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Comparison quality parameters of saffron (*Crocus sativus* L.) produced in Herat, Afghanistan and Torbat Heydarieh, Iran

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Key words: Crocin, picrocrocin, safranal, secondary metabolites, spectrophotometric analysis.

Abstract: Saffron *Crocus sativus* L. (Iridaceae) is one of the most valuable and expensive medicinal plants in the world. In order to compare the quality characteristics of saffron in Afghanistan and Iran, samples of dried *C. sativus* from different saffron-producing regions of Herat province (Afghanistan) and Torbat Heydarieh county (Iran) were collected in the year 2021. The experiment was analyzed in GLM format and Nested method with three replications. The samples of saffron produced from seven different districts of Herat province in Afghanistan, including Injil, Karukh, Guzara, Pashtun Zarghun, Zendeh Jan, Ghoryan, Obeh and nine saffron-producing villages (Fakhrabad, Kadkan, Nasar, Ghaleno, Feizabad, Khorgh, Abrood, Benhang, Shadmehr) of Torbat Heydarieh county in Iran were examined. The results showed that the effect of country and region (districts and villages) on the quality traits of saffron (crocin, picrocrocin and safranal) was significant. Moreover, the comparison of the mean values indicated the presence of significant differences in the qualitative characteristics of saffron among the different investigated villages of Torbat Heydarieh. The saffron produced in Afghanistan had the highest amount of crocin (on average 279.1 ϖ 440nm) and picrocrocin (on average 101 φ 257 nm), while the highest value of safranal (on average 34.2 θ 330 nm) was observed in saffron samples produced in Iran. The highest amount of the above quality traits belonged to the saffron of Zendeh Jan Herat region 5 (Af_5, crocin: 303 ϖ 440 nm, picrocrocin: 106 φ 257 nm, safranal: 33 θ 330 nm), while the five Torbat Heydarieh villages had the lowest quality of saffron (Ir_5, crocin: 164 ϖ 440nm, picrocrocin: 71 φ 257 nm, safranal: 34 θ 330 nm). In the comprehensive analysis of saffron-producing regions in Afghanistan and Iran, in terms of the qualitative attributes (crocin, picrocrocin, and safranal) it was evident that regions 1 to 6 in Herat, Afghanistan (Af1, Af2, Af3, Af4, Af5, Af6) and the Abrood village of Torbat Heidarieh, Iran (Ir7) formed a distinct cluster, demonstrating superior quality compared to other regions. Furthermore, with the exception of the Feizabad village of Torbat Heydarieh (Ir5), all examined samples surpassed the saffron ISO international standard and were categorized as first-grade quality. The exceptional quality of Herat saffron from Afghanistan is likely attributable to the unique geographical features, virgin lands, and specific climatic conditions across diverse cultivation areas.

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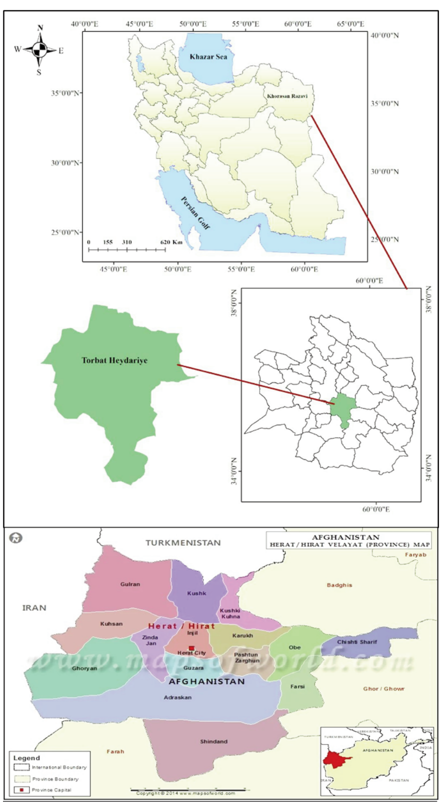
1. Introduction

