Research Article

Received: 29 January 2024 Revised: 21 March 2024 Published online in Wiley Online Library: 22 May 2024

(wileyonlinelibrary.com) DOI 10.1002/jsfa.13594

Comparative study on nutraceutical and sensorial characteristics of saffron (*Crocus sativus* L.) cultivated in Iran, Spain, and Türkiye

Armin Amanpour,[a*] Mostafa Soltani,[b,c*] Leontina Lipan,[d] Jose Miguel Garcia-Garví,[d] Francisca Hernández-García,[e] Ángel A. Carbonell-Barrachina[d] and Esther Sendra Nadal[d]

Abstract

BACKGROUND: Quality properties of 14 saffron samples from Iran, Spain, and Türkiye were compared.

RESULTS: Significant differences were observed between anthocyanins, volatile compounds, fatty acids, total phenolic content, and antioxidant activity of saffron samples ($P < 0.05$). Besides, significant differences in color parameters were observed. Moreover, a total of 13 volatile compounds were identified in the saffron samples using. headspace–solid-phase microextraction–gas chromatography–mass spectrometry, safranal and α-isophorone being the two predominant aroma compounds. Regarding fatty acids, significant differences were seen in the fatty acid profiles of saffron samples ($P < 0.05$), while linoleic acid was the most concentrated fatty acid. In terms of sensory properties, different concentrations of safranal, α-isophorone and 4-ketoisophorone may lead to significant differences in the odor and taste attributes of saffron samples ($P < 0.05$).

CONCLUSION: Changes in corm origin along with climate and agricultural conditions may affect the quality characteristics of saffron cultivated in different geographical areas to a significant degree.
© 2024 The Authors. Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Keywords: saffron; anthocyanins; volatile profile; antioxidant activity; color parameters; sensory properties

INTRODUCTION

Saffron (*Crocus sativus* L.), known as 'red gold', is a perennial herb with various functional properties such as antioxidant, anticarcinogenic, anti-inflammatory, antitumor, and antidepressant characteristics due to the function of bioactive compounds mostly presented in the stigma portion of the *C. sativus* plant.[1,2] Altogether, the chemical composition of saffron includes major and minor components. Among the minor components identified in saffron, crocetin (a natural carotenoid dicarboxylic acid as a precursor of crocin), crocin, picrocrocin (monoterpene glycoside precursor of safranal and a product of zeaxanthin degradation), and safranal are the four main bioactive compounds which contribute not only to the organoleptic features of saffron (pigment, pigment, taste, and odor, respectively), but also have health-progressing properties.[3-5] Indeed, in addition to containing crocetin, crocin, picrocrocin, safranal, anthocyanins, essential oils, and fatty acids as bioactive components, saffron also contain trace amounts of B1 and B2 vitamins, which extend its functional features and makes it attractive for the production of nutraceuticals.[6]

Saffron usage is, nowadays, well known both for flavoring as a color, taste, and/or odor agent of food and for therapeutic benefits even in the mediation of several health disorders.[3] Therefore,

* *Correspondence to: A Amanpour, Department of Gastronomy and Culinary Arts, Faculty of Fine Arts Design and Architecture, Istanbul Medipol University, Istanbul, Türkiye. E-mail: dr.aamanpour@gmail.com; or M Soltani, Department of Food Sciences and Technology, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. E-mail: soltanimstf@gmail.com*

a *Department of Gastronomy and Culinary Arts, Faculty of Fine Arts Design and Architecture, Istanbul Medipol University, Istanbul, Türkiye*

b *Department of Food Sciences and Technology, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*

c *Nutrition and Food Sciences Research Center, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*

d *Research Group 'Food Quality and Safety', Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Department of Agro-Food Technology, Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández de Elche, Alicante, Spain*

e *Grupo de Investigación en Fruticultura y Técnicas de Producción, Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernández University, Alicante, Spain*

© 2024 The Authors. Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

it is widely used in the food industry and especially by the semi-industrial food-producing sector as a food-grade flavoring agent during the manufacture of a wide range of foods, including beverages, bakery and dairy products.[7,8]

Iran is the largest producer worldwide, with almost 80% of total world production (i.e., 220 000 kg); however, the cultivation of saffron is increasing in several countries around the world – for example, in Spain, China, Italy, Türkiye, India, and Switzerland.[2,9] It is well established that the quality properties and yield of saffron, as the final product offered to consumers, are dependent on important parameters such as the geographical origin of the corm, altitude of the cultivation place, agronomic acts, employment of biostimulants, climate (temperature and rainfall rate), conditions of drying (temperature and time), and storage.[8,10,11] Because the climate and cultivation conditions are different in producing countries, there is a range of differences among the quality characteristics of saffron produced in various countries.

Therefore, the current research was conducted to compare and characterize saffron samples collected from Iran, Türkiye, and Spain using anthocyanin profile, antioxidant capacity, total phenolic content (TPC), water activity, color parameters, volatile compounds, fatty acid profile, and sensory properties. Therefore, LC-DAD-ESI-MS/MS (liquid chromatography coupled to diode array detection and electrospray ionization tandem mass spectrometry) and HS-SPME-GC-MS (headspace solid-phase micro-extraction coupled with gas chromatography–mass spectrometry) were used to characterize the anthocyanin and volatile compound profile of samples, respectively. To the best of our knowledge, although there are some studies in the literature conducted on the volatile compounds and antioxidant activity of saffron cultivated in different countries,[4,12-14] comparison and characterization of the product cultivated in different countries based on the various analysis implemented in the present study have not been reported previously.

MATERIALS AND METHODS

Collection of saffron samples

Saffron stigma (Fig. 1(a)) is composed of (i) a red part containing three filaments and can be classified according to their length into three classes – Sargol, Negin, Pushal – and (ii) a yellow part containing one filament known as the Style.[15] Fourteen samples of saffron stigmas – eight belonging to the Pushal class (red parts) and six belonging to the Style class (yellow parts) – were analyzed in this study and are presented in Fig. 1(b). All studied samples were coded and their collected locations are shown in Fig. 1(c), as follows: (i) samples one to six, including both red and yellow parts of saffron stigma – five samples marked with R1/Y1, R2/Y2, R3/Y3, R4/Y4, and R5/Y5 – were collected from different regions of Iran, which were Fariman, Torbat-e-Jam, Khaf, Torbat-e-Heydariyeh, and Roshtkhar, respectively, located in the Khorasan Razavi Province, and one sample labeled with R6/Y6 was collected from the Safranbolu region of Türkiye; (ii) the two remaining samples (including only the red part of saffron), were provided by the Spanish company Jesús Navarro, SA – one, labeled R7, was imported from Iran, and the other, labeled R8, was collected from the Castilla La Mancha region of Spain. This last sample (R8) belonged to the Protected Designation of Origin '*DOP Azafrán de La Mancha*'. Therefore, eight red parts and six yellow parts of saffron samples were provided in 2022 from Iran, Türkiye, and Spain.

Water activity

Water activity was analyzed using an AW meter (Novasina aw-Sprint TH500; Pfaffikon, Zurich, Switzerland). For measurements, 0.5 g samples were used.

Color analysis

Color parameters, expressed in CIE color space, were analyzed by instrumental colorimetry, using a Minolta colorimeter (CFRC400, Minolta Camera Co., Tokyo, Japan) with CIE L^*, a^*, b^* coordinates. An online color converter was used to visually present the saffron color, with L^*, a^*, b^* color coordinates converted via Pantone Nix color sensor (Hamilton, ON, Canada).

Determination of anthocyanins

Extraction and identification of the saffron anthocyanins were carried out following the method described by Hong *et al.*, with some modifications.[16] Briefly, 0.5 g saffron was mixed with 4 mL cold extractant (methanol–water–formic acid; 80:19.9:0.1, v/v) and shaken for 10 min in an orbital bath. Then, the mixture was sonicated using an ultrasonic bath for 10 min and centrifuged (Sigma 2-16K, SciQuip Ltd, Wem, UK) at 1500 × *g* for 10 min. The supernatant was removed, and the pellet residue was re-extracted twice using the same procedure. Two milliliters of the supernatant were filtered through a 0.45 µm nylon Millipore membrane filter and used for analysis.

