Chapter 16

Bioactive ingredients of saffron: extraction, analysis, applications

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Chapter Outline

16.1 Introduction

There are many factors influencing saffron production yield and quality before the flowering stage including the number of irrigations (3−5 times), amount of rainfall, the minimum and maximum temperature in summer and winter, respectively, applying fertilizer, etc. (Mollafilabi, 2003; Negbi, 2003). Saffron quality is also strongly dependent on postharvest parameters as well as crop management in the field. There are several crucial steps in saffron processing, from picking the flowers in farms to drying of stigmas, that are characterized by local traditional practices (Aghaei et al., 2018; Carmona et al., 2006b; Ordoudi and Tsimidou, 2004). Quality characteristics of saffron are directly affected by the postharvest processing stages. Microbial counts and color strength along with apparent features of the stigmas determine the price of each batch and are significantly process-dependent.

The flowering stage of saffron varies from 15 to 25 days and the number of flowers reaches a maximum around 7−10 days after the appearance of the first flower (Mahdavee-Khazaei et al., 2014). The best time for collecting flowers is before sunrise when they are in budding mode, as no sunlight exposure has occurred and the tepals protect stigmas from physical damage during transport to the processing stage (Sepaskhah and Yarami, 2009).

With the dawn of the sun, flowers are surrounded with light and temperatures rise, resulting in opening of the buds. This not only reduces the physical strength of the tepals but also the quality of the stigma, especially its color strength. Extended exposure to light and high temperatures causes flowers to be withered and spoiled (Jafari et al., 2016;

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Khazaei et al., 2016). The stigmas are polluted with pollen from stamens. More importantly stigmas may stick to the stamens, causing the separation process to fail, which downgrades market acceptance.

16.1.1 International classification of saffron stigmas

Saffron stigmas are classified based on the physicochemical characteristics including color strength, appearance, style length, and diameter. Table 16.1 summarizes the specifications of the International Standardization Organization (ISO) 3632 for the physicochemical classifications of saffron in filaments, cut filaments, and powder forms. The ISO 3632 is the guide for international transactions and has been adopted by national standardization organizations in the European Union (e.g., AFNOR, ΕΛΟΤ). As shown in Fig. 16.1A, a stigma consists of a red section containing all three bioactive components of saffron and a white section called the style. The style length, attachment to the stigma, as well as the morphological features of the red section have crucial roles in saffron categorization. For example, in Iran the expression "strong stigma" refers to the ones that are thicker in diameter as well as straight and smooth in length. If a stigma is instead a thin and wavy structure, the stigma is called a "weak stigma" (Fig. 16.1B).

16.1.2 Iranian trade categories

Iran, as the biggest producer of saffron, has set national specifications for characterization of saffron (Table 16.2) in addition to the international specifications. Based on Iranian standards, the different types of traded saffron are Sargol, Pooshal, and Daste as detailed below:

TABLE 16.1 Physicochemical classification of saffron in filaments, cut filaments, and powder forms.

Characteristics	Specification categories			Test method
	1	2	3	
Moisture and volatile matter (mass fraction), % max, filament, and cut filament	12	12	12	ISO 3632-2 (2010), Clause 7
Saffron powder	10	10	10	
Total ash (mass), on dry matter, % max	8	8	8	ISO 928 and ISO 3632-2 (2010), Clause 12
Extraneous matter (mass fraction), % max, floral and plant waste	0.5	3	5	ISO 3632-2 (2010), Clause 8
Foreign matter (mass fraction), % max, from nonanimals (other plants)	0.1	0.5	1	ISO 3632-2 (2010), Clause 9
Acid-insoluble ash (mass fraction), %, on dry matter, max	1	1	1	ISO 930 and ISO 3632-2 (2010), Clause 13
Soluble extract in cold water, (mass fraction), on dry matter, % max	65	65	65	ISO 941 and ISO 3632-2 (2010), Clause 11
Flavor strength (expressed as picrocrocin); $A^{1\%}_{1\ cm}$ 257 nm, on dry matter, minimum	70	55	40	ISO 3632-2 (2010), Clause 14
Aroma strength (expressed as safranal); $A^{1\%}_{1\ cm}$ 330 nm, on dry matter				
Min	20	20	20	
Max	50	50	50	ISO 3632-2 (2010), Clause 14
Coloring strength (expressed as crocin); $A^{1\%}_{1\ cm}$ 440 nm, on dry matter, minimum	200	170	120	ISO 3632-2 (2010), Clause 14
Artificial colorants	Absent	Absent	Absent	ISO 3632-2 (2010), Clause 16 and/or 17

Source: Based on ISO 3632-1 (2011). Spices, Saffron (*Crocus sativus* L.). Part 1: Specification, International Organization for Standardization, Geneva.

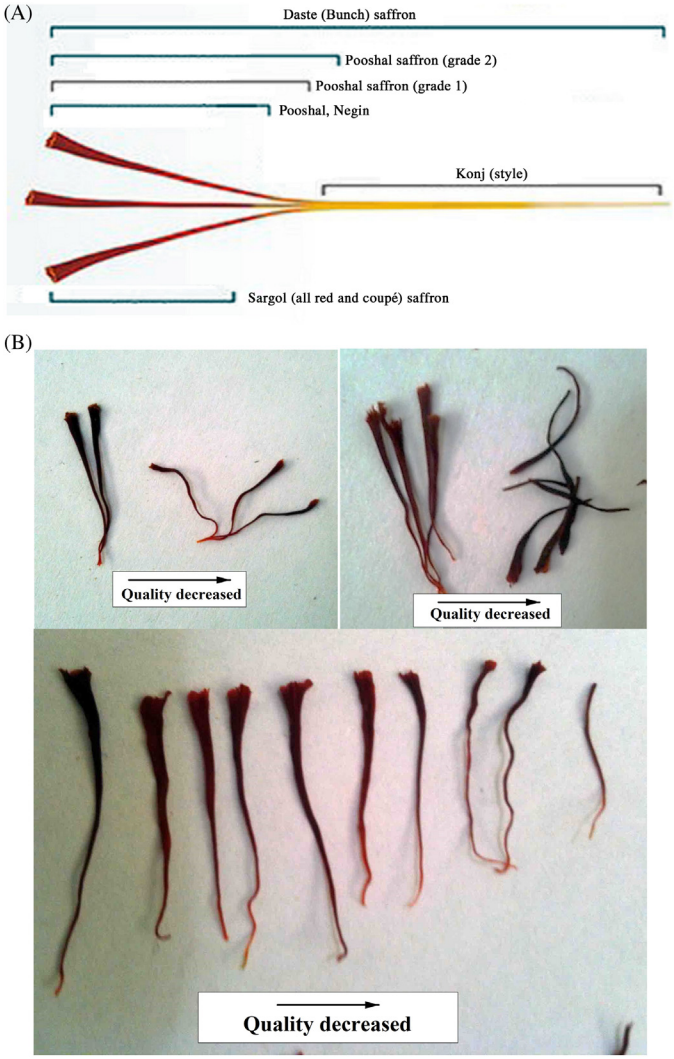


FIGURE 16.1 (A) Different classes (grades) of saffron stigma and (B) physical assessment of saffron stigma.

TABLE 16.2 Classification of saffron according to National Standards of Iran.

INSO classification	Color strength
Sargol Negin	>220
Pooshal Grade 1 (Negin)	>200
Pooshal Grade 2	>180
Pooshal Grade 3	>150
Pooshal Grade 4 (Daste)	>140

Source: Based on INSO, 259-1 (2013). Saffron, Specification. Iranian National Standards Organization (INSO), Iran.

1. Sargol saffron: Saffron Sargol, also known as "All Red" or "Coupé" saffron, is the completely red and cut saffron stigma, which does not have any style section attached to its end. The color strength of this saffron type is in the range of 210−260, depending on the production process and the amount of fragmented stigmas. This type of saffron is categorized in three groups as Sargol Negin, Sargol grade 1, and Sargol grade 2, depending on the color strength, stigma length and thickness, and the amount of fragmented stigmas, as shown in Fig. 16.1A.

   a. Sargol Negin saffron: Saffron categorized in this group means that the collected stigma does not have any style section, foreign materials, or fragmentation. Usually, when the saffron comes from the same farm, the color strength of Sargol Negin is slightly less than Pooshal Negin (see below) due to the heat applied during the production process of Sargol Negin compared to Pooshal. The color strength of this saffron type is in the rage of 240−260.
   b. Sargol grade 1 and 2: The differences between Sargol Negin and Sargol grade 1 and 2 are related to their color strength and stigma appearance. Usually, suppliers differentiate between the saffron grades with the level of fragmented stigmas. It is generally considered that fragile and weak stigmas have inferior coloring strength. The color strength of Sargol grade 1 and grade 2 is in the range of 220−240 and 200−220, respectively.
2. Pooshal saffron: Saffron Pooshal, also known as "Mancha," is a special class of saffron produced directly from fresh stigma along with Daste type (No. 3 below), while Sargol category is derived from these two types by applying further processes (Fig. 16.1A). The reason for the selection of this name is its bulk nature, which results in a lower bulk density compared to the Sargol type. This physical property plays an important role in packaging design. The stigmas in this saffron type are intermeshed and connected to each other. This physical state is considered as a quality index. The color strength of Pooshal saffron is in the range of 170−280, depending on the size of style attached to the reddish stigma as well as the stigma classification as strong or weak.
   a. Pooshal Negin saffron: Thick and smooth stigmas without any style section, foreign materials, or fragmented stigmas are categorized in this group. The highest color strength, the best appearance, and the lowest bulk density belong to this category of saffron. As defined by the Iranian National Standard Organization (INSO), the crocins content of this grade is above 200. Generally, this value ranges from 220 to 280 and this may be elevated up to 300, depending on the harvest time, postharvest handling, field management, etc.
   b. Pooshal grade 1: If the stigmas are thick and smooth in their appearance and a small section of style is attached to them, saffron is called Pooshal grade 1. Based on the ISO and INSO references, the color strength of this grade is approximately 20−30 units less than that of Pooshal Negin, but in practice this difference could be greater.
   c. Pooshal grade 2: Pooshal 2 is saffron with fragile and weak stigmas and a significant style section. The color strength of Pooshal grade 2 is approximately 20−30 units lower than that of Pooshal grade 1.
   d. Pooshal grade 3: Pooshal 3 is saffron with stigmas that are more fragile and weak than Pooshal grade 2. The color strength of Pooshal grade 3 is approximately 20−30 units lower than that of Pooshal grade 2.
3. Daste of saffron: This class of saffron known also as "Bunch" consists of the entire filament (stigma and style) and has the lowest color strength. INSO and ISO standards refer to this class as grade 4 and 3, by the coloring strength of 140 and 120, respectively.

16.2 Saffron drying methods

Similar to the other agricultural crops, postharvest processing plays a crucial role in the quality characteristics of saffron and its physicochemical as well as microbiological properties. The drying process is aimed at protecting the crocins by stopping the enzymatic reactions responsible for its biodegradation, which is achieved with temperatures above 60°C for the minimum possible time (Aghaei et al., 2018, 2019). Selecting the most appropriate drying method is one of the important steps in postharvest processing in order to prepare saffron stigmas with the highest amount of crocins, while obtaining the best morphological features. Generally, during the drying process the moisture content of stigmas decreases between 8% and 10%. The methods of saffron drying are traditional (sun drying, dark-air drying, toasting) and modern (such as freeze drying, microwave drying, etc.) and are selected and applied based on available equipment. The main difference in the methods is related to the temperature applied in the process. Table 16.3 summarizes different studies on the drying of saffron and application of various drying techniques (Acar et al., 2011; Maghsoodi et al., 2012; Raina et al., 1996; Tong et al., 2015a).

