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Quantification of apocarotenoids in commercial Indian (Kashmiri) saffron using UV-Vis spectroscopy and HPLC analysis

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Abstract: Saffron is considered as the most expensive spice in the world. Due to low production, high demand and high cost, saffron is very prone to adulteration for economic benefits while putting public health at risk. The most significant characteristic for determining the quality of the saffron is coloring strength (crocin content), which is determined by measuring UV-Vis absorption at 440 nm in the aqueous preparations of this spice. Picrocrocin and safranal are other key components used to determine saffron quality. This article aims to examine the quality of commercial saffron obtained from various geographical locations of Kashmir (India) by determining their apocarotenoid content using UV-Vis spectrophotometry followed by high-performance liquid chromatography (HPLC) to determine the concentration of saffron metabolites (crocin, picrocrocin and safranal). A total of 31 samples from different origins were used in this study. The UV-Vis spectrophotometric results showed that among 31, only 14 samples fell into grade I, while 9 samples fell in grade II and 5 samples fell in grade III of the ISO category. The remaining 3 samples could not satisfy ISO standards, which indicates that these samples were adulterated. The determination of apocarotenoid content using HPLC analysis varied significantly among samples. These variations may be due to different drying and storage conditions or adulteration.

Key words: saffron; adulteration; crocin; safranal; picrocrocin; UV-Vis spectroscopy; HPLC

Količinsko ovrednotenje apokarotenoidov v komercialnih vzorcih indijskega (kašmirskega) žafrana z analizo UV-Vis spektroskopije in HPLC

Izvleček: Žafran velja za najdražjo začimbo v svetovnem merilu. Zaradi majhne pridelave, velikega povpraševanja in visoke cene je zaradi ekonomskih koristi podvržen ponarejanju, kar povzroča zdravstvena tveganja. Najznačilnejša lastnost za določanje kakovosti žafrana je njegova sposobnost obarvanja (vsebnost krocina), ki se določa z merjenjem UV-Vis absorbcije pri 440 nm v vodnih pripravkih te začimbe. Pikrokrocin in safranal sta ostali klučni komponenti, ki se uporabljata za določanje kakovosti žafrana. V raziskavi smo preučevali kakovost tržnega žafrana pridobljena iz različnih geografskih območij Kašmirja (Indija) z določanjem vsebnosti apokarotenoidov z UV-Vis spektroskopijo, ki ji je sledila analiza z visokotlačno tekočinsko kromatografijo (HPLC), kjer smo v vzorcih žafrana določali koncentracije metabolitov kot so krocin, pikrokrocin in safranal. V raziskavi je bilo analiziranih 31 vzorcev različnega izvora. Rezultati analize z UV-Vis spektroskopijo so pokazali, da se je med 31 vzorci samo 14 uvrstilo v kvaliteto I, 9 vzorcev seje uvrstilo v kvaliteto II in 5 vzorcev v kvaliteto III, glede na ISO kategorije. Preostali 3 vzorci niso izpolnjevali ISO standardov, kar kaže, da so bili ponarejeni. Vsebnost apokarotenoidov v vzorcih se je pri analizi s HPLC značilno razlikovala, kar bi lahko bila posledica različnega sušenja, shranjevanja ali ponarejanja.

Ključne besede: žafran; ponarejanje; krocin; safranal; pikrokrocin; UV-Vis spektroskopija; HPLC

