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Chemical, biochemical and volatile profiles of saffron (*Crocus sativus* L.) from different growing areas of Morocco

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Abstract

Background: The saffron (*Crocus sativus* L.) planted area and production increased in the past decade in Morocco. This crop has been extended to new regions beyond its original main area due to the shift in climate conditions. Therefore, this study aimed to investigate the chemical characterization and the quality of saffron stigma samples collected from 11 different localities.

Results: According to the ISO3632 standard method, all samples were within the ISO36-32.2010 categorization range (category I and II) Contents of crocin responsible for color, picrocrocin responsible for taste, and safranal responsible for the aroma, showed significant differences between samples from different areas. The biochemical analysis revealed that samples from Boulmane (Serghina and El Mers) are rich in polyphenols (5.70 ± 0.34; 5.31 ± 0.004 mg GAE/g DW, respectively) and have an important antioxidant power (IC_{50} for the DPPH: 231.12 ± 1.065; 236.77 ± 2109 respectively) compared to those from Taznakht and Taliouine known for their higher organoleptic quality. Interestingly, volatile profiling by gas chromatography–mass spectrometry identified the safranal, isophorone, ketoisophorone, ß-isophorone, and 1-4-cyclohexanedione, 2,2,6-trimethyl as the main volatile compounds in saffron samples from different regions.

Conclusion: This study is the first to be conducted in Morocco on saffron newly cultivated in new regions (Boulmane, Azilal, and Ain Leuh) and showed the higher potential for growing saffron, thus promoting the adoption of saffron as a substitute crop for the socio-economic development in these new areas.

KEYWORDS
biochemical parameters, saffron (*Crocus sativus* L.), secondary metabolites, volatile profiles by GC–MS

INTRODUCTION

Crocus sativus L. is a perennial plant belonging to the Iridaceae family.[1] It is mainly cultivated in Iran, India, Morocco, Greece, Spain, and Italy.[2] The crop is grown under different pedo-climatic conditions, which significantly affects the produced saffron quality. Iran produces around 90% of the world's production, followed by India, Morocco, Afghanistan, and few southern European countries such as Spain, Italy, and Greece.[2]

Saffron (stigma), is well recognized for its pharmacological benefits such as antioxidant,[3] anti-inflammatory,[4] antihypertensive and hypolipidemic,[5] antidepressant,[6] and antitumor.[7] Interestingly, a new study demonstrated its anti-inflammatory and antiviral potential against severe COVID-19 symptoms.[8] The quality of saffron is closely dependent on the relative amounts of the three important secondary metabolites known as crocins, picrocrocin, and safranal which are responsible for the color, bitter taste, and odor, respectively.[9–11] Their

relative concentrations are impacted by a combination of cultural and harvesting practices as well as post-harvest treatments, especially dehydration techniques.[9–12] Various chemical and biochemical processes occur during drying and storage conditions, resulting in the desired characteristics of the saffron quality, such as aroma, chemical stability, and antimicrobial activity.[13] Crocin, a primary carotenoid of saffron, is the product of the glycosylation of a dicarboxylic carotenoid (crocetin). Picrocrocin, a monoterpene glycoside, is the precursor of safranal. Safranal, volatile monoterpene aldehyde, results from hydrolysis and dehydration of picrocrocin and represents over 65% of the total aroma components in saffron.[2]

Saffron aroma comprises more than 90 volatiles compounds.[14,15] The compound safranal is the most important component of saffron essential oil and is usually determined by the absorbance of aqueous saffron extract at 330 nm (ISO 3632-2010). However, this method is very limited to safranal only, while other key important volatiles such as the isomers of safranal and isophorones, with key odorant effects in saffron, as well as strong therapeutic properties, are not included.[16]

The saffron quality in international commercial agreements can be classified into one of the three categories set by the ISO 3632 (2010) standard. The set price market is based mainly on the color parameter which is measured by using Ultraviolet–visible (UV–vis) spectrometry as established by the ISO 3632 (2010) standard.

A variety of analytical techniques are used to assess the quality of saffron. The most relevant is the ISO 3632: 2010 standard, which is utilized in international trade agreements; however, many studies suggest that such a standard should be revised, in particular, to analyze safranal because it is possible that other compounds may interfere with the determination of safranal content in this analysis.[16] Among established and widely used methods in determining food aroma compounds is gas chromatography–mass spectrometry (GC–MS). This approach has a number of advantages. This method GC–MS has also been developed for the characterization and quantitative determination of volatile saffron markers; it provides the most effective separation in the shortest time for the volatile compounds.[17,18] This method has numerous advantages over the ISO 3632 method: It is used to quantify safranal in a range of saffron samples,[14,17,19–23] for the detection of adulterants,[24] and especially for geographical discrimination of saffron samples.[22,23]

In Morocco, saffron production has increased in recent years.[25] The planted area was tripled (from 610 ha in 2008 to 1944 ha in 2020), and the production increased by 6.2 tonnes in 2020 versus only 1.5 tonnes in 2008 and 3.2 kg/ha instead of 2.5 kg/ha. Before, the cultivation of saffron was limited to two main regions known as Taliouine and Taznakht (the Anti Atlas); however, due to climate change and the increasing demand, its cultivation is being extended beyond the conventional regions to different regions in Morocco with different distinct subclimats. Therefore, the present paper aims to analyze the chemical, biochemical quality, and volatile profiles of saffron from new growing areas, which are characterized by different environmental conditions and assess its potential to produce a good and commercial quality of the saffron.

MATERIALS AND METHODS

Plant materials

Saffron samples were collected from 11 different areas of the anti-Atlas (Taliouine, Timjicht, Siroua, Khouzama), the high Atlas (Ourika), and the Middle Atlas (Azilal, Ain leuh, Serghina, Ain Atia and El Mers) (Table 1 and Figure 1).

Physico-chemical characterization parameters

The moisture, amount of picrocrocin, crocin, and safranal for each sample were determined by following procedures established by ISO3632-2010.[26]

Moisture and volatile matter content

The moisture was measured by drying 500 mg of each powdered saffron sample, then incubated in an oven for 16 h at 103 ± 2°C, and then weighted again. Moisture and volatile content (W_{MV}) were calculated for each sample using the following formula: $W_{MV} = (m_0 - m_4) \times 100/m_0$, where m_0 is the mass of the test portion (g), and m_4 is the mass of the dry residue after incubation (g).[26]

Amount of crocin, picrocrocin and safranal with UV–vis spectrometer

The powdered saffron sample (500 mg) was placed into a volumetric flask for each sample, and 900 mL of distilled water was added. The solution was mixed for 1 h at room temperature, under magnetic stirring at 1000 rpm. Then it was adjusted to 1000 mL with distilled water (1:10). Finally, the extracts were filtered through a polytetrafluoroethylene (PTFE) filter with a pore size of 0.45 µm and directly analyzed by a spectrometer (T60UV-vis, China). The amount of picrocrocin, crocin, and safranal were determined at 275, 440, and 330 nm, respectively. The concentration was calculated using the following formula: $E^{1\%}\ 1\ \text{cm} = D \times 1000/m(100 - W_{MV})$, where D is the measured absorbance value; m is the mass of the sample, and W_{MV} is the moisture and volatile content of the sample expressed as a mass fraction.[26]

Biochemical characterization

Preparation of extracts

Extraction was done using 250 mg of each dried and ground saffron sample and mixed with 10 mL of 80% (v/v) methanol. Afterwards, the solution was shaken slowly at 110 rpm for 8 h. After filtration, the resulting extracts were stored in dark conditions at 4°C until further use.