16.3 Extraction of saffron bioactive components

Scientists in the phytochemical field have tracked a variety of components in saffron stigmas. The researchers revealed that three groups of compounds responsible for color, taste, and aroma are present in dried stigma (Garavand et al., 2019; Sarfarazi et al., 2019). These are crocins, picrocrocin, and safranal, respectively (Fig. 16.2).

The red color of stigmas is due to the presence of uncommon hydrophilic carotenoids named crocins, which are glycosyl and gentiobiosyl esters of crocetin, the 8,8′-diapocarotene-8,8′ dioic acid, not naturally found free/unesterified

TABLE 16.3 Drying methods of saffron stigma.

Drying method	Principles	Advantages/limitations	References
Sun/shade drying	Spreading the fresh saffron stigmas with diameter of 1−10 mm in sun or shade for several hours to 3−5 days.	Prolonged process can destroy the crocins and result in inappropriate morphological features.	Acar et al. (2011), Carmona et al. (2005), Maghsoodi et al. (2012), Raina et al. (1996)
		Nonuniform drying	
Freeze drying	Spreading the fresh stigmas onto the tray of freeze dryer followed by decreasing the temperature to −40°C until complete freezing. Gradually reducing the chamber pressure in parallel to temperature increase dries the stigma.	High quality of produced saffron in terms of crocins content and morphological feature.	Acar et al. (2011), Atefi et al. (2004), Kanakis et al. (2004)
		Cost intensive and long processing time.	
Microwave drying	Spreading of a thin layer of fresh stigmas in microwave tray working at different powers.	The shortest operating time and higher retention of crocins in comparison with long drying time processes, like shade drying.	Maghsoodi et al. (2012), Rajabi et al. (2015), Tong et al. (2015a)
		High microwave power can negatively affect both bioactive components and morphological features in terms of color and structure.	
Electric oven	Fresh stigmas are poured into petri dishes followed by running oven at different temperatures.	Short processing time and higher retention of crocins in comparison with long-time drying processes like shade drying.	Carmona et al. (2005), Maghsoodi et al. (2012), Raina et al. (1996), Tong et al. (2015a)
Vacuum oven drying	Same as electric oven except for the difference in the pressure, which is maintained below the atmospheric level.	Short processing time and higher retention of crocins in comparison with electric oven and long drying process like shade drying.	Raina et al. (1996), Tong et al. (2015a)
Toasting	Putting the fresh stigmas into a silk bottom sieve while heat is applied by various sources.	Nonuniform drying.	Kanakis et al. (2004), Raina et al. (1996)
Refractance-Window (RW) drying	Spreading of a thin layer of fresh stigmas on a membrane/glass on top of boiling water.	RW dryer through Pyrex glass surface and higher temperatures (70°C and 80°C) led to the highest contents of picrocrocin, safranal, and crocins.	Aghaei et al. (2018, 2019)

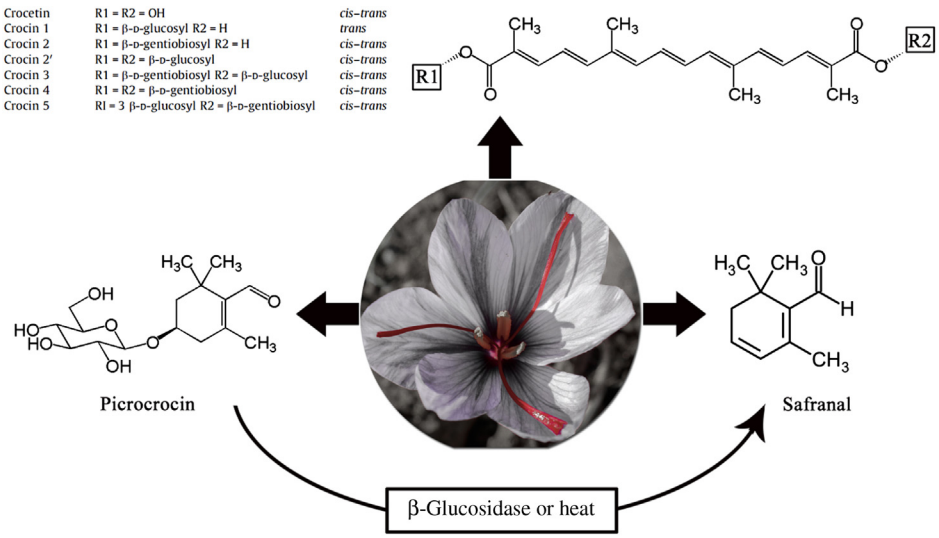


FIGURE 16.2 Main bioactive components of dried saffron stigmas.

(Esfanjani et al., 2015; Faridi-Esfanjani et al., 2017). As shown in Fig. 16.2 the difference in the type of sugar moieties placed in the positions R1 and R2 results in the six classes of crocins including crocin 1, crocin 2, crocin 2′, crocin 3, crocin 4, and crocin 5. In this regard, glycosyl and gentiobiosyl moieties along with −H are indwelled in R1 and R2 at different patterns, making various types of crocins (Sarfarazi et al., 2015). Regarding isomeric structure of crocins occurring in saffron, both isomeric structures of *cis* and *trans* are present in saffron. Total crocins are measured by reading absorbance of saffron extract with a standard concentration using a UV-vis spectrophotometer at wavelength of 440 nm (Mehrnia et al., 2016, 2017).

The bitter taste of saffron comes from a monoterpene glucoside named picrocrocin ($C_{16}H_{26}O_7$, 4-(*b*-D-glucopyranosyloxy) − 2,6,6-trimethyl-1-cyclohexene-l-carboxaldehyde). This component not only brings a charming bitter taste but also acts as a precursor for safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), an essential oil responsible for the saffron aroma (Shahi et al., 2016). Safranal makes up 70% of the total volatile constituents of saffron. It has been reported that this carboxaldehyde volatile component is present in saffron due to the heat applied during the drying process and also because of enzymatic activity of ß-glucosidase on picrocrocin. Researchers have proposed another pathway in which picrocrocin, due to loss of glucose molecule, is converted into oxysafranal (HTCC, 2,6,6-trimethyl-4-hydroxy-1-carboxaldehyde-1-cyclohexene), an intermediate volatile precursor of safranal. HTCC is also converted into safranal through release of a molecule of water.

Several techniques have been developed in order to extract saffron bioactive components with the highest quality while obtaining the highest extraction efficiency (Garavand et al., 2019). Up to now, a variety of methods including high hydrostatic pressure extraction (Shinwari and Rao, 2018), solid-phase microextraction (SPME) (D'Archivio et al., 2018), supercritical fluid extraction (SFE) (Nerome et al., 2016), maceration (Rajabi et al., 2015; Sarfarazi et al., 2015), microwave-assisted extraction (Jafari et al., 2019; Nescatelli et al., 2017), subcritical water extraction (SWE) (Sarfarazi et al., 2019),ultrasonic solvent extraction (Maggi et al., 2011), thermal desorption (TD) (Alonso et al., 1996; Kyriakoudi and Tsimidou, 2015), hydrodistillation (HD) (Kanakis et al., 2004; Maggi et al., 2011), microsimultaneous hydrodistillation−extraction (MSDE) (Rödel and Petrzika, 1991; Tarantilis and Polissiou, 1997), and vacuum headspace (Tarantilis and Polissiou, 1997) have been carried out in order to obtain whole saffron extract or with the aim of purification and separation of a specified saffron bioactive component. Due to the presence of compounds of both hydrophilic and lipophilic nature in saffron, selecting the appropriate solvent as well as other process variables such as extraction time and temperature are crucial determinants of the saffron extract quality and quantity. In this regard, the hydrophilic nature of crocins and picrocrocin results in the selection of water and alcohol as the solvents of choice, while safranal can be extracted well with less polar solvents such as diethyl ether and petroleum ether. Many researchers have investigated the role of extraction technique, solvent type, temperature, saffron stigma physical state, extraction time, ratio of saffron to solvent, etc., on the extraction efficiency (Jalali-Heravi et al., 2009; Kyriakoudi et al., 2012; Kyriakoudi and Tsimidou, 2015, 2018a; Mohajeri et al., 2010; Nerome et al., 2016; Nescatelli et al., 2017; Rubert et al., 2016; Sánchez et al., 2009; Sarfarazi et al., 2015; Sereshti et al., 2014; Shinwari and Rao, 2018; Tong et al., 2018). When the isolation of crocins is desired, the main limitation in designing an extraction system is the sensitivity of crocins to degradation in aqueous media. In this section, some of the extraction methods are reviewed briefly and a summary of extraction studies on saffron bioactive ingredients has been presented in Table 16.4.

16.3.1 Conventional extraction techniques

This class of extraction methods is usually based on the power of different solvents in use and the application of heat and/or mixing. The methods of maceration, Soxhlet, and HD are placed in this category.

16.3.1.1 Maceration

The most common method for extraction of whole saffron extract is maceration. The solvent extraction method is initiated by mixing an appropriate amount of saffron and solvent, followed by stirring over a predetermined time and speed, and ended by filtration and reading the absorbance of the resulting extract using a UV-Vis spectrophotometer. The ISO method for quality control of saffron is based on the maceration method (Sarfarazi et al., 2015). In ISO instructions, assessment of the quality of saffron is conducted by reading the absorbance of saffron extract at 257, 330, and 440 nm after preparation as follows: 500 mg of powdered saffron is transferred into a volumetric flask (1000 mL) containing 900 mL distilled water, stirred (1000 rpm, 1 hour) and the volume brought to 1000 mL. Next, 20 mL of this extract is transferred into a 200 mL volumetric flask and diluted with water to the mark. The results of work by Orfanou and Tsimidou (1995) on the relevant parameters in saffron extract coloring strength prepared via the maceration method

TABLE 16.4 An overview of different studies dealing with extraction of saffron bioactive compounds.

Extraction method	Parameters	Analyte of interest	Results	References
Solid liquid extraction	• Solvent type (water, methanol, acetonitrile, ethanol, methanol/water (50/50, v/v), acetonitrile/water (75/25, v/v) and ethanol/water (70/30, v/v)) • Ultrasound effect on EF	All bioactive components of saffron	• The best solvent was methanol/water (50/50, v/v) followed by ethanol/water (70/30). • Ultrasound increased the extraction efficiency.	Rubert et al. (2016)
Microwave-assisted extraction	• Solvent type (methanol, acetone, diethyl ether, dichloromethane, ethanol, ethylacetate, methanol/water (50/50, v/v), ethanol/water (50/50, v/v)) • Extraction temperature • Extraction time (1, 10, and 19 min) • Extraction volume (2 and 10 mL of solvent)	All bioactive components of saffron	• The best solvent, time, temperature, and volume for obtaining the highest concentration of safranal was ethanol/water (50/50, v/v), 1 min, 40°C and 10 mL, respectively. • The best solvent, time, temperature, and volume for obtaining the highest concentration of crocin 1 were methanol/water (50/50, v/v), 10 min, 40°C, and 10 mL, respectively. • The best solvent, time, temperature, and volume for obtaining the highest concentration of crocin 1 were methanol/water (50/50, v/v), 1 min, 40°C, and 10 mL, respectively.	Jafari et al. (2019), Nescatelli et al. (2017)
Ultrasound-assisted extraction	• Solvent type (hexane and chloroform) • Time of extraction (15, 30, and 60 min) • The concentration of saffron in each organic solvent (20, 40, and 60 g L^{-1})	Safranal	• The best solvent, time, and concentration of saffron in each organic solvent for obtaining the highest concentration of safranal were chloroform, 15 min, and 20 g L^{-1} of saffron, respectively.	Maggi et al. (2011)
Ultrasound-assisted extraction	• Saffron: solvent ratio [182, 425, 1013, 1600, and 1843 (w/v)] • Duration of sonication (5, 9, 18, 26, 30 min)	Total crocetinesters	• The best saffron: solvent ratio and sonication time for obtaining the highest concentration of crocetin were 1600 and 9 min, respectively.	Kyriakoudi and Tsimidou (2015)
Ultrasound-assisted extraction	• Percentage of methanol [0.4, 10.5, 25.2, 40, and 50 (%, v/v)] • Duration of sonication (1.2, 7, 15.5, 24, 29.8 min) • Duty cycles of sonication (active interval) (0.2, 0.3, 0.5, 0.7, and 0.8 s)	Crocins and picrocrocin	• The best methanol concentration, sonication time and duty cycles for obtaining the highest concentration of crocins were 50%, 30 min, and 0.2 s on/0.8 s off, respectively. • The best methanol concentration, sonication time, and duty cycles for obtaining the highest concentration of crocins and picrocrocin were 44%, 30 min, and 0.6 s on/0.4 s off, respectively.	Kyriakoudi et al. (2012)

