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DETERMINATION OF CROCIN CONTENT AND VOLATILE COMPONENTS IN DIFFERENT QUALITIES OF IRANIAN SAFFRON

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ABSTRACT
Saffron, obtained from dried stigmas of *Crocus sativus* L. flowers, is widely used as a food colouring and flavouring spice. The aim of this study was to determine crocin content and volatile constituents of saffron belonging to different quality categories. The quality categories of saffron samples differ in red stigmas (Sargol-I and Sargol-II) and threads with yellow styles (Pushal-I, Pushal-II and Bunch). The total amount of the crocin component was identified with HPLC as highest in the Sargol-I sample (66.67 mg/g) and lowest in the Bunch sample (51.66 mg/g). SPME followed by GC-MS was used to screen of saffron volatile composition. As the result of study, 40 volatile compounds were detected by 3 different fibers (PA, PDMS and CAR/PDMS). GC-MS (PDMS). Safranal, the main volatile compound of saffron, was determined in the Sargol-I, Sargol-II, Pushal-I, Pushal-II and Bunch category samples as 49.64%, 50.29%, 50.42%, 57.02% and 61.31%, respectively.
Keywords: Saffron (*Crocus Sativus* L.); Solid phase micro extraction (SPME); GC-MS; HPLC

FARKLI KALİTEDEKİ İRAN SAFRANLARIN KROSİN İÇERİĞİ VE UÇUCU BİLEŞENLERİNİN BELİRLENMESİ

ÖZ
Safran, *Crocus sativus* L. çiçeklerinin kurutulmuş stigmalarından elde edilen, gıda renklendirici ve lezzet verici olarak yaygın biçimde kullanılan bir baharattır. Bu çalışmanın amacı, farklı kalite sınıflarına ait safranın krosin içeriğini ve uçucu bileşenlerini belirlemektir. Safran örneklerinin kalite sınıfları, içerdikleri kırmızı stigmalar (Sargol-I ve Sargol-II) ve sarı stiluslar (Pushal-I, Pushal-II ve Bunch) bakımından farklılık göstermektedir. HPLC ile belirlenen toplam Krosin miktarı Sargol-I örneğinde en yüksek (66.67 mg / g) ve Bunch örneğinde en düşük (51.66 mg / g) olarak tespit edilmiştir. Safranın uçucu bileşenleri; GC-MS kullanılarak SPME yöntemi ile belirlenmiş, çalışmada 3 farklı fiber (PA, PDMS ve CAR / PDMS) kullanılarak 40 uçucu bileşik tespit edilmiştir. Safranın ana uçucu bileşiği olan Safranal; Sargol-I, Sargol-II, Pushal-I, Pushal-II ve Bunch kategori örneklerinde sırasıyla % 49.64, % 50.29, % 50.42, % 57.02 ve % 61.31 olarak tespit edilmiştir.
Anahtar kelimeler: Safran (*Crocus Sativus* L.); Katı faz mikro ekstraksiyon (SPME); GC-MS; HPLC

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INTRODUCTION

Saffron, obtained from dried stigmas of *Crocus sativus* L. flowers, is widely used as a food colouring and flavouring spice (Negbi, 1997; García-Rodríguez et al., 2017). As it needs specific climatic conditions for growing and skilled labour for harvesting of flowers, it is one of the most expensive spices. Recently, due to the strong flavour and colouring properties of saffron, its consumption has increased with decreasing interest in synthetic food colourants, flavour and aroma agents (Shahi et al., 2016; Anastasaki et al., 2010). The most important saffron-producing countries are Iran, Greece, Morocco, Spain, Italy and India. Among these countries, Iran has special importance, with 240 tonnes of saffron production per year (Shahi et al., 2016; Velasco-Negueruela, 2001; Pitsikas, 2016). As a matter of fact, 1 kg of dried saffron is obtained from about 80 kg fresh flower (Gracia et al., 2009; Husaini et al., 2009; Shahi et al., 2016).

Chemical analysis has shown more than 150 compounds have been extracted from saffron stigmas. These are lipophilic and hydrophilic carbohydrates (63 %), proteins (12 %), amino acids, minerals (5 %) (Ca, PO_4^{-3}, K, Na, Zn and Mn), mucilage, starch, gums, vitamins (especially riboflavin and thiamine), pigments (crocin, α and β carotenes, mangicrocin, xanthonecarotenoid glycosidic conjugate, anthocyanin, lycopene, flavonoids and zeaxanthin), alkaloids, saponins, safranal (aromatic essence terpene) and picrocrocin (bitter flavor) together with other chemical compounds (Negbi, 1997; Melnyk et al., 2010; Shahi et al., 2016).

The compound responsible for the colour of saffron is *cis* and *trans* crocins (Caballero-Ortega et al., 2007; D'Archivio et al., 2016; Tarantilis et al., 1995). The molecular formula of crocin is $C_{44}H_{64}O_{24}$ (Velasco-Negueruela, 2001). Crocins are glucosyl esters of 8,8'-diapocarotene-8,8'dioic acid (crocetin) and water-soluble carotenoids (Hadizadeh, 2010; Pitsikas, 2016; Carmona et al., 2007). α-Crocin (digentiobioside crocetin), crocin-2 (tricrocin or gentioglucoside crocetin), crocin-3 (entiobioside crocetin), crocin-4 (glucoside crocetin), crocin-5 (diglucoside crocetin) are stable under ambient conditions (Christodoulou et al., 2015). Besides its colour, the other features that affect saffron's quality are its taste and aroma. Picrocrocin ($C_{16}H_{26}O_7$), one of the important compound of saffron giving bitterness, turns into 4-hydroxy-2,6,6-trimethyl-1-carboxaldehyde-1-cyclohexene (HTTC) due to acid, alkali, heat and enzyme effects and HTTC turns into safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) which is the main compound of saffron's volatile oil and responsible for saffron's aroma (more than 60% of the essential oil) (Zougagh et al., 2006; Kiani et al., 2018).