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1 INTRODUCTION

Saffron, often referred to as red gold, is obtained from the stigma of *Crocus sativus* L. (Saxena, 2010). *Crocus sativus* is an angiosperm plant, member of the Asparagales family. The flower of *Crocus sativus* is solitary, purple, with six petals, three stamens, one style, and three reddish-orange stigmas. Saffron crocus grows throughout the Mediterranean–Europe, and Western Asia. It is commonly cultivated in Iran, Greece, Spain, Italy, Afghanistan and India (Kashmir) (Kahriz, 2020). Saffron is quite costly because it is used mainly as a flavoring and aromatizing agent (Mir et al., 2022a; Mzabri et al., 2019). Saffron contains approximately 300 volatile and non-volatile metabolites, including crocin, safranal, picrocrocin, monoterpenes, aldehydes, and various other carotenoids of therapeutic potential (Pandita, 2021). It is a well-known spice that is used to cure a variety of medical conditions, including depression (Siddiqui et al., 2018), cardiovascular illness (Kamalipour & Akhondzadeh, 2011), menstrual irregularities (Beiranvand et al., 2016), asthma (Zilaee et al., 2019)lipid profiles, basophils, eosinophils and clinical symptoms in patients with allergic asthma.\nSTUDY DESIGN: Our study was a clinical trial.\nMETHODS: Subjects (N = 80, 32 women and 48 men, 41.25 ± 9.87 years old, insomnia (Taherzadeh et al., 2020), and digestive problems (Khorasany & Hosseinzadeh, 2016). Crocin is a carotenoid chemical compound responsible for the golden yellow-orange color of saffron; picrocrocin gives bitter flavor, and safranal is responsible for the characteristic aroma of saffron. Saffron is currently known as a flavoring agent and a potent natural agent with many health advantages (Azami et al., 2021; Basker & Negbi, 1983; Bolhassani et al., 2014; Mir et al., 2022b). The potential of saffron and its constituents to protect against natural and artificial poisons has enhanced its significance. Due to the high price of saffron and its great demand in the pharmaceutical industry, illegal trafficking and adulteration are prevalent nowadays (Alonso et al., 1998). The common adulterants used in saffron include maize silk, marigold floret, horsehair, wool, saffron stamens, red dried silk fiber, and safflower (*Carthamus tinctorium* L.). Mixing low-grade saffron with high-grade saffron or old stored saffron that has lost its quality with freshly harvested saffron is also a common method of adulteration in saffron (Kumari et al., 2021; Lowell, 1964; Marieschi et al., 2012; Sereshti et al., 2018). The sale and mixing of high-grade Kashmiri saffron with lower-cost Iranian imports is common in India; the resulting mixes are then sold as pure Kashmiri saffron. This trend has deprived saffron cultivators of Kashmir of a significant portion of their revenue (Hussain, 2005). Dyes such as erythrosine, tartrazine, amaranth, sunset yellow, carmoisine, picric acid, ponceau S, methyl orange, and Sudan red are also used as adulterants in saffron (Lozano et al., 1999; Patel et al., 2019; Petrakis et al., 2015).

Internationally, the grading of saffron is based on the standards formulated by the International Organization for Standardization (ISO). The ISO (ISO 3632-1:2011) certification ensures customers that the saffron they purchase is authentic and safe to consume. ISO 3632 has classified saffron into three grades (Grade I, II and III) based on the concentration of crocin, picrocrocin and safranal present in saffron [Table 1]. A greater concentration of these chemicals indicates a better quality of saffron. A quartz cell with a 1 cm pathway is used to measure $E_{1\%}$ at 440, 330, and 257 nm wavelengths. The results are obtained by measuring the absorption at three wavelengths using the equation; $E_{1\,\%}\ 1\ cm = (A \times 10000]/(M \times (100\text{-}H)]$, where $E_{1\%}$ is the specific extinction coefficient, *1 cm* is the path length, *A* is the absorbance, *M* is the mass in grams of the saffron sample, *H* is the moisture and volatile sample material. The moisture and volatile content of the saffron is determined after drying the samples and represented as a mass fraction using the formula: *[(initial mass-constant mass)/initial mass] × 100* (Hadizadeh et al., 2007; ISO - 3632-1:2011).

Despite international standards, various methods have been reported to detect adulteration and determine the quality of saffron viz UV-Vis spectroscopy (Zalacain et al., 2005; Zougagh et al., 2005) HPLC (Haghighi et al., 2007; Hajimahmoodi et al., 2013; Lozano et al., 1999), micellar liquid chromatography, FTIR (Karimi et al., 2016; Ordoudi et al., 2018), H-NMR (Petrakis et al., 2015), gel-electrophoresis (Paredi et al., 2016). Several factors, including geographical conditions, harvesting period, drying procedure employed, temperature and oxygen exposure during storage and adulteration, all have a significant impact on the quality of saffron (Caballero-Ortega et al., 2004). The primary objective of this research was to estimate the quality range and apocarotenoid content of commercial saffron in Kashmir using UV-Vis spectrophotometry and HPLC analysis.