(*Continued*)

TABLE 16.4 (Continued)

Extraction method	Parameters	Analyte of interest	Results	References
Maceration	• Solvent type (hexane, methanol) • Different drying methods [oven (43°C for 100 min, 80°C for 20 min then 43°C for 70 min, 87°C for 20 min then 43°C for 70 min, 75°C for 20 min then 43°C for 70 min, 43°C for 100 min, 92°C for 20 min then 43°C for 70 min), food dryer (46°C for 60 min, 58°C for 20 min then 46°C for 40 min); fresh/no drying; frozen/no drying; frozen, thawed, and then dried (87°C for 20 min then 43°C for 70 min)]	All bioactive components of saffron	• The best solvent and drying method for obtaining the highest concentration of safranal were hexane and oven drying (87°C for 20 min then 43°C for 70 min), respectively. • The best solvent and drying method for obtaining the highest concentration of HCC[a] were hexane and frozen/no drying, respectively. • The best solvent and drying method for obtaining the highest extraction efficiency (%) of picrocrocin were methanol and fresh/no drying, respectively.	Gregory et al. (2005)
Maceration	• Solvent type (ethyl ether, acetone, acetonitrile, methanol, ethanol, isopropanol, ethanol-water (50% v/v), and water)	Picrocrocin, HTCC, and crocins	• The best solvent for picrocrocin, HTCC, and crocins extraction was water followed by ethanol:water (50:50).	Iborra et al. (1992)
Emulsion liquid membrane	• Surfactant type (Span 80, ENJ-3029, plyamine type-surfactants) • Membrane type (CCl4, *N*-decane, CH_3Cl, toluene) • Surfactant concentration (0.5, 2.5, 7, 7.5, 10) • Treat ratio (0.1, 0.2, 0.3, 0.4) • Phase ratio (0.4, 0.6, 0.8, 1.0, 1.2) • Stirring rate (100, 200, 300, 400, 500)	All bioactive components of saffron	• The best surfactant, membrane type, surfactant concentration, treat ratio, phase ratio, and stirring rate for obtaining highest extraction efficiency were Span 80, *N*-decane, 2.5%, 0.3, 0.8, and 300 rpm, respectively.	Mokhtari and Pourabdollah (2013)
Ultrasonic-assisted solvent extraction	• Solvent type (methanol: ethylacetate, methanol: ether, methanol: chloroform) • Solvent volume (10, 20, 30, 40, 50 mL) • Extraction time (15, 37.5, 60, 82.5 min) • Extraction step (1 and 4) • Solvent ratio (10, 30, 50, 70, 90) • Sample amount (0.5, 1.0, 1.5, 2.0, 2.5 g)	Volatile components of saffron	• The best solvent was methanol: ethylacetate and the optimum values of factors were: 2.38 g sample, 29.04 mL solvent, 69.23% methanol solvent ratio, and 71.8 min for the extraction time. • The effect of extraction steps on the response was not significant.	Jalali-Heravi et al. (2009)
Pulsed electric field extraction	• Voltage • Pulse width • Pulse numbers	All bioactive components of saffron	• The optimum condition was voltage of 5 kV, pulse number of 100 pulses, and pulse width of 35 ms.	Pourzaki et al. (2013)
Maceration	• Ethanol concentration (0%–100%) • Extraction time (2–7 h) • Temperature (5°C–85°C)	All bioactive components of saffron	• The optimum conditions in terms of obtaining highest amount of saffron bioactive component was ethanol concentration of 33.33%, extraction time of 2.0 h, and temperature of 85.0°C.	Khazaei et al. (2016), Sarfarazi et al. (2015)

(*Continued*)

TABLE 16.4 (Continued)

Extraction method	Parameters	Analyte of interest	Results	References
Different extraction methods	• Extraction method [solvent-assisted flavor evaporation (SAFE), liquid–liquid extraction, solid-phase extraction, and simultaneous distillation extraction]	Safranal	• According to sensory analysis, the aromatic extract obtained by SAFE was the most representative of saffron odor.	Amanpour et al. (2015)
Supercritical CO_2 extraction	• Temperature (40°C, 60°C, and 80°C) • Pressures (20, 30, 40 MPa) • Enrainer type (water and methanol)	All bioactive components of saffron	• The best entrainer for extraction of picrocrocin, safranal, and HTCC was ethanol. Water was the best type for extraction of deglycosylated crocin and α-crocin. • The optimum pressure for extraction of α-crocin, picrocrocin, and deglycosylated crocin was 30 MPa and for the rest was 40 MPa. • The optimum temperature for extraction of all components was 80°C.	Nerome et al. (2016)
Supercritical CO_2 extraction	• Temperature (35°C–105°C) • Pressure (10–30 MPa) • SC-CO_2 flow rate (0.3–1.5 $cm^3\ min^{-1}$) • Dynamic extraction time (30–150 min)	Safranal and crocins	• The optimal values of variables for safranal extraction were 92°C, 21.3 MPa, 0.9 $cm^3\ min^{-1}$ and 122.0 min. • The optimal values of variables for crocin were obtained at 44°C, 19.3 MPa, 1.0 $cm^3\ min^{-1}$, and 110.0 min.	Goleroudbary and Ghoreishi (2016)
Aqueous two-phase system (ATPS)	• Different types of ATPS (polymer-polymer, polymer-salt, alcohol-salt, and ionic liquid-salt) • Polyethylene glycol (PEG) molecular mass (400, 1000, 3350, 8000, and 10,000 g mol^{-1}) • Volume ratio (0.33–7.25) • PEG concentration (3.5%–20.2% w/w) • Salt (potassium phosphate) concentration (10.4%–20% w/w) • Dextran concentration (8.2%–15.5% w/w) • Ionic liquid concentration (13%–35.2% w/w) • Ethanol concentration (14%–17% w/w) • TLL (15%–50% w/w)	Crocins	• The best system based on their high top phase recovery yield and low cost of system constituents was ethanol–potassium phosphate ATPS. • The best conditions of ATPS were volume ratio = 3.2, ethanol 19.8% (w/w), potassium phosphate 16.5% (w/w), TLL of 25% (w/w), 0.1M NaCl, and 2% (w/w) of sample load.	Montalvo-Hernández et al. (2012)
Macroporous resins	• Resin type (AB-8, Amberlite XAD-1180, XAD-1600, EXA-117, EXA-32, EXA-45, EXA-50, EXA-118, HP20 and HPD-100A)	Crocins of gardenia fruit	• The best resins in terms of adsorptive capacity and selectivity for crocin were XAD-1180, HP20, HPD-100A, and AB-8 (best).	Yang et al. (2009)
Macroporous resins	• Resin type [Polystyrene (D101, X-5, LX60, AB-8, LX38, LX28, LX8) and Acrylate (LX17)]	Crocins of gardenia fruit	• The best resin based on static absorption/desorption experiments was LX60.	Feng et al. (2014)

(*Continued*)

TABLE 16.4 (Continued)

Extraction method	Parameters	Analyte of interest	Results	References
Solid-phase extraction	• Polymer type [gentiobiose imprinted polymer (Gent-MIP) and blank nonimprinted polymer] • Solvent used for binding study [water, acetic acid 2% (v/v), acetic acid 5% (v/v), acetic acid 10% (v/v)] • Type of washing solvents (methanol, THF, water, and ACN)	Crocins of saffron	• The inclusion of gentiobiose in polymer increased the affinity of the Gent-MIP for the crocin than the nonimprinted one. • The best solvent for binding was acetic acid 2% (v/v). • The best washing solvent was ACN.	Mohajeri et al. (2010)

[a] – *4R-hydroxy-ß-cyclocitral.*

showed that the process duration, type of solvent and filter, as well as the stage in which the extract was filtrated played a significant role. In this regard, by increasing the pore size of filter paper or processing time (up to 24 hours), as well as by filtering the extract prior to final dilution, the coloring strength was decreased significantly. Also, it is reported that the best solvent in order to extract the highest amount of saffron active components is methanol (50% v/v) followed by ethanol (50% v/v) and water.

16.3.1.2 Soxhlet extraction

In this method, the dried saffron is placed in an extraction thimble and extracted using an appropriate solvent. Successive and exhaustive Soxhlet extraction is utilized for extraction of picrocrocin using light petroleum, diethyl ether, and methanol as solvents (Tarantilis et al., 1994). In this work, nonglucoside carotenoids and lipids, lipids and picrocrocin, and the glucoside carotenoids were found in light petroleum extract, diethyl ether extract, and methanol extract, respectively. Methanol is the preferred solvent for extraction of saffron components and the resulting extract has been used for medicinal, pharmaceutical, and food purposes (Goleroudbary and Ghoreishi, 2016). On the other hand, Feizzadeh et al. (2008) and Samarghandian et al. (2013) worked on preparation of aqueous saffron extract using Soxhlet by adding 15 g powdered saffron and 100 mL distilled water into the extractor for 18 hours.

16.3.1.3 Hydrodistillation

As its name implies, HD is a process of volatile component isolation in which water vapor penetrates herbal cells and acts as a carrier of volatiles to a condenser rod. In this method, the dried saffron stigma is soaked in water (or a mixture of water and alcohol) for a period of time followed by heating the mixture to the boiling point. Volatiles are carried away in the steam to a condenser that is cooled by a stream of water, liquefying the compounds for collection. Kanakis et al. (2004) worked on quantification of safranal using MSDE. They used diethyl ether as a solvent and a mixture of water/glycol ($-10^{\circ}C$) to cool the condenser. The MSDE procedure was carried out for 2 hours.

16.3.2 Novel extraction methods

16.3.2.1 Supercritical fluid extraction

The cornerstone of this method is based on passing a supercritical fluid through the sample. Carbon dioxide is the most common supercritical fluid due to its characteristics of interest including nontoxicity and nonflammability, liquid-like density, low viscosity, high diffusivity, and selective extraction ability (McHugh and Krukonis, 2013). The SFE based on carbon dioxide (SFE-CO_2) works well with less polar components (safranal in the case of saffron). In order to increase the scope of the process toward polar components extraction, water or an organic solvent is introduced as an entrainer. When water is used in the SC-CO_2 system, two factors are taken into account: (1) the solubility of the components of interest is increased due to the impact of water as a entrainer and (2) water sorption of plant tissue followed by swelling resulted in components of interest escaping more rapidly and easily (Nerome et al., 2016). Lozano et al. (2000) conducted a procedure based on SFE-CO_2 to isolate safranal from saffron and reported extraction of safranal and a small amount of 4-hydroxy-2,6,6-trimethyl-1-carboxaldehyde-1-cyclohexane. Nerome et al. (2016) used a SFE-

CO_2 system with water and methanol as entrainers to extract saffron bioactive compounds. Their results revealed that the efficiency of the SFE-CO_2 system in extraction of both hydrophilic and lipophilic components of saffron was significantly higher than the maceration method via water and methanol. Also, using methanol as an entrainer maximized extraction yield of picrocrocin and safranal, while maximum extraction of crocins was achieved through running the system with water as an entrainer.