Saffron contains more than 160 volatile and aroma compounds (terpenes, terpene alcohol and their esters). Safranal comprises approximately 0.001-0.006% of saffron dry matter (Shahi et al, Kiani et al, 2018). The other saffron volatile constituents are 3,5,5-trimethyl-2-cyclohexene-1-one (isophorone), 3,5,5-trimethyl-3-cyclohexene-1-one (an isomer of isophorone), 2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-ketoisophorone), 2,6,6-trimethyl-2-cyclohexene-1,4-dione and 2,6,6-trimethyl-1,4 cyclohexadiene-1-carboxaldehyde, 2,2,6-trimethyl-1,4-cyclohexanedione, and 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one (Kanakis et al., 2004; Urbani et al., 2015; Amanpour et al., 2015).

In fact, the freshly picked stigmas are nearly odourless, so the typical saffron flavour being developed especially during the drying process. Briefly, all these compounds responsible for saffron's colour, aroma and taste are found intensively in its stigmas (Velasco-Negueruela, 2001; Amanpour et al., 2015). These compounds lose their nature when exposed to light, heat and oxygen during storage (Rajabi et al., 2015). The result of volatile components determined with GC-MS technique has shown components such as 4,4-dimethyl-2-cyclopenten-1-one, heptanal, α-Pinene, 1-carboxaldehyde-5,5-dimethyl-2-methylene-3-cyclohexene, α-Isophorane, eucarvone, tetradecanic acid, hexadecanoic acid, 10,13-octadecadienoic acid, methyl ester, 4,8,1,16-tetramethylheptadecan-4-olide and other

components in low quantities (Sereshti et al., 2014)

Saffron quality, determined by its taste, aroma and colour, depends on its geographical origin, harvesting time (Condursa et al., 2017; Karabagias et al., 2017) and post-harvest treatment such as handling, corm size/weight, corm rate, planting depth, crop density, nutrient type and fertilisation management, weed management, dehydration process and storage conditions (Anastasaki et al., 2010; Rabani-Foroutagheh et al., 2014).

It is clear that, since saffron is a very rare and expensive spice, it is open to adulteration. In order to prevent adulteration and categorise saffron according to its quality, the International Standards Organization (ISO) published a standard ISO-3632 (2011) according to which saffron is categorised in regard to its physical and chemical characteristics. With regard to this standardisation, the limitations of the compounds used in this categorisation, which determine the quality of saffron in terms of colouring strength, flavour strength and aroma strength, have been specified with a spectrophotometric measurement technique by the ISO-3632 (2011). With this technique, it has been declared that the absorbance rate of 1% aqueous solution of saffron at 257 nm for flavour strength must be 70 (Category I), 55 (Category II) and 40 (Category III). It has also been stated that the colouring strength of %1 aqueous solution of saffron at 440 nm must be 200 for Category I, 170 for Category II and 120 for Category III. At the same time, it has been stated that the absorbance grade of the same solution at 330 nm for aroma strength must be between 20 and 50 for all categories.

Numerous works have been performed with different techniques and reported in the literature on saffron quality characteristics depending on origin, drying conditions (Gregory and Menary, 2007) and storage period. But there are few studies focused on the physicochemical properties of different quality Iranian saffron (Anastasaki, et al., 2010; Zougagh et al., 2006; Maggi et al., 2010; Alonso et al., 1996; Anastasaki et al., 2009; Carmona et al., 2006a; D'Auria et al., 2004; Maggi et al., 2009; Masuda et al., 2012; Rödel & Petrzika, 1991; Sarma et al., 1991; Sereshti et al., 2014; Tarantilis & Polissiou, 1997).

In market, it is possible to find different qualities saffron and of course adulterations and similar stigmas from other plants. Generally, saffron has marketed in different quality categories (sargol, pushals and bunch) according to stigma and style mass proportion of the product. In high quality saffron, only the red part of stigma, which is the end of the stigmas, was used. To our best knowledge, there is no study on volatile constituents of Iranian saffron with regard to commercial categorization. Therefore, in this study, saffron samples were sampled according to the ISO categorization of Iranian saffron and some chemical properties (volatile constituents and crocin content) were determined.

MATERIALS AND METHODS

Saffron materials

In this study, different saffron samples from *Crocus sativus* L. marketed in Iran as different quality categories (Sargol-I, Sargol-II, Pushal-I, Pushal-II and Bunch) were used (Figure 1). The samples were provided directly from the commercial producer with a guarantee of freedom from adulteration and were stored at 4 °C in the dark until analysis. The categories of saffron samples differ in red threads (Sargol-I and Sargol-II) and threads with yellow styles (Pushal-I, Pushal-II and Bunch). Sargol saffron consists of only the red part of the stigma. This category of saffron has a strong colouring property. Pushal saffron is longer than Sargol saffron and contains stigmas with styles (Figure 2) (Heidarbeigi et al., 2015). This kind of saffron has a lower colour strength compared to Sargol saffron. In this study, we chose different Sargol (S-I, S-II) and Pushal (P-I, P-II) saffron samples. Bunch consisted completely of stigmas and styles.

