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Influence of drying process on safranal content in the Taliouine Saffron (Morocco): quantification by gas chromatography

L.H. Atyane[1, 2], J. Molinet[3], M.A. Serghini[2], N. Dupuy[3] and L. EL Maimouni[1]*

[1]Materials and Physical Chemistry of the Atmosphere and Climate, Faculty of Science, Ibn Zohr University, BP 8106,80000 Agadir, Morocco.
[2] Laboratory of Biotechnology and Valorization of Natural Resources. Faculty of Science, Ibn Zohr University. BP 8106, 80000 Agadir, Morocco
[3]Aix Marseille Univ, CNRS, IRD, Avignon Université IMBE, UMR 7263, 13397 Marseille Cedex20, France

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elmaimounil@yahoo.fr ;
Phone: +212662078337

Abstract

Dehydration treatment is necessary to convert *Crocus sativus L.* stigmas into saffron spice. To our knowledge, no study has been carried out on the influence of drying process on the Moroccan saffron. In this paper, three drying process were used to study the evolution profiles of the safranal content in Moroccan saffron by UV-spectrophotometry and for the first time by gas chromatography. In addition to shade and sun ambient air treatments, the stigmas were dried in an oven at five temperatures, 30, 45, 60, 75 and 90 ° C. The obtained data of the $E^{1\%}$ of crocin, picrocrocin and safranal determined according to ISO 3632 norm classified the studied saffron into category I. The safranal contents obtained from gas chromatography analysis were considerably greater than those determined by UV spectrophotometry at 330 nm and they were in agreement with those found in saffron from other countries. Characteristics of saffron quality were much better when it dried in oven at 60° C. Finally, further studies on the mechanisms of aroma development during heat treatment and the kinetics of degradation of secondary metabolites during thestorage of saffron are still necessary.

1. Introduction

Saffron, obtained from dried stigmas of *Crocus sativus L.*, is one of the most expensive spices widely appreciated and looked for in the world, not only for its organoleptic characteristics but also for its therapeutic proprieties [1-3]. The composition of saffron is very complex with more than 150 volatile and aromatic compounds and various non-volatile substances such as the carotenoids [4]. The major active components responsible for saffron quality are crocins, glycoside derivatives from the carotenoid crocetin; terpenic aldehydes commonly known as safranal; and picrocrocin, respectively responsible for saffron's coloring power, aroma and taste [5]. A series of analytical methodologies has been developed to determine not only the quality of the saffron,but also the type and the level of adulteration encountered [6,7]. Indeed, the saffron quality is up to now determined according to the norm ISO 3632 (2010) by absorption of an aqueous dried saffron extract at 440 nm, 330 nm and 257 nm respectively for crocin ($C_{44}H_{64}O_{24}$), safranal ($C_{10}H_{14}O$) and picrocrocin ($C_{16}H_{26}O_7$) compounds [8]. Drying process is the most important step that strongly impacts the preparation of saffron. It caused a drastic change in the chemical composition of the samples on which will depend the quality of spice.

Regarding the saffron aroma, safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), has been reported as the major component of volatile fraction of saffron with an amount ranged 60 to 82% [9,10].This monoterpenic aldehyde is a by-product of the chemical and enzymatic conversion of picrocrocin (Fig.1) and appears after heat treatment and during storage of the fresh spice [11-13]. Safranal content commonly measured at 330 nm determines the saffron aroma quality [14]. However, this method is not the most adequate, not only because of the very low solubility of saffron in water, but also because of the interferences of the cis-crocetin esters isomers which absorb at the same wavelength [12, 15-18]. Thus the safranal contents given by this

method are surely overestimated [19]. For these reasons, more specific analytical methods have been proposed and developed [20-25]. The gas chromatography is the most suitable for the quantification of safranal.

The drying process and metabolites degradation kinetics of Moroccan saffron are still an important part of research and further in-depth works have to be performed with the ultimate objective of optimizing the dehydration conditions in order to enhance the quality of this expensive spice.