The analysis was carried in a high-performance liquid chromatograph–triple-quadrupole mass spectrometer (LC-MS/MS 8050, Shimadzu, Kyoto, Japan). Atmospheric pressure ionization or electron spray ionization (ESI) was used for molecular ionization, and a C18 column (Mediterranean SEA 18, 10 mm × 0.21 mm i.d., 2.2 µm particle size) from Teknokroma (Barcelona, Spain) for chromatographic separations. The ESI source was operated with a nebulizer gas flow of 3 L min^{-1}, drying gas flow of 10 L min^{-1}, a desolvation line temperature of 250 °C and heat block temperature of 400 °C. Selected ion monitoring was used with a collision energy of −35 V and full MS scans in positive mode between 100 and 1000 *m/z*. For the analysis a Mediterranea Sea18 column (10 mm L × 0.21 mm i.d., 2.2 µm particle size; Teknokroma, Barcelona, Spain) was used, maintained at a temperature at 50 °C. The mobile phase A was composed of 0.1% (v/v) formic acid (FA) in water (MilliQ), while B consisted of 0.1% (v/v) FA in acetonitrile with a flow rate of 0.400 mL min^{-1}. The injection volume of the sample was 10 µL and the oven temperature was 50 °C. The gradient conditions were as follows: (i) 0–2 min 5% B; (ii) 2–10 min 95% B; (iii) 10–11 min 95% B; (iv) 11–12 min 5% B; and (v) 12–16 min 5% B. For the quantification, four anthocyanins were used as external standards: petunidin-3-*O*-glucoside; delphin-3,5-di-*O*-glucoside; delphinidin-3-*O*-glucoside, and cyanidin-3-*O*-glucoside. The calibration curve consisted of the preparation of the standard stock of 100 ppm, from which five concentrations (0.1, 0.3, 0.5, 0.8 and 1 ppm) were prepared. The analyses were carried out in triplicate.

TPC and antioxidant activity

TPC was analyzed by the Folin–Ciocâlteu method.[17] The antioxidant activity was determined by the $ABTS^+$ and $DPPH^•$ methods.[18,19]

Determination of fatty acids

Fatty acid profiles were obtained by direct transmethylation.[20] A 0.1 g sample (red and yellow parts) was mixed with 100 µL dichloromethane and 1 mL sodium methoxide 0.5 mol L^{-1} and

 © 2024 The Authors.
Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

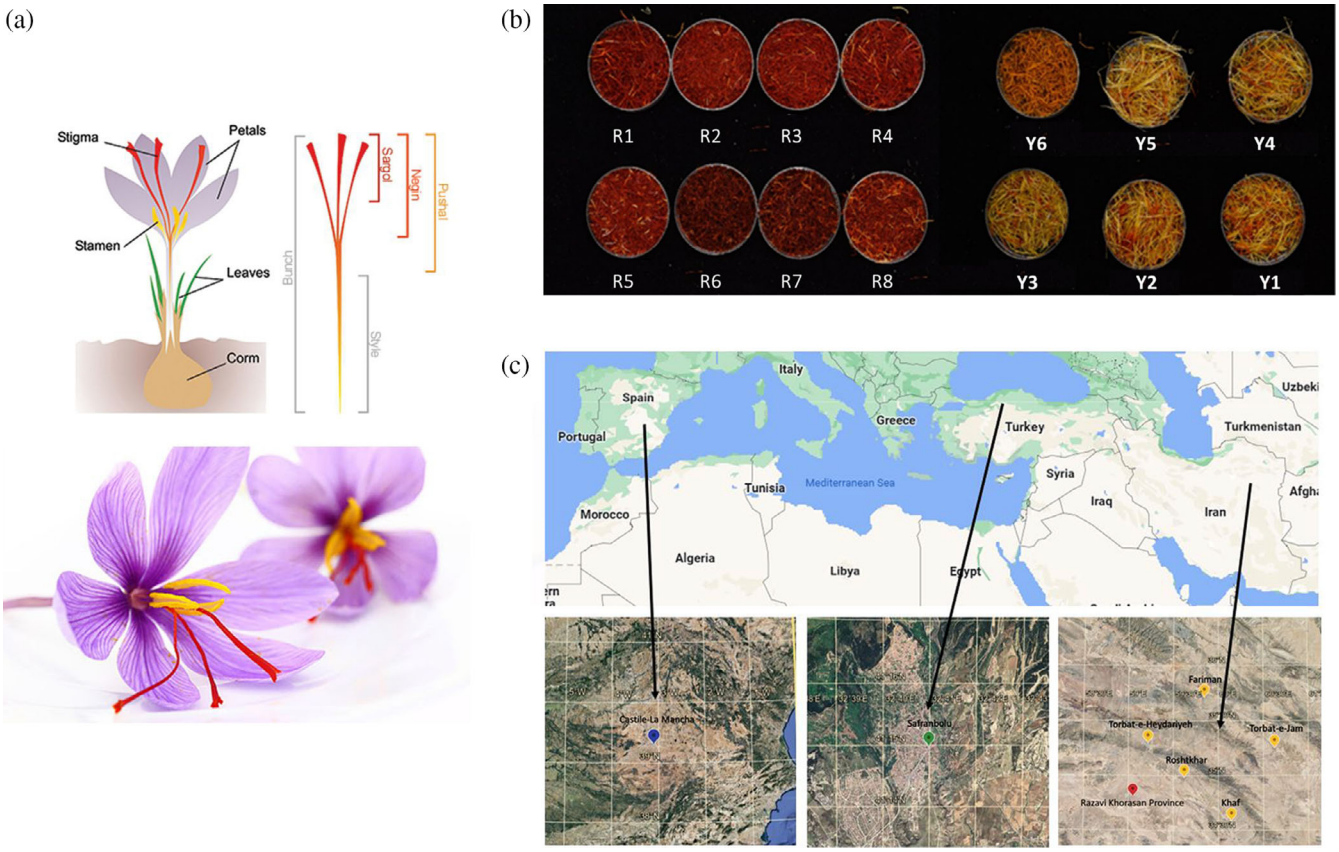

Figure 1. (a) Anatomic scheme of saffron flow. (b) Saffron samples used in the analysis. (c) Sampling sites, adapted from Google Earth.

held for 10 min at 90 °C. Samples were colder in ice by 3 min and 1 mL of BF_3 added, keeping the mixture in darkness for 30 min. Then, 1 mL ultrapure water and 0.6 mL hexane were added; samples were mixed for 1 min and centrifugated for 10 min at 1500 × *g*. The supernatant was recovered and placed in an amber chromatography vial.

For separation, a gas chromatograph (Shimadzu GC-2030) coupled with a flame ionization detector with an automatic injector (AOC-20i) was used. Helium was used as carrier gas, and nitrogen as a make-up gas (40 mL min^{-1}). Flame ionization detection used hydrogen and air at rates of 35 and 350 mL min^{-1}, respectively. The GC system used a Supelco SP-2380 capillary column (length 60 m, internal diameter 0.25 mm, and film thickness 0.20 µm; Supelco, Bellefonte, PA, USA). The injector and detector temperatures were 250 and 260 °C, respectively, and a 1:20 split ratio was used; a helium lineal flow velocity of 28.4 cm s^{-1} was used. The oven temperature started at 70 °C and increased up to 250 °C at a rate of 3 °C min^{-1}. Methyl fatty acids were identified as compared with retention times with Supelco FAME 31MIX (Merck KGaA, Darmstadt, Germany). Results were calculated as a percentage of each fatty acid in the total fatty acid profile.

Volatile compound profile

Volatile compounds were extracted by headspace solid-phase microextraction (HS-SPME). For the extraction, 0.1 g dried sample (only the red part given that the number of samples of the yellow part was extremely small) was placed in a 15 mL glass vial with 10 µL benzyl acetate as internal standard (1000 mg L^{-1}) using 50/30 µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fiber (Supelco). Vials were maintained in a temperature-controlled oven at 40 °C for 60 min (10 min for equilibration and 50 min for extraction) After that, the fiber was inserted into the injector at 250 °C over 3 min, to directly desorb volatile compounds into the gas chromatograph column. A gas chromatograph (GC2030, (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA)), coupled with a triple-quadrupole mass spectrometer detector (Shimadzu TQ8040 NX). To separate volatile compounds, a Sapiens X5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Teknokroma) was used. Injector and detector temperatures were 250 and 230 °C, respectively. The oven program was as follows: initial temperature 35 °C for 5 min; rate of 5 °C min^{-1} up to 150 °C; rate of 10 °C min^{-1} until 280 °C and held for 5 min.

To characterize the volatile compounds, the chromatograms were analyzed using GCMS Postrun Analysis (Shimadzu Corporation, Kyoto, Japan) software, and individual compounds were identified by three methods: (i) mass spectrum (original chemical compound and collection of the Wiley 229 and NIST 14 spectrum libraries); (ii) retention index of standards; and (iii) retention indexes calculated using a C7 to C16 *n*-alkane mix (Sigma-Aldrich, Steinheim, Germany). The experimental retention index was compared with the literature index obtained from the National Institute of Standards and Technology.