Determination of flavour and colouring strength of saffron samples

Saffron samples were analysed according to ISO/International Technical Standard TS 3632 (2011) to identify the quality category of the

samples dependent on flavour and colouring strength. For this purpose, the absorbance of 1% aqueous solution of dried saffron samples was measured at 257 and 440 nm wavelength. Even though this method is also suggested to determine aroma strength, it was not preferred since it was stated in other studies that presented safranal in aqueous solution could interfere with absorbance of crocetin esters (mainly cis-isomers) at suggested wavelength of 330 nm in ISO 3632 (2011) spectrophotometric measurement technique (García-Rodríguez et al., 2017; Hadizadeh et al., 2006; Carmona et al., 2006b).

Figure 1. Saffron samples of different quality categories, which were used in the study (A. S-I, B. S-II, C. P-I, D. P-II and E. Bunch).

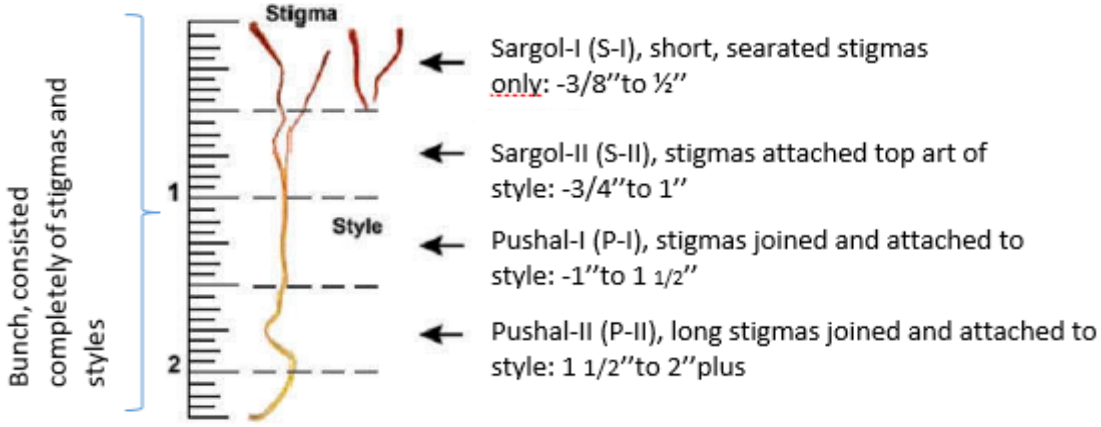

Figure 2. Saffron stigmas with yellow style (Heidarbeigi et al., 2015).

Determination of moisture content

Moisture content of the saffron samples was determined according to ISO-3632 (2011).

Extraction of crosins

The profile of the colour components of the samples was determined with HPLC by partial modification of the technique performed by Masi et al (2016) and Caballero-Ortega et al (2007). According to this techniques, 0.050 ± 0.001 g ground saffron sample was weighed, and then 10 mL methanol:water mixture (50:50) was added and the solution was kept on a magnetic mixer at 4 °C in the dark for 24 h. After that, the acquired extract was transferred into a 15 mL centrifugal tube and centrifuged at 20,000 *g* at 4 °C for 20 min. At the end of this process, after taking 2.5 mL from the obtained top phase, 2.5 mL internal standard (2-nitroaniline) was added, the mixture was transferred into a 25 mL volumetric flask which was then filled with a mixture of methanol:water (50:50).

Evaluation of crosin compounds by High Performance Liquid Chromatography (HPLC)

Evaluation of crosin compounds were analysed by a HPLC (Shimadzu UV-vis 160A) system with a Spherisorb RP ODS-3 (4.6 × 250 mm) column. A, linear gradient of methanol (100) and B, linear

acetonitrile: water (15:85) were used as the mobile phases with a flow rate of 1.0 mL/min for a maximum elution time of 60 min at room temperature and 20 µL injection. The acquired extract was filtered through a 0.45 µm filter, transferred to colourful vials and subjected to HPLC under the conditions described below. The injections were done in parallel. Picrocrocin, HTCC, internal standard and camphor oil were scanned at 257 nm, safranal was scanned at 330 nm and crocins were scanned at 440 nm. The acquired rates were transformed to mg/g safranal by the curve generated with the safranal standard.

Extraction of volatiles by solid phase microextraction (SPME)

A solid phase microextraction (SPME) technique, by fibers with different polarities, 85µm-polyacrylate (PA), 100µm-polydimethylsiloxane (PDMS) and 75µm-carboxenpolydimethylsiloxane (CAR/PDMS), were used to extract the volatile compounds of saffron according to the method reported by D'Auria et al (2006) and Urbani et al (2015). For this purpose, 0.1 g ground saffron sample was weighed into a 20 mL headspace vial, and a Teflon-lined septum was immediately sealed with an aluminium crimp seal. The fibre was maintained over the sample in a vial at 36 °C for 20 min.

Gas chromatography-mass spectrometry (GC-MS) analysis

The volatite constituents of saffron were performed with a GC–MS system (Shimadzu QP2010 Plus) equipped with a TRB-5MS (30 m × 0.25 mm × 0.25 µm) column. Helium was used as carrier gas at 0.8 mL/min and the injection was splitless at 250 °C. The oven temperature was programmed as follows: held at 50 °C for 2 min; then the temperature was raised to 200 °C (3 °C/min, held for 10 min). The ion trap conditions were as follows: transfer line temperature 200 °C, trap temperature 250 °C, scan range 35–450 amu (3 microscans/scan).

Identification of volatile compounds

An alkane standard (C_7–C_{40}) was used to determine the retention index (RI) of each compound. Identification of the volatile compounds was attempted using the mass spectral libraries of Wiley 7 and NIST 02 (2017). They were also confirmed by comparing RI and mass spectra with an online literature library (2017).

