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Investigating the Chemical Composition of Saffron (*Crocus sativus*) Growing in Different Geographic Regions

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ABSTRACT— ***Saffron, the most expensive spice with varying chemical properties, is grown in different parts of the world. The chemistry of different varieties of saffron growing in different geographic regions was studied in this paper. The saffron grown in the Safranbolu and Khorasan regions was analyzed in this study by measuring the amounts of moisture and volatile components, total ash, and undissolved ash in acid within saffron followed by characterization, using UV-Vis spectrophotometry. The results showed that moisture contents in Turkish and Iranian saffron were 6.48% and 5.92% respectively, while the amounts of total ash in Turkish and Iranian saffron were 6.85% and 6.10% respectively. The amounts of ash undissolved in acid were 0.66% and 0.50% respectively in Turkish and Iranian saffron. The results of UV-Vis spectrophotometry showed that equivalent to 1% mixing; the absorbance values for picrocrocin, saffranal, and crocin ingredients were 61.20, 34.08, and 162.12, respectively, in Turkish saffron. On the other hand, the absorbance values for picrocrocin, saffranal, and crocin ingredients were 71.00, 43.81, and 196.56, respectively, in Iranian saffron. All these outcomes conclusively proved that the main reasons behind the differences in chemical composition of saffron are the different conditions of growth and climate. In addition, the effect of fertilizers and bulb size were also important..***

Keywords— *Crocus sativus*, saffron, chemistry of saffron, geography of saffron

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1. INTRODUCTION

Saffron (*Crocus sativus*), the most expensive spice traded in the world, is grown nowadays at diverse places, with different properties and diverse requirements [1]. The economic importance of saffron is immense given the difficulties involved in achieving the demands for the production. The different varieties of saffron that are grown in different geographic regions, relative to climate and conditions of cultivation differ in substance composition [2]. The differences in chemical composition of saffron plants sold in markets can affect the properties of saffron and consequently, the main qualities of these plants such as color, flavor, odor, etc. Therefore, branding and marketing of saffron plants are gaining prominence across various centers and Iran is the world leader in the marketing of saffron [3]. Therefore, the objectives of this study were to investigate the chemical properties of saffron plants grown in different geographic regions of the world and understand the reasons for the differences in substance composition that are associated with the climate or conditions of cultivation. The comparison of various properties such as odor, flavor, and color of Iranian saffron, the market leader with Turkish saffron is an important aim of this study for increasing the quality of saffron farming, depending on the conditions of climate and for obtaining some suggestions regarding saffron farming in Turkey. The amounts of volatile and moisture substances in saffron, undissolved ash in acid, total ash within saffron as well as the main characteristic properties of the Safranbolu and Khorasan varieties are determined, using UV-Vis spectrophotometry and the results obtained from these experiments are evaluated in this study.

2. MATERIAL AND METHODS

2.1 Sampling

Saffron belonging to two different varieties—Iranian (Khorasan region) and Turkish (Safranbolu region), were used in this study. The characteristics of the above-mentioned saffron samples are given in Table 1.

Table 1. Characteristics of saffron samples

	Turkish Saffron	Iranian Saffron
Division	Magnoliophyta	Magnoliophyta
Class	Liliopsida	Liliopsida
Subclass	Liliidae	Liliidae
Order	Liliales	Liliales
Family	Iridaceae	Iridaceae
Genus	Crocus	Crocus
Species	*Crocus sativus* L.	*Crocus sativus* L.
Grown place	Safranbolu	Khorasan
Status	Powder	Powder
Harvest year	2016	2016

2.2 Analytical Methods Used and Sample preparation

Volatile and moisture components were detected in saffron according to technical specifications. Total ash contents were measured according to ISO 928. The investigation of undissolved ash in acid was carried out according to ISO 930. UV-Vis spectrophotometry was used to characterize the amounts of ingredients such as crocin, picrocrocin, and saffranal within saffron with following the methods of TSE 3632–2.

