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Study of qualitative characteristics of saffron cultivated in different regions of Iran

Nasibeh Sharifi, Mohammad Hojjatoleslamy*, Maryam Jafari

Department of food Science and Technology, Islami Azad University, Shahrekord branch, Shahrekord, Iran;
Email: mohojjat@gmail.com

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ABSTRACT

Background & Aim: Saffron is one of the expensive spices known since antiquity for its color, flavor and medicinal properties. Saffron is the dried stigma of *crocus sativus* plant. The three basic components of the stigma on which the qualitative characteristics of saffron depend are crocin (color), picrocrocin (taste), and safranal (aroma). The quality of saffron is a function of climatic conditions, soil type, as well as method of drying, packaging, and storage.

Experimental: In this research, the saffron growing in different regions of Iran including Kerman, Shiraz, Arak, Natanz, Ghaen, Shahrekord, and Dolatabad has been studied with regard to its humidity, ash, phenolic compounds, crocin, safranal, and picrocrocin for its classification.

Results & Discussion: The saffron of Shiraz and that of Kerman showed the maximum and minimum amount for ash with a value of 8.02% and 5.38%, respectively. With a view to moisture content, Natanz and Shahrekord saffron showed the highest and the lowest moisture content with a value of 11.97% and 9.7%, respectively. The phenolic compounds were determined by spectrophotometry and ranged from 17.43 mg gallic acid/g dry weight of saffron in Kerman sample to 8.87mg gallic acid/g dry weight in Dolatabad saffron. The highest level of safranal, crocin, and picrocrocin belonged to Shahrekord, Dolatabad, and Shahrekord saffron with a rate of 84.43, 234.4 and 38.4 (strength/ g dry matter) based on the most absorption over three wavelengths of 257, 440, and 330nm, respectively.

Industrial and practical recommendations: With regard to variable applications of saffron as flavoring and coloring in food industry, the results of this study can be used for appropriate usage of saffron cultivated in different regions according to their specifications.

1. Introduction

Saffron is one of the most expensive seasonings all over the world. It is obtained by drying the stigma of saffron plant, scientifically named *Crocus sativus* (Shahidi & Bolandi, 2008). Saffron is a perennial plant of Iridacea family flowering in fall season (Caballero-Ortega *et al*, 2007). This plant often has 1 to 3 violet-colored flowers and its stalk is very short hiding beneath soil (Shahidi & Bolandi, 2008). Saffron grows in Mediterranean climate with mild cold winters and dry and hot summers; the suitable soil for its growth is clay (Khoddami *et al*., 2013). There are two different types of saffron, i.e. strand and cut strand saffron. Strand saffron consists of stigma along with a section

of style, while the style is separated from the stigma in the cut strand saffron (Nazari & Keifi, 2011). The origin of saffron whose reputation dates back to about 2500 years ago is the Alvand and Zagross mountainsides (Caballero, 2007). Iran is one of the greatest producers of saffron in the world, and other countries such as Turkey, Greece, Italy, Spain, France, Egypt, Israel, Iraq, and Pakistan also cultivate saffron (Nazari & Keifi, 2011). Saffron contains carbohydrate, vitamins, carotenoid, protein, fat, flavonoids, and one triterpene aromatic named Safranal (Shahidi & Bolandi, 2008). The phenolic compounds that contain one aromatic ring with one or several more hydroxyl groups in plants are generated as a response to physiological pressures such as pathogens, attack of insects, and ultraviolet light (Hadizadeh *et al*., 2010). Flavonoids are one of the most important phenolic compounds and mostly play a role along with carotenoids in the formation of orange, yellow, and red colors (Caballero- Ortega *et al*., 2007). Stigma contains flavones, flavanols, iso-flavanols, anthocyanins and catechin (Mohamad *et al*., 2015).