16.3.2.2 High pressure extraction

The principles of high pressure extraction (HPE) and high pressure processing of food are similar and based on applying a pressure between 100 and 1000 MPa for a determined time. Like other modern methods, HPE brings advantages of lower energy consumption, and the produced extract meets the highest quality indexes. Unlike thermal extraction methods, in the HPE process the covalent bonds remain intact and hydrophobic, hydrogen, and ionic bonds are affected (Li et al., 2012; Shinwari and Rao, 2018; Shouqin et al., 2004). Shinwari and Rao (2018) assessed extraction of nutraceuticals from saffron using the thermal-assisted high hydrostatic pressure process by applying pressure of 100−600 MPa in combination with elevated temperatures (30°C−70°C). In this work, saffron powder and water were mixed in a ratio of 1:100w/v and vacuum-packed followed by hydration and exposure to high pressure. They revealed that the extraction efficiencies of crocin, picrocrocin, and safranal were increased significantly (52%−63%, 54%−85%, and 55%−62%, respectively) when compared to the maceration method. The pressure is transmitted through a solution of monopropylene glycol (30%). Buzrul et al. (2008) used water, ethylene glycol, and ethanol as pressure transmitting fluids to treat liquid foods by HPP.

16.3.2.3 Ultrafiltration

Ultrafiltration (UF) is a separation process in which membranes with pore size of 0.1−0.001 μm are used to remove high-molecular-weight substances, colloidal materials, and organic and inorganic polymeric molecules. The attractive properties of UF include its mild operating conditions and relatively high selectivity, making it a popular method toward conventional one. UF efficiency is affected by a variety of factors including total soluble solid content, nominal molecular weight limit, fouling behavior, and cross-flow rate (Zeman and Zydney, 2017). Sánchez et al. (2009) investigated the effect of a centrifugal UF process on saffron bioactive components. In that work, first, the hydrophobic components of saffron were removed, then aqueous saffron extract was prepared. The extract was then centrifuged and subjected to UF. Successive dead-end microfiltration and cellulose acetate membrane filters were finally used to clarify the end product.

16.3.2.4 Microextraction methods

One of the most important features of the microextraction method is use of negligible amounts of solvent, resulting in their reputaiton as "green technology." Other attractive characteristics of this category are simplicity, high efficiency, and short processing time (Ocaña-González et al., 2016). The methods of SPME, stir-bar sorptive extraction (SBSE), and dispersive liquid−liquid microextraction (DLLME) are discussed in the following subsection.

16.3.2.5 Solid-phase microextraction

SPME is a comparatively new extraction method and is a well-known solvent-free technique with characteristics such as high speed, ease of use, and sensitivity. SPME is the process in which the components of interest (in gaseous or liquid phase) are absorbed on a fiber coating. Identification of these components can be achieved successively through gas chromatography (Pawliszyn, 2011). D'Archivio et al. (2018) analyzed the aroma profile of saffron using SPME as follows: saffron hydration was performed, followed by conditioning of fiber and exposure to SPME. Gas chromatography (GC) was used to identify the analyte at 250°C for 5 minutes.

16.3.2.6 Stir-bar sorptive extraction

Like SPME, SBSE requires only a small amount of solvent to extract biochemicals from the sample. In this method, the desired component is moved into a nonmiscible liquid phase. Polydimethylsiloxane (PDMS) is the predominant sorptive extraction phase due to its thermo-stable properties and possession of desired diffusion manner (Prieto et al., 2010). Maggi et al. (2011) used SBSE to assess semivolatile organic contaminants and pollutants in saffron as follows. PDMS coated stir bars were stirred (1000 rpm, 25°C, 14 hours) through the saffron aqueous solution containing methanol,

sodium sulphate anhydrous, and methanolic solutions. After that, the stir bar was separated followed by rinsing and drying. The analytes were assessed by introducing them into a TD tube for GC/MS/MS analysis.

16.3.2.7 Dispersive liquid–liquid microextraction

DLLME as a novel extraction method is at the center of attention due to its positive characteristics such as simplicity, low cost, and ease of running and development. DLLME is conducted through formation of a ternary solvent system consisting of dispersing solvent, extracting solvent, and the aqueous medium that contains the analyte. Each part of this system plays a specified role in order to extract the maximum amount of analyte. Accordingly, the extraction solvent is scattered through the aqueous medium by acting as a dispersing solvent. DLLME is also applied as a sample preparation technique because of its ability to give high enrichment factors by treating a relatively negligible amount of aqueous sample. This process includes the following steps (Rezaee et al., 2006, 2010):

1. The binary system of extracting and dispersing solvents is introduced to an aqueous medium.
2. Dispersion of analyte and extracting solvent is achieved in a short time, named cloudy state.
3. Centrifugation is applied to isolate dispersion solvent enriched with analyte followed by analysis with a microsyringe.

Sereshti et al. (2014) aimed to isolate and enrich saffron volatile components through ultrasound-assisted extraction (UAE) in conjunction with DLLME. In this work, a mixture of methanol and acetonitrile was added into a specified amount of ground saffron and exposed to an ultrasonic process. Henceforth, preconcentration solvent (chloroform) is introduced into the centrifuged extract followed by quick injection into aqueous NaCl solution. Accordingly, cloudy state appeared and the dispersion solvent was separated using centrifugation. Finally, GC was used to analyze the extracted analyte.

16.4 Characterization of saffron bioactive compounds

16.4.1 UV-Vis spectrophotometry

Detection and estimation of the content of crocins, picrocrocin, and safranal in a saffron sample has been carried out until now almost exclusively with the aid of UV-Vis spectrophotometry. This technique is included in the ISO 3632-2 (2010), which is related to the determination of the major saffron constituents (Rajabi et al., 2019). In particular, according to this standard, after the preparation of an aqueous saffron extract, a spectrum is recorded in the range of 200–700 nm. Absorbance values at specific wavelengths of 440, 250, and 330 nm are used to estimate of the content of crocins, picrocrocin, and safranal in a saffron sample as measures of "coloring," "flavor," and "aroma" strength, respectively, using Eq. (16.1).

$$E^{1\%}_{\lambda\max} = \frac{D \times 10,000}{m(100 - H)} \tag{16.1}$$

where D is the absorbance value at 440 nm (coloring strength), 330 nm (aroma strength), and 250 nm (flavor strength); m, the mass of the test portion (g); and H, the moisture and volatile content of the sample (%, w/w).

Orfanou and Tsimidou (1996) proposed the exploitation of the entire UV-Vis spectrum instead of only specific wavelengths using derivative spectroscopy. Especially a second derivative spectrum can be a valuable tool for the accurate determination of λ_{max} and for the resolution of overlapping peaks. Representative zero order and second derivative spectra of authentic Greek, Spanish, and Iranian authentic saffron samples are shown in Fig. 16.3. Derivative UV-Vis spectrophotometry has been used by Zalacain et al. (2005) to detect the presence of various artificial colorants, namely naphthol yellow, tartrazine, quinoline yellow, Sunset yellow, Allura red, amaranth, azorubine, Ponceau 4R, and Red 2G, in saffron. Masoum et al. (2015) exploited the second derivative to simultaneously detect the presence of two colorants with a high degree of overlapping UV-Vis spectra, tartrazine and sunset yellow, in adulterated saffron. In another work, Ordoudi et al. (2017) found that the second derivative spectrum of authentic saffron changed substantially with the addition of 2% w/w of carminic acid, a natural colorant that can be illegally added to saffron to enhance coloring strength. The authors suggested that the second derivative could provide a clue about the presence of carminic acid in saffron even at relatively low amounts.

Doubts about whether or not absorbances at 257 and 330 nm are representative of picrocrocin and safranal content, respectively, have been expressed by various researchers working in the field of saffron since the 90s (e.g., Orfanou and Tsimidou, 1996). In particular, absorbance at 257 nm has been frequently criticized by numerous researchers as

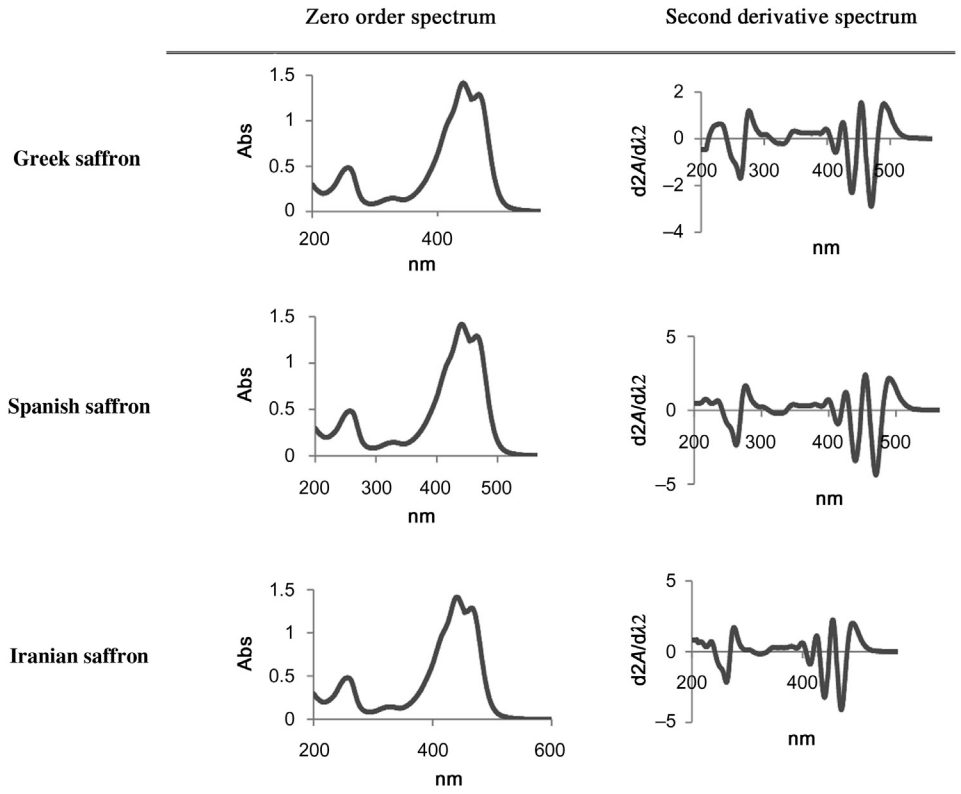


FIGURE 16.3 Representative zero order and second derivative spectra of Greek, Spanish, and Iranian authentic saffron samples. *Data from the saffron sample collection of LFCT.*

being related to glycosidic bonds of crocins rather than to picrocrocin and absorbance at 330 nm as being related to *cis*-crocins rather than to safranal. Garcia-Rodriguez et al. (2017) prepared aqueous extracts of 390 saffron samples and analyzed them both by UV-Vis spectrometry and high performance liquid chromatography—diode array detector (HPLC-DAD). Quantification of safranal in both cases was carried out with proper calibration curves at 330 nm. The authors observed no correlation between the safranal content found with UV-Vis and HPLC-DAD analysis and suggested that this was due to an overestimation in safranal content using the UV-Vis that was related to the simultaneous absorbance of *cis*-crocins at 330 nm.