TABLE 1 Characteristic of the origin of different studied samples.

Samples	Locality	Altitude	Temperature range (min–max)	Region
Z1	Serghina	2029 m	3°–28°	Fès-Meknès
Z2	Ain Leuh	1300 m	2°–28°	Fès-Meknès
Z3	Ain Atia	2015 m	3°–28°	Fès-Meknès
Z4	El Mers	2018 m	3°–28°	Fès-Meknès
Z5	Timjicht (Taznakht)	1561 m	1°–35°	Darâa-Tafilalet
Z6	Siroua	2010 m	1°–35°	Darâa-Tafilalet
Z7	Khouzama	1427 m	1°–35°	Darâa-Tafilalet
Z8	Ourika	1100 m	6°–38°	Marrakech-Safi
Z9	Azilal	1577 m	2°–33°	Beni Mellal-Khénifra
Z10	Taliouine (Coop-Dar Zafaren)	NI	7°–37°	Souss-Massa
Z11	Askaoun (Taliouine)	2000 m	7°–37°	Souss-Massa

Abbreviation: NI, not identified.

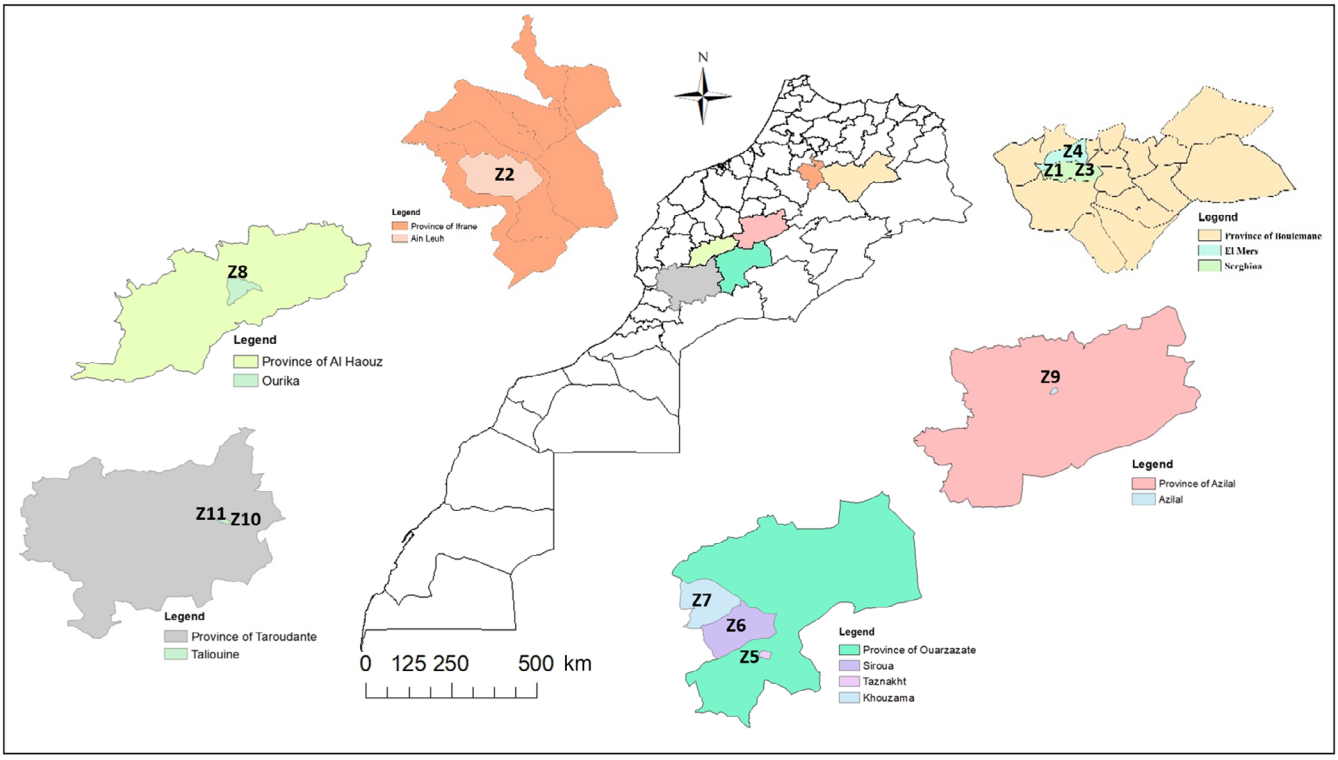

FIGURE 1 Map showing the samples location zones.

Total phenolic contents

The total phenolic content (TPC) of each sample was determined by a colorimetric method based on the procedure described by Ghanbari et al.[27] Briefly, 0.5 mL of each sample was added to 2.5 mL of Folin–Ciocalteu (FC) reagent (1:10) and incubated for 5 min at room temperature. Afterwards, 2 mL of 7.5% sodium carbonate solution was added. After the shake, the mixture was incubated in a hot water bath at 45°C for 15 min. Finally, the absorbance was recorded at 765 nm. The results were expressed as mg of gallic acid equivalent (GAE/g sample dry weight [DW]).

Total flavonoid content

The total flavonoid content (TFC) was measured by the aluminum chloride method using quercetin as a standard and described by Ghanbari et al.[27]: 0.3 mL of 5% $NaNO_2$ solution was added to 0.5 mL of methanolic extract. The mixture was incubated in the dark at room temperature for 6 min. Thereafter, 0.6 mL of 10% $AlCl_3$ was added and incubated for 5 min. Finally, 3 mL of NaOH 1 M was added, and the final volume was adjusted to 10 mL with distilled water. The absorbance was read at 510 nm after 15 min incubation. The total

flavonoid content values were expressed as mg of quercetin equivalent (QE) per g DW.

DPPH radical scavenging activity

Methanolic DPPH solution 0.5 mM (1.5 mL) was added to 0.75 mL of prepared 50, 100 and 300 µg/mL extract concentrations.[27,28] After 20 min, the absorbance was determined at 517 nm with 80% methanol as blank. The same concentrations of ascorbic acid were used as a positive control. The percentage of inhibition was determined according to the following formula: Inhibition (%) = $((A_{control} - A_{sample})/A_{control}) \times 100$, where A_{sample} is the absorbance values of the sample and $A_{control}$ is the absorbance of the control. After calculating the percentage of inhibition, a linear regression model was established based on concentration and percentage of inhibition.