Saffron (*Crocus sativus* L.) is one of the world's most valuable and expensive medicinal and industrial plants. Originally confined to limited geographic habitats, its significance has led to its widespread cultivation in various parts of the world (Mathew, 1999). In addition to Iran, saffron is also cultivated in other countries such as Afghanistan, India, Spain, Italy, Morocco, Turkey and Greece. In 2019, Afghanistan, as the fourth producer in the world, exported about six tons of saffron (ASYB, 2021), while Iran was the world largest producer with 281 tons of saffron in 2021 (SCI, 2021). Due to the high economic value of saffron, its cultivated area is expanding rapidly in the neighboring countries of Iran, such as Afghanistan and Pakistan (FAO, 2018). Recently, Afghan farmers have developed saffron cultivation in Herat province. Also, the Afghan government is encouraging farmers to grow saffron instead of poppy (*Papaver samniferum* L.) to improve their livelihoods. Herat province is geographically located in the neighborhood of Khorasan province in Iran. In addition Khorasan is the largest saffron production center in the world (Nazarian *et al.,* 2018). Khorasan-Razavi and South Khorasan provinces in Iran are considered the main saffron production pole in the world (Mollafilabi and Khorramdel, 2016). Herat province of Afghanistan is the main center of saffron production in the country, so farmers are engaged in saffron production in almost all the cities of this province (Nazarian *et al.,* 2018). The production method of saffron is slightly different in Iran and Afghanistan. Compliance with the technical principles of cultivation, the use of inputs, and the method of drying saffron are not the same in the two aforementioned countries. So that these factors may have been able to affect the quantity and quality of saffron production.

The stigma of three branches of saffron, which has a red color, contains three main compounds: crocin, picrocrocin and safranal (Tajik *et al.,* 2012). Saffron is widely used in the food and pharmaceutical industries, and due to its therapeutic effects on cancer and depression, it is receiving increasing attention from consumers and processing industries (Lechtenberg *et al.,* 2008). The medicinal and nutritional importance of saffron is related to the existence of a set of secondary metabolites in the stigma. Due to the presence of these metabolites and high amounts of crocin, picrocrocin, and safranal, saffron has the ability to provide color, a special taste and a pleasant aroma in food products. Limited spices have a similar ability to simultaneously provide color, aroma and flavor in the food product to which they are added (Valle Garcia-Rodriguez *et al.,* 2014). Considering the importance of secondary metabolites such as crocin, picrocrocin, and safranal in determining the nutritional and medicinal value of saffron; the measurement of these three parameters can be used as a suitable indicator of quality (Zalacain *et al.,* 2005). To determine the quality of saffron in Iran, in addition to using the ISO international standard (ISO/TS 3632-1 and 3632-2), the national standard (No. 1/259 and 2/259) is also used. Saffron is widely used in the food and pharmaceutical industries. Saffron quality is usually determined by three important secondary metabolites in the stigma i.e. crocin, picrocrocin and safranal, known as effective substances. It has been confirmed that temperature is the main factor controlling the flowering behavior of saffron (Koocheki *et al.,* 2009). In a prior study conducted by Feizi and Moradi (2019), it was revealed that the saffron stigma yield exhibited a positive correlation with regional rainfall, organic matter, nitrogen, phosphorus, and soil sand percentage. Conversely, there was a negative correlation with average temperature and soil clay percentage. The authors also highlighted that altitude above sea level serves as a significant spatial parameter influencing the production of secondary metabolites. Additionally, their findings indicated positive correlations between altitude and picrocrocin, safranal and crocin. Similarly, Kaveh and Salari (2018) reported a positive correlation between crocin and both picrocrocin and safranal. Conversely, both crocin and safranal exhibited a negative correlation with altitude. Moreover, it has been stated that soil pH within the neutral to slightly alkaline range is positively correlated with saffron quality (Gresta *et al.,* 2008). In general, the crop quality is influenced by various factors, exhibiting variation across different regions, such as agricultural practices, nutrition, soil type, water management and air conditions, and ultimately the method employed for saffron drying. On this basis, the purpose of this research was to compare the quality characteristics of saffron produced in Herat province of Afghanistan and Torbat Heydarieh county of Iran.