Moreover, considering the differences in polarity among crocins and picrocrocin as well as safranal, the first two are indeed expected to be present in an aqueous saffron extract prepared according to ISO 3632-2. In contrast, nonpolar safranal is not expected to be fully extracted with water (Kyriakoudi and Tsimidou, 2018b). Nevertheless, currently many researchers continue to use the extraction protocol proposed by ISO 3632-2 followed by UV-Vis analysis for the determination of crocins, picrocrocin, and safranal. To overcome the limitations regarding the extraction of safranal with water, different nonpolar solvents in combination with various extraction means have been reported in the literature. In particular, Maggi et al. (2011) prepared saffron extracts by means of UAE using diethyl ether or chloroform as extraction solvents. The authors compared the content of safranal obtained by measuring the absorbance of the prepared extracts at 330 nm using UV-Vis spectrometry to its content obtained by GC-MS and found a good correlation.

It is worth mentioning that besides the abovementioned limitations, UV-Vis spectrophotometry finds many applications still today for various purposes. For instance, Cossignani et al. (2014) examined the effect of drying conditions of saffron produced in the Umbria region (Italy) on the content of crocins, picrocrocin, and safranal using the $E^{1\%}$ values obtained by UV-Vis spectrophotometry. Moreover, D'Archivio and Maggi (2017) applied principal component analysis (PCA) to the UV-Vis spectra of aqueous extracts of 81 saffron samples from various areas in Italy for their geographical classification.

16.4.2 High performance liquid chromatography (HPLC)

In the context of the revision of the ISO 3632 standard in 2003 (ISO 3632-2, 2003), HPLC coupled to a UV-Vis detector was introduced for the first time. However, it is worth mentioning that its use was restricted only to the detection of artificial acidic colorants in saffron and was not proposed for apocarotenoid analysis. According to the standard,

TABLE 16.5 Gradient elution system for saffron analysis.

Stage	Time (min)	Solvent A	Solvent B	Solvent C
Equilibration	10	90	10	0
1	0	90	10	0
2	7	48	52	0
3	10	48	52	0
4	14	0	60	40
5	24	0	60	40
6	25	90	10	0

Source: Based on ISO 3632-2 (2010). Saffron (*Crocus sativus* L.). Part 2: Test Methods. Organization for Standardization, Geneva.

chromatographic separation was carried out on a C18 column (25 cm × 4.0 mm, 5 μm). As mobile phase, either an aqueous solution of 1 mM tetra-*n*-butylammonium hydrogen sulfate and 1 mM potassium dihydrogen phosphate (pH = 4.5) (A) and acetonitrile (B) (70:30, v/v) or an aqueous solution of 1.4 mM tetra-*n*-butylammonium hydrogen sulfate and 1.4 mM potassium dihydrogen phosphate (pH = 4.5) (A) and acetonitrile (B) were proposed. The flow rate was 1 mL min^{-1}. Separation of saffron compounds could be performed with either isocratic or gradient elution [0−14 minutes, 0% (B)], 14−30 minutes, 100% (B), 30−40 minutes, 100% (B). In the latest revision of the standard in 2010 (ISO 3632-2, 2010), the use of a C18 chromatographic column (150 mm × 4.6 m, 3 μm) was proposed along with a guard column (10 mm × 4.6 mm, 4 μm). As mobile phase, an aqueous solution of 0.01 M potassium dihydrogen phosphate (pH = 7) (A), methanol (B), and acetonitrile (C) were proposed. Separation was carried out at a flow rate of 0.8 mL min^{-1} using gradient elution as described below (Table 16.5).

It is quite strange that although HPLC has been the main analytical tool for the analysis of saffron apocarotenoids in literature, it has not yet been introduced in the standard for this purpose. Alterations regarding the chromatographic column characteristics as well as the elution gradient system are numerous. Table 16.6 gives an overview of the chromatographic systems and conditions that are reported in literature for the analysis of crocins, picrocrocin, and safranal up to today.

More specifically, separation of saffron apocarotenoids is carried out on RP C18 chromatographic columns with length that ranges from 100 to 250 mm, internal diameter from 2.1 to 4.9 mm, and particle size in the range of 4−10 μm. Analysis is usually carried out at ambient temperature, even though higher temperatures of 30°C have been also reported. Gradient elution is almost exclusively used for the separation of saffron apocarotenoids. The mobile phase generally includes water and polar organic solvents such as methanol or acetonitrile. The addition of acid such as acetic, phosphoric, or formic acids (0.25%−1%) are suggested to regulate the pH in order to improve peak resolution by inhibiting the ionization of compounds during analysis. UV-Vis and DADs, coupled or not to a mass detector (MS), are usually used for the detection of crocins, picrocrocin, and safranal. The characteristic wavelengths for the detection of these compounds are usually 440, 250, and 330 nm, respectively. A typical RP-HPLC-DAD profile is shown in Fig. 16.4. Spectral characteristics, λ_{max} values, as well as the major ions of the individual apocarotenoids are presented in Table 16.7.

It is worth mentioning that the exploitation of UHPLC has been reported in literature for the rapid separation and identification of saffron compounds. In particular, Rocchi et al. (2018) used a UHPLC-MS/MS system equipped with a reversed-phase Kinetex C18 column (100 mm × 2.1 i.d., 1.7 μm) packed with Core Shell particles in order to separate the *trans*- and *cis*-isomers of crocins of 42 saffron samples with different origin, age, and drying conditions. The mobile phase consisted of water (5 mM formic acid) (A) and acetonitrile (5 mM formic acid) (B) with a flow rate of 0.3 mL min^{-1}. The authors managed to separate crocins within 10 min. In the same content, Moras et al. (2018) proposed a UHPLC-DAD/MS method for the quality assessment of saffron as well as for the detection of adulteration with *Gardenia jasminoides* Ellis. A Core-Shell reversed-phase Kinetex C18 column (150 mm × 2.1 i.d., 2.6 μm) was used for the analysis. The mobile phase consisted of water (0.01% formic acid) (A) and acetonitrile (B) with a flow rate of 0.6 mL min^{-1}. 35 compounds including *trans*- and *cis*-crocins, picrocrocin, as well as kaempherol derivatives were identified. The authors suggested that with this method it was possible to separate and identify compounds that are not

TABLE 16.6 Overview of the chromatographic systems and analytical conditions for crocetin sugar esters, picrocrocin, and safranal determination.

Aim of the study	Chromatographic column	Mobile phase	Elution conditions	Detector	Reference
Development of an HPLC/PDA/ESI-MS method for the identification of safflower, marigold, and turmeric in saffron	Gemini C18	Gradient elution	0 min, 95%–5% B	DAD (520, 440, 425, 410, 350, 250 nm)	Sabatino et al. (2011)
	150 mm × 2.1 i.d, 3 μm	A: H_2O (0.3%, v/v, formic acid), B: ACN (0.3%, v/v, formic acid),	0–50 min, 72%–28% B		
			50–60 min, 57%–43% B	MS (ESI, negative ion mode)	
		Flow rate 0.2 mL min^{-1}	60–65 min, 57%–43% B		
			65–80 min, 95%–5% B		
Development of a UAE method for the recovery of crocetin sugar esters and picrocrocin from saffron	LiChroCART Superspher 100 C18 125 × 4 mm i.d., 4 μm	Linear gradient elution	20 min, 20%–100% B	DAD (440, 330, 250 nm)	Kyriakoudi et al. (2012)
		A: H_2O (0.1%, acetic acid),			
		B: ACN			
		Flow rate 0.5 mL min^{-1}			
Characterization of secondary metabolites in saffron from central Italy	Gemini C18	Gradient elution	0–5 min, 90%–10% B	DAD (440, 250 nm)	Cossignani et al. (2014)
	100 mm × 2.0 i.d, 5 μm	A: H_2O, B: ACN	5.25 min, 20%–80% B		
		Flow rate 0.8 mL min^{-1}	25–30 min, 20%–80% B	MS (ESI, negative and positive ion modes)	
Development of a HPLC-DAD method for the determination of the major saffron apocarotenoids from an aqueous extract prepared according to ISO 3632	Phenomenex Luna C18	Gradient elution	0–5 min, 80%–20% B	DAD (440, 330, 250 nm)	Garcia-Rodriguez et al. (2014)
	150 mm × 4.6 i.d., 5 μm	A: H_2O, B: ACN	5–15 min, 20%–80% B		
		Flow rate 0.8 mL min^{-1}	15–20 min, 20%–80% B		
Analysis of bioactive saffron constituents	C18	Gradient elution	0–3 min, 20%–40% B	DAD (440, 330, 250 nm)	Chaharlangi et al. (2015)
	150 mm × 4.6 i.d., 5 μm	A: H_2O, B: ACN	3–8 min, 40%–50% B		
		Flow rate 1 mL min^{-1}	8–12 min, 50%–50% B		
			12–15 min, 50%–80% B		
Development of a HPLC-DAD method for the determination of the safranal content	Phenomenex Luna C18	Gradient elution	0–5 min, 80%–20% B	DAD (440, 330, 250 nm)	Garcia-Rodriguez et al. (2017)
	150 mm × 4.6 i.d., 5 μm	A: H_2O, B: ACN	5–15 min, 20%–80% B		
		Flow rate 0.8 mL min^{-1}	15–20 min, 20%–80% B		

(*Continued*)

TABLE 16.6 (Continued)

Aim of the study	Chromatographic column	Mobile phase	Elution conditions	Detector	Reference
Development of a method for the determination of saffron compounds using HPLC-DAD	KNAUER Eurospher C18 250 mm × 16, 10 μm	Gradient elution	0–5 min, 10%–90% B	DAD (440, 308, 250 nm)	Kabiri et al. (2017)
		A: H_2O, B: ACN	5–15 min, 80%–20% B		
		Flow rate 0.8 mL min^{-1}	15–20 min, 80%–20% B		
Quality assessment of saffron using UHPLC-DAD-MS and detection of adulteration with gardenia fruit extract	Kinetex C18	Gradient elution	0–1 min, 95%–5% B	DAD (440, 330, 310, 250 nm)	Moras et al. (2018)
	150 mm × 2.1 i.d., 2.6 μm	A: H_2O (0.01%, v/v, formic acid), B: ACN	1–9 min, 60%–40% B		
			9–15 min, 0%–100% B		
		Flow rate 0.6 mL min^{-1}	15–17 min, 0%–100% B	MS (ESI, positive ion mode)	
			17–17.1 min, 95%–5% B		
Development of an UHPLC-MS/MS procedure to determine crocins as a marker of quality	Kinetex C18	Gradient elution	0–0.1 min, 95%–5% B	MS (ESI, negative ion mode)	Rocchi et al. (2018)
	100 mm × 2.1 i.d, 1.7 μm	A: H_2O (5 mM formic acid), B: ACN (5 mM formic acid)	0.1–13 min, 1%–99% B		
			13–17 min, 1%–99% B		
		Flow rate 0.3 mL min^{-1}	17–20 min, 95%–5% B		
Quantitative HPLC-based metabolomics of Iranian saffron samples	KNAUER Eurospher C18	Gradient elution	0–5 min, 10%–90% B	DAD (440, 308, 250 nm)	Vahedi et al. (2018)
	250 mm × 4.6 i.d., 5 μm	A: H_2O, B: ACN	5–25 min, 80%–20% B		
		Flow rate 1 mL min^{-1}	25–30 min, 80%–20% B		