Volatile profile analysis

Extraction of volatile compounds by HS-SPME was realized using a 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) fiber that was preconditioned according to the instructions of the manufacturer. Extraction was performed with 300 mg of saffron powder placed in a 20 mL vial incubated at 30°C for 5 min. The sampling time with the SPME fiber was 10 min at 30°C and the injection temperature in the gas chromatograph 270°C.

The volatile components analysis of saffron was performed by GC–MS using Agilent 7890A system (Wilmington, DE, USA) with mass selective detector 5975Network MSD and coupled to an MPS automatic sampling system. Chromatographic separation was performed on HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.17 mm), and the following temperature program was used: 60°C held for 3 min, then increased to 210°C at a rate of 4°C/min, then held at 210°C for 15 min, then increased to 300°C at a rate of 10°C/min, and finally held at 300°C for 15 min. Helium was used as the carrier gas at a constant flow of 1 mL/min. For quantification, the results are presented as a percentage of the peak area considering a response factor of the fiber.

Statistical analysis

Statistical analysis was performed by SPSS V25 software, and the results were reported as the mean ± standard deviation. A variance analysis was performed to check for a significant difference between sites. Duncan's multiple analysis was used to compare the mean of sample absorbances at a significance level of $p < 0.05$. The correlation coefficients and their signification levels were calculated using the Pearson model. The principal component analysis (PCA) was carried out according to the correlation coefficients. The Varimax rotation method with Kaizer normalization was applied in order to identify the most discriminating variables and the total inertia expressed by the model. A dendrogram (hierarchical tree) was developed based on the Euclidean distance to understand similarity levels between the different sites better and classify them to show the variation between samples according to their different origins.

RESULTS AND DISCUSSION

Physico-chemical characterization parameters

Moisture and volatile content

As the saffron is a very hygroscopic product making it very sensitive to moisture, thus increasing the risk of spoilage, the process of drying stigmas as a post-harvest treatment is widely practiced to reduce the moisture to a value below 12%, and to guarantee a microbiologically stable product. The moisture of saffron samples varied between 6% and 10% (Table 2), which is below the established threshold of 12%. The data showed that all collected samples were within limits. According to Mzabri et al.,[29] the difference observed in the moisture content between the different samples may be related to the environmental conditions in their corresponding locations, particularly when the flowers are harvested under wet conditions, making the drying operation more difficult. In addition, the traditional drying parameters such as drying time, ambient temperature, and ambient moisture content can increase the moisture content of the product.

Levels of picrocrocin, safranal, and crocin

Contents of crocin responsible for color, picrocrocin responsible for taste, and safranal responsible for the aroma, showed significant differences between samples from different areas. The means of crocin, picrocrocin, and safranal ranged from 165.31 to 261.94; 67.19 to 118.09, and 28.07 to 38.99, respectively. These data align well with those reported by Lage et al., Ghanbari et al., and Tabibian et al.[27,30,31] who assessed the quality of stigmas from corms grown in different environments. The highest levels of crocin (261.94)/(Z5), picrocrocin (118.09)/(Z5), and safranal (38.99)/(Z2) were observed respectively in samples of Timchijt (Taznakht) (Z5) and Ain Leuh (Z2). In contrast, the lowest level of crocin (165.31)/(Z2), picrocrocin (67.19)/(Z8), and safranal (28.07)/(Z1) were observed, respectively, in samples from Ain leuh, Ourika, and Serghina. According to the ISO3632 standard method, the results showed that samples from Boulmane (Seghina-El Mers-Ain Atia), Taznakht (Timjicht–Siroua), Azilal and Taliouine (Dar Zaefaran) belong to the category I, while the others four from Askaoun, Ain Leuh, Khouzama, and Ourika are situated in category II (Table 2).

The results show that samples rich in crocins and picrocrocins are poor in safranal and vice versa (Table 2). This is consistent with the finding by Sereshti et al.,[32] who reported a negative correlation between safranal, picrocrocin, and crocin content in stigmata following 2 years of storage. Generally, the variability in the quality parameters (crocins, picrocrocins, and safranal) could be attributed to the

TABLE 2 Comparison of the picrocrocin, safranal, and crocin contents of the studied samples.

Sample	Moisture (%)	Picrocrocin $E^{1\%}$ 257 nm	Safranal $E^{1\%}$ 330 nm	Crocin $E^{1\%}$ 440 nm	Quality* category
Z1	9.9	79.68 ± 3.409^{c}	28.07 ± 0.132^{a}	226.79 ± 0.11^{c}	I
Z2	9.9	78.57 ± 0.183^{c}	38.99 ± 0.755^{f}	165.31 ± 0.425^{a}	II
Z3	10	69.10 ± 0.573^{b}	33.13 ± 0.138^{de}	223.22 ± 3.198^{c}	I
Z4	6	98.13 ± 0.105^{g}	31.00 ± 0.105^{c}	257.34 ± 0.542^{e}	I
Z5	6.6	118.09 ± 0.276^{h}	33.40 ± 0.171^{e}	261.94 ± 0.934^{e}	I
Z6	6.7	97.96 ± 1.064^{g}	29.04 ± 2.087^{ab}	236.43 ± 0.678^{d}	I
Z7	6.6	91.27 ± 0.561^{f}	32.97 ± 0.248^{de}	178.90 ± 0.866^{b}	II
Z8	8.4	67.19 ± 1.058^{a}	33.72 ± 0.954^{e}	171.86 ± 15.83^{ab}	II
Z9	8.3	99.72 ± 1.210^{g}	32.11 ± 0.272^{d}	243.17 ± 0.838^{d}	I
Z10	6	86.06 ± 0.276^{e}	30.12 ± 0.495^{bc}	221.11 ± 0.585^{c}	I
Z11	9.6	83.23 ± 0.110^{d}	29.64 ± 0.179^{b}	177.26 ± 0.609^{b}	II

Note: Duncan' significant difference p <0.05. In each column samples having the same letter are not signficantly different according to Duncan' s test. *ISO 3632 limits for the first quality category (saffron filaments) are picrocrocin>70, 20 < safranal <50, crocins >200. ISO 3632 limits for the second quality category (saffron filaments) are picrocrocin >55, 20 < safranal <50, crocins >170. ISO 3632 limits for the third quality category (saffron filaments) are picrocrocin >40, 20 < safranal <50, crocins.

genetic material used, the quality of the seed cromes, the environment condition as well as to agronomic, harvest and post-harvest (drying and storage) practices.[29,33–35]

The breakdown of picrocrocin gives rise to safranal during the drying and storage of saffron.[36,37] Drying methods and practices (naturally in shade, sunlight, electric ovens, etc) are known to affect the final composition of saffron.