The most important compounds present in saffron stigma are crocin, picrocrocin and safranal (Mozzafar *et al*., 2014). Crocin is a 20-carbon carotenoid of the glycoside derivatives of crocetin producing a red-orange color when dissolved in water forming about 6 to 16 percent of the dry weight of saffron; it is the effective material responsible for the saffron color (Nazari & Keifi, 2011). Crocin is used for the production of color in beverage industry and flour products (Khodadadi, 2014). The bitter taste of saffron is derived from glycoside picrocrocin, which is a colorless monoterpene aldehyde (Caballero-Ortega *et al*., 2004). Due to the binding of one molecule of glucose to hydroxy trimethyl cyclohexene carboxaldehyde, picrocrocin is produced and over the process of saffron drying stages, picrocrocin is hydrolyzed and changed into volatile Safranal, which is the factor for the aroma of saffron and consisting of about 30 to 72% of the aroma compounds of the stigma (Emadi & Saiedirad, 2011). The saffron stigma contains the carotenoids of hydrophile and lipophile. Due to the presence of antioxidants in its stigma, saffron has anti-cancer properties and reduces heart attacks and depression (Karimi *et al*., 2010). The quality of saffron is a function of different factors such as climate conditions, harvesting conditions, storing conditions, method of drying, and packaging (Caballero-Ortega *et al*, 2004). Saffron usually tolerates high temperatures, but it is sensitive to light and oxygen. Therefore, it is necessary to place it in suitable packages like polyethylene with cellophane covering (Hadizadeh *et al*., 2010). The quality of saffron is decided by the rate of its color, and compounds of aroma and taste which are affected by different factors such as climate conditions, harvesting conditions, storing conditions, method of drying, and packaging (Caballero-Ortega *et al*., 2004; Hadizadeh *et al*., 2010). Zarrinkamar *et al.* (2011) showed that the great altitude and the average low temperature of a region had positive effects on the qualitative specifications of Ghaen and Tabas saffron. The phenolic contents of Natanz Karcass saffron was measured on the basis of gallic acid by Goli *et al.* (2012) who showed that the methanol extract of saffron had the most phenol compounds. Joki *et al*. (2012) studied the rate of Picrocrocin and Safranal of different regions in Khorasan Province and showed that the most crocin and Picrocrocin belonged to Torbateheidarieh. In another research, Tajik (2011) specified that the qualitative specifications of regions with 3 years of saffron cultivation were better than those of regions with 6 years of saffron cultivation (Sariri *et al*., 2011). Atefi (2012) showed that different methods of drying affected the qualitative specifications of saffron. With regard to the importance of Iranian saffron in the world trade and the spread of this product in different regions of Iran, no comprehensive research has yet been conducted to study the qualitative specifications of saffron in different regions of Iran. The goal of this research is to compare the saffron of different regions of Iran in terms of color, aroma, taste, phenolic compounds, moisture, and ash.

2. Materials and Methods

2.1. Sampling

Saffron samples were taken from each of the provinces of Isfahan (Natanz and Dolatabad), Fars (Estahban), Kerman (Baft), Yazd (Beniz), Markazi (Arak), Khorasan (Ghaen), and Chaharmahal and Bakhtiari (Farrokhshahr).

2.2. Chemicals

All solvents and chemicals used were of analytical grade and obtained from Sigma Chemical Co. (St Louis, MO).

2.3. Determination of moisture and ash contents

259-2 National Standard was employed for determination of moisture and ash. The moisture content was measured by drying the sample in an oven at 100–105ºC to constant weight and ash by incinerating in a furnace at 550ºC.

2.4. Determination of qualitative specifications with spectrophotometer

Extract preparation: About 500 g of saffron was mixed with 900 ml distilled water and placed on a magnetic stirrer at 1000 rpm for one hour. Then, 20 ml of the solution brought to volume of 200 ml with distilled water. The solution was filtered with filer paper and the absorption of the solution was read with spectrophotometer (Shimadezo, 2100). The results of the solution absorption reading of the three wavelengths of 257, 330, and 440 nm were calculated according to the following formula for safranal, picrocrocin and crocin, respectively.

$$E^{1\%}(\lambda_{max}) = \frac{D*10000}{m(100-w)} \quad (1)$$

Where D is the absorbance of the sample, m and w are the mass and moisture content of saffron samples. The results were expressed as strength/ g dry matter.

2.5. Determination of total phenolics (Folin-Ciocalteu)

Extract preparation: About 50 mg of the saffron sample were mixed with 5 ml distilled water and 5ml of 98% methanol and placed in a refrigerated incubator (German Aqualytic) equipped with a shaker for 24 hours in a dark space. The supernatant was then separated and placed in a centrifuge at 1500 rpm for 20 minutes. The solution was filtered with filter paper, its surrounding was covered with aluminum foil, and it was placed in a refrigerator.