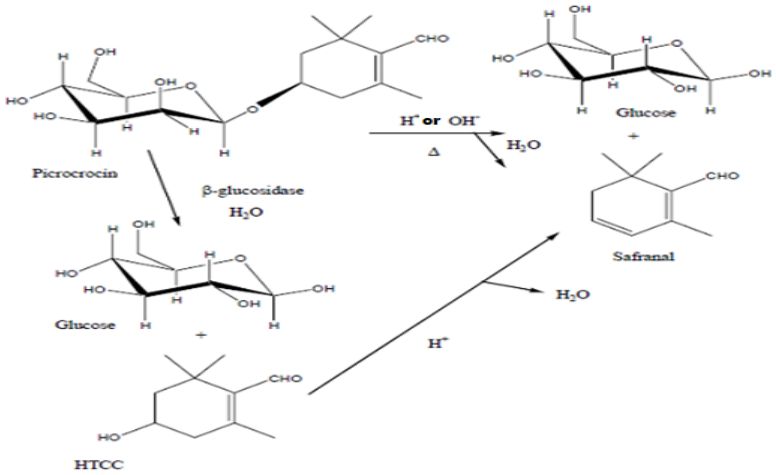

Figure 1: Simplified scheme of the safranal mechanism formation by chemical and enzymatic conversion of picrocrocin

The aim of this work was to determine for the first time by gas chromatography the safranal concentration in Moroccan Saffron originating from Taliouine zone (Morocco). Furthermore, its aim is to determine the optimal condition of the dehydration of the spice, realized traditionally under shade or sun, in ambient air. The evolution of the content of safranal, determinate by CG andUV at 330 nm, according to temperature of the drying process, is discussed in this work (paper).

2. Material and Methods

2.1. Samples and drying process.

Fresh saffron was harvested in the Askaoun village of Taliouine Commune (Taroudant Province) in Morocco on November 2016. The saffron samples were directly obtained from the producers with the guarantee of their origin. The same day, the stigmas were separated from a random selection of the picked flowers, by hand in the laboratory of Agadir city. The stigmas were then dehydrated by spreading them on a clean cloth at ambient air for 7 days under the shade, or 7 hours under the sun, or were dried in an oven at five different temperatures carefully selected. Table 1 shows the details of those dehydration conditions. After being cooled in ambient air, the dried stigmas were then stored in sealed vials and kept at 4°C in darkness until analysis. A total of seven samples (S1 to S7) were studied under different drying processes.

Table 1: Drying process conditions of samples saffron studied in this work

Saffron sample	Dehydration conditions			
S1	Shade		6 days	
S2	Sun		7 h	
S3	T_{oven}(°C)	30	Time (min)	220
S4		45		150
S5		60		100
S6		75		60
S7		90		30

2.2. Sample extractions

In this work, two extraction types were performed under darkness conditions. The first one is classic where the distilled water is used to extract secondary metabolites of saffron, followed by UV-spectrophotometry analysis. The second one is specific, with methanol as solvent, followed by a gas chromatographic analysis of safranal.

Thus the saffron aqueous extracts were prepared according to ISO 3632 (2010):50 mg of dried stigmas were powdered with pestle and porcelain mortar, sieved at 0.5 mm diameter before being placed with 90 mL of bi-distilled water in a 100 mL volumetric flask. The obtained solution was magnetically stirred in darkness for 1hour before being completed with 10 mL of bi-distilled water and then homogenized. The solution was then filtered using a 0.45µm porosity filter (PTFE) and the filtrate liquid was immediately analyzed by UV to determine respectively $E^{1\%}$ of crocin, picrocrocin and safranal.
With the aim of analyzing safranal in the same sample of saffron, 20 mL of methanol (purity > 98%, Sigma Aldrich) were added to 50 mg of powdered product prepared as described below, in 25 mL volumetric flask. After magnetic stirring of the solution for 1 hour, it was completed to 25 mL and centrifuged at 3900 tours/min for 10 min. The solution was finally filtered in this case with a polypropylene membrane filter of 0.22 µm (VWR Int.).

2.3. Samples UV and GC Analysis
This study involved both of the analysis: the secondary metabolitesof the Moroccan saffron according to ISO/TS 3632 method and the analysis of safranal for the first time by GC-FID. The quantification of safranal is obtained from an external calibration curves realized from safranal standard solutions (purchased from Sigma Aldrich, purity > 88%) and respectively prepared in water and methanol for UV-spectrophotometry and CG analysis. Finally a comparison with an IGP commercial saffron sample, which was quantified in the same analytical conditions, has been studied.