Maggi et al.[38] reported that the safranal content of stigmata increased during 3 years of storage and then decreased afterwards. A longer storage period induced a decrease in the HTCC (4-hydroxy-2,-6,6-trimethylcyclohex-1-enecarbaldehyde) which is considered as a possible reservoir for safranal production during storage. Also, crocins and picrocrocins naturally degrade in stigma cells during drying, storage, and extraction depending on temperature, humidity, light irradiation, and other media compounds.[39]

Analysis of groups and establishment of the dendrograms of similarity

The dendrogram analysis based on chemical features (three components of crocin, picrocrocin, and safranal) showed variability among studied areas. The similarity level (d) based on the squares of Euclidean distances ranged from 1 to 25. Two clusters have been identified (I and II). The first group (I) was identified at $d = 8$, comprises seven zones, and is subdivided into two distinct subgroups A and B. The first subgroup A contains four zones (Z5, Z9, Z4, and Z6) from Tamjicht, Azilal, Elmers and siroua respectively, and is essentially characterized by strong color, taste, and aroma in this cluster, the Z5 (Tamjicht/Taznakht) is distinguished from neighboring subsets, have the highest level of color and taste, and forms a branch far from the A subgroup.

The second sub-group B contains three areas (Z1, Z10, and Z3) from Serghina, Taliouine, and Ain Atia, respectively, which are closer in terms of color, taste, and aroma. There are characterized by strong color and medium taste and aroma.

The second group was identified at $d = 25$ and is made up of four samples Z11, Z2, Z7, and Z8 from Askouan, Ain Leuh, Kouzama, and Ourika, respectively, which are also closer in crocin, picrocrocin, and safranal levels (Figure 1) also they are characterized by medium color, taste, and strong aroma.

The difference observed between the samples collected from the same region can be explained by the altitude factor and the drying/storage practices. According to Mzabri et al.[29] and Lage and Cantrell[34] altitude is among the factors that influence color, confirming the results found particularly in the Boulmane zone. Also, it should be noted that drying is an important step to have a good saffron quality, but the poor preservation of the stigmata can significantly alter its aroma, color properties, and taste as well. Saffron is very hygroscopic and humidity makes it lose its aroma and induces its blackening hence the need to keep it in a dry place and apply appropriate drying and storage modes to guarantee saffron that meets the requirements of quality.[29,34,40]

Biochemical characterization parameters

Descriptive analysis

Highly significant variability for total polyphenols and flavonoids (p < 0.001) and slightly significant variability for antioxidant activity (p < 0.05) were among the samples. The contents of total polyphenols (TPC) varied between 4.17 and 6.05, with an average of 5.05 mg GAE/g DW. The highest values were recorded in Serghina, El Mers, Khouzama, Taliouine-Siroua and Askaoun with mean values of 5.70 ± 0.34; 5.31 ± 0.004; 5.30 ± 0.034; 5.25 ± 0.004–5.25 ± 0.012 and 5.24 ± 0.359 mg GAE/g DW respectively. However, Ourika and Ain

Atia samples were the least rich in polyphenols, with average contents of 4.33 ± 0.008 and 4.45 ± 0.012 mg GAE/g DW, respectively (Table 3 and Figure 2). These results are consistent with several previous works.[27,41,42]

The concentrations of total flavonoids (TFC) varied between 1.05 and 2.91 with an average of 1.80 ± 0.680 mg QE/g DM. It was high in samples from Khouzama (2.86 ± 0.006 mg QE/g DW), Siroua (2.84 ± 0.068 mg QE/g DW), Taliouine (2.62 ± 0.131 mg QE/g DW), and Azilal (2.03 ± 0.020 mg QE/g DW), but low in sample from Ain Atia (1.07 ± 0.013 mg QE/g DW), and Ourika (1.15 ± 0.006 mg QE/g DW). These values are consistent with studies by Ghanbari et al.,[27] Goli et al.,[42] and Baba and Malik.[43]

Half maximal inhibitory concentration (IC_{50})

The IC_{50} is a variable that provides information on the quality of radical scavenging for each antioxidative test. Inversely proportional to

TABLE 3 Average levels of biochemical properties of Saffron stigmata collected from different regions (total phenolic content [TPC], total flavonoid content [TFC], and DPPH radical scavenging activity).

Samples	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	DPPH (IC_{50}) (μg/mL)
Z1	5.70 ± 0.347^{c}	1.19 ± 0.14^{ab}	231.12 ± 1.065^{a}
Z2	5.20 ± 0.312^{b}	1.29 ± 0.002^{bc}	246.09 ± 1.971^{ab}
Z3	4.33 ± 0.008^{a}	1.07 ± 0.013^{a}	249.71 ± 16.828^{b}
Z4	5.31 ± 0.004^{bc}	1.42 ± 0.07^{d}	236.77 ± 2.109^{ab}
Z5	4.73 ± 0.012^{a}	1.96 ± 0.006^{e}	239.61 ± 4.330^{ab}
Z6	5.25 ± 0.012^{bc}	2.84 ± 0.068^{g}	241.89 ± 7.966^{ab}
Z7	5.30 ± 0.034^{bc}	2.86 ± 0.006^{g}	231.80 ± 1.301^{a}
Z8	4.45 ± 0.012^{a}	1.15 ± 0.006^{a}	249.17 ± 17.709^{b}
Z9	4.74 ± 0.572^{a}	2.03 ± 0.020^{e}	239.08 ± 4.490^{ab}
Z10	5.25 ± 0.004^{bc}	2.62 ± 0.131^{f}	234.15 ± 5.128^{ab}
Z11	5.24 ± 0.359^{bc}	1.33 ± 0.034^{cd}	239.29 ± 6.639^{ab}

Note: Duncan' significant difference $p < 0.05$. Values are presented as mean ± standard deviation. The different letters (a–g) represent statistically significant differences between samples at $p < 0.05$.

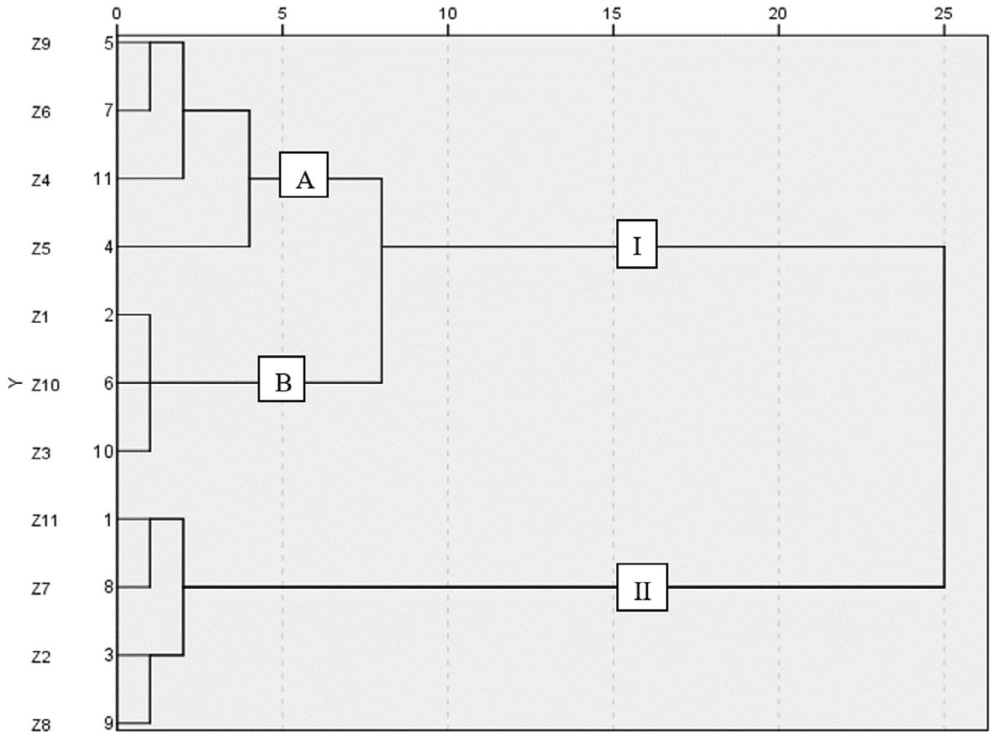

FIGURE 2 Dendrogram classifying samples based on chemical characteristics.

the IC_{50} value, the antioxidant potential is greater when very small concentrations are required to scavenge half of the radicals.