To perform the test, first, 0.5 ml of the extract was diluted with 20 ml of distilled water; then, 2.5 ml of folinciocalteu (10% by volume) and 0.5 ml of 7.5% sodium carbonate solution were added to it and kept for 30 minutes at ambient temperature until the light blue color appeared. Then, the absorption of the solution was measured along the wavelength of 765nm. Total polyphenol content was expressed as mg of gallic acid per g of dry matter. A calibration curve of gallic acid in methanol was performed in a concentration range of 25–100 mg/l.

2.6. Statistical analysis

Results were shown as the mean ±SD of three separate determinations. The data were statistically analyzed by ANOVA program in SPSS software, version 22. The means evaluation was done using Duncan test at 5% probability level.

3. Results and discussion

3.1. Moisture and ash contents

The results for the moisture and ash contents of saffron samples are presented in Table 1. As seen, no significant difference was observed between the moisture content in the samples of Yazd and Natanz saffron at the probability level of 5%. There was also no significant difference ($p<0.05$) among the samples taken from Arak, Shahrekord, Dolatabad, Ghaen, and Kerman. Among samples, Natanz saffron showed significantly ($p<0.05$) higher moisture content with a value of 11.97% and Shahrekord saffron showed the lowest value with a rate of 9.7%. The most important factors affecting the moisture content are the method of drying, duration of drying, and storage conditions. There are different methods of drying such as drying in the shade, toasting, freeze drying, microwave, and solar drying. These values were higher than those obtained by Atefi (2012) in which the saffron moisture was measured through three methods of drying, i.e. roasting, oven, and shade (5.26%, 6.4%, and 5.5%). Since the saffron drying method is traditionally done in the shade in the two mentioned regions, we may conclude that the duration of drying process was probably different and unsuitable packing cause to an increase in moisture of saffron during storage and transportation.

In terms of ash, no significant difference ($p<0.05$) was observed among the saffron samples of Shahrekord, Arak, Natanz, and Dolatabad. Ash content of the samples ranged from 5.3% to 8.02% showing that ash level of Shiraz saffron was significantly($p<0.05$) higher than that of the other samples which implies higher amount of minerals in this saffron. Ash

is indicative of the presence of minerals in saffron depending on the type of soil and climatic conditions as well as the rate of the pollutants existing in air. Values found in this study were similar to the range reported by some researchers (Atefi *et al.*, 2012).

Table 1. Moisture and ash contents of saffron samples

Region	Moisture (%)	Ash (%)
Shiraz	10.96±0.15^{b}	8.02±0.15^{a}
Shahrekord	9.70±0.26^{d}	5.90±0.08^{d}
Kerman	10.13±0.23^{c}	5.38±0.07^{e}
Yazd	11.86±0.11^{a}	6.39±0.06^{c}
Arak	9.90±0.10^{cd}	5.95±0.16^{d}
Natanz	11.97±0.05^{a}	5.77±0.01^{d}
Dolatabad	10.03±0.05^{cd}	5.81±0.06^{d}
Ghaen	9.83±0.28^{cd}	7.10±0.07^{b}

Lowercase letters in the same column represent significant difference ($p<0.05$).

Table 2. Crocin, picrocrocin and safranal level (strength/ g of dry matter) of saffron samples**.**

Region	Crocin	Picrocrocin	Safranal
Shiraz	185.5±0.50^{g}	79.66±0.57^{d}	30.83±0.76^{e}
Shahrekord	234.36±1.70^{b}	84.43±0.40^{a}	38.40±0.52^{a}
Kerman	218.73±0.46^{e}	83.03±0.25^{b}	36.86±0.23^{b}
Yazd	140.96±0.15^{h}	72.06±1.00^{e}	21.16±0.20^{f}
Arak	229.26±0.66^{c}	84.40±0.52^{a}	37.53±0.50^{b}
Natanz	211.76±0.76^{f}	70.60±0.52^{f}	30.33±0.57^{e}
Dolatabad	236.40±0.52^{a}	81.93±0.90^{c}	35.70±0.26^{c}
Ghaen	225.80±0.80^{d}	83.83±0.28^{ab}	33.73±0.46^{d}

Lowercase letters in the same column represent significant difference ($p<0.05$).