The details of the test methods employed are described in Table 2. Colorimetric methods were employed in which an amount of 0.50 g of each of the saffron samples was placed in a crucible, containing diphenylamine solution until the reddish brown coffee color of the solution changed to blue. The determination of undesirable substances was not performed as the saffron samples were well packed and found to be pure according to the standards.

Table 2. Methods used to determine the ash acid

Principle	The colorimetric reaction
Reactive	Reagents only in laboratory purity
Sulfuric acid	Density 1.19 g/L
Diphenylamine Solution	1 g of diphenylamine was added to 20 mL of sulfuric acid and 4 mL of water.

2.3 Determination of volatile components and moisture

An amount of 2.50 g of each of the two saffron samples was weighed, and dried at a temperature of 103 °C for 16 h. The samples were kept on a watch glass covered with a lid and cold air was supplied to the desiccator. Following the cooling step, the samples were weighed. After that, moisture, volatile substance contents (WMV), sample weight (m_0/g) and dry impurity (m_4/g) were calculated as percentages (%) using Equation 1.

$$W_{MV} = (m_0 - m_4) \times 100/m_0 \qquad (1)$$

2.4 Determination of total ash content

Empty crucibles (diameter: 50 mm, height: 50 mm, and volume: 75 mL) were dried in ash furnaces as the first step followed by cooling and weighing. In the following step, 2.0 g of each of the saffron samples were added to these empty crucibles and kept in furnaces until white ashes were obtained. In the final step, these ashes were weighed, and the results were recorded.

2.5 Determination of undissolved ash in acid

The ratio of amounts of total ash, obtained after filtration of ash samples that were added to a solution, containing approximately 10% dilute hydrochloric acid by mass (ρ20 = 1.045 g/mL–1.050 g/mL) to total sample weight was calculated and expressed as a percentage. The calculation was done using Equation 2.

$$\text{Amount of insoluble ash in acid (\%)} = [(M_2 - M_1)/m] \times 100 \qquad (2)$$

Where M_2 represents the weight of insoluble ash and crucible, M_1 represents the weight of empty crucible, and m represents the weight of the saffron sample, respectively.

2.6 UV-Vis spectrophotometer analysis

Saffron samples were kept in 1000 mL flasks, containing 900 mL distilled water and were protected from light. The residual volume was made up with distilled water. The glass lids of the flasks were closed and samples were then mixed with a magnetic stirrer for an hour till homogeneous solutions were obtained. The undetermined quantities of samples were taken and diluted in 200 mL flasks, filtered through membranes (kept away from light), and measured using UV-Vis spectrophotometry. Subsequently, the results were calculated using Equation 3.

$$E^{\%1} = D \times 10000/m\ (100-H) \qquad (3)$$

where $E^{\%1}$ represents absorbance corresponding to 1% mixing, D denotes specific absorbance, m represents the weight of the saffron sample, and H denotes the content of moisture and volatile substances in saffron sample respectively. The absorbance maxima for picrocrocin, saffranal, and crocin ingredients are given in Table 3.

Table 3: Maximum ($E^{\%1}$) absorbance values for picrocrocin, saffranal, and crocin

$E^{\%1}$ (nm)	Component
250	Picrocrocin
330	Saffranal
440	Crocin

3. RESULTS

3.1 Results of volatile components and moisture

The amounts of moisture and volatile substances present in the saffron samples are given in Table 4.

Table 4. Results of Volatile Components and Moisture

Parameter	Turkish saffron	Iranian saffron
Initial amount	2.5 g	2.5 g
Dried sample	2.33 g	2.35 g
Moisture and volatile substance	6.48%	5.92%

3.2 Results of total ash content

The amounts of total ash in saffron were calculated and expressed as percentages (%), using Equation 4.

$$[W_{Fcrucible+saffron} - W_{crucible}]/[W_{Icrucible+saffron} - W_{crucible}] \times 100 \quad (4)$$

where $W_{Fcrucible}+_{saffron})$ denotes the final weight of the crucible and saffron sample after taking out from the furnace, $W_{(Icrucible+saffron)}$ denotes the initial weight of crucible and saffron sample before placing in the furnace, and $W_{crucible}$ denotes the weight of crucible. The results obtained are shown in Table 5.