The IC_{50} for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test varied significantly between 228.75 and 269.54 μg/mL with an average of 239.88 ± 9.346 μg/mL (Table 4). The lowest IC_{50} were recorded in samples from Serghina and Khouzama (231.12 ± 1.065 and 231.80 ± 1.301 μg/mL) followed by Taliouine (234.15 ± 5.128 μg/mL) and El Mers (236.77 ± 2.109 μg/mL), which reflects the antioxidant power of saffron from these origins. The values obtained are consistent with studies conducted by Ghanbari et al.[27] and Karimi et al.[41]

Saffron stigmas have been shown to possess antioxidant activity, therefore, it is a promising natural product that, in addition to being a colorant, it plays a role of the antioxidant source, which could improve product quality in functional foods, beverages, pharmaceuticals and cosmeceutical industries.

The antioxidant activity in the saffron stigmas could be attributed to the presence and synergistic effects of phenolic compounds and flavonoids in addition to the presence of other active compounds. Siddhuraju and Becker[44] noted that plants with higher levels of total phenols and flavonoids exhibited greater reduction activity. Also, it was reported that increasing the antioxidant activity can be related to the maintained crocins in the stigma. Therefore crocins potentially quenches free radicals.[40,45,46]

Some of the observed variability in antioxidant capacity could be related to genotypic and environmental variables within species, as well as the analytical methodologies used.[33]

Correlation between secondary metabolites and biochemical parameters

The chemical (secondary metabolites) and biochemical variables were analyzed by the bivariate correlation analysis of the Pearson model in order to identify the relationship between them. Correlation coefficients could provide information on potentially important variables in evaluating different saffron samples. The analysis revealed significant correlations at the 0.05 and 0.01 levels, marked in bold in Table 4. Polyphenols are negatively correlated with IC_{50} (DPPH) at the level of $p < 0.01$ and with safranal at the level of $p < 0.05$. These correlations are expressed by coefficients of ($r = -0.473$) and ($r = -0.394$), respectively. The antioxidant capacity of saffron is mainly due to its richness in polyphenols (the richer the saffron is in polyphenols, the more powerful its antioxidant activity).

On the other hand, crocins are positively correlated with picrocrocins ($r = 0.655$) and negatively with safranal ($r = -0.413$), since crocins and picrocrocins naturally degrade in saffron stigmas during drying and storage and dehydration of picrocrocins gives rise to safranal.

Also, flavonoids were found to be positively correlated with picrocrocins at a level of $p < 0.01$ with a coefficient of 0.529. This correlation could be explained by the influence of the picrocrocin content measured as $E^{1\%}$ 275 nm by the presence of other water-soluble compounds such as flavonoids.[47]

Principal component analysis

A PCA based on the Varimax method was performed to determine the variables that express the maximum of the total variance. In our study, only a principal component load greater than 0.5 was considered significant for each factor. The PCA revealed two components that express 70.75% of the variance (Table 5). The first component represented 47.76% of the total variance and was mainly influenced by polyphenols, antioxidant activity, and safranal content. As for the second component, PC2 represented 22.98% of the total variance and was positively correlated with picrocrocins and crocins.

The scatter plot was established based on two principal components, PC1 and PC2, whose respective expressed variances are 47.76 and 22.98% (Figure 3). It revealed three groups. Starting from negative to positive values, the distribution of samples around PC1 indicates an increase in the polyphenols content and antioxidant capacity and a decrease in the safranal content. While, in the same direction on PC2, the distribution of samples provides information on an increase in crocins and picrocrocins.

This analysis showed great similarity in polyphenols and antioxidant capacity between the samples from Boulmane (Z1, Z4) and those from Taznakht and Taliouine, known for their quality and renown. In addition, similarity in terms of crocin and picrocrocin was revealed between the samples of Taznakht and Azilal. Finally, another similarity in terms of safranal richness is shown in samples from Ain Leuh and Ourika.

TABLE 4 Correlation matrix between secondary metabolites, biochemical compounds, and antioxidant activity.

	Flavonoides	Polyphenols	IC_{50} (DPPH)	Crocines	Picrocrocines	Safranal
Flavonoides	1					
Polyphenols	0.223	1				
IC_{50} (DPPH)	−0.307	**−0.473**[a]	1			
Crocines	0.172	−0.040	−0.217	1		
Picrocrocines	**0.529**[a]	0.145	−0.280	**0.655**[a]	1	
Safranal	−0.262	**−0.394**[b]	0.333	**−0.413**[b]	−0.157	1

Note: Significant correlations at a level of $p < 0.01$ and $p < 0.05$ are marked in bold.
[a]The correlation is significant at the 0.01 level (bilateral).
[b]The correlation is significant at the 0.05 level (bilateral).

To our knowledge, this study is the first carried out in Morocco on saffron to compare different and new locations, particularly Boulmane and Ain Leuh. It reveals very encouraging results leading to the extension of this precious crop to new sites.

Determination of volatile components by GC–MS

Volatiles analysis carried out by GC–MS chromatography identified several volatile compounds in the different saffron samples from the different regions (Table 6). Most of identified VOC have been previously reported in saffron from diverse geographical origins by different extraction and detection methods.[13,15,19,20,23,24,27,48–51] The terpenes were the most abundant in all samples, with monoterpenic ketones (such as β-isophorone, α-isophorone, and 4-ketoisophorone) and monoterpenic aldehydes (such as Safranal) being the most represented groups which are line with reported data.[19,52]

Safranal is the most volatile component of saffron and constitutes about 60% of the volatile fraction.[38] It is the most influential aroma compound that contributes to the overall aroma of *C. sativus*.[17] Our study revealed that the content of safranal varied between 66.06% in Z2 (Ain Leuh) and 22.96% in Z7 (Khouzama). The highest value was recorded in the Ain Leuh samples, which is consistent with UV-spectroscopy results. Also, the amount of safranal increased depending on the temperature of the drying process,[53] thus confirming our result. In previous works, high values of safranal (mean 69.3%) were reported by Urbani et al.[20] also values between 8.7% and 41% were found by Kanakis et al.[15]

Saffron minor volatiles are described as isophorone analogues (C9 and C10 groups of compounds) with a similar structure to safranal such as β-isophorone, α-isophorone, 4-ketoisophorone were reported in almost all collected samples from all geographical areas .