3.2. Safranal, crocin and picrocrocin contents in saffron samples

According to Table 2, there is a significant difference ($p<0.05$) among all the saffron samples in terms of crocin content. The highest level of crocin was found in Dolatabad saffron (234.4 based on the strength/g of dry matter) and Yazd saffron showed the lowest level (140.96 strength/g of dry matter). On Our results were less than that found by Salari *et al*. (2012) who measured the crocin of saffron as 250.26 (strength/g of dry matter). There was no significant difference ($p<0.05$) in safranal concentration between the saffron samples of Arak and Kerman. Furthermore, no significant difference was found between Natanz and Shiraz samples. The highest value for safranal was found in Shahrekord saffron (38.4, based on strength/ g of dry matter) and the lowest amount was found in Yazd saffron (21.16 based on strength/ g of dry matter). In terms of the picrocrocin content, no significant difference ($p<0.05$) was observed among the saffron samples of Shahrekord, Arak, and Ghaen and also among Ghaen and Kerman saffron samples. The highest level of picrocrocin was found in Shahrekord saffron sample (84.43 strength/g of dry matter) and the lowest level was found in Natanz saffron (70.6, strength/g of dry matter). The level of these three compounds in saffron is a function of different factors such as method of drying, climatic conditions, altitude, plant age, time of harvest, storage ambient temperature, type of packaging, and plant species. The amount of picrocrocin and safranal in Shahrekord saffron was more than that presented by Atefi *et al.* (2012) who reported it 81.25 and 17.62 (strength/ g of dry matter) for picrocrocin and safranal, respectively. In addition, Salari *et al.* (2012) also measured the picrocrocin and safranal content of Southern Khorasan saffron and reported them as 112.9 and 27.24 (strength/ g of dry matter), respectively. Regarding the effect of altitude, Zarrinkamar *et al.* (2011) studied two regions of Ghaen with an altitude of 1400 meters and Tabas with an altitude of 700 meters. The results showed that the amount of the three factors was more in Ghaen with a higher altitude than that in Tabas. According to Zarrinkamar *et al.* (2011) it is expected that Shahrekord shows the most rate of crocin while having the highest altitude, but only the two factors of safranal and picrocrocin of Shahrekord saffron are higher than those of Dolatabad. Therefore, it can be concluded that since the drying method for all the saffron is traditional (in shade); therefore, other factors such as average temperature of the region, plant age, type of soil, type of saffron bulb, rate of rainfall, climate, and the time of saffron flower harvest could affect the range of these three factors of color, aroma, and taste.

3.3. Total phenolic compounds

The results of phenolic compounds of saffron samples are presented in Table 3. Total phenol content ranged from 17.43 mg gallic acid/g of dry weight (Kerman saffron) to 8.87 mg/g of dry weight (Dolatabad saffron). No significant difference ($p<0.05$) was observed between the saffron samples of Shiraz and Dolatabad and also between Shiraz and Arak. Phenolic compounds produced in plants as a response

to physiological pressures such as pathogens, insect attacks, and ultraviolet irradiation. According to the results obtained by Karimi *et al.* (2010) the highest amount of phenolic compounds based on gallic acid was found in Kerman saffron with a value of 17.43 mg/g and the lowest amount was found in Dolatabad saffron with a value of 8.89 mg/g which were similar to our results. They also reported that phenolic compounds of methanol and ethanol extracts of Kashmar saffron were 6.5 and 6.3mg gallic acid/g of dry weight of saffron, respectively. phenolic compound based on caffeic acid of saffron reported by Gismondi *et al.* (2012) was 53.52 µg/mg. Asymopolo *et al*. (2005) concluded that the saffron with higher crocin and safranal had higher antioxidant property since the methanol solution of crocin had 65% antioxidant activity (DPPH free radical scavenging) and safranal had 36% antioxidant activity.

Table 3. Total phenolic content of saffron samples.

Region	TPC(mg gallic acid/g of dry matter)
Shiraz	9.24 ± 0.24^{fg}
Shahrekord	9.89 ± 0.20^{e}
Kerman	17.43 ± 0.18^{a}
Yazd	10.78 ± 0.11^{d}
Arak	9.32 ± 0.10^{f}
Natanz	15.88 ± 0.20^{b}
Dolatabad	8.89 ± 0.11^{g}
Ghaen	13.90 ± 0.47^{c}

Lowercase letters in the same column represent significant difference ($p<0.05$).

4. Conclusion

With regard to the importance of Iranian saffron and the spread of the planting of this valuable plant in different regions of the country, it is necessary to classify and compare the saffron of different regions with regard to their qualitative specifications. The results of this research showed that the qualitative specifications of saffron of different regions of Iran are influenced by different factors such as the quality of saffron bulb, type of soil, altitude of the region, average temperature of the region, age of saffron plant, climatic conditions, storage conditions, packaging, and method of drying.

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