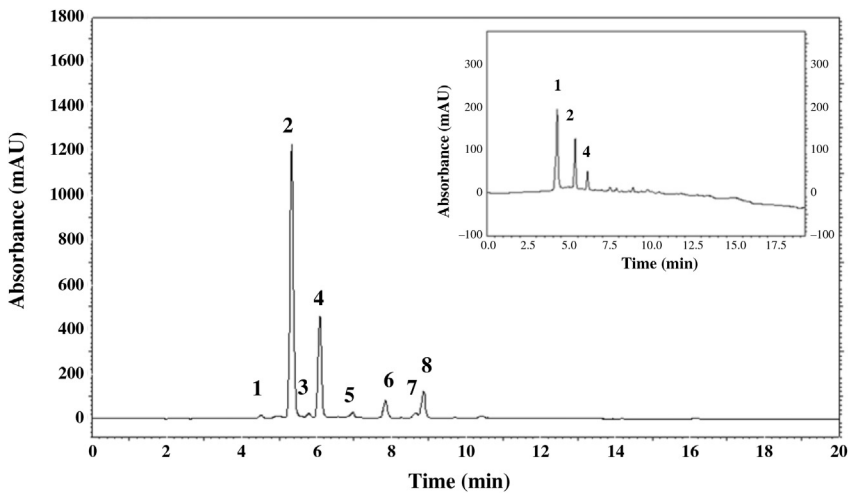
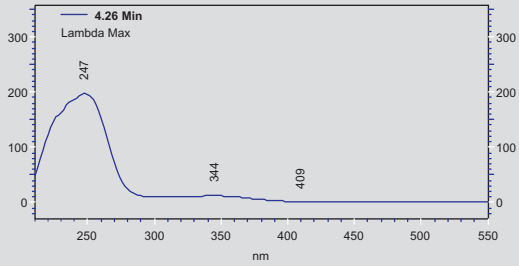
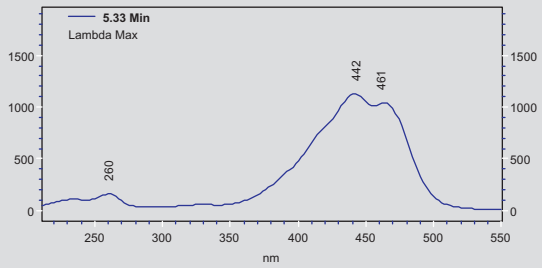
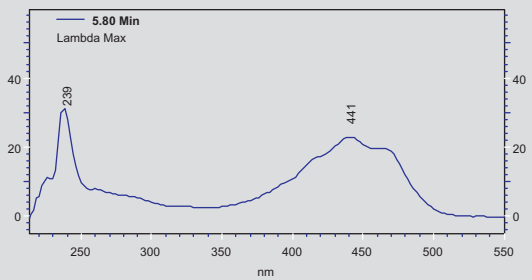
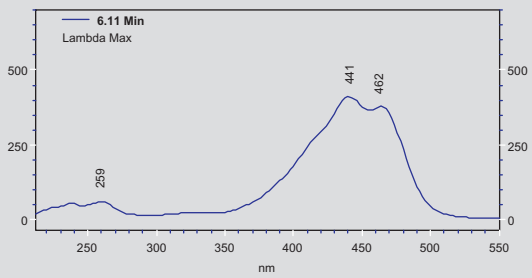
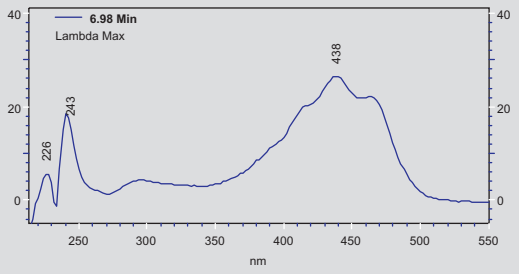


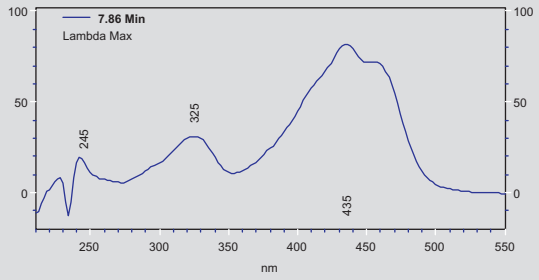
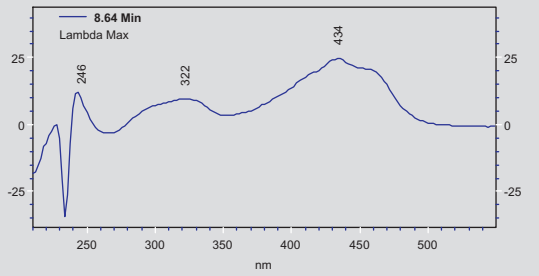
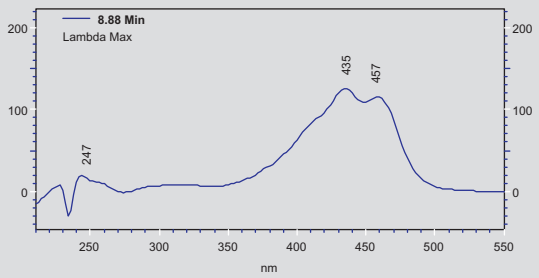
FIGURE 16.4 Representative RP-HPLC-DAD profile of an aqueous saffron extract at 440 nm; Insert, Representative RP-HPLC-DAD profile at 250 nm.
Peak assignment as described in Table 16.3. *Data from the saffron sample collection of LFCT.*

TABLE 16.7 UV-Vis spectra, λ_{max} values and major ions (m/z) of individual apocarotenoids of an aqueous saffron extract at 440 and 250 nm.[a]

Peak	UV-Vis spectra	λ_{max}	Major ions (m/z)	Nomenclature[b]
1		247	375 $[M\text{-}H + HCOOH]^-$	Picrocrocin
2		442, 461	329 $[\{(M + Na + H)\text{-}G\}\text{-}G]^+$, 508 $[(M + K)/2]^+$, 1015 $[M + K]^+$	*trans*-di-(β-D-gentiobiosyl) crocetin ester (*trans* − 4-GG)
3		441,461	–[c]	*trans*-(β-D-neapolitanosyl)-(β-D-glucosyl) crocetin ester (*trans* − 4-ng)
4		441,462	329 $[\{(M + Na + H)\text{-}g\}\text{-}G]^+$, 427 $[(M + K)/2]^+$, 837 $[M + Na]^+$,	*trans*-(β-D-gentiobiosyl)-(β-D-glucosyl) crocetin ester (*trans* − 3-Gg)
5		438,460	–[c]	*trans*-di-(β-D-glucosyl) crocetin ester (*trans* − 2-gg)

(*Continued*)

TABLE 16.7 (Continued)

Peak	UV-Vis spectra	λ_{max}	Major ions (m/z)	Nomenclature[b]
6		325, 435,456	329 [{(M + Na + H)-G}-G]$^+$, 508 [(M + K)/2]$^+$, 1015 [M + K]$^+$	*cis*-di-(β-D-gentiobiosyl) crocetin ester (*cis* − 4-GG)
7		322, 434,457	329 [{(M + Na + H)-g}-G]$^+$, 837 [M + Na]$^+$	*trans*-(β-D-gentiobiosyl)-(β-D-glucosyl) crocetin ester (*cis* − 3-Gg)
8		435,457	—[c]	*trans*-mono-(β-D-gentiobiosyl) crocetin ester (*trans* − 2-G)

[a]*Chromatographic conditions as described in detail by Kyriakoudi et al. (2012).*
[b]*G, gentiobiose; g, glucose; n, neapolitanose (nomenclature is in accordance with Carmona et al., 2006b).*
[c]*Not detected under the applied chromatographic conditions.*

well separated using common C18 columns with particle sizes greater than 3 μm. Taking into consideration all the above, UHPLC is a promising tool in saffron analysis. The adoption of a UHPLC rather than a HPLC protocol for the analysis of saffron apocarotenoids should be considered in a forthcoming revision of the ISO 3632-2 standard.

The main weakness regarding quantification of saffron apocarotenoids is the lack of commercially available standards or their high price. Regarding crocins, commercially available standards are either of uncertified purity (microscopy grade) or expensive and available only through a small number of companies [LGC Standards (United Kingdom), ALB Materials Inc (United States), Tauto Biotech Co. Ltd. (China), ChemFaces (China), Fluka (Germany)]. This renders their determination with accuracy very difficult. To overcome this limitation, quantification of crocetin sugar esters after separation was attempted using (1) artificial colorants (e.g., 4-nitroaniline) as internal standards (Lozano et al., 2000) and (2) values of the molecular coefficient absorbance (ε) of *trans*- and *cis*- crocetin esters at 440 nm (89,000 and 63,350, respectively) (Alonso et al., 2001; Caballero-Ortega et al., 2007; Carmona et al., 2006a). In this context, a mathematical equation (Eq. 16.2) based on the peak area of each crocetin sugar ester with respect to the total peak area at 440 nm and also on the molecular coefficient absorbance of each compound has been suggested by Sánchez et al. (2008) for the quantification of total and individual crocetin esters.

$$\%\ \text{of crocetin ester}_i\ \text{on dry basis} = \frac{[\text{MW}_i \times (E^{1\%}{}_{440\ \text{nm}}) \times A_i]}{10} \times \varepsilon \tag{16.2}$$

where MW_i is the molecular weight of the crocetin ester$_i$, $E^{1\%}{}_{440\ \text{nm}}$ is the coloring strength, A_i is the percentage peak area of the crocetin ester$_i$ at 440 nm, and ε is the molecular coefficient absorbance value.

The same research group (Del Campo et al., 2010) proposed a similar mathematical equation (Eq. 16.3) for the quantification of picrocrocin some years later.

$$\%\ \text{of picrocrocin on dry basis} = \frac{[\text{MW}_i \times (E^{1\%}{}_{250\ \text{nm}}) \times A_i]}{10} \times \varepsilon \tag{16.3}$$

where Mw_i stands for the molecular weight of the picrocrocin, $E^{1\%}{}_{250\ \text{nm}}$ is the flavor strength, A_i is the percentage peak area of picrocrocin at 250 nm, and ε is the molecular coefficient absorbance value of picrocrocin (10,100).

Some efforts to use laboratory isolated standards for the quantification of total and individual crocins as well as picrocrocin have been described in the literature. In particular, Sánchez et al. (2009) isolated picrocrocin with column chromatography via a C18 adsorbent. The chromatographic purity of the isolated picrocrocin was calculated as the percent of the total peak area at 250 nm and was found to be 96%. Kyriakoudi et al. (2012) quantified the total and the two main individual crocins of saffron (i.e., *trans* − 4-GG and *trans* − 3-Gg crocetin esters) after their separation with the aid of an appropriate calibration curve of in-house isolated *trans* − 4-GG crocetin ester. Its isolation was carried out by semipreparative RP-HPLC, its identity was confirmed by LC-ESI-MS analysis and by NMR spectroscopy, and its purity was found to be 98%. The isolation of picrocrocin using thin layer chromatography was reported by Cossignani et al. (2014). Koulakiotis et al. (2015) reported the development of an HPLC-DAD method for the quantification of *trans* − 4-GG and *trans* − 3-Gg crocetin ester as well as their *cis*-isomers and *trans* − 2-gg crocetin ester. The respective compounds were isolated using semipreparative HPLC, identified by UPLC-ESI-MS and MS/MS analysis, and their purity was found to be 98%. In addition, Tong et al. (2015b) also used in-house isolated standards for the quantification of *trans* − 4-GG and *trans* − 3-Gg crocetin esters, their *cis*-isomers, and *trans* − 2-G crocetin ester with purity of >95%. Kabiri et al. (2017) also used semipreparative HPLC to isolate *trans* − 4-GG and picrocrocin. The purity of the isolated compounds was found to be 97.2% and 91.1%, respectively. Moreover, a molecularly imprinted polymer using gentiobiose as a template (G-MIP) has been reported as the sorbent in a solid-phase extraction method for the selective extraction of *trans* − 4-GG from a methanolic saffron extract (Mohajeri et al., 2010). The authors observed that the G-MIP had significantly higher affinity to *trans* − 4-GG than other compounds and allowed its selective extraction with a recovery of ∼84%. For the quantification of safranal with HPLC, commercially available standards are usually used (e.g., Garcia-Rodriguez et al., 2017; Moras et al., 2018; Vahedi et al., 2018).