Statistical analysis

All assays were conducted on duplicate samples of homogenates (n=4). Analysis was conducted using the SAS software (Statistical Analysis System, Cary, NC, USA). When main effects or interactions were significant, Duncan's multiple range test was used.

RESULTS AND DISCUSSION

Determination of the quality category of saffron samples

As a result of the analysis, it was found that the colouration ability of 1% aqueous solution of saffron at 440 nm for S-I sample was 244.32, S-II sample 137, P-I sample 126.75, P-II sample 123.50 and Bunch sample 103.75. The absorbance grade of the same solution at 257 nm for flavour strength of the S-I sample was 80, S-II sample 46.2, P-I sample, 43.6 P-II sample 42 and Bunch sample 39.3. As a result of these obtained data, the S-I sample was identified as Category I, S-II, P-I and P-II were Category III. On the other hand, the Bunch sample cannot be categorized due to its too low colouration ability and flavour strength. The lower colouration ability and flavour strength of Bunch sample may be related to both high style content of and excessive drying. The moisture and safranal results, presented below, also verifies excessive drying of this sample.

Moisture content of saffron samples

The moisture content of S-I, S-II, P-I, P-II and Bunch samples were determined as 7.18%, 7.22%, 7.41%, 7.35% and 4.64%, respectively. The results showed that all samples of saffron, except for the Bunch sample, had a very similar moisture content of around 7%, with the Bunch sample at around 4%. According to the ISO 3632 (2011) saffron standard, the moisture content of saffron must be a maximum of 12%. In this regard, the saffron samples used in this research correspond

to the standard. According to the results, the moisture content of the Bunch sample differs from the other samples ($p < 0.05$). This difference makes us think that the Bunch sample was exposed to a higher heat and/or longer drying process, although the supplying firm declared that all samples were exposed to equal drying processes.

Profile of the crosin components of saffron samples

The component amounts of saffron samples determined by HPLC are given in Table 1; the chromatograms for the components are depicted in Appendices 1–5. Each component was identified by comparing its retention period with that stated in the literature. When the results are examined, it can be seen that the components forming the total crocin that gives its colour to the saffron (picrocrocin, trans-crocin 3, trans-crocin 2, trans-crocin 2', cis-crocin 2 and cis-crocin 4) diminished at a significant level ($p < 0.01$), except for trans-crocin 4. The highest crocin amount was found in the S-I sample (66.67 mg/g) and the lowest in the Bunch sample (51.66 mg/g). It can be seen in Table 1 that the most crucial crocin isomers are trans-crocin 3 and trans-crocin 4.

Table 1. Duncan's multiple range test results for the averages of identified components (HPLC)

Component	Class				
	S-I	S-II	P-I	P-II	Bunch
Picrocrocin	$5.21^{a} \pm 0.00$	$4.99^{b} \pm 0.00$	$4.59^{c} \pm 0.00$	$4.49^{d} \pm 0.00$	$3.01^{e} \pm 0.00$
HTCC	0.13 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.15 ± 0.00
3-Gentiobiosy-kaempferol	0.20 ± 0.04	0.24 ± 0.01	0.22 ± 0.01	0.20 ± 0.00	0.20 ± 0.00
Safranal	0.65 ± 0.00	0.70 ± 0.01	0.75 ± 0.01	0.80 ± 0.00	1.06 ± 0.00
Trans-crocin 4	$35.93^{a} \pm 0.02$	$34.56^{b} \pm 0.01$	$32.54^{c} \pm 0.12$	$30.08^{d} \pm 0.02$	$29.50^{e} \pm 2.21$
Trans-crocin 3	$23.66^{a} \pm 0.01$	$23.06^{a} \pm 0.41$	$21.50^{ab} \pm 0.08$	$19.02^{b} \pm 0.00$	$18.86^{b} \pm 0.40$
Trans-crocin 2'	$1.10^{a} \pm 0.00$	$1.07^{b} \pm 0.00$	$0.88^{c} \pm 0.00$	$0.72^{d} \pm 0.00$	$0.46^{d} \pm 0.00$
Cis-crocin 4	$4.64^{a} \pm 0.00$	$4.08^{b} \pm 0.00$	$3.30^{c} \pm 0.00$	$2.70^{d} \pm 0.00$	$1.52^{e} \pm 0.00$
Trans-crocin 2	$1.20^{a} \pm 0.00$	$1.19^{b} \pm 0.00$	$1.19^{b} \pm 0.00$	$1.170^{c} \pm 0.00$	$1.15^{d} \pm 0.00$
Cis-crocin 2	$0.14^{b} \pm 0.01$	$0.16^{ab} \pm 0.00$	$0.15^{b} \pm 0.00$	$0.18^{a} \pm 0.010$	$0.17^{ab} \pm 0.00$
Total	$72.86^{a} \pm 0.08$	$70.19^{ab} \pm 0.44$	$65.26^{ab} \pm 0.22$	$59.51^{ab} \pm 0.12$	$57.18^{c} \pm 2.61$

Values within a line with different superscript letters are significantly ($P < 0.01$) different.

When Table 1 is examined, the amount of picrocrocin, which gives the taste of bitterness to saffron, significantly diminishes ($p < 0.01$) from the S-I sample (5.21 mg/g) to the Bunch sample (3.01 mg/g) depending upon the categorisation. As a matter of fact, not only the colour components (crocin), but also the amount of picrocrocin is one of the criteria assessed in categorisation (Velasco-Negueruela, 2001).

The broad range of values is reported by different researchers for saffron components such as crocin, picrocrocin and safranal from country to country. According to the some studies' results, crocins ranged between 0.85- 32.4 %, picrocrocin ranged between 0.79- 12.94 % and the safranal values range between 1.07- 6.15 % on a dry weight basis. Different drying methods, storage and extraction conditions of saffron degrade these compounds and the degree of degradation depends on temperature, humidity, light irradiation and other compounds in the environment (Lage and Cantrell, 2009).