2 MATERIAL AND METHODOLOGY

Saffron in India is cultivated and commercialized in Kashmir. The main local commercial zones of saffron in Kashmir are Srinagar, Pampore and Budgam. Besides local markets, the saffron in Kashmir is also commercialized by government-operated commercial emporiums (e.g., Government Kashmir Art Emporiums). Twenty-four samples of saffron were collected from Kashmir, among which six samples were collected from Government operated commercial emporiums (KAE), six sam-

Table 1: Grades of saffron based on ISO 3632-1:2011

Component	λ_{max}	Category I	Category II	Category III
Crocin	440 nm	> 200	170-200	120-170
Safranal	257 nm	20-50	20-50	20-50
Picrocrocin	330 nm	> 70	55-70	40-55
Moisture and volatile matter % (m/m),	-	10	10-12	10-12

ples were collected from the local market of Srinagar (SXR), six samples were collected from Pampore (PAM) district, and six samples were collected from Budgam (BUD). The samples collected were supposed to be yielded from the crop year 2019 and processed in 2020 as per their packing. Besides these samples, four samples were collected from Afghanistan (AFG), and two samples were collected from Iran (IRN). The samples collected from KAE, AFG and IRN had an origin certificate and were assured free from any adulteration. One sample was collected from Sigma Aldrich (SIG). A total of 31 samples were used for this study. Crocin and safranal standards were purchased from Sigma Aldrich. Picrocrocin was obtained from BioMall. HPLC-grade reagents (methanol, acetonitrile) were obtained from Loba Chemie.

2.1 DETERMINATION OF FLORAL WASTE CONTENT

About 1 g of each sample was taken, and each filament was spread on the paper. With the help of forceps, different floral waste components were separated, and the samples were weighed again. The floral waste was taken in shoe glass and weighed. The floral waste content of the sample (*wF)* was expressed as per ISO guidelines as a percentage by mass, using the relation:

$$wF = (m_2 - m_1)\ x\ 100/m_o\ \%$$

Where m_0 is the mass, in grams, of the test portion; m_1 is the mass, in grams, of the shoe glass; m_2 is the mass, in grams, of the shoe glass containing the floral waste.

2.2 DETERMINATION OF MOISTURE AND VOLATILE CONTENT

The samples collected needed to be examined for their authenticity. For such purposes, ISO 3632 has provided guidelines for conducting UV-Vis spectroscopy. To calculate E_1 %, first, the moisture content of all the samples was calculated. One gram of saffron from each sample was placed in a Petri dish and kept in the oven for 18 hours at 70 ^{0}C. After that, samples were weighed again to measure the moisture and volatile matter content (*wMV)* and is expressed as:

$$wMV = (m_0 - m_1)\ x\ 100/m_0\ \%$$

where m_0 is the mass, in grams, of the test portion; m_1 is the mass, in grams, of the dry residue.

2.3 UV-VIS SPECTROSCOPY

The UV-Vis spectroscopy for samples was performed according to ISO guidelines with slight modifications in order to get a greater yield of apocarotenoid compounds. Briefly, 100 mg mass of dried saffron samples was extracted with 5 ml cold 50 % (v/v) ethanol in mortar and pastel. The extract was then transferred to a screw-capped 50 ml tube, and a total amount of 20 ml 50 % (v/v) ethanol was added. Tubes were sonicated for 20 minutes on ice, centrifuged for 15 minutes at 4000 rpm, and washed twice with 5 ml of 50 % (v/v) ethanol. Spectrophotometric technique was employed to analyze the supernatant. For analysis, the supernatant (1 ml) was diluted to 5 ml with 50 percent (v/v) ethanol. The absorption of crocin, safranal, and picrocrocin at 440 nm, 330 nm, and 257 nm, respectively, was used to create a standard curve. The sample supernatants were diluted 100 times, and direct absorbance readings were obtained using a Shimadzu spectrophotometer (1 cm pathway quartz cell) at 440 nm, 330 nm, and 257 nm, respectively. A UV-Vis scan was also obtained to observe peaks in samples of different geographical locations. The results obtained were used to measure E_1 % of aqueous saffron extract using the following relation:

$$E_{1\%}\ 1\ cm = (A \times 10000/\ [(M \times (100\text{-}H)]$$

2.4 HPLC ANALYSIS

For HPLC analysis, 50 mg of powdered saffron samples were extracted with 10 ml of 50 % methanol-water (v/v) and magnetically stirred for 24 hours at 4 °C in the