2. Materials and Methods

In order to compare the qualitative characteristics of saffron in Afghanistan and Iran, samples of dried saffron from seven different districts of Herat province (Injil, Karukh, Guzara, Pashtun Zarghun, Zendeh Jan, Ghoryan, Obeh) of Afghanistan and nine saffron-producing villages (Fakhrabad, Kadkan, Nasar, Ghaleno, Feizabad, Khorgh, Abrood, Benhang, Shadmehr) of Torbat Heydarieh county of Iran (Fig. 1) were collected in the year 2021. The experimental data underwent analysis through the General Linear Model (GLM) procedure. Within the GLM, seven samples from Afghanistan (Af) and nine samples from Iran (Ir) were defined, each with three replications, and the saffron samples were nested over countries. Unlike a factorial model, the nested model differs in that the levels of the second factor (saffron samples) vary within each level of the first factor (country), making it unfeasible to estimate interaction effects in this model. Mean values of qualitative characteristics were compared using the Fisher LSD test. To assess similarities both between and within samples from the two countries, the collected data underwent cluster analysis. All statistical analyses were conducted using Minitab®21 (https://www.gmsl.it/minitab-analisi-dati/).



Districts	Coordinates	Annual rainfall (mm)	Altitude (m)
Ghoryan	34°20′24″N 61°28′12″E	201.0	790
Zenda Jan	34°20′44″N 61°43′12″E	201.0	835
Injil	34°18′00″N 62°15′00″E	238.9	920
Guzara	34°13′0″N 62°13′18″E	238.9	920
Pashtun Zarghun	34°17′0″N 62°37′0″E	241.0	1150
Obeh	34°22′11″N 63°10′35″E	288.0	1277
Karukh	34°29′32″N 62°35′32″E	301.0	1320
City			
Torbat Heydarieh	35°16′26″N 59°13′10″E	247.0	1450

Fig. 1 - Map and geographical characteristics of the studied regions in Herat province of Afghanistan and Torbat Heydarieh county of Iran.

The quality characteristics of the saffron samples were determined by the laboratory of the Saffron Institute of University of Torbat Heydarieh. To determine the amount of crocin, picrocrocin and safranal, the spectrophotometer method (ultraviolet-visible spectrophotometry) and the ISO/TS 3632 standard were used. For this purpose, 500 mg of each saffron sample was ground and after transferring to a 1000 ml volumetric flask, 900 ml of distilled water was added to it. Then it was stirred by a magnetic stirrer for one hour at a speed of 1000 rpm. The volume of the resulting solution was brought up to 1000 ml with distilled water and again placed in the mixer to obtain a uniform solution. The amount of 20 ml of the resulting solution was brought to a volume of 200 ml and stirred until a uniform solution was formed. The solution was filtered away from light, and after obtaining a clear solution, the amount of light absorption in the wavelengths of 200 to 700 nm was recorded compared to the reference (distilled water). The absorption value in the range of 257 nm was related to picrocrocin, in the range of 330 nm to safranal, and in the range of 440 nm to crocin. The reported number was obtained from the following equation (Kaveh and Salari, 2018):

$$E = D \times 1000 / m\,(100 - H)$$

where E is the value of each color compound, D is the absorption value (the number recorded in the spectrophotometer), m is the weight of the saffron sample in grams, and H is the weight percentage of moisture and volatile substances in the sample.

Minitab software was used for statistical analysis, and the means were compared by lest significant difference test (LSD).

3. Results and Discussion

The results of nested analysis of variance showed that the effect of country and regions on quality traits of saffron (picrocrocin, safranal, and crocin) was significant (Table 1). Moreover, the comparison of mean values indicated the presence of significant differences in the qualitative characteristics of saffron among the investigated regions of Herat, and Torbat Heydarieh (Table 2). The saffron produced in Afghanistan had the highest amount of crocin (with an average of 279.1 ϖ 440 nm) and picrocrocin (with an average of 101 φ 257 nm), while the highest amount of safranal (with an average of 34.2 θ 330 nm) was observed in the samples of saffron produced in Iran. The highest amount of crocin (303 ϖ 440 nm) and picrocrocin (106 φ 257 nm) belonged to the saffron sample of Zendeh Jan region and the highest amount of safranal (39 θ 330 nm) was observed in the 6thsaffron sample of Torbat Heydarieh (Ir_6) (Table 2).

