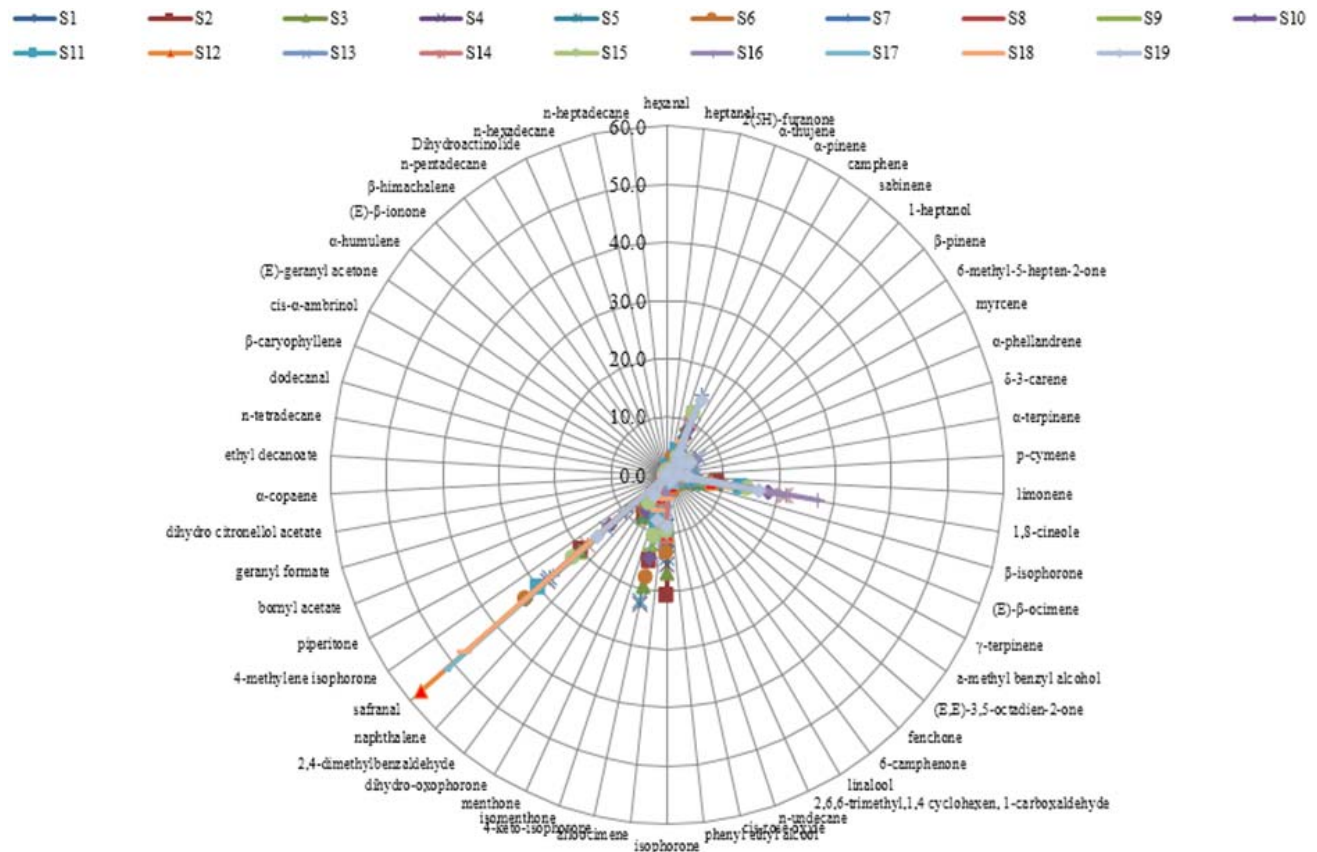


Fig 1: Representative chart of phytochemical composition (%) of accessions analysed