The isophorone compound oscillated between 45.70% in Z10 (Dar zaefaran/Taliouine) and 11.58% in Z2 (Ain Leuh), followed by ketoisophorone, which varied from 22.39% in Z9 (Azilal) to 5.46% in Z6 (Siroua/Taznakht) and ß-isophorone whose values varied between 16.49% in Z9 (Azilal) and 0.29% in Z11 (Askaoun/Taliouine). In

TABLE 5 Principal component analysis: the first two components (PC1 and PC2).

	Components	
	PC1	PC2
Polyphenols	**0.916**	−0.096
Flavonoids	0.430	0.497
IC_{50} (DPPH)	**−0.900**	−0.265
Picrocrocins	0.179	**0.890**
Safranal	**−0.640**	−0.276
Crocins	0.031	**0.880**
Variance %	**47.76**	**22.98**
Cumulative %	47.76	**70.75**

Note: Eigen values higher than |0.5| are mentioned in bold.

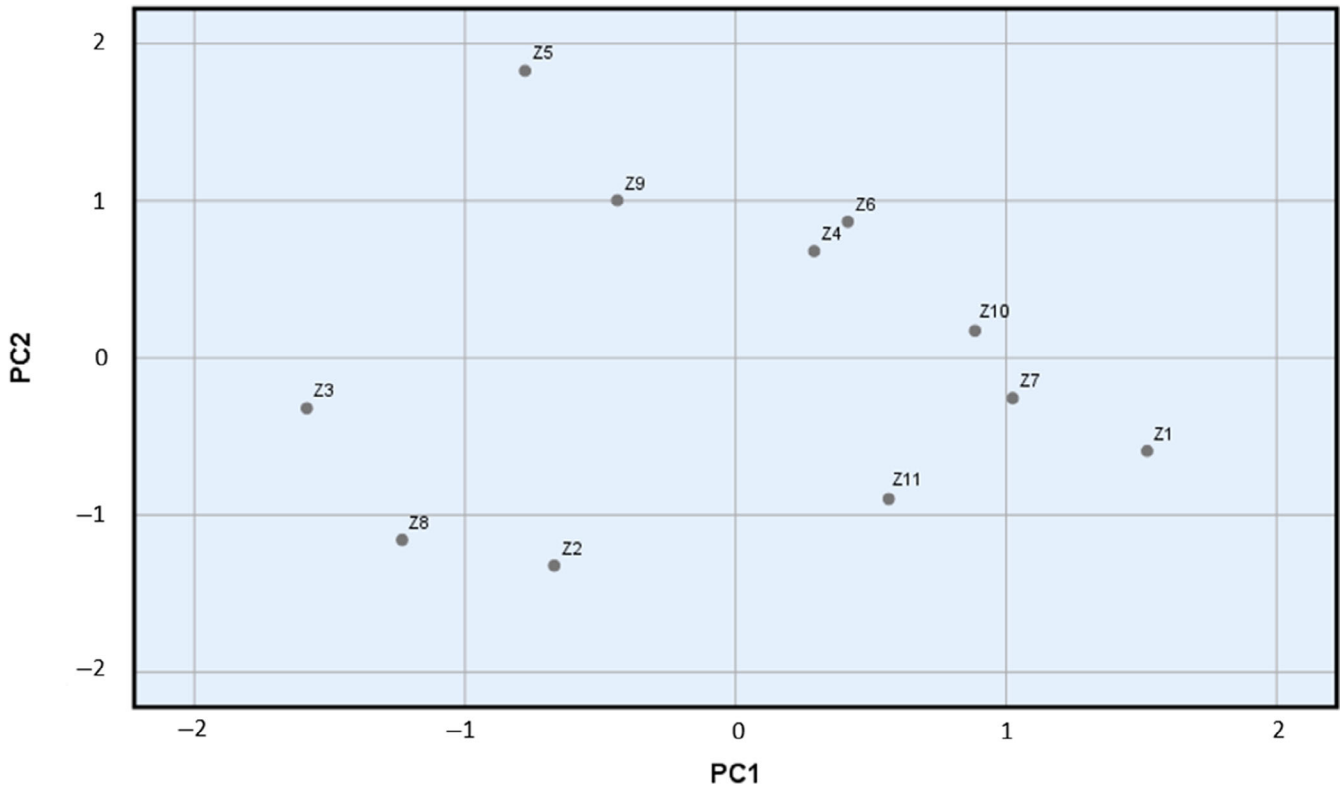

FIGURE 3 Scatter plot of saffron samples.

TABLE 6 Volatile compounds of saffron samples from different geographical origins.

Sample	Compound	CAS number	RI calculated	RI lit	% peak area
Z1	Acetic acid	64-19-7	647	710	1.03
	Acetic acid, methoxy-, ethyl ester	3938-96-3	696	845	0.44
	Acetoin	513-86-0	786	711	0.10
	2(5H)-furanone	497-23-4	921	951	1.12
	3-cyclohexen-1-one, 3,5,5-trimethyl-(ß-isophorone)	471-01-2	1037	1429	3.07
	3,5,5 trimethyl-2-cyclohexen-1-one (Isophorone)	78-59-1	1126	1118	24.28
	2-cyclohexen-1-one, 2-hydroxy-6-methyl-3-(1-methylethyl)-	54783-36-7	1133	1395	0.28
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	1125-21-9	1150	1142	14.82
	2-hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	28750-52-9	1166	1164.9	1.10
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1183	1258	12.24
	1,3-cyclohexadiene-1-carboxaldehyde,2,6,6-trimethyl-(Safranal)	116-26-7	1207	1201	34.07
	4-hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde (HTCC)	35692-94-5	1226	1322	0.03
	(4aR,7S,7aS)-4,7-dimethyl-5,6,7,7a-tetrahydrocyclopenta[c]pyran-1(4aH)-one	21651-53-6	1314	1289	0.69
	1-cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	1000196-69-4	1356	1309	0.58
	Di-epi-.alpha.-cedrene-(I)	21996-77-0	1386	1388.2	0.01
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1397	1396.4	3.14
	1h-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	469-61-4	1413	1403.3	0.15
Z2	Acetic acid	64-19-7	646	710	0.75
	Butyrolactone	96-48-0	918	915	0.23
	2(5H)-furanone	497-23-4	924	951	0.11
	2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde	162376-82-1	1101	1108.7	1.08
	3,5,5 trimethyl-2-cyclohexen-1-one (Isophorone)	78-59-1	1122	1118	11.58
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	1125-21-9	1148	1142	11.40
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1183	1258	3.79
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- (Safranal)	116-26-7	1211	1201	66.07
	2,4-cycloheptadien-1-one, 2,6,6-trimethyl-	503-93-5	1225	1245	1.25
	2-hydroxy-3,5,5-trimethylcyclohex-2-ene-1,4-dione	35692-98-9	1250	1250	1.13
	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-3,6-dimethyl-	13341-72-5	1310	1314	0.93
	Carbonicacid, monoamide, N-octyl-, propyl ester	1000415-25-3	1334	1824	0.29
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1396	1396.4	0.89
	4-hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde (HTCC)	35692-94-5	1465	1322	0.52
Z3	Acetic acid	64-19-7	647	710	1.01
	Acetoin	513-86-0	780	711	0.09
	2(5H)-furanone	497-23-4	793	951	0.80
	3-cyclohexen-1-one, 3,5,5-trimethyl-(ß-isophorone)	471-01-2	1037	1429	2.80
	Isophorone	78-59-1	1111	1118	18.15
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	1125-21-9	1147	1142	15.20
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1173	1258	10.24
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- (Safranal)	116-26-7	1202	1201	28.18
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1391	1396.4	2.75
	2-hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	28750-52-9	1164	1164.9	9.15
	Di-epi-.alpha.-cedrene-(I)	21996-77-0	1382	1388.2	0.01