dark. The samples were then centrifuged for 30 minutes at 5000 rpm. The supernatant was collected and filtered through 0.2 µm syringe filters. For quantitative analysis of crocin, picrocrocin and safranal, 1 ml of 2-nitroaniline was added as an internal standard to each sample before analysis (Caballero-Ortega et al., 2007). The analysis was carried out in a Shimadzu HPLC equipped with quaternary pumps; coupled to a photo-diode-array detector. Ethanol (50 %, v/v) and acetonitrile (15 %, v/v) were used as the mobile phase. Detection was carried out with an injection volume of 20 µl, a flow rate of 1 ml min^{-1} with 35-40 min of run time. Crocin was detected at 440 nm, picrocrocin at 250 nm and safranal at 330 nm. A calibration curve was constructed for internal standard using concentrations of 0.125, 0.25, 0.5, and 1.0 mg ml^{-1}. Quantitative analysis was carried out in accordance with the molecular absorption coefficient of each peak obtained at the wavelength of maximum absorbance of the components. The R2 values ranged from 0.9722to 0.9890, and results were expressed in milligrams per gram of saffron stigmas.

2.5 STATISTICAL ANALYSIS

One-way ANOVA was used to compare means and Duncan's Multiple Range Test (DMRT) was used to assess significance using IBM SPSS (version-20). Two tailored Pearson correlations between apocarotenoid levels with floral waste content and moisture levels were done using IBM SPSS (v. 20). The results were also analyzed using the multivariate analysis technique principal component analysis (PCA) using Origin-2021b (version-9.8b). PCA is a dimensionality-reduction technique often used to decrease the dimensionality of big data sets by converting a large collection of variables into a smaller one that still retains most of the information in the large set.

3 RESULTS AND DISCUSSION

The determination of floral waste in the samples was performed by physical separation of floral waste and then measuring its weight. The floral waste in the samples varied in range, with samples obtained from SXR and BUD showing a high range of floral waste. KAE samples and sigma samples showed the lowest range of floral waste, while IRN and AFG samples showed a medium to low range of floral waste. Floral waste in the Sigma sample was not detected (Table 2).

The moisture/volatile matter content was performed to analyze if the samples had been properly dried and processed. The average moisture level in KAE samples was found to be 6.26 %. Samples from SXR, BUD and PAM showed high levels of moisture and volatile content matter (12.45 %, 7.90 %, and 7.26 %, respectively). The average moisture and volatile content in the AFG and IRN samples was 6.35 % and 542 %, respectively, while in the SIG sample, it was found to be 4.49 % (Table 2).

Apocarotenoid content (E_1 %) was determined using UV-Vis spectrophotometry. The main objective of this measure analysis was to analyze the quality range of commercial saffron sold in Kashmir. The saffron samples were evaluated in accordance with the ISO 3632-2:2010 guidelines. One-way ANOVA and DMRT were used to compare means and assess the level of significance. The results showed significant variation in all the samples (Table 2). Results showed that average crocin content varied from 198.5 in KAE samples, 135.16 in SXR samples, 184.5 in PAM samples, 166 in BUD samples, 197.25 in AFG samples, 200.5 in IRN samples and 203 in sigma samples. Based on crocin content, it was found that among thirty-one samples, fourteen samples fell in category I, nine fell into category II, five fell in category III and three were counterfeit or adulterated samples as they showed E1 % less than 110. Similarly, picrocrocin expressed as direct reading of the absorbance at 257 nm showed an average concentration of 36.5 in KAE samples, 23.83 in SXR, 33 in PAM, 28.16 in BUD, 34 in AFG, 38.5 in IRN and 32 in SIG sample (Table 3). The safranal content in twenty-nine samples was found to be above 20, thus falling in the optimum range under ISO criteria. Three samples resulted in a safranal content range below 20, which is not optimal as per ISO guidelines. The floral waste and moisture/volatile content in saffron samples were negatively correlated with crocin content values (-0.87, -0.81, respectively). The results were analyzed using PCA analysis. PC1 (76.23 %) and PC2 (18.06 %) accounted for 94.29 % of the total variance of the data. The coefficient for both the principal components is given in Table 5. A biplot of samples was obtained to distinguish between adulterated and pure saffron (Figure 1).