16.4.3 Gas chromatography-mass spectrometry (GC-MS)

Besides HPLC, GC coupled to MS has also been used over the years for the determination of safranal content, taking into consideration its volatile nature. Modifications regarding the characteristics of the chromatographic column, the oven temperature program, as well as the sample preparation procedures are numerous. An overview of the gas chromatographic conditions for the analysis of safranal and other volatiles in saffron up to today is given in Table 16.8. In particular, capillary chromatographic columns are used for the analysis of safranal and other saffron volatiles, with lengths that range from 30 up to 60 m, internal diameter from 0.22 to 0.32 mm, and particle size from 0.25 to 0.5 μm. Various techniques have been reported in the literature for the extraction or isolation of volatile compounds from saffron including HD, MSDE, TD, SPME, UAE, etc. Regarding safranal, as in the case of HPLC, its quantification is typically carried out with the aid of appropriate calibration curves of commercially available standard of high purity (e.g., Anastasaki et al., 2009; Kanakis et al., 2004; Liu et al., 2018).

16.4.4 Electronic nose technique

Electronic nose (e-nose) is a rapid and powerful technique that does not require any special sample preparation and allows the determination of the volatile profile of a product as a whole (Gliszczyńska-Świgło and Chmielewski, 2017). In particular, e-noses are devices that consist of an array of various types of sensors, which are able to mimic the sense of smell. The sensors are treated with various odor-sensitive chemical compounds, each one of them yielding a specific fingerprint, the so called smellprint. These patterns are then used to create a database that can be used in order to identify unknown odors (Peris and Escuder-Gilabert, 2016). Taking into consideration that the volatile profile of a product

TABLE 16.8 Overview of the extraction techniques and the chromatographic conditions for the analysis of safranal and other volatiles in saffron using GC-MS.

Aim of the study	Extraction technique	Chromatographic column	Chromatographic conditions	Reference
Qualitative determination of volatile compounds of saffron and quantitative determination of safranal.	Microsimultaneous hydrodistillation extraction, ultrasound-assisted extraction	HP-5 ms capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness)	Carrier gas: Helium Flow rate: 1 mL min^{-1} Column temperature: 50°C (3 min), 3°C/min to 180°C, 15°C/min to 250°C (5 min) Injection volume: 1 μL (splitless mode)	Kanakis et al. (2004)
Analysis of the volatile profile of saffron	Thermal desorption	BP21 capillary column (50 m, 0.22 mm i.d., 0.25 μm film thickness)	Carrier gas: Helium Column temperature: 100°C (5 min), 18°C/min 210°C (15 min)	Carmona et al. (2006a)
Study of the seasonal variation of saffron based on aroma constituents	Solid-phase microextraction	ZB-5 MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness)	Carrier gas: Helium Flow rate: 0.8 mL min^{-1} Column temperature: 40°C (2 min), 8°C/min to 250°C (splitless mode)	D'Auria et al. (2006)
Quantitative structure-retention relationship study of saffron aroma compounds based on the projection pursuit regression method	Solid-phase microextraction	ZB-5 MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness)	Carrier gas: Helium Flow rate: 0.8 mL min^{-1} Column temperature: 40°C (2 min), 8°C/min to 250°C (splitless mode)	Du et al. (2008)
Geographical differentiation of saffron	Ultrasound-assisted extraction	HP-5 ms capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness)	Carrier gas: Helium Flow rate: 1 mL min^{-1} Column temperature: 50°C (3 min), 3°C/min to 180°C, 15°C/min to 250°C (5 min) Injection volume: 1 μL (splitless mode)	Anastasaki et al. (2009)
Characterization of volatile compounds of Iranian saffron	Ultrasound-assisted extraction	HP-5 ms fused silica capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness)	Carrier gas: Helium Flow rate: 1 mL min^{-1} Column temperature: 60°C (1 min), 5°C/min to 200°C (1 min), 20°C/min to 280°C (21 min) Injection volume: 1 μL (split ratio 1:5)	Jalali-Heravi et al. (2009)

(*Continued*)

TABLE 16.8 (Continued)

Aim of the study	Extraction technique	Chromatographic column	Chromatographic conditions	Reference
Determination of safranal for the quality control of saffron	Ultrasound-assisted extraction	HP-5 ms capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness)	Carrier gas: Helium	Maggi et al. (2011)
			Flow rate: 1 mL min^{-1}	
			Column temperature: 50°C (3 min), 3°C/min to 180°C, 15°C/min to 250°C, 250°C (5 min)	
			Injection volume: 1 μL (splitless mode)	
Determination of aroma compounds of Iranian saffron	Liquid–liquid extraction (LLE), solid-phase extraction (SPE), simultaneous distillation/extraction (SDE), solvent-assisted flavor extraction (SAFE)	DB-Wax capillary column (30 m × 0.25 mm i.d., 0.5 μm thickness)	Carrier gas: Helium	Amanpour et al. (2015)
			Flow rate: 1.5 mL min^{-1}	
			Column temperature: 5°C/min to 200°C, 8°C/min to 260°C, 260°C (5 min)	
			Injection volume: 3 μL (splitless mode)	
Geographical discrimination and commercial categorization of saffron	Steam distillation	Optima-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm)	Carrier gas: Helium	Liu et al. (2018)
			Flow rate: 1.5 mL min^{-1}	
			Column temperature: 5°C/min from 80°C to 150°C, 25°C/min to 250°C	
			(split ratio 4:1)	

is specific and allows its discrimination from adulterated ones, e-nose is a very promising tool not only for monitoring food quality but authenticity as well (Gliszczyńska-Świgło and Chmielewski, 2017). The use of e-nose has been already reported for the quality assessment or authenticity control of various products such as coffee (e.g., Buratti et al., 2015; Severini et al., 2015), milk (e.g., Yu et al., 2007), olive oil (e.g., Cerrato Oliveros et al., 2002), honey (e.g., Benedetti et al., 2004; Dymerski et al., 2014), tea (e.g., Bhattacharyya et al., 2008; Dutta et al., 2003) etc. However, the applications of the e-nose for saffron samples are limited. Carmona et al. (2006a) were the first to use an e-nose based on 27 metal oxide semiconductor (MOS) gas sensors coupled to principal component analysis (PCA) in order to discriminate saffron samples with different geographic origins (i.e., Iran, Morocco, Greece, Spain). In this way, discrimination with 90% confidence was achieved. An e-nose based on 6 MOS gas sensors coupled to PCA has also been used by Heidarbeigi et al. (2015) to detect adulteration in saffron samples. In particular, the authors examined the volatile fingerprint of pure saffron, saffron with yellow stamens, and safflower. This e-nose system allowed discrimination among pure and adulterated saffron samples at adulteration above 10%. Kiani et al. (2016) reported the use of a portable e-nose based on 10 MOS gas sensors coupled to a multilayer perceptron artificial neural network for the discrimination of saffron samples from various regions of Iran. The authors suggested that the developed system is inexpensive, nondestructive, and allows discrimination with a 100% success rate. The same research group (Kiani et al., 2017) used a similar e-nose system based on gas sensors for the quantitative characterization of safranal and other saffron volatile compounds. For the prediction of the $E^{1\%}_{330\ nm}$ values, an unsupervised pattern recognition model was used. The authors suggested that the e-nose is a nondestructive technique appropriate for the analysis of safranal content in saffron samples without the need for prior extraction.

16.5 Applications of saffron bioactive ingredients: from prehistory up to 21st century

Saffron has been used for various purposes such as spice, medicine, dye, and perfume by different nations throughout history. Its particular significance is illustrated in the famous fresco fragments of the Bronze Age found in Thira (Santorini, Greece) that depict a female goddess figure surrounded by a young girl and a monkey in a landscape filled with *Crocus* plants. More specifically, the young girl seems to gather *crocus* flowers in a basket, whereas the monkey extends some stigmas toward the goddess. Moreover, the lower part of the fresco shows a young woman who seems to use saffron for the treatment of her bleeding foot. Analysis of the fresco (Ferrence and Bendersky, 2004) suggests that not only a description of the saffron production line is illustrated, but also its medicinal and healing properties are exhibited. The first report regarding the use of saffron as an aid in dyspnea, urination, menstrual disorders, and childbirth is dated to the Bronze Age. Subsequently, Hippocrates, Erasistratus, Diokles, and Dioscorides used saffron in different historical periods for various medicinal purposes such as treatment of eye diseases (e.g., cataract) and toothache, as an aphrodisiac and emmenagogue, as well as for its styptic and soothing properties. The use of saffron for cosmetic purposes is also reported since ancient times. Saffron was also used as a dye for expensive royal fabrics such as silk, cotton, and wool.

Up to today, saffron is highly valued in the food industry for the unique color, taste, and aroma that it imparts to food preparations. It is used as a spice, either in filaments or in powder form, in many traditional European dishes such as the Spanish paella, the French soup bouillabaisse, the risotto alla Milanese, as well as in many Greek bakery products found in the Cyclades and Dodecanese islands. Saffron consumption is more common in Iran, where $\sim$90% of the world's total annual saffron production originates from. Moreover, countries of the Arabian Peninsula as well as Egypt, consume high quantities of saffron throughout the year even though most of them do not produce this spice but import it either in bulk form or packed in small quantities. Apart from its use in food preparations, saffron is used in Arabic coffee, the most popular and common drink in these countries. Saffron is also used in the preparation of distinct bakery products meant to be consumed during a certain time period [e.g., "saffron buns" that are traditionally prepared on St. Lucia Day celebrated during Christmas (13th of December) in Sweden or Labrokouloura that are consumed during the Easter period in Astypalaia (Cyclades, Greece)] (Kyriakoudi and Tsimidou, 2018a; Ordoudi and Tsimidou, 2004).

In the following sections, emphasis is given only on applications of saffron for which scientific evidence exists.

16.5.1 Food industry

Dairy products: Due to its sensorial attributes, saffron finds applications in dairy products and more specifically in certain types of cheese such as the Piacentinu Ennese, a sheep's milk hard Protected Destination of Origin (PDO) cheese from Sicily, the semihard cheese Pecorino allo Zafferano from Italy made from pasteurized sheep's milk, as well as an Austrian cow's milk cheese called Lüneberg. Effects of the concentration of saffron on the chemical, sensorial, textural, and microbiological characteristics of a pressed sheep's milk cheese during ripening have been examined by Licón et al. (2012). The authors found that cheeses containing saffron were more yellow, firmer, more elastic, and microbiologically more stable compared to the ones without saffron. The research group of Polysiou (Aktypis et al., 2018) supplemented a fresh ovine cheese with saffron and examined its microbiological, physicochemical, antioxidant, color, and sensory characteristics. The authors found that even though no significant changes in the physicochemical properties of the cheese were observed, its antimicrobial and antioxidant activity was enhanced due to the presence of saffron.

Cereal products: Saffron has been added to the formulation of fresh pasta, which after cooking was evaluated in terms of textural, physicochemical, and sensory properties (Armellini et al., 2018). The authors found that the addition of saffron increased the acceptability of the saffron enriched pasta in terms of visual aspect, color, aroma, taste, chewiness, hardness, gumminess, and overall acceptability. Moreover, the authors claim that the use of saffron enhanced the antioxidant activity of the final product based on the DPPH and ABTS assays.