Volatile compounds of saffron samples

To determine the best fiber properties, volatile compounds of S-I saffron sample determined with 3 different fibers with different polarities by GC-MS. Result shows that; 14, 34, 32 compounds were identified by PA, PDMS and CAR/PDMS,

respectively. The volatile compounds of S-I samples with 3 different fibers are shown in Table 2. It can be seen in that the acetic acid, 2(5H)-furanone, isophorone, 4-ketoisophorone, 2,6,6-trimethyl-1,4-cyclohexanedione, safranal, HTCC were the main volatile compounds detected in all tested fibers (Figure 3). Since PDMS fiber yielded higher number of volatile compounds, it was also used in the analysis of other saffron samples.

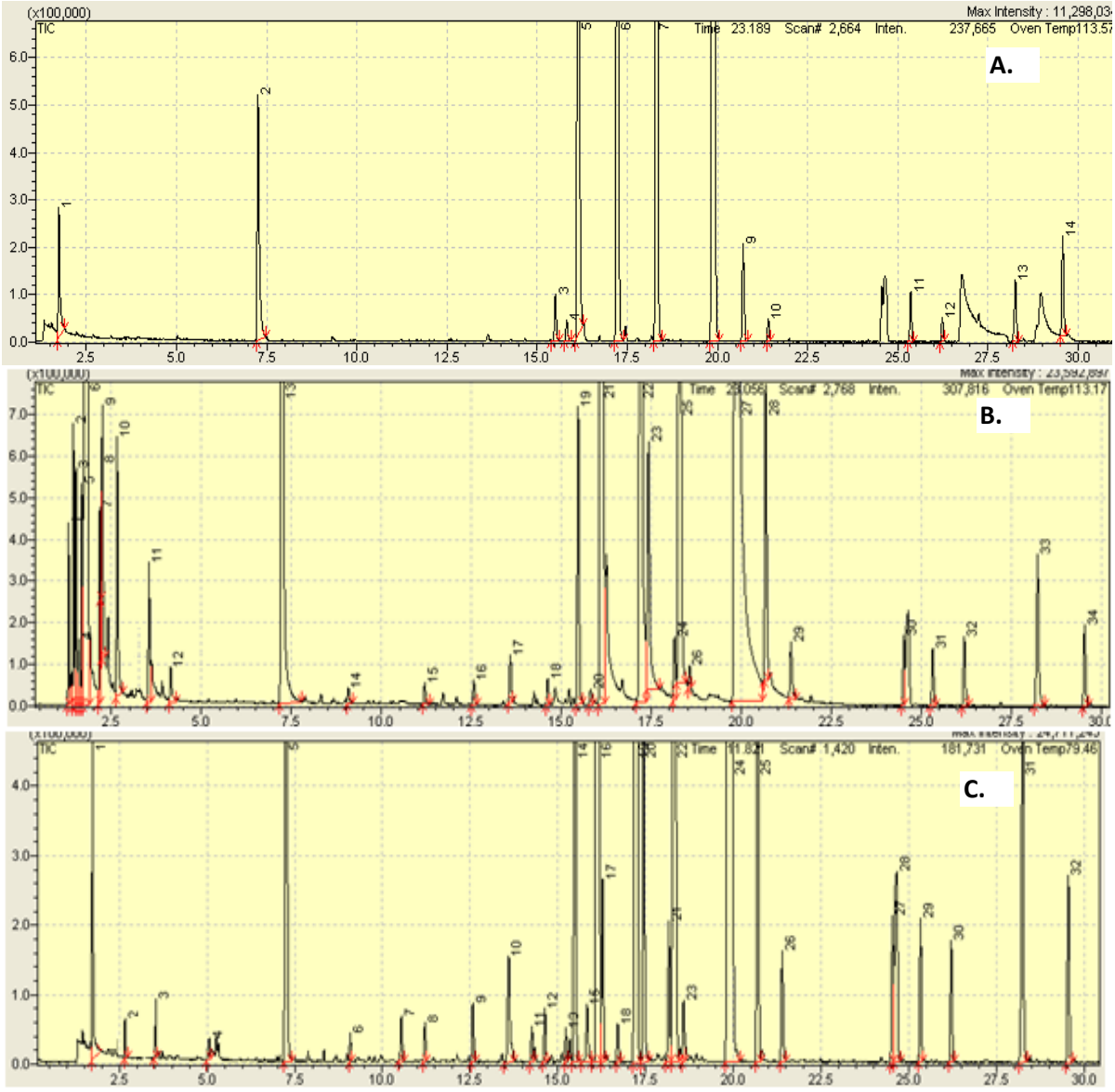

Figure 3. GC-MS fingerprint chromatograms of saffron samples (S-I) (A:PA, B:PDMS, C:CAR/PDMS).

Table 2. A solid phase microextraction (SPME) technique was used to extract the aroma components of S-I saffron sample with 3 different fibers (PA, PDMS and CAR/PDMS) by GC-MS (X ± SE).