Descriptive sensory analysis

Sensory analysis was done using a trained panel of the Food Quality and Safety (CSA) research group of Miguel Hernández University. This trained panel consisted of eight panelists (four males and four females, between 25 and 50 years old) with more than 1000

 © 2024 The Authors.
Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

training hours in food sensory analysis, specifically in vegetables and fruits. Before analysis, the panel worked together to establish a list of principals and most important sensory attributes based on previous literature[21] and panel experience. The developed list consisted of six odor attributes: odor intensity, saffron ID, fruity, floral, grassy/vegetal, and dried tomato; and nine flavor descriptors: taste intensity, saffron ID, fruity, floral, grassy, sweetness, bitterness, astringency, and pungency. Only the red parts of samples were analyzed. Samples were presented in two ways: (i) a dilution of 1 g L^{-1} was placed in a transparent glass tube to analyze the colorant and intensity of colored water; and (ii) the sensory analysis was done directly on the fresh sample. In this case, 0.2 g of sample was served in a transparent tub with lid. All samples were codified with three-digit codes and were presented according to a randomized block designed to avoid biases. The scale used by the panelists to quantify the intensity of saffron sensory attributes ranged from 0 to 10 with increments of 0.5 (where 0 represents no intensity and 10 extremely high intensity).

Statistical analysis

One way analysis of variance (ANOVA), Tukey's multiple range test, and principal component analysis (PCA regression map) were carried out using XLSTAT Premium 2016 (Addinsoft Inc., New York, NY, USA).

RESULTS AND DISCUSSION

Water activity

Water is known as the main factor in the prevention and/or controlling of food spoilage caused by both microbial and enzymatic agents. Thus, awareness of the rate of water availability in the food structure can help in considering appropriate strategies for extending the preservation of food for longer times. Thus, a significant relationship has been reported between increasing the water activity value and a decrease in the quality characteristics of saffron, especially regarding reduction in the desirable color of the product.[1,22] As shown in Table 1, significant differences were determined in the water activity values of the red parts of saffron samples ($P < 0.05$). In this sense, R7 was the sample with the highest value (0.548), and R1 and R2 presented the lowest values (0.429 and 0.443, respectively) of water activity. It has been established that some harmful reactions – that is, lipid oxidation, enzymatic activity, and Maillard reaction – could be retarded in the range between 0.2 and 0.4 of water activity.[1] Therefore, all experimental saffron samples (except sample R7) were desirable in terms of water activity.

Color parameters

Both crocetin (a natural carotenoid dicarboxylic acid as a precursor of crocin) and crocin together with high water solubility are the main compounds responsible for color production in saffron.[1,14] Color parameters obtained by colorimetry are shown in Table 1. This method showed significant differences in all parameters ($P < 0.05$). With respect to the Pushal class (red parts) of samples, the highest value for brightness ($L = 30.1$) was reached by sample R5 and the darkest sample was R6 ($L = 22.2$). This sample, obtained from Türkiye, also had the lowest a^* (23.8) and b^* values (15.6), meaning that, besides being the darkest sample, it was also less red and yellow. Sample R8 had the highest b^* value (23.7). Chroma (C^*) was lower in samples R6 and R7 (28.4 and 30.9, respectively) and hue angle (h^*) was higher in sample R8 (38.8) than R1 (30.2). On the other hand, in the yellow parts of saffron samples, L^* values were determined between 67.5 (Y5) and 50.2 (Y6). Y6 obtained the highest a^*, b^*, and C^* values (16.4, 55.2, and 57.6, respectively). The values of a^* and b^* in all saffron

Table 1. Water activity and instrumental color and of Pushal (red) and Style (yellow) parts of saffron samples

	a_w	L^*	a^*	b^*	Chroma	Hue	a/b
ANOVA test[a]							
Red saffron	***	***	***	***	***	**	**
Yellow saffron	NS	**	***	***	***	**	**
Tukey multiple range test[b]							
Red saffron							
R1	0.429c	26.9c	34.4a	20.1c	39.9a	30.2c	1.7a
R2	0.443c	28.2bc	35.0a	21.0bc	40.8a	31.0bc	1.7ab
R3	0.447bc	28.1bc	34.7a	21.0bc	40.5a	31.2bc	1.7ab
R4	0.447bc	28.8ab	31.3ab	22.7ab	38.6a	36.0ab	1.4bc
R5	0.460bc	30.1a	32.5ab	21.3abc	38.9a	33.3abc	1.5abc
R6	0.454bc	22.2e	23.8d	15.6d	28.4b	33.3abc	1.5abc
R7	0.548a	23.9d	25.6cd	17.3d	30.9b	34.0abc	1.5abc
R8	0.487b	28.7ab	29.6bc	23.7a	37.9a	38.8a	1.2c
Yellow saffron							
Y1	0.499	58.4b	10.0b	42.1b	43.3b	76.7bc	0.2ab
Y2	0.495	59.5b	10.5b	38.9bc	40.3bc	74.9c	0.3a
Y3	0.486	59.5b	5.2c	35.5c	35.9cd	81.7ab	0.1bc
Y4	0.437	57.8b	9.7b	36.4c	37.7cd	75.1c	0.3a
Y5	0.474	67.5a	3.9c	33.7c	33.9d	83.5a	0.1c
Y6	0.443	50.2c	16.4a	55.2a	57.6a	73.5c	0.3a

[a] NS, not significant at $P < 0.05$; asterisks indicate significance at $^{**}P < 0.01$ and $^{***}P < 0.005$.
[b] Values followed by the same letter, within the same column, are not significantly different ($P > 0.05$), according to Tukey's least significant difference test.

 © 2024 The Authors.
Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

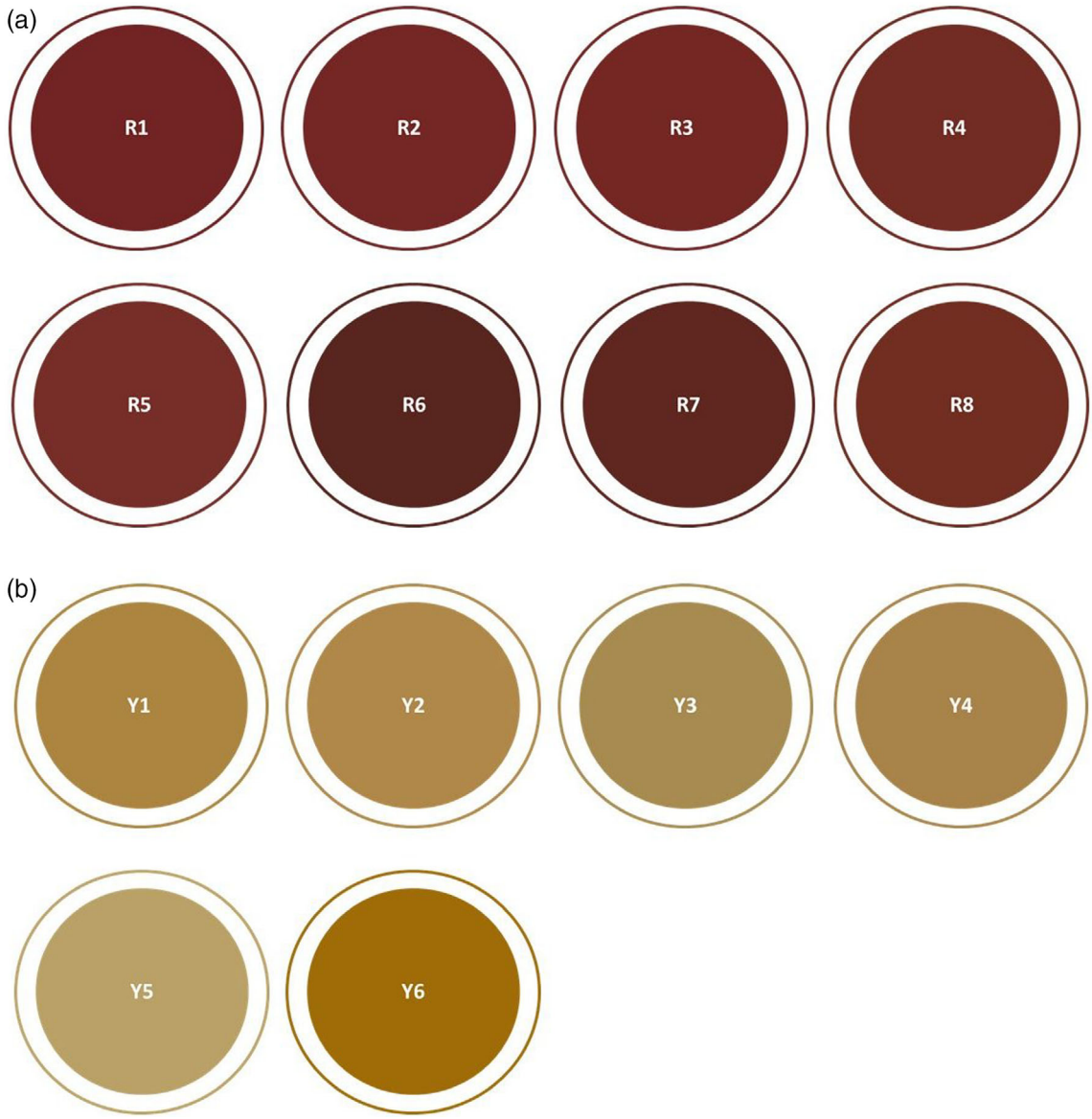

Figure 2. Pantone of each sample combining L^*, a^*, b^* color coordinates values in a color converter: (a) Pushal (red part) and (b) Style (yellow part) saffron classes.