Desserts: Almodóvar et al. (2018) used a commercial saffron extract with standardized (by HPLC) amount of crocins to prepare two cold saffron flavored desserts, namely white chocolate soup with yogurt and saffron as well as a cheese cake with orange jam and saffron. The authors suggested that the use of the commercial standardized saffron extract allowed more precise dosage control resulting in increased consumer acceptability compared to the use of saffron stigmas.

Alcoholic and nonalcoholic beverages: Extracts of saffron are also used in alcoholic and nonalcoholic beverages such as Strega, Benedictine, vermouth, and other bitter drinks, as well as in herbal teas (e.g., http://www.krocuskozanis.com/). Saffron bitterness is a limiting factor for consumer acceptance in nonalcoholic beverages (Chrysanthou et al., 2016).

Ordoudi et al. (2015) examined a variety of infusions prepared from commercially available herbal tea blends with saffron in terms of chemical characterization as well as determination of the bioaccessibility of crocins. Results showed that the copresence of strong phenolic antioxidants from the other herbs enhanced the bioaccessibility of crocins, which was determined using an in vitro gastrointestinal digestion model. The authors suggested that the findings of this study could be exploited in the design of novel saffron-based functional beverages. It is worth mentioning that to the best of our knowledge, information concerning the bioaccessibility and bioavailability of saffron apocarotenoids is rather limited (Asai et al., 2005; Kyriakoudi et al., 2013, 2015a; Lautenschläger et al., 2015; Ordoudi et al., 2015). Such data are of particular importance in order to establish oral intake limits and recommendations, since only a certain amount of a bioactive compound is actually absorbed in the blood circulation and reaches the target tissues after ingestion.

In general, a critical factor for the use of saffron apocarotenoids not only in food but also in pharmaceutical and cosmetics applications, as shown below, is their stability. Being unsaturated compounds, saffron apocarotenoids are prone to degradation. Their stability has been examined under the influence of various parameters such as temperature, pH, water activity, oxygen, and light (Alonso et al., 1990; Tsimidou and Biliaderis, 1997; Tsimidou and Tsatsaroni, 1993). There is a growing interest of the scientific community in the protection of these precious apocarotenoids via encapsulation. In particular, saffron extracts as well as isolated crocetin sugar esters and crocetin have so far been encapsulated in different matrices such as polyvinylpyrrolidone, pullulan, maltodextrin, gum Arabic, deoxycholic acid, etc. using various techniques such as freeze drying (Jafari et al., 2016; Chranioti et al., 2015; Selim et al., 2000), spray drying (Esfanjani et al., 2015; Kyriakoudi and Tsimidou, 2018c; Rajabi et al., 2015; Zhou et al., 2013), and inclusion complexation (Kyriakoudi and Tsimidou, 2015).

16.5.2 Pharmaceutical industry

Even though saffron is mainly used in the food industry as a spice, a variety of pharmacological properties (e.g., anticarcinogenic, antioxidant, neuroprotective, cardioprotective, antiinflammatory, antidiabetic, etc.) have been attributed either to saffron extracts or to its major apocarotenoids. This is the reason why saffron has been characterized as a functional spice (Kyriakoudi et al., 2015b). There are numerous book chapters and review articles that summarize or critically focus on its most important biological actions, which are examined with either in vivo, ex vivo, or in vitro studies (Alavizadeh and Hosseinzadeh, 2014; Bhandari, 2015; Bukhari et al., 2018; Finley and Gao, 2017; Giaccio, 2004; Leone et al., 2018; Pitsikas, 2016; Singla and Bhat, 2011; Ulbricht et al., 2011). From the wide spectrum of biological actions of saffron, in the present work, emphasis is given to the most promising applications against critical chronic diseases such as Alzheimer's disease, opthalmological diseases, as well as depression and anxiety.

Alzheimer's disease: Many researchers have focused on the use of saffron extracts or its bioactive apocarotenoids for the potential treatment of Alzheimer's disease, which is the most common form of dementia among people over the age of 65 worldwide (Shahi et al., 2016). Alzheimer's is an irreversible progressive neurodegenerative disease characterized by behavioral disturbances, cognitive deterioration, and functional disability (Adalier and Parker, 2016). In particular, consumption of 30 mg day^{-1} saffron in two doses of 15 mg each for a period of 22 weeks by 54 adults elicited similar effects as consumption of 10 mg day^{-1} donepezil (5 mg twice per day) in treatment of mild to moderate Alzheimer's disease (Akhondzadeh et al., 2010a). In another study, the same research group (Akhondzadeh et al., 2010b) carried out a randomized, double-blind, placebo-controlled trial and showed that consumption of 30 mg day^{-1} (15 mg twice/day) saffron for 16 weeks by 46 patients with probable Alzheimer's disease resulted in better outcomes compared to placebo. Based on these results, the authors suggested that saffron is safe and effective in the treatment of mild to moderate Alzheimer's disease, at least in the short-term. Another randomized, double-blind clinical trial was conducted in 68 patients with moderate to severe Alzheimer's disease (Farokhnia et al., 2014). Patients received saffron extract (30 mg day^{-1}) or memantine (20 mg day^{-1}) for 12 months. The study showed that the 1-year saffron administration exhibited comparable results to memantine in terms of reducing cognitive decline in patients with moderate to severe Alzheimer's disease. Tiribuzi et al. (2017) investigated in vitro the ability of *trans*-crocetin to restore the reduced ability of monocytes of Alzheimer's disease patients to degrade amyloid-$\beta_{(1-42)}$ ($A\beta_{42}$). This protein is the main component of the amyloid plaques found in the brains of Alzheimer patients. *Trans*-crocetin (5 μM) was found to enhance $A\beta_{42}$ degradation in monocytes after incubation for 24 hours. The authors suggested that *trans*-crocetin could be potentially used as a new antiamyloid agent. In another study (Finley and Gao, 2017), the multifunctional protective activities of crocins in brain cells as well as its potential to improve learning and memory are presented. Based on scientific evidence, the authors suggested that crocins could be a promising agent for the prevention or treatment of Alzheimer's disease.

Visual impairment: Incubation of photoreceptors in retinal cell cultures with *trans* − 4-GG (30 μM) was found to increase their viability against blue and white light induced death (light stress) (Laabich et al., 2006). Based on these results, the authors suggested that *trans* − 4-GG as well as other crocins could be used as possible therapeutic agents for degenerative diseases of the retina. In a similar in vivo study, crocetin (100 mg kg^{-1}) was found to inhibit photoreceptor degeneration and retinal dysfunction, indicating that crocetin has protective effects against retinal damage (Yamauchi et al., 2011). In an in vivo study in rats, 8 weeks of administration of crocins by intraperitoneal injection showed beneficial effects on prevention of diabetic cataracts (Bahmani et al., 2016). This effect was attributed by the authors to fact that crocins, as antioxidant and hypoglycemic agents, decreased protein glycation and prevented cataract formation.

Depression and anxiety: Depression and anxiety are the most common phycological disorders. Saffron has been reported to relieve their symptoms and even treat them. In particular, Tabeshpour et al. (2017) examined the effects of saffron on postpartum depression with a double-blind and placebo-controlled clinical study. In particular, 60 breastfeeding mothers with mild to moderate depression were randomly divided into two groups. Half women received 30 mg day^{-1} in the form of a capsule and the other half received an equivalent dose of placebo for 8 weeks. The results of this study showed that saffron was more effective than the placebo in treating postpartum depression in new mothers. The authors suggested that saffron could be used as an alternative medication for reduction of the symptoms of postpartum depression. Another double-blind and placebo-controlled clinical trial examined the effects of an alcoholic saffron extract on mild-to-moderate comorbid depression-anxiety and sleep quality of 54 patients (Milajerdi et al., 2018). Saffron (30 mg day^{-1}) or placebo capsules of similar dose were administered to the patients for 8 weeks. The authors found that saffron relieved the symptoms of mild to moderate depression, anxiety, and sleep disturbances. Lopresti and Drummond (2014) carried out a randomized, double-blind, placebo-controlled trial to examine the effects of saffron on anxiety and depressive symptoms of 80 patients ages of 12−16. Half of them were given a tablet containing 14 mg of a standardized saffron extract, and the other half was given a placebo tablet. The authors suggested that after 8 weeks saffron was found to relieve the anxiety and depressive systems.

16.5.3 Cosmetics and other sectors

Taking into account all of the pharmacological actions of saffron, a renewed interest in its exploitation in various types of cosmetics has come about. However, to the best of our knowledge, the relevant research articles found in the literature are still limited. More specifically, Fekrat (2004) reported the use of an ethanolic saffron extract in sun protection creams, body lotions, shampoos, hair care products, moisturizing creams, as well as liquid soaps. Some years later Vyas et al. (2010) used a concentrated saffron dry extract as an ingredient in a cream, a lotion, and a face powder. The properties of these formulations were examined with patch tests on various subjects ages 18−28. The results showed increased shining and lightening of the skin compared to a control product. The authors attributed this observation to the presence of crocins. Taking into consideration that the process of ageing involves not only reactive oxygen species but also inflammatory mediators, saffron could be potentially used against ageing. Toward this end, a group of researchers from L'Oréal Company (Fagot et al., 2018) examined the antioxidant and antiinflammatory effects of crocin on normal human keratinocytes and fibroblasts in vitro. The researchers found that the production of proinflammatory mediators (e.g., IL-6, IL-8, PGE_2, TNFα) is inhibited in the presence of *trans* − 4-GG crocetin ester, whereas antioxidant defense is increased. Based on their findings, the authors suggested that *trans* − 4-GG crocetin ester could be exploited as a promising skin ageing prevention cosmetic agent. The UV protective effects of saffron have been also highlighted. In particular, Zarkogianni and Nikolaidis (2016) prepared oil-in-water emulsions containing saffron, which were examined for their antisolar activity by measuring their sun protection factor (SPF). The authors suggested that saffron could be used as a natural UV-absorbing agent in sunscreen products. In the same context, Ntohogian et al. (2018) prepared sunscreen oil-in-water emulsions based on chitosan nanoparticles with saffron. The authors concluded that the prepared chitosan-saffron nanoparticles showed thermal and color stability for up to 90 days as well as minimal sunscreen protection (SPF 2.15−4.85) compared to blank emulsion (SPF = 1.00).

Even though there are only a few relevant published research articles, numerous cosmetic products containing saffron are already commercially available [e.g., a serum that claims to correct all signs of aging (https://www.korresusa.com/skincare/golden-krocus-ageless-saffron-elixir) and face creams to promote skin glow and nourishment (https://www.stylecraze.com/reviews/lever-ayush-natural-fairness-saffron-face-cream/#gref, https://www.alibaba.com/product-detail/Saffron-Whitening-Cream-100g-Paraben-Free_50018430835.html)].

Applications regarding the use of saffron as a natural dye for textiles have also been reported in the literature. However, such reports are limited due to saffron's high price. In particular, Tsatsaroni and Eleftheriadis (1994)

used aqueous saffron extracts to dye cotton and wool, which constitute suitable substrates for natural dyes. Dyeing of cotton and wool by a methanolic saffron extract has also been reported by Liakopoulou-Kyriakides et al. (1998) after enzymatic treatment with α-amylase, which is said to minimize wool shrinkage and improve the dyeing properties.

16.6 Conclusion

Evidence in this chapter demonstrates the continuing interest of scientists, mainly from saffron producing countries, to update and modernize means of extraction and analysis of saffron bioactive compounds. The interest in bioactivity studies and applications is rather universal, though once again most of the studies come from scientists in saffron producing countries. Scientific results support the multidisciplinary interest in the exploitation of the properties and uses of this most precious commodity.

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