(Continues)

TABLE 6 (Continued)

Sample	Compound	CAS number	RI calculated	RI lit	% peak area
Z4	Acetic acid	64-19-7	645	710	1.03
	Acetoin	513-86-0	705	711	0.13
	2(5H)-furanone	497-23-4	925	951	1.10
	3-cyclohexen-1-one, 3,5,5-trimethyl-(ß-isophorone)	471-01-2	1038	1429	3.04
	Isophorone	78-59-1	1123	1118	20.24
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (ketoisophorone)	1125-21-9	1149	1142	14.80
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1180	1258	11.14
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- (Safranal)	116-26-7	1205	1201	30.24
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1396	1396.4	3.08
	2-hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	28750-52-9	1180	1164.9	1.25
	2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde	162376-82-1	1100	1108.7	0.80
Z5	2,3-butanedione	431-03-8	635	572	0.09
	Acetic acid	64-19-7	646	710	1.98
	2-Propanone, 1-hydroxy-	116-09-6	699	652	0.10
	Acetoin	513-86-0	704	711	1.15
	Butanoicacid, 4-hydroxy-	591-81-1	922	933	0.30
	2(5H)-furanone	497-23-4	926	951	0.41
	3-cyclohexen-1-one, 3,5,5-trimethyl- (ß-Isophorone)	471-01-2	1037	1429	5.35
	2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde	162376-82-1	1101	1108.7	1.01
	Isophorone	78-59-1	1121	1118	41.04
	2-cyclohexen-1-one, 2-hydroxy-6-methyl-3-(1-methylethyl)-	54783-36-7	1132	1274.1	0.27
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	1125-21-9	1146	1142	16.10
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1174	1258	7.11
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- (Safranal)	116-26-7	1201	1201	23.15
	(4aS,7S,7aR)-4,7-dimethyl-5,6,7,7a-tetrahydrocyclopenta[c]pyran-1(4aH)-one	21651-62-7	1315	1377	0.18
	1-cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	1000196-69-4	1356	1309	0.20
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1395	1396.4	1.04
	2-butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	17283-81-7	1446	1414	0.02
	Isoelemicin	487-12-7	1456	1644	0.06
	5,9-undecadien-2-one, 6,10-dimethyl-, (E)-	3796-70-1	1466	1455	0.04
	6-methoxy-4-methylcoumarin	6295-35-8	1509	-	0.01
	Benzaldehyde, 6-hydroxy-4-methoxy-2,3-dimethyl-	34883-12-0	1616	-	0.36
Z6	2,3-butanedione	431-03-8	636	572	0.27
	Acetic acid	64-19-7	645	710	1.75
	2-propanone, 1-hydroxy-	116-09-6	701	652	0.10
	Acetoin	513-86-0	706	711	1.55
	1-octanamine	111-86-4	922	1038	0.18
	2(5H)-furanone	497-23-4	925	951	1.46
	3-cyclohexen-1-one, 3,5,5-trimethyl-(ß-isophorone)	471-01-2	1037	1429	4.63
	2-isopropylidene-3-methylhexa-3,5-dienal	1000191-76-5	1058		0.19
	2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde	162376-82-1	1101	1108.7	1.34
	Isophorone	78-59-1	1120	1118	29.27
	Dehydroacetic acid	520-45-6	1132		0.36
	(+)-2-bornanone	464-49-3	1137		0.15
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	1125-21-9	1147	1142	23.31

TABLE 6 (Continued)

Sample	Compound	CAS number	RI calculated	RI lit	% peak area
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1174	1258	5.47
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- (Safranal)	116-26-7	1201	1201	27.18
	(4aR,7S,7aS)-4,7-Dimethyl-5,6,7,7a-tetrahydrocyclopenta[c] pyran-1(4aH)-one	21651-53-6	1315	1289	0.44
	1-cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	1000196-69-4	1356	1309	0.47
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1396	1396.4	1.43
	2-butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	17283-81-7	1446	1414	0.10
	Isoelemicin	487-12-7	1456	1644	0.21
	Dodecane, 5,8-diethyl-	24251-86-3	1542		0.01
	Benzaldehyde, 6-hydroxy-4-methoxy-2,3-dimethyl-	34883-12-0	1618		0.14
Z7	2,3-butanedione	431-03-8	635	572	0.03
	Acetic acid	64-19-7	647	710	2.23
	1-Butanol	71-36-3	696	670	0.37
	Acetoin	513-86-0	703	711	1.06
	Butanoicacid, 4-hydroxy-	591-81-1	919	933	0.43
	2(5H)-Furanone	497-23-4	922	951	0.37
	3-cyclohexen-1-one, 3,5,5-trimethyl-(ß-Isophorone)	471-01-2	1037	1429	5.34
	2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde	162376-82-1	1102	1108.7	0.79
	Isophorone	78-59-1	1122	1118	22.75
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	1125-21-9	1147	1142	19.73
	2-hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	28750-52-9	1165	1164.9	0.01
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1175	1258	15.10
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- (Safranal)	116-26-7	1200	1201	22.96
	(4aS,7S,7aR)-4,7-Dimethyl-5,6,7,7a-tetrahydrocyclopenta[c] pyran-1(4aH)-one	21651-62-7	1318	1377	0.66
	1-cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	1000196-69-4	1355	1309	1.08
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1396	1396.4	6.95
	Caryophyllene	87-44-5	1423		0.08
	2-butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	17283-81-7	1446	1414	0.03
	6-methoxy-4-methylcoumarin	6295-35-8	1507		0.03
Z8	Acetic acid	64-19-7	650	710	0.29
	Butanoicacid, 4-hydroxy-	591-81-1	926	933	0.13
	2(5H)-furanone	497-23-4	795	951	1.77
	3-cyclohexen-1-one, 3,5,5-trimethyl-(ß-Isophorone)	471-01-2	-	1429	1.448
	Isophorone	78-59-1	1119	1118	15.82
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	1125-21-9	1144	1142	13.07
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1171	1258	8.42
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- (Safranal)	116-26-7	1200	1201	58.97
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1396	1396.4	2.91
	Thymoquinone	490-91-5	1315	1276	1.55
	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	20307-83-9	1533		0.19
Z9	2,3-butanedione	431-03-8	634	572	0.16
	Acetic acid	64-19-7	645	710	1.68
	1-Butanol	71-36-3	694	670	0.18
	Acetic acid, methoxy-, ethyl ester	3938-96-3	698	845	0.30