samples were positive and represented a range of colors from red to yellow that are specific to saffron. Determination of red to yellow colors in the experimental samples also indicated the presence of carotenoids, including crocin and crocetin, which might be responsible for the red, orange, and yellow colors in saffron.[14,23] Furthermore, the degradation rate of carotenoids (in particular, crocin) and, in consequence, the intensity of saffron specific color, is dependent on the growing and processing parameters – that is, temperature, water activity, pH, light, and oxygen.[22] For ease of understanding, L^*, a^*, and b^* color coordinates were converted to Pantone, as presented in Fig. 2. Here, the difference in color, mainly between R5 (the brightest sample) and R6 (the darkest sample) for the Pushal and Style class of saffron, can be visibly appreciated.

Anthocyanins

Anthocyanins are natural pigments belonging to the flavonoid group and possess antioxidant characteristics. Saffron petals are considered to be an important source of natural anthocyanin and can be used in the formulation of functional foods because of their antioxidant capacity.[24] pH, temperature, oxygen, and light are destructive factors that can have negative effects on these natural coloring agents.[2,14,25] Anthocyanin compounds and their amounts are presented in Table 2. Significant differences were observed in all anthocyanin components of saffron samples ($P < 0.05$). Petunidin-3-*O*-glucoside content ranged between 0.39 and 1.11 mg kg^{-1}, the sample cultivated in Türkiye (R6) having the lowest content, and the sample cultivated in the Khaf area of Iran (R3) the highest content. Similar results were observed for delphin-3,5-di-*O*-glucoside, which ranged from 0 for sample R6 from Türkiye (Safranbolu city) to 5.02 mg kg^{-1} for sample R4 from Iran (Torbat-e-Heydariyeh city). In addition, the R3 and R5 samples from Iran, belonging to the areas Khaf and Roshtkhar, also registered high concentrations of this compound (4.59 and 4.09 mg kg^{-1}). Regarding delphinidin-3-*O*-glucoside, this only appeared in samples R3 (0.80 mg kg^{-1}), R4

 © 2024 The Authors.
Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Table 2. Anthocyanin profile of Pushal class of saffron (red part) and total phenolic content and antioxidant activity of both Pushal (red part) and Style (yellow part) saffron classes

Anthocyanin	Petunidin-3-*O*-glucoside (mg kg^{-1})	Delphin-3,5-di-*O*-glucoside (mg kg^{-1})	Delphinidin-3-*O*-glucoside (mg kg^{-1})	Cyanidin-3-*O*-glucoside (mg kg^{-1})
ANOVA[a]	*	***	***	***
Tukey multiple range test[b]				
R1	0.66abc	1.69 cd	0.00d	78.2b
R2	0.82abc	2.69bc	0.00d	77.8b
R3	1.11a	4.59ab	0.80a	89.6ab
R4	0.98ab	5.02a	0.64b	90.0ab
R5	0.59abc	4.09ab	0.61b	85.7ab
R6	0.39c	0.00d	0.00d	89.2ab
R7	0.45bc	0.31d	0.00d	76.8b
R8	0.67abc	0.48cd	0.13c	105a

	TPC(mg GAE 100 mg^{-1})	ABTS(mmol Trolox g^{-1})	DPPH(mmol Trolox g^{-1})
ANOVA test[a]			
Red part	***	***	NS
Yellow part	***	***	**
Tukey multiple range test[b]			
Red part			
R1	4796ab	58.6b	295
R2	5735a	88.3a	320
R3	4623ab	58.9b	316
R4	5045ab	57.7b	321
R5	5529a	67.2b	310
R6	3256c	28.6c	282
R7	4200ab	34.1c	303
R8	4289ab	35.0c	289
Yellow part			
Y1	1787b	10.8c	243ab
Y2	1256b	14.9bc	251ab
Y3	1130b	22.9b	248ab
Y4	1144b	15.6bc	268a
Y5	1181b	9.9c	247ab
Y6	4627a	46.8a	216b

[a] NS, not significant at $P < 0.05$. Asterisks indicate significance at *$P < 0.05$, **$P < 0.01$, and ***$P < 0.005$.
[b] Values followed by the same letter, within the same row, were not significantly different ($P > 0.05$), according to Tukey's least significant difference test.

(0.64 mg kg^{-1}), and R5 (0.61 mg kg^{-1}) from Iran, and R8 (0.13 mg kg^{-1}) from Spain. Finally, cyanidin-3-*O*-glucoside ranged from 77.8 to 105 mg kg^{-1} and was the compound found in the greatest amount, representing 96% of the total anthocyanins. Spanish saffron from PDO '*DOP Azafrán de La Mancha*', showed the highest content of cyanidin-3-*O*-glucoside, followed by samples R3 (89.6 mg kg^{-1}), R4 (90.0 mg kg^{-1}), and R5 (85.7 mg kg^{-1}) from Iran, and R6 from Türkiye (89.2 mg kg^{-1}), while R1 (78.2 mg kg^{-1}), R2 (77.8 mg kg^{-1}), and R7 (76.8 mg kg^{-1}) from the other areas of Iran showed the lowest amounts of this compound. The differences in the anthocyanin value of saffron samples may be due to differences in climatic conditions such as rainfall rate and temperature, agricultural practices, drying, and storage processes.[8,26]

TPC and antioxidant activity

Saffron has a remarkable free radical scavenging activity and antioxidant activity owing to the presence of bioactive compounds, including polyphenols.[8,14] The TPC and antioxidant activity of the experimental saffron samples are presented in Table 2. Significant differences were observed in TPC of both red and yellow parts of saffron samples ($P < 0.05$). In addition, red parts of saffron samples had higher TPC compared to the yellow parts. In red parts of saffron samples, higher concentrations were obtained in R2 and R5, with 5735 and 5529 mg gallic acid equivalents (GAE) 100 mg^{-1}, respectively. It is important to highlight that sample R6, cultivated in Türkiye, had the lowest amount of TPC. This aspect might be related to the anthocyanins, as explained in the paragraph above. On the other hand, in yellow parts of saffron samples, the highest concentration of TPC was achieved in sample Y6, with 4627 mg GAE 100 mg^{-1}, and all the other samples were similar among themselves. Phenolic compounds are secondary metabolites with high antioxidant capacity that can eliminate or prevent the creation or activity of oxidizing agents such as singlet oxygen free radicals.[27] Cultivation, climate, and soil conditions along with genetic background are parameters that

 © 2024 The Authors.
Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

influenced the content of phenolic compounds in saffron and caused differences in TPC of experimental saffron samples.[28]

Regarding antioxidant activity determined using both ABTS and DPPH methods, the red part of studied saffron samples had higher values than the yellow part, except in Y6. According to the results obtained by the ABTS method, while in the red part of the saffron samples the highest antioxidant activity was observed in the R2 sample with 88.3 mmol Trolox g^{-1}, in the yellow part of the saffron samples antioxidant activity reached the highest value, with 46.8 mmol Trolox g^{-1} in sample Y6. On the other hand, based on the DPPH method result, the red part did not show statistical differences, and in the yellow part, Y4 with 268 mmol Trolox g^{-1} showed the highest value of antioxidant activity among the experimental samples. It was reported that the existence of antioxidant capacity in saffron is due to the presence of bioactive compounds like polyphenols, flavonoids, and carotenoids. In this context, anthocyanins, which are a group of flavonoids, have a remarkable role in the antioxidant properties of saffron. Moreover, carotene, a main group of carotenoids, consists of two major types of α-carotene and β-carotene and influences positively the antioxidant capacity of saffron.[14,23,29]