HPLC analysis provides quick and simple measurement of the three major saffron components, with excellent linearity, selectivity, sensitivity, and accuracy. The crocetin, picrocrocin, and safranal were determined by HPLC at three wavelengths 440, 250, and 330 nm, respectively. The results were analyzed by one-way ANOVA to compare means, and Duncan's Multiple Range Test (DMRT) was used to assess significance. The concentration of these metabolites varied significantly (Table 2). The variations may be attributable to the geographical origin of samples, different drying procedures, storage conditions and adulteration (Biancolillo et al., 2020; Delgado et al., 2005; Maghsoodi et al., 2012). The average concentration of crocin varied from 40.64 mg g^{-1} in

Table 2: Floral waste percentage (wF %), moisture and volatile percentage (wMV %), UV-Vis analysis, HPLC analysis of saffron samples

			UV-Vis Analysis			HPLC analysis		
Sample	wF %	wMV %	Crocin (E1 %) 440 nm	Picrocrocin (E1 %) 257 nm	Safranal (E1 %) 330 nm	Crocin (mg g^{-1})	Picrocrocin (mg g^{-1})	Safranal (mg g^{-1})
KAE1	0.77	5.26	205^{a}	85^{a}	42^{a}	39.32^{a}	5.36^{a}	0.26^{bc}
KAE2	0.41	6.10	212^{a}	86^{a}	44^{a}	43.51^{a}	5.89^{a}	0.31^{a}
KAE3	0.66	5.15	178^{a}	62^{b}	28^{c}	45.36^{a}	6.21^{a}	0.26^{bc}
KAE4	2.73	7.09	203^{a}	74^{a}	35^{a}	39.95^{a}	6.01^{a}	0.29^{a}
KAE5	1.43	6.43	188^{a}	69^{b}	31^{ab}	42.29^{a}	4.03^{b}	0.3^{a}
KAE6	6.17	7.53	205^{a}	79^{a}	39^{a}	33.43^{ab}	4.91^{a}	0.27^{abc}
SXR1	11.51	9.34	134^{b}	56^{c}	24^{c}	38.49^{a}	4.02^{b}	0.28^{ab}
SXR2	4.93	6.73	179^{a}	59^{c}	27^{c}	26.36^{b}	3.16^{c}	0.17^{c}
SXR3	5.61	10.3	184^{a}	62^{b}	28^{c}	32.41^{ab}	3.98^{b}	0.2^{c}
SXR4	20.49	14.68	80^{b}	43^{c}	23^{c}	ND	ND	0.18^{c}
SXR5	9.49	11.35	162^{b}	57^{c}	25^{c}	34.24^{ab}	3.23^{c}	0.29^{a}
SXR6	25.3	22.35	72^{b}	40^{c}	16^{c}	18.26^{b}	2.79^{c}	0.23^{c}
PAM1	4.53	12.08	195^{a}	69^{b}	32^{ab}	33.32^{ab}	3.63^{b}	0.29^{a}
PAM2	5.68	7.84	152^{b}	65^{b}	29^{b}	38.43^{a}	3.23^{c}	0.26^{bc}
PAM3	7.85	7.63	201^{a}	74^{a}	34^{bc}	42.45^{a}	4.02^{b}	0.28^{ab}
PAM4	0.60	6.26	163^{b}	72^{ab}	33^{bc}	30.44^{ab}	3.62^{bc}	0.32^{a}
PAM5	1.93	8.23	189^{a}	67^{b}	30^{b}	29.4^{ab}	3.14^{c}	0.26^{bc}
PAM6	6.25	5.39	207^{a}	82^{a}	40^{a}	33.31^{ab}	4.11^{b}	0.27^{abc}
BUD1	2.48	6.19	202^{a}	79^{a}	38^{a}	32.43^{ab}	3.89^{b}	0.29^{a}
BUD2	2.80	7.26	185^{a}	60^{c}	29^{b}	26.38^{b}	3.77^{b}	0.27^{abc}
BUD3	3.91	8.15	149^{b}	56^{c}	25^{c}	27.39^{b}	3.56^{c}	0.28^{ab}
BUD4	16.60	10.28	105^{b}	53^{c}	24^{c}	20.2^{b}	3.04^{c}	0.21^{c}
BUD5	11.29	5.33	181^{a}	59^{c}	27^{c}	30.3^{ab}	3.69^{b}	0.23^{c}
BUD6	10.24	6.35	176^{a}	58^{c}	26^{c}	32.41^{ab}	3.51^{c}	0.25^{bc}
AFG1	1.56	5.68	201^{a}	78^{a}	37^{a}	41.34^{a}	5.1^{a}	0.27^{abc}
AFG2	1.34	5.34	208^{a}	81^{a}	39^{a}	30.49^{ab}	4.25^{b}	0.3^{a}
AFG3	1.97	6.63	192^{a}	68^{b}	31^{b}	31.42^{ab}	4.07^{b}	0.29^{a}
AFG4	3.81	7.76	188^{a}	64^{b}	29^{bc}	38.38^{a}	3.81^{b}	0.25^{bc}
IRN1	2.10	5.26	206^{a}	84^{a}	41^{a}	35.42^{ab}	5.33^{a}	0.26^{bc}
IRN2	1.92	5.59	195^{a}	75^{a}	36^{a}	35.12^{ab}	5.1^{a}	0.29^{a}
SIGMA	ND*	4.49	203^{a}	72^{ab}	32^{ab}	34.41^{ab}	4.46^{ab}	0.31^{a}