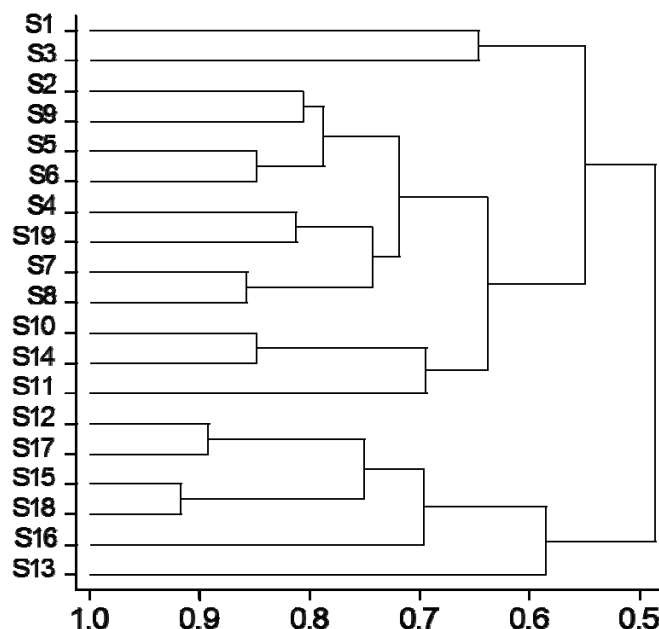


Fig 2: Dendrogram obtained by hierarchical clustering analysis based on saffron volatile components of 19 Moroccan accessions

4. Conclusion

The volatile profiles of different Moroccan saffron samples, collected from different regions were described for the first time in this study. The site-to-site variability in saffron volatile composition is essential to understanding geographical patterns of accession diversity and the variable controlling the volatile composition. A difference has been observed on some accessions depending on the environment and drying method, which might be useful for a first discrimination. A match between chemical identification and environment was noticed for samples with natural drying. Jaccard similarity method allowed a discrimination of the samples, based on their chemical composition.

This study could form the basis to other incoming studies on saffron product based on multivariate analysis including complete climatic data analysis that influence volatiles during natural drying in order to produce an original product with some peculiarities to protect quality, sustainability, and safety of saffron production in small farms.

5. Acknowledgement

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Table 1: Samples origin and site identification

Serial Numbers	Site Origin	Lat./Long.	Altitude (m)	Soil Ph (Water)	Organic Matter (%)	Clay (%)	Drying Mode
S1	Taliouin	30.53 /-7.9	1404	8.11	0,05	20.00	Natural/Sun
S2	Taliouin	30.53 /-7.9	1393	8.19	0.39	20.00	Natural/Shade
S3	Taliouin	30.53 /-7.9	1477	7.93	0.81	35.00	Natural/Sun
S4	Taliouin	30.53 /-7.9	1764	8.08	1.03	15.00	Natural/Shade
S5	Taliouin	30.53 /-7.9	1783	8.25	1.15	10.00	Natural/Shade
S6	Taliouin	30.53 /-7.9	1715	7.90	1.18	10.00	Natural/Shade
S7	Taliouin	30.53 /-7.9	1733	7.80	3.29	10.00	Natural/Shade
S8	Taliouin	30.53 /-7.9	1792	7.48	5.18	5.00	Natural/Shade
S9	Taznakht	30.57/-7.20	1500	8.61	1.26	NI	Natural/Shade
S10	Taznakht	30.57/-7.20	1604	8.45	0.59	NI	Natural/Shade
S11	Taznakht	30.57/-7.20	2100	7.90	2.40	NI	Natural/Shade
S12	Marrakech	31.63/-8	1100	8.28	3.9	NI	Oven
S13	Errachidia	31.93/-4.42	1500	8.48	0.14	NI	Natural/Shade
S14	Taznakht	30.57/-7.20	1590	7.80	1,80	NI	Natural/Shade
S15	Errachidia	31.93/-4.42	1906	8.31	3.90	NI	Oven
S16	Tetuan	35.57/-5.36	100	8.11	0.05	22.5	Natural/Shade
S17	Kosovo	42.60/20.90	NI	8.19	0.39	> 35%	Oven
S18	PDO (Cooperative)	NI	NI	7.93	0.81	NI	Oven
S19	Saffron Market	NI	NI	8.08	1.03	NI	Natural/Shade

NI: Not identified, Lat./Long. : Latitude and Longitude

Table 2: Volatile Components of 19 Saffron accessions: GC-MS analytical results of saffron spice accessions