3.3 Results of undissolved ash in acid

The amount of undissolved ash obtained from 0.30 g of Turkish saffron was 0.002 g, while that obtained from 0.20 g of Iranian saffron was 0.001 g. Therefore, the amounts of undissolved ash in acid, obtained from both the saffron varieties were less than 1% (Table 5).

Table 5. Results of Total Ash Content and Undissolved Ash in Acid

Type of saffron	Total ash content (%)	Undissolved ashes in acid (%)
Turkish	6.85	0.66
Iranian	6.10	0.50

3.4 Results of UV-Vis Spectrophotometer Analysis

The absorbance maxima for picrocrocin, saffranal, and crocin in the powdered saffron were determined. Furthermore, spectrophotometric values of picrocrocin, saffranal and crocin for both samples are given in Table 6.

Table 6. Results of UV-Vis Spectrophotometer Analysis

Component	Type of saffron			
	Turkish		Iranian	
	Level (mg/g)	E%1 (nm)	Level (mg/g)	E%1 (nm)
Picrocrocin	3.30	61.20	3.69	71.00
Saffranal	0.59	34.08	0.65	43.81
Cosine	33.10	162.12	37.80	196.56

4. DISCUSSION AND CONCLUSION

Iranian saffron is classified into five groups according to ISO Standards. Iranian saffron, coming from the All Red Group is considered to be the first group. The moisture content of this saffron variety was found to be (7.18 ±0.13 %) in previous experiments [4]. In our experiments, the amounts of moisture and volatile substances were 6.48% and 5.92%, respectively, in Turkish and Iranian saffron. Hence, it can be said that the results of the present study are different from previous ones, thereby showing that different saffron grown in different geographic regions have different properties even if they belong to the same class. According to the previous studies, saffron belonging to the first four groups has 7% moisture content, while that belonging to the fifth group has 4% moisture content, clearly establishing that there is no relation between moisture content and quality class of saffron [4].

Iranian saffron has a longer shelf life than Turkish saffron. The relative humidity and amounts of volatile matters in a food ingredient are inversely proportional. Less humidity, depending on the size of saffron buds, results in a longer shelf-life of Spanish saffron, which contains about 5–8% ash according to ISO Standards [5]. The amounts of ash in saffron were found to be 5.40% and 6.00%, respectively in previous studies [6, 7]. In our study, the amounts of ash were 6.10% and 6.85% in Iranian and Turkish saffron respectively, showing that less undesirable substances are there in the Iranian saffron than in the Turkish saffron. The main reason behind this observation is the type of soil where saffron are cultivated. Besides, the amount of ash increases linearly with the mineral content of the soil and with the minerals contained in fertilizers. The amounts of undissolved ash in acid within Iranian and Turkish saffron were found to be 0.50% and 0.66% respectively in the present study and this outcome varied linearly with the composition of soil and fertilizers, similar to the results obtained in relation to amounts of total ash in saffron.

The results from UV-Vis spectrophotometry revealed that the absorbance values for crocin corresponded to 1% mixing were 162.12 and 196.56 in Turkish and Iranian saffron, respectively. The absorbance values for saffranal were noted to be 34.08 and 43.81 in Turkish and Iranian saffron, respectively, while that for picrocrocin in Turkish and Iranian saffron were detected as 61.20 and 71.00, respectively. Thus, it can be said that the Iranian saffron has more intense color, pigment, and flavor than Turkish saffron due to higher saffranal content. The comparison of results between our study and the previous ones for the amounts of saffranal, picrocrocin and crocin in Turkish and Iranian saffron reveal that the saffron grown in Greece has more saffranal content than saffron growing in Turkey or Iran. In fact, the saffron grown in Greece has the highest saffranal content (488.60 g/kg) compared to other saffron grown worldwide. The saffron verities grown in Italy (403.90 g/kg), Iran (346.90 g/kg), and Spain (335.90 g/kg) are behind Greece in respect to saffranal content [8]. Saffranal content mainly affects the odor and color of saffron as mentioned.