Fatty acid profile

Fatty acids are distributed in various parts of saffron, such as stigmas, stamens, petals, and stems.[9] The fatty acid profile of both red and yellow parts of saffron samples is presented in Table 3. A total of 34 fatty acids were identified in all saffron samples. The most predominant fatty acids in all samples were linoleic acid (C18:2), linolenic acid (C18:3), and palmitic acid (C16:0). This result is consistent with the results reported by Chichiriccò *et al*. (2019)[30] and Zara *et al*. (2021),[31] who reported high quantities of the abovementioned fatty acids in saffron. In the red part of saffron, 12 fatty acids presented significant differences. Linoleic acid was higher in R8, with 41.4% of total fatty acids, and lower in R3, with 38.1%. Linolenic acid was higher in R1 saffron sample (15.8%) and lower in R8 (12.12%). Pentadecanoic acid also appeared in higher concentration in R1, R3, and R7, with 11.330%, 13.3%, and 13.1%, respectively. In the yellow part, significant differences were found in 12 fatty acids. Linoleic acid, the most concentrated fatty acid, appeared in concentrations between 33.8% (Y4) and 47.6% (Y2). The second most concentrated fatty acid was palmitic acid, with the highest concentration in Y6 (19.8%) and lowest concentration in Y4 (13.9%). The third most important fatty acid was linolenic acid, with concentrations between 8.75% and 15.1% (Y4 and Y6, respectively). The differences in quantity of fatty acids in saffron samples may be due to the initial content of fat as a consequence of differences in origin of the corm, agronomic practices, temperature, and rainfall rate.[10,11]

Volatile compound profile

Volatile profiles of the red part in saffron samples are presented in Table 4. A total of 13 compounds were characterized and quantified in the red part of saffron and the results are similar to those reported in saffron by previous studies.[4,32,33] However, there were differences between the present study and previous research regarding quantities of volatile compounds identified in the saffron samples.[4,12-14] Safranal, with saffron note, was the main volatile compound with the highest concentration among the identified compounds in all red saffron samples, followed by α-isophorone and 4-ketoisophorone, related to saffron and herbal notes. In this sense, the highest and lowest concentrations of safranal were observed in R7 (107 mg kg^{-1}) and R8 (32.9 mg kg^{-1}) samples, respectively. Based on the previous studies, 30–70% of the different saffron essential oils are mostly composed of safranal.[34,35] Regarding the aroma chemistry of saffron spice, safranal as the key aroma compound is formed from picrocrocin and 4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde (HTCC) during the drying process of saffron.[36] On the other hand, the highest concentration of α-isophorone was observed in sample R7 (16.3 mg kg^{-1}), while samples R3 and R8 had the lowest concentrations (4.54 mg kg^{-1} and 6.08 mg kg^{-1}, respectively). 4-Ketoisophorone concentrations ranged between 1.96 and 9.99 mg kg^{-1} in this study. Derivatives of isophorone are the other key aroma compounds responsible for the characteristic notes of whole saffron aroma.[4,37] According to the biosynthesis mechanism of β-isophorone, it can occur simultaneously with the degradation of zeaxanthin and the creation of both (1*R*)-3,5,5-trimethyl-3-cyclohexen-1-ol *O*-*b*-D-glucopyranoside and of (4*R*)- and (4*S*)-4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one *O*-*b*-D-glucopyranoside. The latter two glycosides are possible precursors of isophorone derivatives such as α- and β-isophorones.[38]

Sensory analysis

A range of bioactive compounds existing in the matrix of saffron effectively influence the sensory characteristics of the final product besides their potential health properties. In this context, while compounds like crocin, crocetin, lycopene, and carotene have an important role in the color of saffron, the odor and taste of the product are affected by safranal, isophorone, and picrocrosin.[2,39] Descriptive sensory profiles of the red parts of saffron samples relating to their appearance and flavor attributes are shown in Table 5. Odor attributes of samples are indicated by six odor descriptors: odor intensity, saffron ID, fruity, floral, grassy/vegetal, and dried tomato. Flavor attributes consisted of nine descriptors: taste intensity, saffron ID, fruity, floral, grassy, sweetness, bitterness, astringency, and pungency. As seen, only five descriptors presented significant differences among the saffron samples. Sample R8 was the most intense saffron in the nose and R5 was the least intense. Samples R6 and R7 recalled dried tomato (5.2 and 5.9 scores, respectively). Regarding flavor attributes, the sample with highest taste intensity was R6 (6.1) and the lowest intensity was R4 (2.9).

Another interesting point was the detection of relatively high bitterness in samples R6 and R7. Picrocrocin, with a chemical structure of 4-(β-D-glucopyranosyl)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, is known as the main compound responsible for the creation of bitter taste in saffron. This chemical compound is created via the degradation of zeaxanthin and described as the precursor of safranal. It was reported that oxidative hydrolysis of zeaxanthin leads to the creation of picrocrocin. Safranal with high aromatic characteristics is also formed by changes in picrocrocin under drying temperatures and the action of glycosidases.[3]

Color attribute of saffron samples was analyzed as saffron capacity to color water, and was evaluated from orange (the lowest value) to red (the highest value). In this sense, the sample with the highest capacity was R2 (9.0), and the lowest value was achieved by R4 sample (4.1). Comparing these results with the instrumental color, we observed that the samples with the highest value of a^* color coordinate (red notes) were R1, R2, and R3 which corresponded to the sample with the highest saffron capacity to color the sample, while the samples with the lowest values of a^* color coordinate were R6, R7, and R8, which

 © 2024 The Authors.
Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Table 3. Fatty acid profile of Pushal (red) and Style (yellow) parts of saffron samples, expressed as percent of total fatty acids

Fatty acid	ANOVA[a] Red saffron	Red saffron[b] R1	R2	R3	R4	R5	R6	R7	R8	ANOVA[a] Yellow saffron	Yellow saffron[b] Y1	Y2	Y3	Y4	Y5	Y6
C8:0	NS	0.049	0.032	0.045	0.034	0.034	0.045	0.072	0.026	NS	0.026	0.018	0.063	0.031	0.024	0.091
C10:0	NS	0.318	0.479	0.548	0.418	0.531	0.345	0.598	0.356	*	0.039b	0.094a	0.039b	0.045b	0.035b	0.058ab
C11:0	***	0.193ab	0.173b	0.276a	0.256ab	0.275a	0.061c	0.066c	0.198ab	NS	0.484	0.479	0.642	8.312	0.452	0.239
C12:0	NS	0.745	0.392	0.407	0.431	0.405	0.574	0.448	0.427	***	0.252b	0.212b	0.231b	0.409b	0.205b	3.075a
C13:0	*	0.693a	0.242a	0.350a	0.266a	0.253a	0.936a	0.591a	0.322a	*	0.079b	0.109b	0.090b	0.216ab	0.081b	0.275a
C14:0	NS	0.423	0.295	0.270	0.313	0.314	0.423	0.367	0.243	***	0.268b	0.132b	0.187b	0.181b	0.137b	1.163a
C14:1	NS	0.252	0.235	0.338	0.234	0.241	0.422	0.640	0.266	NS	0.033	0.057	0.050	0.041	0.052	0.038
C15:0	*	11.3a	9.75ab	13.3a	8.37b	9.49ab	7.58b	13.1a	9.06ab	NS	0.575	1.08	0.672	0.847	0.680	0.141
C15:1	*	0.037b	0.034b	0.044ab	0.050ab	0.060ab	0.038b	0.059ab	0.096a	NS	0.190	0.186	0.256	0.304	0.196	0.089
C16:0	NS	12.1	12.5	12.1	12.7	12.3	13.7	12.5	12.2	**	14.6b	14.6b	16.7ab	13.8b	14.6b	19.8a
C16:1	**	0.436ab	0.354b	0.400b	0.469ab	0.416ab	0.349b	0.408b	0.585a	**	0.937ab	1.212a	0.876b	0.812bc	1.058ab	0.532c
C17:0	NS	0.338	0.175	0.167	0.205	0.160	0.166	0.177	0.167	NS	0.168	0.161	0.188	0.144	0.128	0.151
C17:1	*	3.15a	0.909b	0.737b	0.838b	0.808b	0.431b	0.369b	0.486b	**	0.669a	0.668a	0.557ab	0.478ab	0.798a	0.258b
C18:0	NS	0.739	1.06	1.21	1.51	0.870	1.43	0.584	0.861	NS	2.35	1.87	2.44	2.56	2.43	2.15
C18:1t9	**	3.66ab	0.033b	0.033b	6.90a	7.04a	0.029b	5.83ab	4.89ab	NS	2.70	2.50	3.59	4.06	4.72	2.86
C18:1c9	***	1.20c	8.11a	8.45a	2.38bc	2.36bc	8.78a	2.75bc	4.36b	NS	6.35	6.07	4.82	5.67	3.77	3.11
C18:2n6t	NS	0.301	0.432	0.395	0.558	0.485	0.452	0.554	0.587	NS	1.93	1.40	1.18	2.42	1.76	0.504
C18:2n6c	*	39.9ab	41.3ab	38.2b	40.4ab	39.7ab	40.5ab	39.1ab	41.4a	*	43.0a	47.6a	46.5a	33.8b	46.6a	35.5b
C18:3n6 gamma	NS	0.228	0.171	0.190	0.196	0.197	0.248	0.208	0.308	***	0.416a	0.396a	0.379a	0.328a	0.440a	0.157b
C20:0	NS	0.258	0.267	0.303	0.332	0.333	0.319	0.327	0.345	NS	1.66	1.44	0.985	2.00	1.56	0.576
C18:3n3 alpha	*	15.8a	14.9ab	14.0ab	14.2ab	14.0ab	12.9ab	12.9ab	12.1b	**	11.4a	10.3a	12.6a	8.75b	9.91b	15.1a
C20:1n9	NS	0.152	0.068	0.057	0.068	0.092	0.159	0.101	0.171	***	0.215b	0.219ab	0.170c	0.209bc	0.259a	0.074d
C21:0	NS	0.577	0.210	0.224	0.216	0.305	0.202	0.229	0.449	NS	1.18	0.562	0.573	1.35	1.05	0.318
C20:2	NS	0.851	1.22	1.44	1.59	1.59	1.56	1.29	1.79	NS	3.10	2.34	1.51	3.99	2.85	3.85
C20:3n3	**	0.188abc	0.147bc	0.138c	0.190abc	0.165bc	0.185abc	0.250ab	0.281a	***	0.491b	0.564b	0.433b	0.504b	0.607b	4.62a
C22:0	NS	0.651	1.17	1.04	1.29	1.37	1.36	1.44	1.34	NS	0.937	1.01	0.463	1.081	0.932	0.486
C20:3n6	NS	0.246	0.379	0.341	0.311	0.433	0.391	0.328	0.391	NS	0.471	0.483	0.399	0.422	0.491	0.476
C22:1n9	NS	0.204	0.115	0.173	0.200	0.199	0.155	0.219	0.464	NS	0.342	0.129	0.115	0.819	0.144	0.149
C23:0	NS	0.611	0.307	0.180	0.568	0.472	0.504	0.471	0.735	NS	1.215	0.650	0.347	0.898	0.661	0.481
C22:2	**	1.25cd	1.79a	1.75ab	1.26cd	1.39bcd	1.51abc	1.09d	1.30cd	*	1.36ab	1.06ab	0.982b	2.40a	1.34ab	0.898b
C20:5n3	NS	0.486	0.455	0.377	0.389	0.494	0.981	0.494	0.626	NS	0.504	0.565	0.680	0.944	0.382	1.61
C24:0	NS	0.898	1.32	1.44	1.26	1.45	1.45	1.35	1.25	NS	1.42	1.37	0.83	1.57	1.36	0.819
C24:1	NS	0.928	0.540	0.593	0.656	0.792	0.978	0.690	0.744	NS	0.328	0.344	0.182	0.260	0.231	0.386
C22:6	NS	0.683	0.252	0.445	0.234	0.556	0.376	0.312	0.891	NS	0.216	0.173	0.195	0.297	0.106	0.048