Volatile component	RI*	Minimum %	Maximum %	% of samples containing this components
Hexanal	802	tr	1.1	70
Heptanal	900	tr	0.3	70
2(5H)-furanone	918	0.3	1.9	100
α -thujene	931	tr	0.5	74
α -pinene	939	1.2	15.0	100
camphene	953	0.2	4.4	100
sabinene	977	tr	0.8	91
1-heptanol	970	0.2	0.2	4
β -pinene	980	0.6	4.5	96
6-methyl-5-hepten-2-one	986	tr	0.3	43
myrcene	992	1.1	6.4	100
α -phellandrene	1005	0.2	1.2	100
δ -3-carene	1011	0.2	1.1	100
α -terpinene	1018	0.2	1.1	100
<i>p</i> -cymene	1027	0.5	3.3	100
limonene	1031	0.9	5.3	91
1,8-cineole	1034	3.0	27.4	100
β -isophorone	1044	0.6	6.0	70
(<i>E</i>)- β -ocimene	1051	tr	0.3	39
γ -terpinene	1062	tr	1.8	96
α -methylbenzylalcohol	1064	0.9	2.1	13
(<i>E,E</i>)-3,5-octadien-2-one	1068	tr	0.4	26
fenchone	1087	0.4	0.9	35
6-camphenone	1093	1,8	1.9	9
linalool	1099	0.1	0.5	74
<i>n</i> -undecane	1100	0.1	0.2	9
<i>cis</i> -rose oxide	1111	0.4	1.4	26
2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (isomer of safranal)	1114-1120	0.78	3.18	100
isophorone	1120	2.1	20.9	100
alloocimene	1130	tr	tr	13
4-keto-isophorone	1144	2.9	22.9	100
isomenthone	1148	0.7	0.7	4
menthone	1154	tr	0.5	78
dihydro-oxophorone	1169	0.6	8.6	96
2,4-dimethylbenzaldehyde	1180	tr	tr	13
naphthalene	1182	tr	0.2	22
safranal	1200	9.6	57.3	100
4-methylene isophorone	1219	tr	0.5	100
piperitone	1253	tr	tr	4
bornylacetate	1285	tr	tr	4
geranylformate	1298	tr	0.6	4
dihydrocitronellol acetate	1319	tr	tr	4
α -copaene	1376	tr	tr	4
ethyldecanoate	1395	tr	tr	4
<i>n</i> -tetradecane	1400	tr	0.2	43
dodecanal	1408	tr	tr	9
β -caryophyllene	1418	tr	tr	4
<i>cis</i> - α -ambrinol	1438	tr	0.1	87
(<i>E</i>)-geranylacetone	1454	tr	0.2	87
α -humulene	1456	tr	tr	4
(<i>E</i>)- β -ionone	1485	tr	tr	74
β -himachalene	1499	tr	tr	4
<i>n</i> -pentadecane	1500	tr	tr	4
Dihydroactinolide	1537	tr	tr	4
<i>n</i> -hexadecane	1600	tr	0.1	39
<i>n</i> -heptadecane	1700	tr	0.2	39

tr= traces; *RI, linear retention indices relative to C6-C28 n-alkanes on theHP-5 column,

Table 3: Jaccard similarity matrix of distances based on the presence-absence between all pairs of 19 saffron accession

Sites	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19
S1	1																		
S2	0,742	1																	
S3	0,647	0,667	1																
S4	0,606	0,727	0,639	1															
S5	0,742	0,813	0,714	0,781	1														
S6	0,676	0,794	0,658	0,765	0,848	1													
S7	0,676	0,743	0,750	0,765	0,848	0,829	1												
S8	0,611	0,722	0,641	0,743	0,771	0,806	0,857	1											
S9	0,677	0,806	0,568	0,719	0,806	0,788	0,788	0,818	1										
S10	0,618	0,788	0,564	0,657	0,735	0,722	0,722	0,750	0,781	1									
S11	0,600	0,667	0,590	0,639	0,714	0,800	0,750	0,684	0,706	0,694	1								
S12	0,625	0,697	0,526	0,618	0,697	0,735	0,686	0,765	0,6875	0,727	0,611	1							
S13	0,622	0,561	0,500	0,500	0,600	0,634	0,595	0,619	0,590	0,625	0,571	0,632	1						
S14	0,600	0,765	0,550	0,639	0,714	0,750	0,703	0,730	0,813	0,848	0,722	0,657	0,610	1					
S15	0,690	0,710	0,486	0,625	0,710	0,697	0,697	0,727	0,759	0,800	0,618	0,821	0,639	0,719	1				
S16	0,618	0,639	0,525	0,657	0,686	0,676	0,722	0,800	0,727	0,714	0,605	0,727	0,585	0,649	0,8	1			
S17	0,594	0,667	0,541	0,588	0,667	0,706	0,657	0,735	0,656	0,697	0,541	0,893	0,649	0,629	0,852	0,697	1		
S18	0,621	0,645	0,514	0,563	0,645	0,636	0,636	0,667	0,690	0,733	0,559	0,750	0,629	0,656	0,917	0,733	0,846	1	
S19	0,618	0,735	0,649	0,813	0,788	0,771	0,824	0,800	0,781	0,818	0,743	0,676	0,585	0,743	0,742	0,765	0,647	0,677	1

Table 4: Grouping (G) at 75% of similarity

G1	G2	G3	G4	G5
S2, S9 S5 S6	S4, S19	S7 S8	S10 S14	S12 S17 S15 S18

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