No	RI	Chemical name	Fibers					
			PA		PDMS		CAR-PDMS	
			R_T	Area (%)	R_T	Area (%)	R_T	Area (%)
1	629	*Ethanol*	-	-	1.443	0.44 ± 0.05	-	-
2	633	*Undefined*	-	-	1.513	0.48 ± 0.09	-	-
3	638	*Undefined*	-	-	1.591	0.15 ± 0.54	-	-
4	643	*2-Methylpropanal*	-	-	1.678	0.37 ± 0.58	-	-
5	653	*Acetic acid*	1.743	1.11 ± 0.06	1.84	9.49 ± 0.25	1.737	0.42 ± 0.06
6	674	*3-Methylbutanal*	-	-	2.172	0.34 ± 0.54	-	-
7	677	*2-Methylbutanal*	-	-	2.215	0.18 ± 0.09	-	-
8	678	*3-Methyl-2-butanone*	-	-	2.246	0.47 ± 0.10	-	-
9	704	*Acetoin*	-	-	2.657	0.52 ± 0.09	2.664	0.04 ± 0.01
10	760	*1-Pentanol*	-	-	3.542	0.34 ± 0.65	3.542	0.05 ± 0.01
11	797	*Hexanal*	-	-	4.144	0.11 ± 0.79	-	-
12	831	*Isovaleric asid*	-	-	-	-	5.066	0.04 ± 0.02
13	906	*2(5H)-Furanone*	7.265	2.73 ± 0.09	7.258	3.46 ± 0.25	7.259	1.84 ± 0.07
14	953	*Octanal*	-	-	9.08	0.04 ± 0.14	9.088	0.04 ± 0.03
15	990	*Benzaldehyde*	-	-	-	-	10.542	0.07 ± 0.01
16	1006	*β-Phellandrene*	-	-	11.199	0.06 ± 0.64	11.215	0.06 ± 0.01
17	1036	*6-Methyl-5-hepten-2-one*	-	-	12.56	0.07 ± 0.24	12.576	0.09 ± 0.01
18	1059	*β-Phorone*	-	-	13.581	0.15 ± 0.37	13.599	0.27 ± 0.07
19	1074	*Undefined*	-	-	-	-	14.258	0.06 ± 0.01
20	1082	*Undefined*	-	-	14.619	-	14.636	0.09 ± 0.00
21	1095	*Isophorone isomer*	-	-	-	-	15.231	10.05 ± 0.02
22	1101	*Linalol*	15.508	0.42 ± 0.53	15.466	0.87 ± 0.65	15.485	1.17 ± 0.02
23	1108	*5-Methylfurfural*	1582	0.21 ± 0.07	15.811	0.06 ± 0.58	15.84	0.12 ± 0.54
24	1115	*Isophorone*	16.133	11.48 ± 0.67	16.118	8.20 ± 0.03	16.151	14.95 ± 0.03
25	1118	*2-Hydroxy-isophorone*	-	-	-	-	16.279	0.31±0.05
26	1127	*1,3,3-trimethyl-7-oxabicyclo[4.1.0]heptane-2,5-dione*	-	-	-	-	16.712	0.07 ± 0.09
27	1138	*4-Ketoisophorone*	17.255	10.66 ± 0.08	17.206	8.72 ± 0.03	17.241	10.52 ± 0.12
28	1142	*2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one*	-	-	17.418	0.96 ± 0.68	17.445	0.68 ± 0.56
29	1158	*Undefined*	-	-	18.143	0.22 ± 0.17	18.166	0.26 ± 0.56
30	1161	*2,6,6- Trimethyl-1,4-cyclohexanedione*	18.311	8.35 ± 0.08	18.287	4.90 ± 0.24	18.328	7.51 ± 0.37
31	1167	2-Isopropylidene-3-methylhexa-3.5-dienal	-	-	18.557	0.06 ± 0.64	18.583	0.12 ± 0.12
32	1196	*Safranal*	19.894	59.32 ± 0.07	19.914	49.64 ± 0.29	19.958	55.51 ± 0.07
33	1213	*Eucarvone*	20.714	0.93 ± 0.41	20.675	0.91 ± 0.03	20.701	1.00 ± 0.06
34	1228	*3,5,5-Trimethyl-2-hydroxy-1,4-cyclohexadione-2-ene*	21.411	0.2 ± 0.54	21.377	0.20 ± 0.43	21.401	0.21 ± 0.14
35	1297	*Undefined*	-	-	24.512	0.21 ± 0.06	24.538	0.27 ± 0.68
36	1301	*Undefined*	-	-	-	-	24.657	0.59 ± 0.45
37	1316	*3,5,5-Trimethyl-4-hydroxy-1-cyclohexanone-2-ene*	25.357	0.44 ± 0.65	25.317	0.18 ± 0.71	25.339	0.26 ± 0.84
38	1336	*Undefined*	26.229	0.22 ± 0.06	26.187	0.21 ± 0.06	26.212	0.22 ± 0.24
39	1383	*2,4,4-Trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadien-1-one*	28.26	0.63 ± 0.05	28.223	0.54 ± 0.08	28.25	0.76 ± 0.31
40	1414	*HTCC*	29.572	1.01 ± 0.07	29.532	0.25 ± 0.02	0.76	0.35 ± 0.60

RI: retention index; R_T: retention time

According to the obtained results, 39 different volatile compounds were detected saffron samples (Table 3). Acetic acid, 2-(5H)-furanone, isophorone, 4-ketoisophorone, 2,6,6-trimethyl-1,4-cyclohexanedione and safranal were the main volatile compounds detected in all saffron categories. Similarly, safranal has been determined as the main volatile compound of saffron in previous studies (Urbani et al., 2015; Maggi et al., 2009; D'Auria et al., 2006; del Campo et al., 2009; Amanpour et al., 2015). The safranal amount in the Bunch sample was found as the highest and quite different from the other categories of saffron. This diversity and high amount is thought to related to excessive drying of this sample. It was stated in a study by Carmona et al. (2006a) that the amount of safranal in saffron increases depending on the increase of drying temperature at practices higher than 90°C. They hypothesized that picrocrocin turns into safranal at high temperature. The same researchers also stated that the amounts isophorone and 2,6,6-trimethyl-1,4cyclohexanedione increased at high drying temperatures. In present study, it was determined that the Bunch sample also had large amounts of these compounds. Moreover, high content of 2(5H)-Furanone in Bunch sample shows the higher non-enzymatic browning of these sample.