[a] NS, not significant at $P < 0.05$. Asterisks indicate significance at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$.
[b] Values followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

© 2024 The Authors.

Table 4. Volatile profiles of Pushal (red) class of saffron samples (mg kg^{-1})

No.	Volatile compound profile	ANOVA[a]	R1	R2	R3	R4	R5	R6	R7	R8
			Tukey multiple range test[b]							
1	2(5*H*)-Furanone	**	0.892ab	0.995ab	1.07ab	1.28a	1.66a	0.232b	1.24a	1.04ab
2	*β*-Isophorone	**	0.256abc	0.498a	0.279abc	0.410ab	0.400ab	0.045c	0.049c	0.190bc
3	Linalool	NS	0.042a	0.024a	0.022a	0.057a	0.029a	0.043a	0.029a	0.044a
4	Nonanal	NS	0.080ab	0.084ab	0.094a	0.075ab	0.075ab	0.073ab	0.033b	0.083ab
5	Alloocimene	***	0.732bc	0.876b	1.31a	0.924ab	0.935ab	0.628bc	1.30a	0.372c
6	Phenylethyl alcohol	***	0.471bc	0.167e	0.461bc	0.422cd	0.192e	0.538b	1.42a	0.334d
7	*α*-Isophorone	***	10.1bc	8.69bc	4.54c	12.9ab	12.2ab	13.4ab	16.4a	6.08c
8	4-Ketoisophorone	***	4.99c	4.74cd	1.96d	5.61c	6.25bc	9.99a	8.66ab	4.62cd
9	2-Hydroxyisophorone	***	0.281c	0.350bc	0.138c	0.320c	0.396bc	0.667b	1.09a	0.315c
10	2,3-Dehydro-1,8-cineole	**	0.453bc	0.560bc	0.275c	0.621bc	0.680abc	1.09a	0.862ab	0.49bc
11	Safranal	***	51.1cd	49.0cd	58.6bc	58.9bc	56.1 bc	71.3b	106.7a	32.9d
12	Eucarvone	***	0.996b	0.766bc	0.334c	0.893b	0.900bb	2.34a	1.97a	0.785bc
13	Diosphenol	***	0.735de	1.32cd	0.415e	0.938cde	1.5c	3.56a	2.70b	3.477a
	Total	***	71.1cd	68.1cd	69.5cd	83bc	81.3bc	103.9b	142.5a	50.7d

[a] NS, not significant at $P < 0.05$. Asterisks indicate significance at *$P < 0.05$, **$P < 0.01$, and ***$P < 0.005$.
[b] Values followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

Table 5. Descriptive sensory profiles of Pushal (red) class of saffron samples

Attribute	ANOVA[a]	R1	R2	R3	R4	R5	R6	R7	R8
		Tukey multiple range test[b]							
Odor attributes									
Odor intensity	**	5.1bc	6.7ab	6.1abc	5.2abc	5.0c	6.5abc	6.4abc	6.8a
Saffron ID	NS	4.0	5.6	4.9	4.1	4.4	3.1	3.9	4.3
Fruity	NS	0.4	0.6	0.5	0.6	0.4	0.6	0.7	0.7
Floral	NS	1.1	1.6	1.6	1.2	0.9	0.8	1.1	0.9
Grassy/vegetal	NS	1.4	1.7	2.1	2.3	1.8	2.4	2.4	1.9
Dried tomato	**	3.0b	4.2ab	4.8ab	4.1ab	3.1b	5.2a	5.9a	3.9b
Flavor attributes									
Taste intensity	***	5.0ab	5.9a	4.0ab	2.9c	3.1bc	6.1a	5.4a	4.2ab
Saffron ID	NS	4.6	5.4	3.9	2.4	2.7	4.5	3.9	3.3
Fruity	NS	0.1	0.2	0.2	0.2	0.4	0.2	0.2	0.2
Floral	NS	0.7	1.1	0.7	0.5	0.6	1.1	0.8	0.8
Grassy	NS	0.2	0.4	0.3	0.1	0.2	0.2	0.3	0.2
Sweetness	NS	0.8	0.9	1.1	0.6	0.6	0.8	0.6	0.6
Bitterness	NS	0.1	0.1	0.1	0.1	0.1	0.8	0.4	0.3
Astringency	**	0.1b	1.3a	0.3ab	0.1b	0.1b	1.1ab	0.9ab	0.6ab
Pungency	NS	0.0	0.6	0.1	0.0	0.1	0.6	0.5	0.4
Color attribute	***	7.1b	9.0a	6.6b	4.1f	6.0c	4.9e	5.0de	5.4d

[a] NS, not significant at $P < 0.05$. Asterisks indicate significance at *$P < 0.05$, **$P < 0.01$, and ***$P < 0.005$.
[b] Values followed by the same letter, within the same row, were not significantly different ($P < 0.05$).

corresponded to the samples with the lowest capacity. This tendency was applied to almost all samples, but not for R4, which was between the samples with the highest a^* values; however in the sensory analysis, this was the sample with the lowest capacity of water coloring. In this context, it was reported that reduction in the drying rate and time could decrease some reactions like decomposition of carotenoids and have a positive effect on color intensity in saffron.[40]

PCA after varimax rotation and agglomerative hierarchical clustering (AHC)

Classification of Pushal (red) and Style (yellow) parts of saffron samples using PCA and AHC is presented in Fig. 3(a,b), respectively. Placing of the red and yellow parts of the saffron samples in different clusters and on different sides of the corresponding PCA and AHC figures confirmed the differences among them in terms of properties analyzed. As shown in Fig. 3(a) and based on

© 2024 The Authors.