In the cluster analysis of qualitative traits of saffron produced in different regions of Herat, four clus-

Table 1 - Analysis of variances (MS) for saffron quality parameters in different regions of Herat Province – Afghanistan and Torbat Heydarieh, Iran

Source of Variation	Df	Picrocrocin (φ 257 nm)	Safranal (θ 330 nm)	Crocin (ϖ 440 nm)
Country	1	1547.15 **	89.76 **	20539.1 **
Region	14	168. 05 **	21.47 **	2545.5 **
Error	32	0.98	0.95	0.98
Total	47			

** and * are significant at the 0.01 and 0.05 levels of probability, respective.

Table 2 - Mean comparison of quality parameters of saffron from different regions of Herat Province - Afghanistan and Torbat Heydarieh, Iran

Country	Region name	Picrocrocin (φ 257 nm)	Safranal (θ 330 nm)	Crocin (ϖ 440 nm)
Afghanistan		101 a	31.4 b	279.1 a
Iran		89.6 b	34.2 a	237.4 b
LSD 0.05		0.291	0.285	0.291
Af1	Injil	103 c	30 f	281 d
Af2	Karukh	104 bc	29 f	289 b
Af3	Guzara	101 d	34 cd	270 f
Af4	Pashtun Zarghun	104 bc	29 f	283 c
Af5	Zendeh Jan	106 a	33 de	303 a
Af6	Ghuryan	97 e	29 f	275 e
Af7	Obeh	92 gh	36 b	253 i
Ir1	Fakhrabad	93 fg	30 f	252 i
Ir2	Kadkan	88 k	36 b	236 j
Ir3	Nasar	91 hi	33 de	259 h
Ir4	Ghaleno	90 ij	32 e	236 j
Ir5	Feizabad	71 m	34 cd	164 m
Ir6	Khorgh	89 jk	39 a	227 k
Ir7	Abrood	105 ab	35 bc	289 b
Ir8	Bengang	94 f	34 cd	265 g
Ir9	Shadmehr	85 l	35 bc	209 l
LSD 0.05		0.816	0.799	0.816

Means with the same letters are not significantly different based on the LSD test at 1% probability level P≤0.01.

ters were observed. The highest amount of the above qualitative traits belonged to zone 5 of Zendeh Jan (Af_5, crocin: 303 ϖ 440 nm, picrocrocin: 106 φ 257 nm, safranal: 33 θ 330 nm), and the lowest amount was belonged to zone 7 of Obeh (Af_7, crocin: 253 ϖ 440 nm, picrocrocin: 92 φ 257 nm, safranal: 36 θ 330 nm). Also, the above qualitative traits in saffron were obtained from Torbat Heydarieh region of Iran in four different clusters including (Fakhrabad: Ir_1, Nasar: Ir_3, Benhang: Ir_8), (Kadkan: Ir_2, Ghaleno: Ir_4, Khorgh: Ir_6, Shadmehr: Ir_9), Abrood: Ir_7 and Feizabad: Ir_5, so that region 5 had the lowest quality of saffron (Ir_5, crocin: 164 ϖ 440 nm, picrocrocin:71 φ 257 nm, safranal:34 θ 330 nm). It should be noted that in terms of quality, the different saffron producing regions of Torbat Heydarieh were ranked after the saffron of different regions of Herat. In general, in the examination of the regions of the two countries in terms of the three qualitative attributes of picrocrocin, crocin, and safranal, it was observed that regions 1, 2, 3, 4, 5 and 6 of Herat (Af_1, Af_2, Af_3, Af_4, Af_5, Af_6) and region Abrood of Torbat Heydarieh (Ir_7) was in a cluster and was superior to other regions (Fig. 2).

Also, all the studied samples, except for Torbat Heydarieh Feizabad (Ir_5), were qualitatively higher than the ISO international standard and placed in the first place among first-class saffron (Table 3).