UV-Vis Spectrophotometry: The UV-Vis absorption spectra of all the aqueous extracts after proper dilution were measured at 330 nm with a spectrophotometer (UV2300 model) equipped with quartz cells (pathway 1 cm). Absorbance measurements were obtained for each solution and ranged from 0.1 to 1.5. The results were expressed according to ISO/TS 3632(2010). The moisture and volatile matter contents (W_{MV}) were determined by successive weighing of 2.5 g of stigmas introduced in an oven set at 103 ± 2°C for 16 hours[26]. The results expressed in % were calculated by the following ratio: (initial mass - constant mass)/initial mass) ×100 for each determination.
Safranal GC analysis :1 uL of the alcohol extracted solution of Safranal was injected an Agilent Technology 7890 gas chromatograph with a auto-sampler Agilent Technologies injector 7683B. The injectior temperature was 230 °C with a split ratio of 1:20. A HP5 capillary column (J&W Scientific) of 30m x 0.32mm I.D, film thickness 0.25um, was used. H_2 was the carrier gas at flow rate of 1.0 mL/min. Column temperature was initially held at 50 °C for 3 min, and raised at a rate of 15°C/min to 150 °C and then increased to 240°C with the rate of 80°C/min and finally kept constant at this temperature for 32.5 min. The FID detector temperature was set at 250 °C.

Under those chromatographic conditions the standard of safranal has a retention time of 22.95 min. For the analysis of Moroccan saffron, 11 or 12 compounds were usually appear at different retention times.
The retention time (RT in min) of safranal was 22.945 minas presented in Figure 2. The concentrations of the safranal compound was determinated from external calibration curves obtained with safranal standard solutions, prepared in methanol (purity > 98%), between 0.01 and 0.1 mg/mL. Figures 3a and 3b present an example of calibration curve with a regression coefficient (r^2) greater than 0.99. Examples of chromatograms arepresented for samples dried under shade and sun in ambient air (Fig.4) and dried in oven at 60°C and at 90°C (Fig.5).

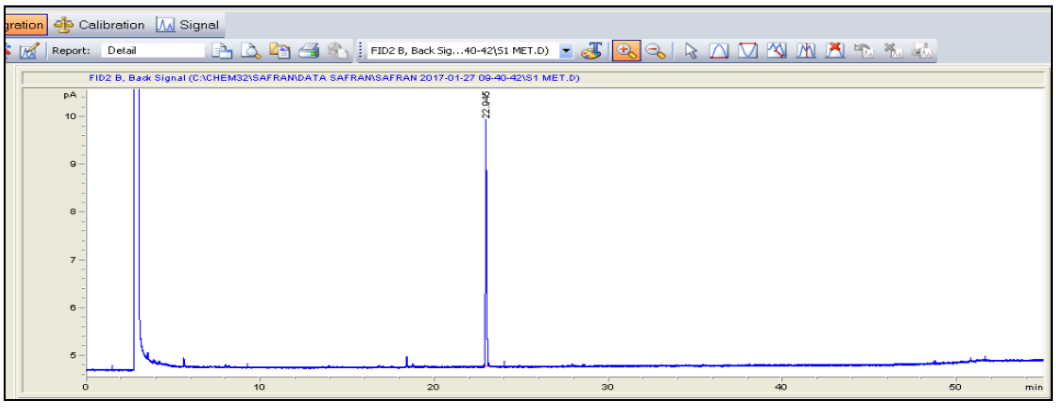

Figure. 2: Example of Chromatograms of safranal used for calibration under analysis conditions of this work