On the other hand, Spanish saffron has the highest picrocrocin content (8.14 mg/g) compared to other types [9]. Picrocrocin is the main ingredient responsible for the color of saffron. The amounts of picrocrocin, as determined by UV-Vis spectrophotometry and HPLC methods, were found to be 150.78 g/kg and 171.30 g/kg in Italian saffron, 150.01 g/kg and 162.40 g/kg in Spanish saffron, 140.35 g/kg and 148.40 g/kg in Greek saffron, and 120.59 g/kg and 102.10 g/kg in Iranian saffron, respectively [8]. In general, saffron contains 13–14% of sugar approximately. Although the ratios of sugars in saffron are similar in every region, these ratios are affected by soil conditions. The soil properties change even within the same region and this is the main reason for classifying Iranian saffron, grown in the Khorasan region into five groups. In addition, fertilizers also affect various soil properties. Therefore, it can be said that climate and conditions of cultivation are the most important factors responsible for the differences in chemical composition of saffron. The climate of Iran is most suitable for saffron farming, which is effortless and offers more quality in Iran. Fall rains, hot summers, and warm winters are appropriate for higher yields and the quality of saffron. Saffron has low water requirement and therefore, clayey and sandy soils are the most suitable for its cultivation [2]. Irrigation is necessary for regions not receiving enough rain. Cold air and high rainfall decrease the yield of saffron and consequently, saffron plants cannot grow in the regions that receive 800–1000 mm of rain. In fact, Khorasan and Safranbolu regions have the most favorable climate for saffron farming.

Moreover, conscious agriculture is the second factor that is distinguished as a growing condition for saffron. Planting patterns are performed consciously according to climatic conditions. Italian farmers cultivate bulb at 2–3 cm space, 15 cm in depth affecting the product quality. On the other hand, Greek, Moroccan, and Spanish farmers cultivate at different spaces and depths in accordance with climatic conditions [5]. Furthermore, fertilizers are significant factors that also affect agriculture. Finally, the sizes of bulbs should be mentioned because the cultivation of big-sized bulbs affects the quality of products with increasing average yields per hectare [10].

The fact that Turkish saffron has lower values of all parameters analyzed in this study than Iranian saffron except for humidity is also related to growing conditions as well as climatic conditions. The high levels of humidity observed in Turkish saffron are linearly associated with climatic conditions. Furthermore, traditional drying methods are available in Turkey, while various drying methods are performed in Iran and this factor significantly changes the moisture ratios in saffron. In Turkey, not much emphasis is laid on topics such as soil analysis, amounts of fertilizer to be added to soils, and the importance of planting. The depth and frequency of saffron planting should be adjusted based on the Greece model, where yield is higher than the Safranbolu region. Irrigation methods should be modernized and periods should be regulated with the help of new research techniques. Saffron should be dried in hot rooms similar to the methods adopted in Spain and Iran. The types of packaging of saffron should also be noted and developed as in Spain.

It can be concluded from the results that total amounts of picrocrocin and crocin, effectively control the quality and color of saffron. These are higher in Iranian saffron compared to Turkish saffron. Hence, Iranian saffron contains more color ingredient than Turkish saffron. The conditions of climate and cultivation are the significant reasons behind these differences in saffron composition. Climate, temperature, and rainfall are the important factors within ecological properties, which affect the quality of products. Irrigation, fertilizers, and type of cultivation are the other major agricultural factors.

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