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

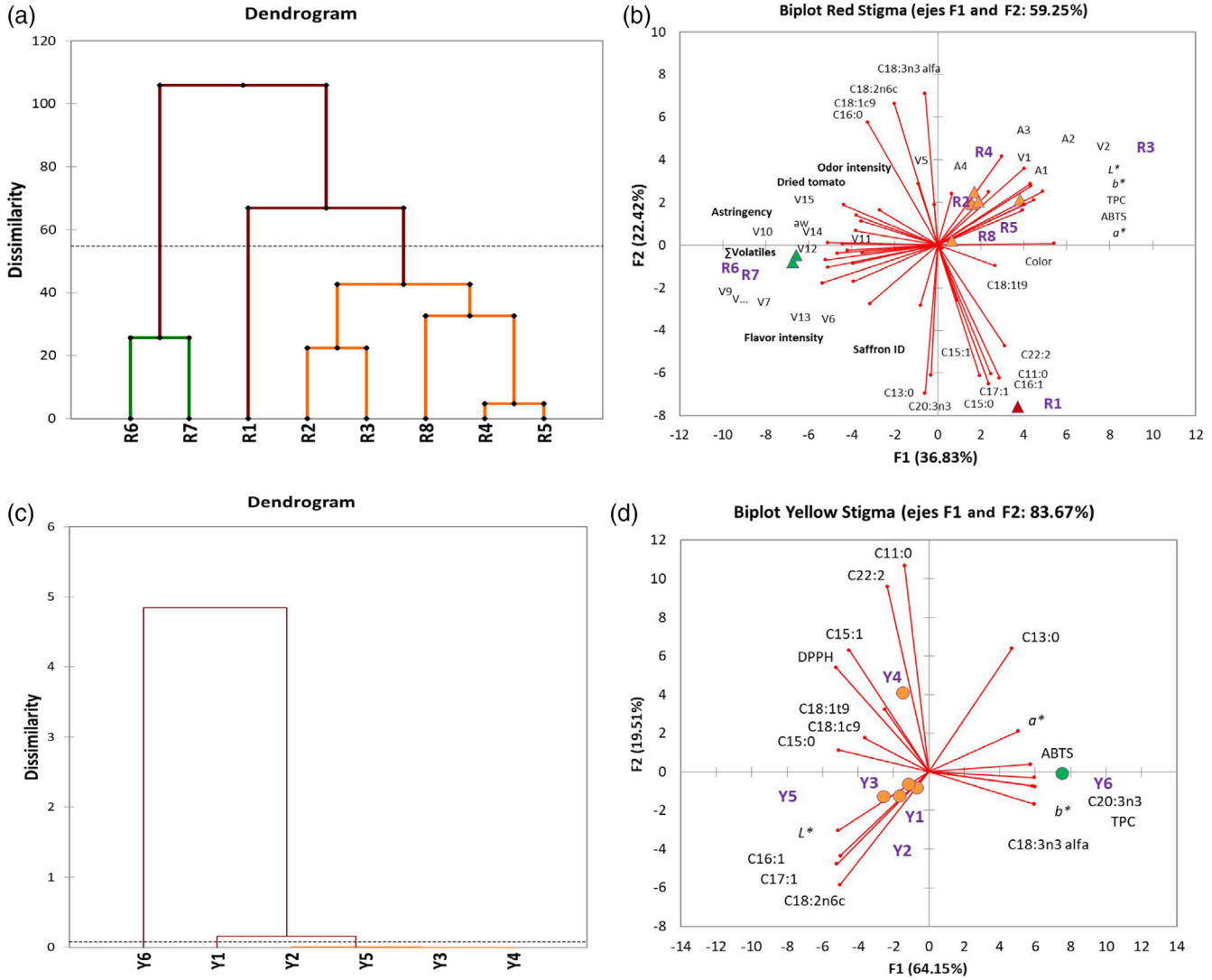

Figure 3. Principal component analysis and agglomerative hierarchical clustering (a) for the Pushal part of the saffron stigma (red part) and (b) for the Style part of the saffron stigma (yellow part).

PCA, red parts of saffron samples have been clustered as a result of the differences in their physicochemical properties, including mainly water activity, color parameters, TPC, ABTS, and odor and taste intensity values. In this context, while R2, R3, R4, R5, and R8 were placed in the same cluster, R6 and R7 were placed in a different cluster. However, R1 was not grouped in a cluster and is seen individually. A similar trend can be observed in the AHC analysis of the red part of saffron samples (Fig. 3(b)). R2, R3, R4, R5, and R8 were placed on the positive side and R6 and R7 on the negative side of AHC, due to the difference among the samples in color parameters, TPC, antioxidant activity, fatty acid profiles, and sensory characteristics.

Regarding the yellow part of saffron samples and according to the PCA dendrogram (Fig. 3(c)), Y1, Y2, Y3, and Y5 samples were placed in the same cluster, probably because of their similar quality properties in terms of water activity, color parameters, TPC, ABTS, and DPPH values compared to other samples. In this context, while Y6 was placed in a different cluster, Y4 is seen individually. On the other hand, according to the AHC of the yellow part of the saffron samples (Fig. 3(d)), while Y1, Y2, Y3, and Y5 were placed on the negative side, Y6 was seen on the positive side because of the differences in color parameters, fatty acid profiles, TPC, and antioxidant activity.

CONCLUSIONS

The quality properties of saffron samples collected from Iran, Spain, and Türkiye were identified. Changes in the anthocyanins, volatile compounds, fatty acids, TPC, and antioxidant activity were observed among saffron samples. While the highest antioxidant activity was determined in the samples collected from Iran, the sample cultivated in Türkiye had the lowest value. On the other hand, more red and yellow color values were observed in the samples cultivated in Iran and Spain, respectively. Safranal and α-isophorone were determined as the most dominant volatile compounds in the red part of saffron samples. The intensity of odor and taste were significantly different among the red part of saffron samples. Linoleic, linolenic, and palmitic acids were the most dominant fatty acids in all saffron samples. Significant differences in water activity, color parameters, TPC, ABTS, DPPH,

J Sci Food Agric 2024; **104**: 7580–7591 © 2024 The Authors. wileyonlinelibrary.com/jsfa
Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

and odor and taste intensity values led to classification of saffron samples in different clusters of PCA and different sides of AHC. In conclusion, changes in the quality characteristics of the experimental saffron samples were expected due to the corm origin, climate, and agricultural conditions. Therefore, according to the current survey, samples cultivated in Iran were characterized by their highest anthocyanins (such as petunidin-3-*O*-glucoside, delphin-3,5-di-*O*-glucoside, and delphinidin-3-*O*-glucoside), TPC amount and antioxidant activity, *L**, *a**, and chroma color parameters, total volatile compounds, odor and flavor attributes, as well as some fatty acids in the red part of saffron. In the case of saffron samples cultivated in Spain, these were characterized by their highest anthocyanin (cyanidin-3-*O*-glucoside), *b**, chroma and hue color parameters, and some fatty acids in the red part of saffron. Finally, those cultivated in Türkiye were characterized by their highest TPC amount and antioxidant activity, *a**, *b**, and chroma color parameters, and some fatty acids in the yellow part of saffron.

AUTHOR CONTRIBUTIONS

Armin Amanpour: supervision, conceptualization, visualization, investigation, methodology, writing, original draft preparation, design of the study, data curation, and validation and approval of results. Mostafa Soltani: visualization, investigation, methodology, writing, original draft preparation, reviewing, and editing. Leontina Lipan: formal analysis, investigation, methodology, data curation and validation, approval of results, reviewing and editing. Jose Miguel Garcia-Garví: formal analysis, methodology, data curation and validation and approval of results. Francisca Hernández-García: formal analysis, methodology, data curation and validation and approval of results. Ángel A Carbonell-Barrachina: supervision, investigation, methodology, software, formal analysis, funding acquisition, reviewing, and editing. Esther Sendra Nadal: supervision, investigation, methodology, software, formal analysis, funding acquisition, reviewing, and editing.

ACKNOWLEDGEMENTS

Chromatographic equipments (LC-MS/MS; GC-MS, GC-FID) were acquired thanks to Grant EQC2018–004170-P funded by MCIN/AEI/10.13039/501,100,011,033 and by ERDF A way of making Europe. The study was financed by the Generalitat Valenciana, Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital project AICO/2021/326. The author Leontina Lipan was founded by the "Ministerio de Universidades" and Europe Union-Next Generation EU in the framework "Ayudas para la Recualificación del Sistema Universitario Español", in the modality "MARGARITA SALAS".