Table 3. Composition of saffron sample aroma components (X ± SE)

No	RI	R_T	Chemical name	Area (%)				
				S-I	S-II	P-I	P-II	Bunch
1	625	1.4	*Undefined*	-	0.07 ± 0.45	0.09 ± 0.01	0.08 ± 0.04	0.10 ± 0.01
2	629	1.443	*Ethanol*	0.44 ± 0.24	0.35 ± 0.24	0.27 ± 0.22	0.43 ± 0.27	0.12 ± 0.29
3	633	1.513	*Undefined*	0.48 ± 0.30	0.68 ± 0.35	0.74 ± 0.32	0.66 ± 0.30	0.66 ± 0.24
4	638	1.591	*Undefined*	0.15 ± 0.07	0.24 ± 0.07	0.25 ± 0.07	0.24 ± 0.06	0.14 ± 0.07
5	643	1.678	*2-Methylpropanal*	0.37 ± 0.01	0.42 ± 0.12	0.67 ± 0.17	0.41 ± 0.01	0.51 ± 0.03
6	653	1.840	Acetic acid	9.49 ± 0.25	7.05 ± 0.28	6.82 ± 0.27	7.70 ± 0.06	5.63 ± 0.20
7	659	1.925	Undefined	-	0.03 ± 0.01	0.06 ± 0.02	0.10 ± 0.01	0.05 ± 0.01
8	674	2.172	3-Methylbutanal	0.34 ± 0.05	0.39 ± 0.31	0.46 ± 0.04	0.30 ± 0.05	0.44 ± 0.05
9	677	2.215	2-Methylbutanal	0.18 ± 0.01	0.81 ± 0.11	0.26 ± 0.12	0.23 ± 0.31	0.16 ± 0.12
10	678	2.246	3-Methyl-2-butanone	0.47 ± 0.19	0.64 ± 0.25	0.76 ± 0.18	0.58 ± 0.57	0.68 ± 0.16
11	690	2.425	Undefined	-	-	0.35 ± 0.17	-	0.17 ± 0.38
12	704	2.657	Acetoin	0.52 ± 0.32	1.15 ± 0.16	0.97 ± 0.24	0.69 ± 0.38	1.20 ± 0.04
13	760	3.542	1-Pentanol	0.34 ± 0.06	0.18 ± 0.05	0.45 ± 0.06	0.93 ± 0.06	0.26 ± 0.06
14	766	3.65	Undefined	-	-	0.23 ± 0.01	-	0.25 ± 0.01
15	783	3.913	Undefined	-	-	-	-	0.12 ± 0.01
16	797	4.144	Hexanal	0.11 ± 0.00	0.05 ± 0.00	0.20 ± 0.00	-	0.14 ± 0.00
17	906	7.258	2(5H)-Furanone	3.46 ± 0.02	3.76 ± 0.06	4.09 ± 0.09	4.34 ± 0.07	8.67 ± 0.07
18	953	9.080	Octanal	0.04 ± 0.00	-	-	-	-
19	1006	11.199	β-Phellandrene	0.06 ± 0.01	-	-	-	0.23 ± 0.02
20	1036	12.56	6-Methyl-5-hepten-2-one	0.07 ± 0.01	0.07 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.03
21	1059	13.581	β-Phorone	0.15 ± 0.02	0.19 ± 0.07	0.23 ± 0.09	0.19 ± 0.06	-
22	1101	15.466	Linalol	0.87 ± 0.05	0.81 ± 0.03	0.86 ± 0.07	1.17 ± 0.04	1.18 ± 0.043
23	1108	15.811	5-Methylfurfural	0.06 ± 0.00	0.06 ± 0.00	-	-	-
24	1115	16.118	Isophorone	8.20 ± 0.03	10.22 ± 0.07	12.32 ± 0.07	12.51 ± 0.26	13.52 ± 0.07
25	1119	16.333	2-Hydroxy-isophorone	-	-	-	-	0.21 ± 0.02
26	1138	17.206	4-Ketoisophorone	8.72 ± 0.21	9.19 ± 0.41	10.39 ± 0.21	10.53 ± 0.34	15.98 ± 0.12

Table 3. Continued

No	RI	R_T	Chemical name	Area (%)				
				S-I	S-II	P-I	P-II	Bunch
27	1142	17.418	2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one	0.96 ± 0.06	1.02 ± 0.04	0.48 ± 0.19	0.82 ± 0.43	0.66 ± 0.54
28	1151	17.821	Undefined	-	-	-	-	0.19 ± 0.34
29	1158	18.143	Undefined	0.22 ± 0.21	0.32 ± 0.53	0.19 ± 0.67	0.19 ± 0.23	0.09 ± 0.76
30	1161	18.287	2,6,6- Trimethyl-1,4-cyclohexanedione	4.9 ± 0.04	4.92 ± 0.04	4.99 ± 0.23	5.19 ± 0.12	7.23 ± 0.02
31	1167	18.557	2-Isopropylidene-3-methylhexa-3.5-dienal	0.06 ± 0.01	-	0.10 ± 0.01	-	0.07 ± 0.01
32	1196	19.914	Safranal	49.64 ± 0.29	50.29 ± 0.38	50.42 ± 0.26	57.02 ± 0.66	61.31 ± 0.25
33	1213	20.675	Eucarvone	0.91 ± 0.11	0.73 ± 0.15	0.51 ± 0.24	1.04 ± 0.25	0.53 ± 0.35
34	1228	21.377	3,5,5-Trimethyl-2-hydroxy-1,4-cyclohexadione-2-ene	0.20 ± 0.01	-	0.10 ± 0.01	0.16 ± 0.01	-
35	1297	24.512	Undefined	0.21 ± 0.11	-	-	-	-
36	1316	25.317	3,5,5-Trimethyl-4-hydroxy-1-cyclohexanone-2-ene	0.18 ± 0.06	-	0.07 ± 0.04	0.14 ± 0.06	0.06 ± 0.01
37	1336	26.187	Undefined	0.21 ± 0.32	0.25 ± 0.34	0.12 ± 0.23	0.27 ± 0.34	0.07 ± 0.01
38	1383	28.223	2,4,4-Trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadien-1-one	0.54 ± 0.34	0.79 ± 0.25	0.57 ± 0.05	0.62 ± 0.04	0.21 ± 0.01
39	1414	29.532	HTCC	0.25 ± 0.07	0.31 ± 0.13	0.29 ± 0.11	0.27 ± 0.01	0.5 ± 0.02