(Continues)

TABLE 6 (Continued)

Sample	Compound	CAS number	RI calculated	RI lit	% peak area
	Butanoicacid, 4-hydroxy-	591-81-1	921	933	0.21
	2(5H)-Furanone	497-23-4	925	951	0.23
	3-cyclohexen-1-one, 3,5,5-trimethyl-(ß-Isophorone)	471-01-2	1039	1429	16.49
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	116-26-7	1059	1201	0.26
	2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde	162376-82-1	1101	1108.7	1.52
	Isophorone	78-59-1	1122	1118	18.54
	2-cyclohexen-1-one, 2-hydroxy-6-methyl-3-(1-methylethyl)-	54783-36-7	1132	1395	0.44
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	1125-21-9	1148	1142	22.39
	2-hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	28750-52-9	1164	1164.9	1.22
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1174	1258	9.72
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- (Safranal)	116-26-7	1200	1201	19.17
	(4aS,7S,7aR)-4,7-Dimethyl-5,6,7,7a-tetrahydrocyclopenta[c] pyran-1(4aH)-one	21651-62-7	1315	1377	0.59
	1-cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	1000196-69-4	1355	1309	0.83
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1396	1396.4	6.05
Z10	Acetic acid	64-19-7	647	710	2.39
	1-Butanol	71-36-3	700	670	0.10
	Acetoin	513-86-0	708	711	0.46
	2(5H)-furanone	497-23-4	920	951	2.16
	3-cyclohexen-1-one, 3,5,5-trimethyl-(ß-Isophorone)	471-01-2	1036	1429	1.22
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	116-26-7	1058	1201	0.10
	2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde	162376-82-1	1101	1108.7	1.14
	Isophorone	78-59-1	1123	1118	45.70
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	1125-21-9	1146	1142	13.03
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1176	1258	6.57
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- (Safranal)	116-26-7	1203	1201	30.88
	2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-3,6-dimethyl-	13341-72-5	1316	1314	0.16
	1-cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	1000196-69-4	1357	1309	0.20
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1395	1396.4	0.83
	Benzaldehyde, 6-hydroxy-4-methoxy-2,3-dimethyl-	34883-12-0	1617	-	0.16
Z11	Acetic acid	64-19-7	649	710	1.16
	1-butanol	71-36-3	715	670	0.03
	2(5H)-furanone	497-23-4	793	951	1.76
	3-cyclohexen-1-one, 3,5,5-trimethyl-(ß-isophorone)	471-01-2	1042	1429	0.29
	2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde	162376-82-1	1101	1108.7	1.15
	3,5,5 trimethyl-2-cyclohexen-1-one (isophorone)	78-59-1	1122	1118	11.75
	2,6,6-trimethyl-2-cyclohexene-1,4-dione (ketoisophorone)	1125-21-9	1150	1142	14.07
	1,4-cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	1186	1258	6.48
	1,3-cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-(Safranal)	116-26-7	1212	1201	60.76
	2,4-cycloheptadien-1-one, 2,6,6-trimethyl-	503-93-5	1250	1245	0.21
	(4aS,7S,7aR)-4,7-Dimethyl-5,6,7,7a-tetrahydrocyclopenta[c] pyran-1(4aH)-one	21651-62-7	1310	1377	0.99
	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	35692-95-6	1396	1396.4	1.25
	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	487-11-6	1455	1585	0.10

Note: RI lit: The RI theoretical value was found in Pherobase on the same column.

addition, a higher percentage of 1,4-cyclohexanedione, 2,2,6-trimethyl is recorded in Z7 (Khouzama/Taznakht).

Isophorone, one of the main components, is an element that plays an important role in generating new compounds during the aging and drying processes.[38] Also, it is more abundant in samples dried under more excessive conditions (high temperature and for a long time).[20] Sereshti et al.[32] reported that safranal and isophorone are especially important, contributing to the distinctive saffron aroma.

As for ketoisophorone, it is generated from isophorone during the drying process, and its concentration has been shown to decrease with increased drying time.[20] Ketoisophorone and ß-isophoroneconstitute a marker of the freshness of saffron and indicate the impact of its storage.[54] Sevindik[55] observed that the amount of safranal and isophorone sharply increased while β-isophorone, β-ionone and HTCC levels decreased as a result of 1 year storage period. These chemical compounds, similar to safranal, have been reported as the most abundant volatile compounds while profiling the volatiles of the saffron stigmas sampled from different geographical zones with different pedo-climatic conditions.[15,19,20,24,27,38,48,49,51,55] Their quantity is reported to increase as a factor of drying temperature and long storage duration, resulting in an enhanced of the spicy and floral notes of the saffron.[32,38,51,55]

Besides, all saffron samples contain a medium to high acetic acid content (2.39% in Z10 [Taliouine] and 2.23% in Z7 [Khouzama/Taznakht]). In fact, saffron from Morocco and Iran is characterized by a high amount of acetic acid.[56]

Other components were found in small quantities in all the samples such as4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde, Furanoneand 2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde. According to Jalali-Heravi et al.,[57] Furanone was never detected in Spanish or Greek saffron samples. It was one of the main components of Iranian saffron that could be used as a fingerprint for Iranian saffron. Moroccan saffron yielded the same results.[30,58] While, other components were identified in the majority of samples, in particular2-hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone, Butanedione, Acetoin, Butanol, and Di-epi-alpha-cedrene.

The differences noted between safranal and the other main volatile compounds in saffron depend mainly on the post-harvest conditions (particularly the drying), storage, isolation, and analysis of volatile compounds. Previous studies have shown that safranal decreases with increasing the pretreatment time. The environmental impact is not noticeable when samples are dried in an oven; however, when done naturally, the difference between samples' aroma from the different environments is obvious.[30,58]

CONCLUSION

This study is the first to be carried out in Morocco on saffron newly cultivated in new sites. These new areas which are characterized by an intensity of the cold and altitude factor showed a higher potential for growing saffron. Its chemical and biochemical quality revealed good results particularly in samples from Boulmane, Azilal and Ain leuh sites. All samples were within the ISO36-32.2010 categorization range (categories I and II), reflecting the saffron quality from the different studied Moroccan areas. In terms of volatile compounds, different regions (Ain leuh, Boulmane, and Azilal) have shown good results. Therefore, saffron cultivation in new sites will contribute to ensuring a stable source of income and improving the quality of life of producers and women involved in the picking, pruning, and drying of *C. sativus* stigmas.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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