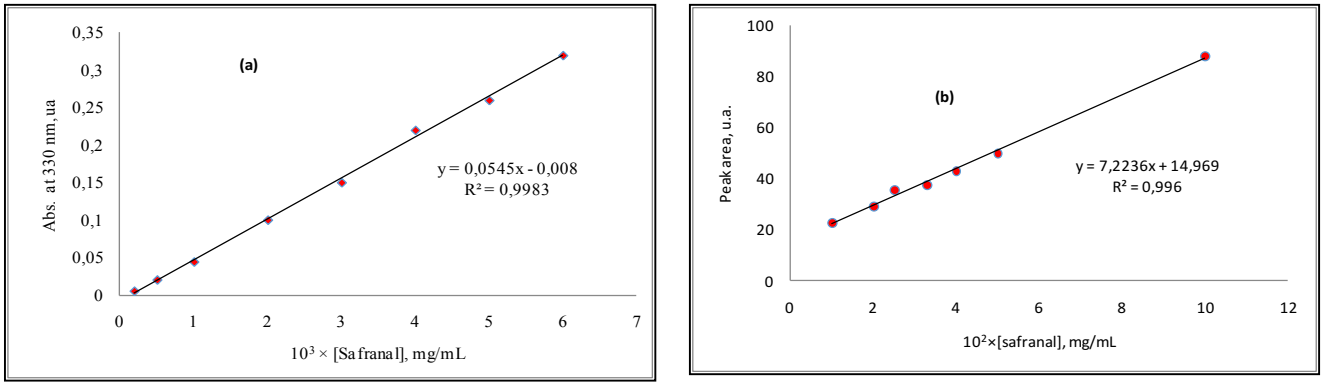

Figure.3: Calibration curves used for calculating concentration of safranal analyzed by UV-330 nm (a) and CG (retention times of Safranal: 22.935 min) (b), correlation coefficient (r^2) greater than 0.99 for the two analysis methods.