Means followed by the same letter within the columns are not significantly different ($p < 0.05$) using DMRT
*ND- Not Detected

KAE samples, 29.952 mg/g in SXR samples, 34.55mg/g in PAM samples, 28.18 mg/g in BUD samples, 35.40 mg g^{-1} in AFG samples, 35.27 mg g^{-1} in IRN samples and 34.41 mg g^{-1} in sigma sample. Safranal, one of the main components responsible for the fragrance of the spice, is soluble in polar solvents and poorly soluble in nonpolar solvents. The safranal content as per the ISO 3632 (2011) method cannot be categorized in any grade as the ISO

Table 3: Quality characteristics of saffron obtained from different geographical locations using the ISO-3632 method

Sample Origin	ISO Category	Crocin (E1 %) 440 nm	Safranal (E1 %) 257 nm	Picrocrocin (E1 %) 257 nm
KAE	I (4)	203-212	28-31	62-69
	II (2)	168-178	35-42	74-84
SXR	II (2)	179-184	27-28	59-62
	III (2)	134-162	24-25	56-57
	IV* (2)	72-80	16-23	40-43
PAM	I (3)	195-207	32-40	64-82
	II (1)	189	30	67
	III (2)	152-163	29-33	65-73
BUD	I (1)	202	38	79
	II (3)	176-185	26-29	58-60
	III (1)	149	25	56
	IV* (1)	105	24	53
AFG	I (3)	192-208	31-39	68-81
	II (1)	188	29	64
IRN	I (2)	36-41	75-24	75-24
SIG	I (1)	203	32	72