RI: retention index; R_T: retention time

Amanpour et al. (2015) were determined 4-ketoisophorone, dihydrooxophorone and isophorone (ketones) in the Iranian saffron samples. Isophorone is formation occurred by a mechanism involving the degradation of zeaxanthin and it important in saffron to provide odour (Culleré et al., 2011; Amanpour et al., 2015). Two glycosides ((1R)-3,5,5-trimethyl-3-cyclohexen-1-ol O-β-D-glucopyranoside and of 4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one O-β-D-glucopyranoside) are, respectively, the possible precursors of β-isophorone and α-isophorone (Condurso et al., 2017). When Table 3 is examined, isophorone, 4-ketoisophorone, 2-hydroxy-isophorone and 2,6,6- trimethyl-1,4-cyclohexanedione were detected in saffron samples and Bunch sample also had large amounts of these compounds.

In a study by Karabagias et al. (2017) a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber 50/30 μm was used to extract headspace volatiles from saffron. They were determined aldehydes (2-Methyl-butanal, 3-Methyl-butanal, Hexanal, Heptanal, Octanal, 2-Isopropylidene-3-methylhexa-3.5-dienal, 2,4-Dimethyl-benzaldehyde, 2,4,5-Trimethyl-benzaldehyde) and terpenoids (α-phellandrene,

dL-limonene, 2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one, 2,2,6-Trimethyl-1,4-cyclohexandione, 5-Hydroxy-2,5-cyclohexadien-1-one-2,4,4-trime-thyl-3-carboxaldehyde (2,4,4-Trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadien-1-one)) as volatile compounds in Morocco, Greece, Spain and Iranian saffron samples.

In another study (Carmona et al. (2006a) analysing the volatile compounds of saffron with TD-GC–MS, determined acetic acid, linalool, 2,6,6-trimethyl-1,4-cyclohexene-1 carboxaldehyde, isophorone, safranal and 2,6,6-trimethyl-2-cyclohexene-1,4-dione compounds. D'Auria et al. (2004) determined 3,5,5-trimethyl-2-cyclohexenone-1-one, 3,5,5-trimethyl-2-cyclohexene-1,4-dione, 2,4,4-trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadien-1-one, 3,5,5-trimethyl-1,4-cyclohexadione, 3,5,5-trimethyl-2-cyclohexenone-1-one, safranal, acetic acid, hexanal, 2(5H)-furanone, 3,5,5-trimethyl-4-hydroxy-1-cyclohexanone-2-ene and 5,5-dimethyl-1-ethyl-1,3-cyclopentadiene α-isophorone in Iranian saffron, in parallel with our study. Also, α-isophorone, β-isophorone, safranal, ketoisophorone, HTCC, 2,4,4-trimethyl-3-carboxaldehyde-5-hydroxy-2,5-cyclohexadien-1-one, 3,5,5-trimethyl-4-hydroxy-1-cyclohexanone-2-ene and eucarvone are essential volatile compounds of saffron as well as HTCC, mint furanone and n-hexadecanoic acid compounds (Urbani et al., 2015; Jalali-Heravi, et al., 2009; Jalali-Heravi et al., 2010). Condurso et al. (2017) determined 6-methyl-5-heptene-2-one as component in Italian, Spanish, and Moroccan saffron. This compound is derived from the carotenoids degradation.

In the present study, suitability of commercial Iranian saffron to ISO standard and the volatile compounds and some chemical properties of the samples was determined. According to the results, the S-I sample was identified as Category I while S-II, P-I and P-II were Category III. The Bunch sample did not categorized in any category and could not be marketed in International trade. The moisture values of saffrons except for the Bunch sample were determined as 7% but the moisture value of the Bunch sample was determined as 4.64% and it is thought to have been exposed excessive drying. The total amount of the crocin component which gives saffron its characteristic colour was identified with HPLC as highest in the S-I sample (66.67 mg/g) and lowest in the Bunch sample (51.66 mg/g). This rate decreased in parallel with the decrease in quality and the amount of picrocrocin, which gives saffron its bitterness; the larger amount of it, which shows the high quality of saffron, decreased dramatically due to the classification when going from the S-I sample to the Bunch sample.

As a result of the volatile compounds analysis performed by GC–MS using the SPME method, in all the saffron samples, acetic acid, 2-(5H)-furanone, isophorone, 4-ketoisophorone, 2,6,6-trimethyl-1,4-cyclohexanedione, eucarvone and safranal were found as significant compounds with regard to percentage. The study showed that percentage amount of safranal increased by decreasing of saffron colouring quality.

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