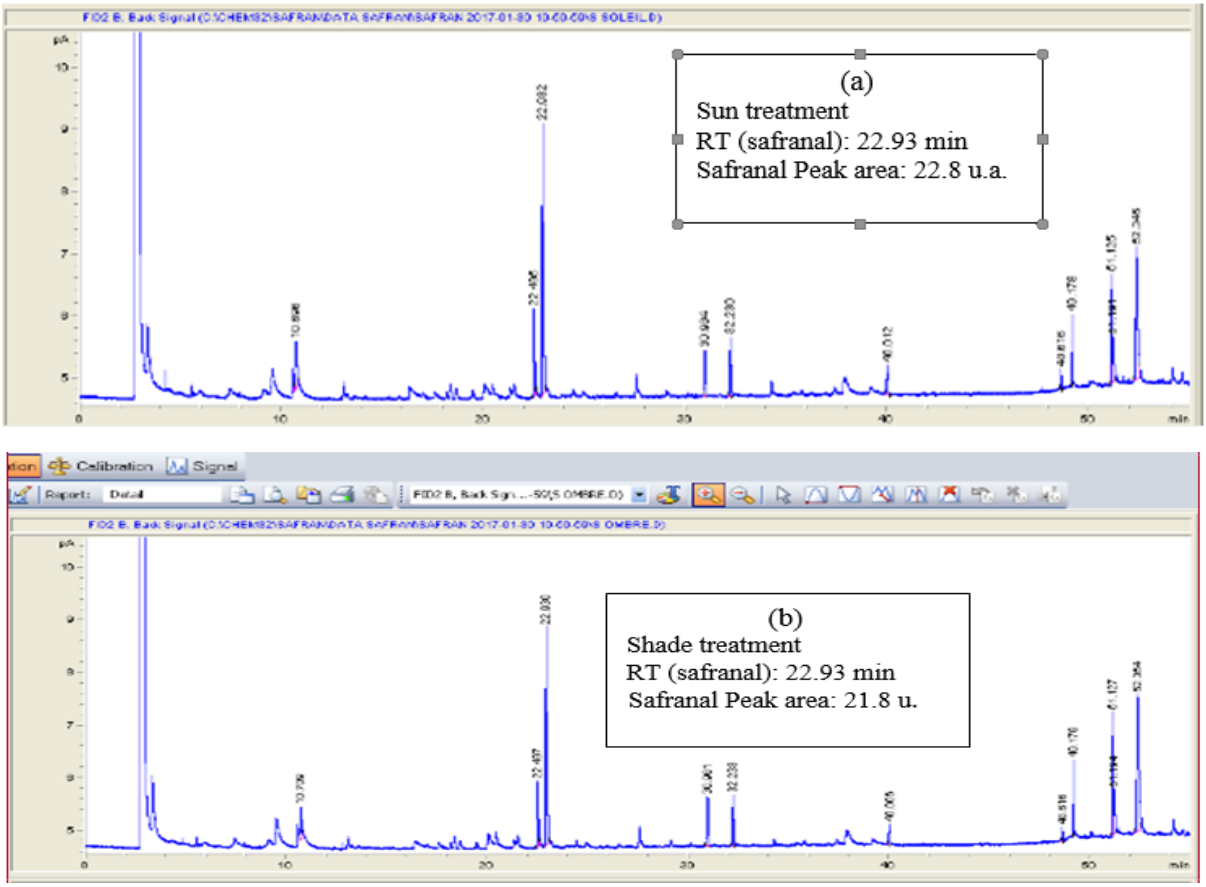

Figure. 4: Example of chromatograms of saffron samples dried under sun (a) and shade (b) in ambient air

3. Results and discussion

In this study, the fresh samples of saffron are dried by three different methods including oven drying, sun and shade drying in ambient air. The $E^{1\%}$ average values of aqueous saffron extract determined at 440, 330 and 257 nm with those processes of drying are summarized in Table 2. Table 2 also gives the moisture and volatile matter contents (%) and the safranal contents (mg/g) obtained from the external calibration curves for the UV-spectrosphotometry and the gas chromatography analysis. The moisture and volatile matter contents are in all samples less than 12% as limited by ISO 3632 (2010).

According to GC-analysis data, saffron samples do not differ in their chemical composition (11 or 12 components appear on each GC chromatogram) except two peaks : the peak associated to the retention time (RT): 18.37 min appeared into chromatograms recorded at 30, 60, 75 and 90°C and the peak associated to RT : 52.35 min which disappeared at 90°C. The saffron samples differ in the concentration of their compounds and especially that of safranal(Figure 5(a) and (b)).