*Highly adulterated or counterfeit saffron samples

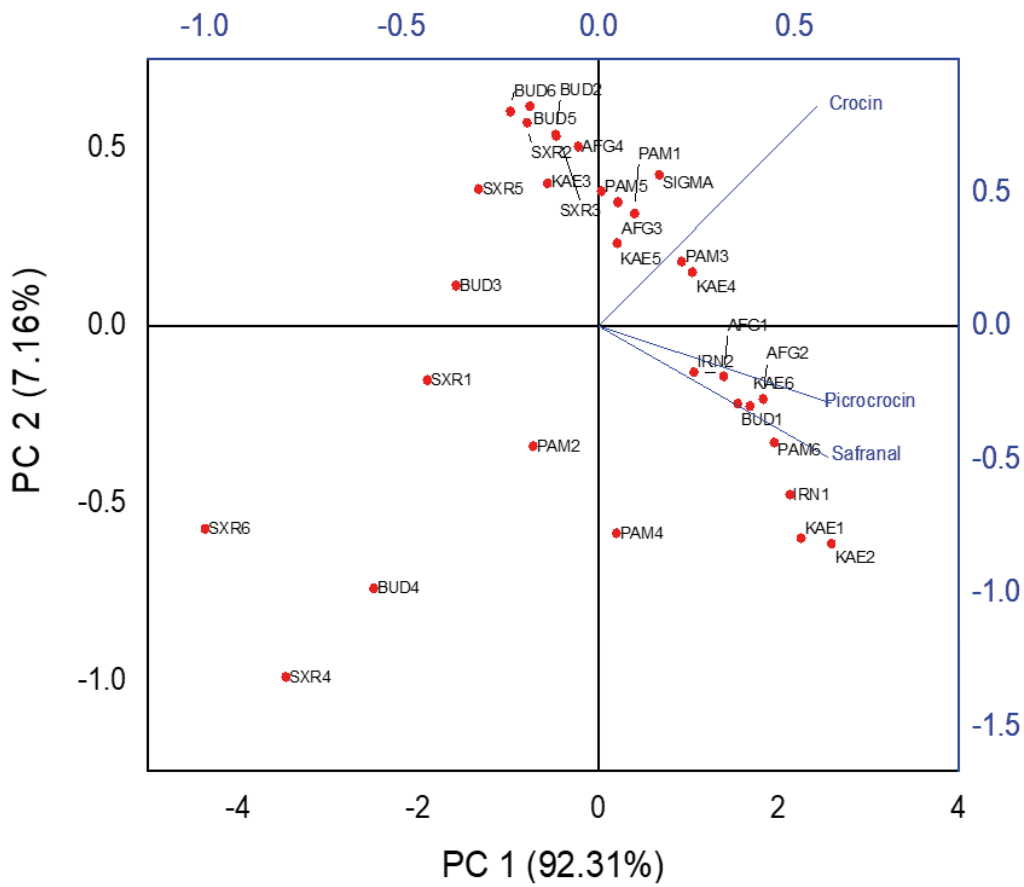

Figure 1: PCA analysis (biplot) of saffron samples (UV-Vis analysis)

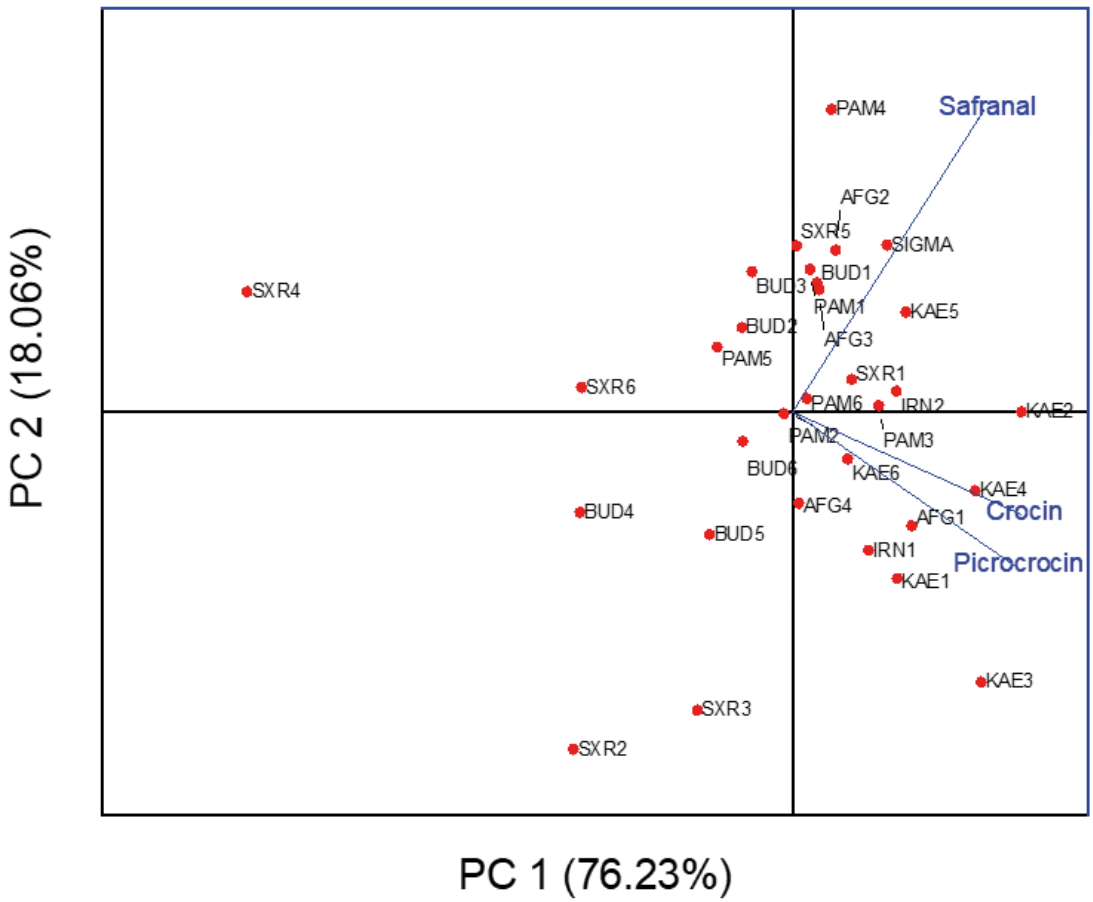

Figure 2: PCA analysis (biplot) of saffron samples (HPLC analysis)