In the review and comparison of the quality of saffron produced in the major production centers of Razavi and South Khorasan provinces, it was reported that out of 14 production centers (Safi Abad, Qaynat, Kashmar, TorbatHydarieh, Khalil Abad, Bardeskan, Shahn-Abad, Chakhmaq, Torbat-Jam, Feiz-Abad, Sabzevar, Sarayan, Taghi-Abad and Birjand) the amount of safranal was lower than the ISO and national standards of Iran in all the investigated villages. For picrocrocin, all regions had values higher than the ISO standard, but regions 2, 10, 11, and 12 could not achieve the minimum national standard, and the product produced in regions 5, 8, and 9 was within the standard limit. Region 12 (Ir_5: Feizabad) had the lowest amount of crocin, which was lower than the ISO standard. Regions 2, 5, 10 and 14 also had crocin values lower than the national standard of Iran (Kaveh and Salari, 2018). The study of the quality characteristics of saffron in Herat-Afghanistan showed that the difference between picrocrocin and safranal was significant in different regions. The highest amount of picrocrocin (104/50 φ 257 nm) and safranal (34/95 θ 330nm) was observed in the saffron

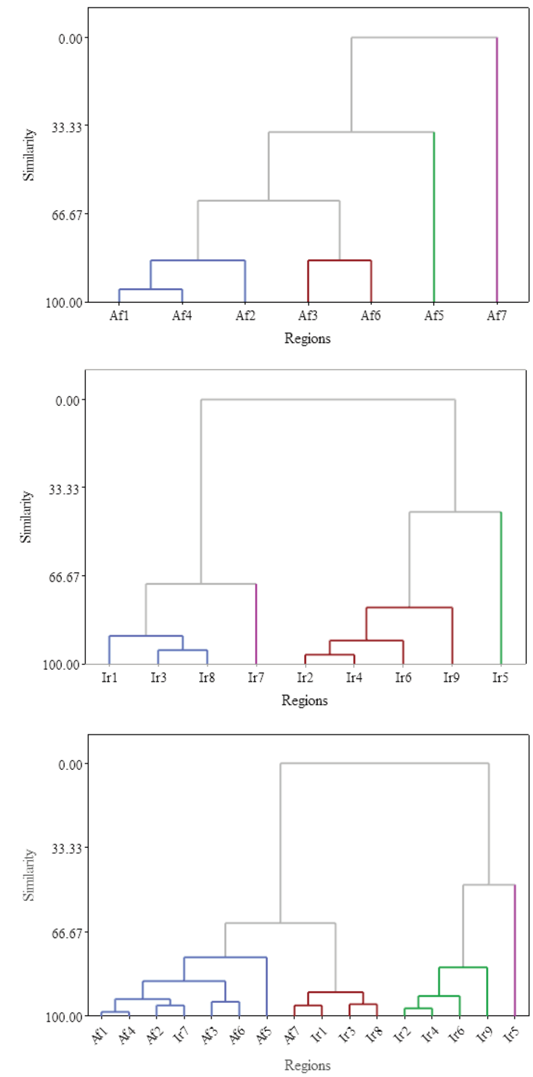


Fig. 2 - Dendogram for hierarchical cluster analysis of 16 studied locations based on crocin, picrocrocin and safranalin Heart, Afghanistan and Torbat Heydarieh, Iran. Ir1- Ir9 (Ir1: Fakhrabad, Ir2: Kadkan, Ir3: Nasar, Ir4: Ghaleno, Ir5: Feizabad, Ir6: Khorgh, Ir7: Abrood, Ir8: Benhang, Ir9: Shadmehr) are 9 saffron producing regions of Torbat Heydarieh, Iran and 7 different district of Heart, Afghanistan are: Af1: Injil, Af2: Karukh, Af3: Guzara, Af4: Pashtun Zarghun, Af5: Zendeh Jan, Af6: Ghuryan, Af7: Obeh.