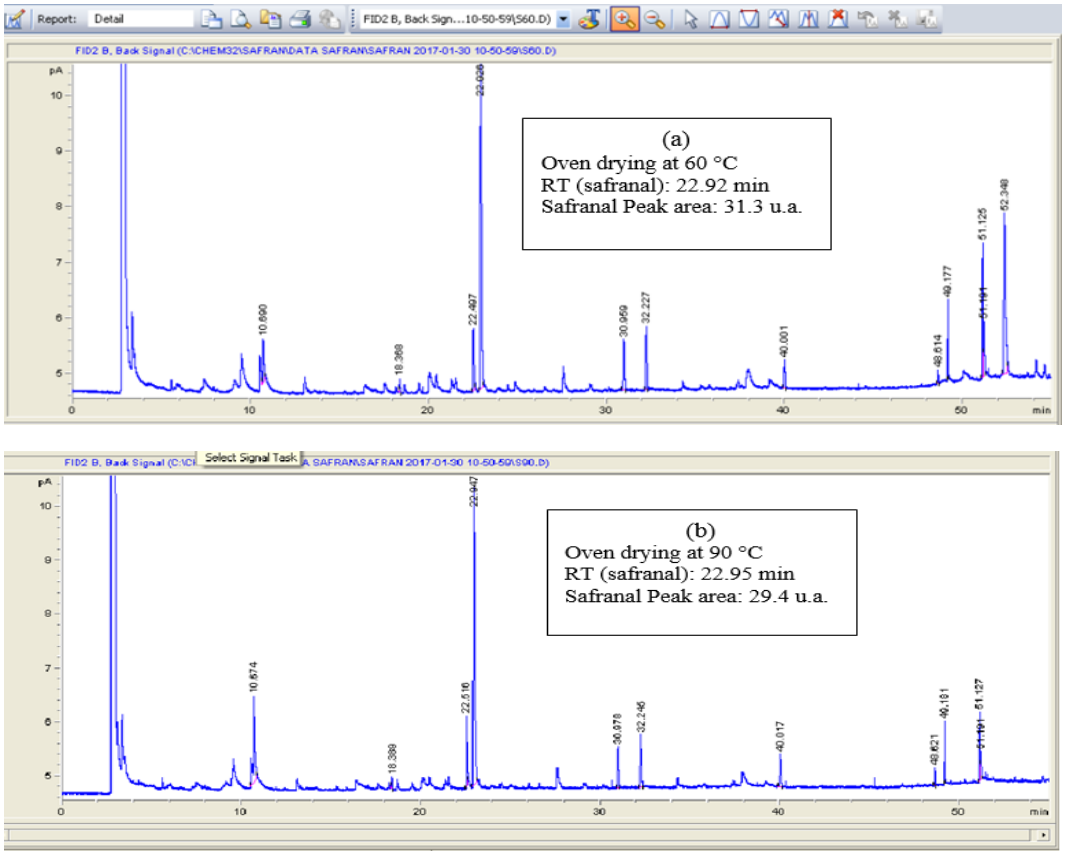

Figure. 5: Example of chromatograms of saffron samples dried in oven at 60°C (a) and at 90°C (b)

Additionally, all oven dried samples studied here belong to category I, for safranal, according to ISO 3632 norm. The only exception is for safranal UV values in the samples treated at 75°C and 90°C where $E^{1\%}$ (330 nm) exceed the norm limit value ($20<E^{1\%}<50$). As regards the drying process in ambient air, under shade and sun, the $E^{1\%}$ for crocin (440 nm) and picrocrocin (257 nm) are very low, probably due to high amount of humidity in Agadir city when the samples were treated. In contrast, the concentration of safranal, determined by UV absorption increases between 30°C and 90°C, probably due to the increase in of the solubility of safranal and its interfering absorbents, in water when the temperature increases. An increase in crocin and picrocrocin levels was also observed between 30°C and 60°C. But these values for these two compounds decrease between 60°C and 90°C. For the crocin the $E^{1\%}$ is below the limiting value ($E^{1\%}$ = 200 category I) of the norm ISO 3632(2010) for the drying treatment at 30°C, 75°C, 90°C and for drying method at shade and sun.

Fig.6(b) presents the safranal profile determined by gas chromatography versus dry temperature values. The lower amount of safranal was observed at 30°C (3.8 mg/g). The maximum safranal content was obtained above 60°C (11.5 mg/g) before decreasing from 75°C to 90°C (respectively 10.6 and 10.3 mg/g). The amounts of safranal obtained by the drying process, between 45°C and 90°C, are largely higher than those obtained with shade and sun treatments in ambient air. Indeed, for the treatment in shade the amount of safranal is 5.1mg/g and for the treatment in the sun 5.9mg/g. The diminution observed between 60°C and 70°C can be attributed to high dispersion and vaporization of safranal compound.

The safranal content increased by 46%, **67**%, 64 and 63% when the heat temperature was changed from 30 to 45, 60, 75 and 90°C respectively**.** When the drying temperature was changed from shade to under sun in ambient air, the safranal content increased by 14% and by 28%, **56**%, 52 and 50% when the drying process was changed from shade in ambient air to oven drying method at 45, 60, 75 and 90 °C respectively. It is clear that the oven drying process at 60°C appear to be the most effective treatment.

For the crocin and picrocin,when the oven drying temperature passes from 30 to 45, 60, 75 and 90°C, the $E^{1\%}$ (440 nm) and $E^{1\%}$ (257 nm) increases by 27% to **38**%, 21% and 6% for crocin and by 23% to 26%, 18% and 13% for picrocrocin. While for shade tosun treatments, the value of $E^{1\%}$ (440 nm) and $E^{1\%}$ (257 nm) increase is respectively 30% and 23%. Finally, when the drying temperature changes from shade to oven treatment 45, 60, 75 and 90°C, the $E^{1\%}$ (440) and $E^{1\%}$ (257) increased by 54, **61**% , 50% and 41% for crocin and by 39% ; **41**% , 35% and 32% for picrocrocin respectively.

Table 2: Average values of UV-spectrophotometry measurements of secondary metabolites and safranal content (mg/g) determined by UV-Vis spectrophotometry and by GC-FID.