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES

1 Jafari SM, Bahrami I, Dehnad D and Shahidi SA, The influence of nanocellulose coating on saffron quality during storage. *Carbohydr Polym* **181**:536–542 (2018).
2 Garavand F, Rahaee S, Vahedikia N and Jafari SM, Different techniques for extraction and micro/nanoencapsulation of saffron bioactive ingredients. *Trends Food Sci Technol* **89**:26–44 (2019).
3 Amanpour A, Kelebek H and Selli S, GLC/HPLC methods for saffron (*Crocus sativus* L.), in *Bioactive Molecules in Food. Reference Series in Phytochemistry*, ed. by Mérillon JM and Ramawat KG. Springer, Cham, pp. 1987–2035 (2019).
4 Amanpour A, Sonmezdag AS, Kelebek H and Selli S, GC–MS–olfactometric characterization of the most aroma-active components in a representative aromatic extract from Iranian saffron (*Crocus sativus* L.). *Food Chem* **182**:251–256 (2015).
5 Razavi BM and Hosseinzadeh H, Saffron: a promising natural medicine in the treatment of metabolic syndrome. *J Sci Food Agric* **97**:1679–1685 (2017).
6 Shahi T, Assadpour E and Jafari SM, Main chemical compounds and pharmacological activities of stigmas and tepals of 'red gold'; saffron. *Trends Food Sci Technol* **58**:69–78 (2016).
7 Rahaiee S, Moini S, Hashemi M and Shojaosadati SA, Evaluation of antioxidant activities of bioactive compounds and various extracts obtained from saffron (*Crocus sativus* L.): a review. *J Food Sci Technol* **52**:1881–1888 (2015).
8 Cardone L, Castronuovo D, Perniola M, Cicco N and Candido V, Saffron (*Crocus sativus* L.), the king of spices: an overview. *Sci Hortic (Amsterdam)* **272**:109560 (2020).
9 Abu-Izneid T, Rauf A, Khalil AA, Olatunde A, Khalid A, Alhumaydhi FA *et al.*, Nutritional and health beneficial properties of saffron (*Crocus sativus* L): a comprehensive review. *Crit Rev Food Sci Nutr* **62**:2683–2706 (2022).
10 Cardone L, Castronuovo D, Perniola M, Cicco N and Candido V, Evaluation of corm origin and climatic conditions on saffron (*Crocus sativus* L.) yield and quality. *J Sci Food Agric* **99**:5858–5869 (2019).
11 Caser M, Demasi S, Victorino ÍMM, Donno D, Faccio A, Lumini E *et al.*, Arbuscular mycorrhizal fungi modulate the crop performance and metabolic profile of saffron in soilless cultivation. *Agronomy* **9**:232 (2019).
12 Karabagias IK, Koutsoumpou M, Liakou V, Kontakos S and Kontominas MG, Characterization and geographical discrimination of saffron from Greece, Spain, Iran, and Morocco based on volatile and bioactivity markers, using chemometrics. *Eur Food Res Technol* **243**:1577–1591 (2017).
13 Kosar M, Demirci B, Goger F, Kara I and Baser KHC, Volatile composition, antioxidant activity, and antioxidant components in saffron cultivated in Turkey. *Int J food Prop* **20**:S746–S754 (2017).
14 Cerdá-Bernad D, Clemente-Villalba J, Valero-Cases E, Pastor J-J and Frutos M-J, Novel insight into the volatile profile and antioxidant properties of *Crocus sativus* L. flowers. *Antioxidants* **11**:1650 (2022).
15 Mohamadzadeh Moghadam M, Taghizadeh M, Sadrnia H and Pourreza HR, Nondestructive classification of saffron using color and textural analysis. *Food Sci Nutr* **8**:1923–1932 (2020).
16 Hong HT, Netzel ME and O'Hare TJ, Optimisation of extraction procedure and development of LC–DAD–MS methodology for anthocyanin analysis in anthocyanin-pigmented corn kernels. *Food Chem* **319**:126515 (2020).
17 Gao X, Ohlander M, Jeppsson N, Björk L and Trajkovski V, Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *J Agric Food Chem* **48**:1485–1490 (2000).
18 Brand-Williams W, Cuvelier M-E and Berset C, Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol* **28**:25–30 (1995).
19 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* **26**:1231–1237 (1999).
20 Park PW and Goins RE, In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *J Food Sci* **59**:1262–1266 (1994).
21 Predieri S, Magli M, Gatti E, Camilli F, Vignolini P and Romani A, Chemical composition and sensory evaluation of saffron. *Foods* **10**:2604 (2021).
22 Hosseini A, Razavi BM and Hosseinzadeh H, Pharmacokinetic properties of saffron and its active components. *Eur J Drug Metab Pharmacokinet* **43**:383–390 (2018).
23 Rubio Moraga A, Ahrazem O, Rambla JL, Granell A and Gómez Gómez L, Crocins with high levels of sugar conjugation contribute

 © 2024 The Authors.
Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

to the yellow colours of early-spring flowering crocus tepals. *PLoS One* **8**:e71946 (2013).
24 Khazaei KM, Jafari SM, Ghorbani M, Kakhki AH and Sarfarazi M, Optimization of anthocyanin extraction from saffron petals with response surface methodology. *Food Anal Methods* **9**:1993–2001 (2016).
25 Castañeda-Ovando A, de Lourdes Pacheco-Hernández M, Páez-Hernández ME, Rodríguez JA and Galán-Vidal CA, Chemical studies of anthocyanins: a review. *Food Chem* **113**:859–871 (2009).
26 Njuguna SM, Makokha VA, Yan X, Gituru RW, Wang Q and Wang J, Health risk assessment by consumption of vegetables irrigated with reclaimed waste water: a case study in Thika (Kenya). *J Environ Manage* **231**:576–581 (2019).
27 Wong C-C, Li H-B, Cheng K-W and Chen F, A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chem* **97**:705–711 (2006).
28 Tajik S, Zarinkamar F, Soltani BM and Nazari M, Induction of phenolic and flavonoid compounds in leaves of saffron (*Crocus sativus* L.) by salicylic acid. *Sci Hortic (Amsterdam)* **257**:108751 (2019).
29 Serrano-Díaz J, Sánchez AM, Maggi L, Martínez-Tomé M, García-Diz L, Murcia MA *et al*., Increasing the applications of *Crocus sativus* flowers as natural antioxidants. *J Food Sci* **77**:C1162–C1168 (2012).
30 Chichiriccò G, Ferrante C, Menghini L, Recinella L, Leone S, Chiavaroli A *et al*., *Crocus sativus* by-products as sources of bioactive extracts: pharmacological and toxicological focus on anthers. *Food Chem Toxicol* **126**:7–14 (2019).
31 Zara S, Petretto GL, Mannu A, Zara G, Budroni M, Mannazzu I *et al*., Antimicrobial activity and chemical characterization of a non-polar extract of saffron stamens in food matrix. *Foods* **10**:703 (2021).
32 Cadwallader KR, Baek HH and Cai M, Characterization of saffron flavor by aroma extract dilution analysis, in *Spices Flavor Chemistry and Antioxidant Properties*, ed. by Risch SJ and Ho C-T. ACS Publications, Washington, DC, pp. 66–79 (1997).
33 Culleré L, San-Juan F and Cacho J, Characterisation of aroma active compounds of Spanish saffron by gas chromatography–olfactometry: quantitative evaluation of the most relevant aromatic compounds. *Food Chem* **127**:1866–1871 (2011).
34 Carmona M, Zalacain A, Salinas MR and Alonso GL, A new approach to saffron aroma. *Crit Rev Food Sci Nutr* **47**:145–159 (2007).
35 Maggi L, Carmona M, Del CCP, Kanakis CD, Anastasaki E, Tarantilis PA *et al*., Worldwide market screening of saffron volatile composition. *J Sci Food Agric* **89**:1950–1954 (2009).
36 Lozano P, Delgado D, Gomez D, Rubio M and Iborra JL, A non-destructive method to determine the safranal content of saffron (*Crocus sativus* L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography. *J Biochem Biophys Methods* **43**:367–378 (2000).
37 Iborra JL, Castellar MR, Cánovas MA and Manjón AR, TLC preparative purification of picrocrocin, HTCC and crocin from saffron. *J Food Sci* **57**:714–716 (1992).
38 Winterhalter P and Straubinger M, Saffron—renewed interest in an ancient spice. *Food Rev Int* **16**:39–59 (2000).
39 Finley JW and Gao S, A perspective on *Crocus sativus* L. (saffron) constituent crocin: a potent water-soluble antioxidant and potential therapy for Alzheimer's disease. *J Agric Food Chem* **65**:1005–1020 (2017).
40 Tabibian SA, Labbafi M, Askari GH, Rezaeinezhad AR and Ghomi H, Effect of gliding arc discharge plasma pretreatment on drying kinetic, energy consumption and physico-chemical properties of saffron (*Crocus sativus* L.). *J Food Eng* **270**: 109766 (2020).

 © 2024 The Authors.
Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.