Table 3 - Sample classification based on ISO 3632/1-2 Normative (ISO, 2003)

ISO category	E 1% 257 nm	E 1% 440 nm
I Grade	70	190
Grade II	55	150
Grade III	40	100

Accordingly, to the absorbance readings at different wavelengths of solutions of the same concentration E1% ($w.v^{-1}$) at 257 and 440 nm. Source: Gresta *et al.*, 2008.

samples of the saffron-producing regions, while the highest amount of crocin (236/95 ꞷ 440nm) was recorded in the saffron samples of Pashtun Zarghun city. Also, by comparing the studied samples with the international saffron standard (ISO 3632), it was observed that all the samples were higher than the group grade 1 (Nazarian *et al.,* 2021).

In the world, there are many differences in product quality parameters from one region to another. The reported values for crocin range from 0.85 to 32.4 percent of dry matter (Alonso *et al.,* 1999), safranal from 0.026 to 0.29 mg/dry weight (Hadizadeh *et al.,* 2006), picrocrocin 2.18 to 6.15 percent of dry matter (Straubinger *et al.,* 1998) has been reported for Iranian saffron. The results showed that there is a positive correlation between picrocrocin with safranal, crocin and altitude above sea level (Kaveh and Salari, 2018). The correlation of traits with altitude was consistent with the results of (Lage and Cantrell, 2009). Studies conducted on the correlation of saffron quality and geographical conditions of various factors such as soil, altitude above sea level and temperature have been reported as factors with positive or negative correlation. In a study on the qualitative characteristics of saffron in Morocco, it was reported that the quality of the soil had no effect on the quality of saffron (Lage and Cantrell, 2009). In another study, it has been stated that soil acidity in the range of neutral to slightly alkaline has a positive correlation with saffron quality (Gresta *et al.,* 2008). In many countries, researchers reported that the chemical composition of saffron samples can be strongly influenced by weather conditions, agricultural management practices, harvesting methods, and methods used to dry saffron stigma (Lozano *et al.,* 2000; Zareena *et al.,* 2001; Kanakis *et al.,* 2004; Behdani and Fallahi, 2015). Based on the above reports, it can be said that the amount of saffron compounds in different countries may be very different. During the last two decades, saffron cultivation in Afghanistan has developed significantly, and it has been promoted in most provinces of the country. Afghan farmers do not pay enough attention to saffron nutrition management due to the bad economic situation. Therefore, they use the least amount of animal manure and chemical fertilizers in saffron cultivation. The saffron samples examined in this research were selected from different regions of Herat province with different geographical characteristics (altitude, amount of rainfall, latitude, etc.). The results showed that these different geographical characteristics can affect the quality of saffron produced. Zendeh Jan region with an altitude of 835 m and an annual rainfall of 201 mm was produced the best quality of saffron, while the quality of saffron decreased with the increase of the altitude and the amount of rainfall, so that Obeh region in Herat and samples of saffron produced in Torbat Heydarieh were have the lowest quality of saffron. On the other hand, due to the simultaneous growth of saffron with winter rainfall, the water required by saffron can be supplied by irrigating 2 to 3 times in this way. The method of drying saffron, which affects the quality of saffron, is completely traditional in Afghanistan, and without the use of machines. Therefore, according to the prosperity and development of saffron cultivation in different regions and the virgin lands of Herat province, which have not been under the cultivation of this new and economic product, the qualitative superiority of saffron produced in different regions of Herat compared to the samples of Torbat Heydarieh county, for the amount of secondary metabolites such as crocin, picrocrocin and safranal does not seem unlikely.

4. Conclusions

The chemical composition of saffron stigma can be strongly influenced by weather conditions, altitude, farm management practices, harvesting methods, and the approach to stigma drying. In general, the samples from regions 1 to 6 (Anjil, Karukh, Guzarah, Pashtun Zarghun, Zendeh Jan and Ghoryan) of Herat province, Afghanistan and Abrood region (Ir_7) of Torbat Heydarieh, Iran, exhibited higher levels of crocin and picrocrocin compared to the ISO standard.These samples not only secured the first-place ranking but also surpassed saffron samples from various regions in Torbat Heydarieh, Iran. This superiority can be attributed to the unique geographical features, the virgin nature of the land dedicated to saffron cultivation for the first time, and the different climatic conditions in the cultivated regions.

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