Drying Methods		W_{MV}	$E^{1\%}$ Safranal	$E^{1\%}$ Crocin	$E^{1\%}$ Picrocrocin	$[C_{10}H_{14}O]$ (mg/g) "by UV-Vis"	$[C_{10}H_{14}O]$ (mg/g) " by GC"
Commercial saffron		8.5	48.5	215.8	120.5	0.8	8.8
Electric oven	30°C	9.5	26.5	148.8	90.6	0.5	3.8
	45°C	8.1	47.9	202.4	117.5	0.8	7.1
	60°C	7.9	**49.9**	**238.9**	**121.6**	**0.9**	**11.5**
	75°C	7.5	**69.2**	188.1	110.3	1.2	10.6
	90°C	7.4	**75.6**	157.7	103.7	1.3	10.3
Shade		10.2	24.5	93.5	71.3	0.4	5.1
Sun		9.9	31.1	133.2	93.2	0.5	5.9

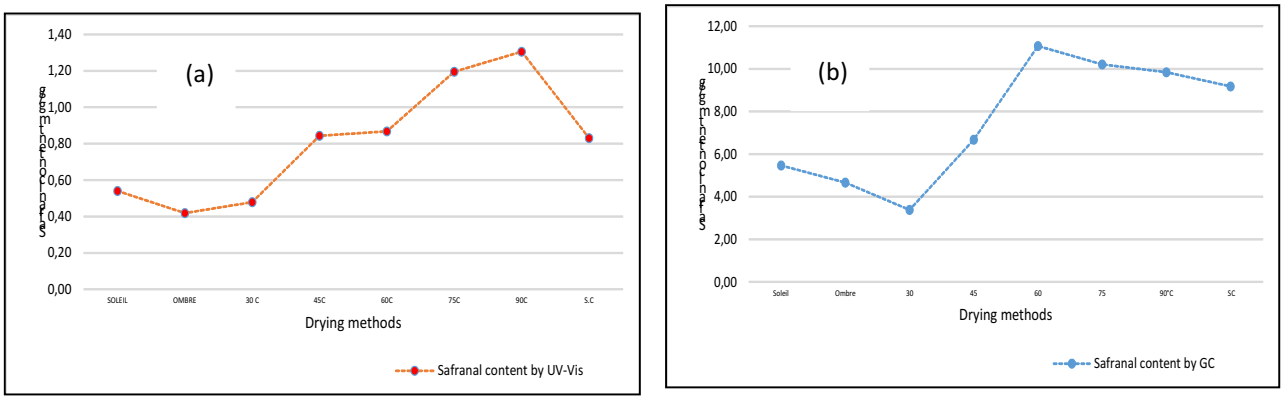

Figure. 6: Safranal profiles by UV absorption at 330 nm (a) and gas chromatography (b) analysis

The safranal values observed in the present work, in the range of previous studies, confirm that the heat treatment protocol used is valuable. These safranal values were ranged from 3.8 to 11.5 mg/g in this work, 5.0 to 8.5 mg/g for Iranian saffron [27], 3.6 to 4.2 mg/g for Indian saffron [27] and 6.8 to 10.4 mg/g for Greece saffron [27]. More important, the oven drying process at 60 °C leads to higher levels of crocin and picrocrocin indicating a better quality of the saffron.

Conclusion

The aim of this study was the determination, for the first time by gas chromatography, of the content of safranal in Moroccan Saffron and to find others drying process of the spice up to now practiced under shade or sun in ambient air. Three processes of drying were used, and all have confirmed the great influence of drying on the secondary metabolites contents of saffron. In addition to shade and sun treatments in ambient air, the samples were dried in an electric oven at different temperatures, namely 30, 45, 60, 75 and 90°C. The values obtained of $E^{1\%}$ of the main secondary metabolites, determined according to ISO 3632 norm, show that in every case, the saffron is of category I. The contents of safranal obtained by chromatographic analysis are greater than those determined by UV spectrophotometry at 330 nm, and they are in agreement with the safranal content of saffron from other countries. Characteristics of saffron quality are much better when it dried in an electric oven at 60 °C. Finally, other studies on the mechanisms of aroma development during drying and the kinetics of degradation of secondary metabolites during the storage of saffron are still necessary.

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