method doesn't provide a precise classification of grades of saffron based on safranal content. The average safranal content differed significantly across KAE (0.28 mg g^{-1}), SXR (0.22 mg g^{-1}), PAM (0.28 mg g^{-1}), BUD (0.25 mg g^{-1}), AFG (0.27 mg g^{-1}), IRN (0.27 mg g^{-1}), and sigma samples (0.31 mg g^{-1}). Meanwhile, average picrocrocin content ranged from 5.40 mg g^{-1} in KAE, 3.43 mg g^{-1} in SXR, 3.62 mg g^{-1} in PAM, and 3.57 mg g^{-1} in BUD samples, 4.30 mg g^{-1} in AFG, 5.21 mg g^{-1} in IRN and 4.46 mg g^{-1} in SIG sample (Table 4). Crocin and picrocrocin were not detected in SXR4 sample. A biplot of samples was obtained to analyze the relation between metabolites in samples using PCA (Figure 2). PC1 (92.31 %) and PC2 (7.16 %) accounted for 99.47 % of the total variance of the data. The coefficient for both the principal components is given in Table 5.

The results obtained from UV-Vis spectroscopy showed that saffron samples from KAE were of the highest grade compared to other saffron obtained from other commercial sites. The saffron from PAM and BUD commercial sites showed a moderate range of quality. The samples from AFG and IRN fell in grade I and II as per ISO parameters. The saffron from SXR markets showed the lowest grade compared to other samples. The HPLC analysis showed a higher concentration of apocarotenoid content in KAE samples, followed by AFG and IRN samples. The SXR samples showed the lowest quality and apocarotenoid content, which indicates an indication of adulteration.

4 CONCLUSION

The evaluation of the quality of saffron selections was done by UV-Vis Spectroscopy according to the limit set by the ISO 3632, and the determination of apocarotenoid content was analyzed by HPLC analysis. The UV-Vis spectrophotometric results categorized the sample into different grades as per standards formulated by ISO. Only fourteen samples were identified as Grade I, and 13 samples were either grade II or grade III. The remaining 3 samples were found to be highly adulterated. The results obtained from HPLC analysis showed significant variation. The highest concentration of apocarotenoids

Table 4: HPLC-based concentration range of crocin, safranal and picrocrocin of saffron samples obtained from different geographical locations

Sample Origin	Crocin (mg g^{-1})	Safranal (mg g^{-1})	Picrocrocin (mg g^{-1})
KAE	33.43-45.36	0.26-0.31	4.03-6.21
SXR	18.26-38.49	0.17-0.29	2.79-4.02
PAM	29.40-42.45	0.26-0.32	3.14-4.11
BUD	20.20-32.43	0.21-0.29	3.04-3.89
AFG	30.49-41.34	0.25-0.30	3.81-5.10
IRN	35.12-35.42	0.26-0.29	5.10-5.33
SIG	34.41	0.31	4.46

Table 5: Loading of the first two principal components (PC's) for concentration of metabolites

Variable	Coefficients of PC1	Coefficients of PC2
UV-Vis analysis		
Crocin	0.5555	0.82244
Safranal	0.58313	-0.49034
Picrocrocin	0.59278	-0.28836
HPLC analysis		
Crocin	0.61407	-0.28918
Safranal	0.51423	0.85245
Picrocrocin	0.59875	-0.43554

was found in KAE samples, followed by AFG, IRN, PAM and BUD samples. Samples from SXR showed the least concentration of apocarotenoids, indicating a high level of adulteration. Adulteration in saffron is a big concern and needs to be addressed scientifically. It has an adverse effect on the saffron industry since counterfeit or adulterated saffron accounts for considerable market share. Although various instrumental methods (HPLC, GC-MS, FTIR, Raman Spectroscopy, etc.) for detecting adulteration in saffron, these methods are laboratory-based and cannot be used on a large sample size. Consequently, improvements to the existing ISO methods are suggested, and the development of a technique for on-the-spot detection of adulteration in saffron is recommended for future studies.

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