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REVIEW

Novel Extraction Techniques: An Effective Way to Retrieve the Bioactive Compounds from Saffron (*Crocus Sativus*)

Abdul Rahaman[a,b], Ankita Kumari[c], Muhammad Adil Farooq[d], Xin-An Zeng[a,b], Sadia Hassan[e], Ibrahim Khalifa[f], Rana Muhammad Aadil[g], Muhammad Farhan Jahangir Chughtai[d], Adnan Khaliq[d], Nabeel Ahmad[h], and Mohd Anas Wajid[i]

[a]School of Food Science and Engineering, South China University of Technology, Guangzhou, China; [b]Overseas Expertise Introduction Centre for Discipline Innovation of Food Nutrition and Human Health (111 Centre), Guangzhou, China; [c]School of Medicine, South China University of Technology, Guangzhou, China; [d]Department of Food Science and Technology, Faculty of Engineering and Technology, Khwaja Fareed University Engineering and Information Technology, Rahimyar, Pakistan; [e]Department of Nutritional Sciences, Faculty of Science and Technology, Government College Women University, Faisalabad, Pakistan; [f]Food Technology Department, Faculty of Agriculture, Benha University, Egypt; [g]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan; [h]School of Biotechnology, Iftm University, Moradabad, India; [i]Department of Computer Science, Aligarh Muslim University, Aligarh, India

ABSTRACT
Saffron is considered as most prominent food product used in medicine, flavouring and colouring substances due to its bioactive compounds that have health-boosting characteristics. Saffron has been cultivated to get flower and obtain metabolites from dried stigma. The main phytochemicals found in saffron are picrocrocin, safranal, kaempferol, phenol, flavonoid, delphinidin and crocetin which shows good bioactivity and antioxidant capacity. However, the quantity and quality of bioactive compounds vary with extraction techniques used. Extraction of bioactive compounds from saffron requires continued searching for extraction techniques that are ecologically and economically feasible. Conventional extraction methods are time-consuming and require a huge quantity of solvents. Therefore, novel extraction techniques have been developed to extract bioactive compounds from saffron, which can decrease the extraction time as well as solvent consumption to improve the extract quality and extraction yield. This review discusses the development of promising techniques for the extraction bioactive compounds from saffron, which could be utilized in the value-added products (food, pharmaceutical, and cosmetic industries).

KEYWORDS
Saffron; extraction techniques; bioactive compounds; conventional techniques; novel techniques

Introduction

Medicinal plants and herbs are the sources of bioactive compounds, which play a major role in the maintenance of human health.[1] Among these, saffron (*Crocus sativus*) is one of the traditional herbs, which is majorly grown in Iran, Italy, India, Greece, and Spain. While it is also cultivated with minimal production in Australia, Afghanistan, Azerbaijan, China, Egypt, France, Israel, Iraq, Japan, Mexico, Morocco, Pakistan, Switzerland, Turkey, Tasmania, and UAE.[2] The main structural components of saffron include corm, flower, and stigma that are responsible for flavor, color, and taste (Fig. 1). It is

CONTACT Xin-An Zeng xazeng@scut.edu.cn School of Food Science and Engineering, South China University of Technology, Guangzhou, China; Overseas Expertise Introduction Centre for Discipline Innovation of Food Nutrition and Human Health (111 Centre), Guangzhou, China; Sadia Hassan sadiahassan88@gmail.com Department of Nutritional Sciences, Faculty of Science and Technology, Government College Women University, Faisalabad, Pakistan.

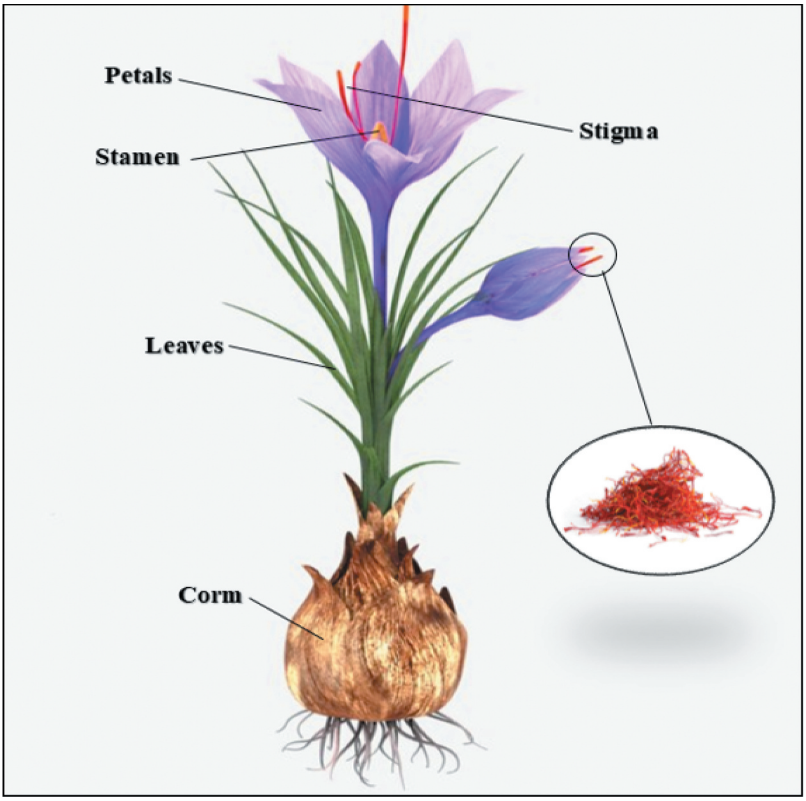


Figure 1. Structural components of the saffron plant.

also the source of natural additives and widely used to produce nutraceutical food products.[3,4] Bioactive compounds in saffron attribute to a range of therapeutic properties including antioxidant, antidepressant, anticarcinogenic, anti-inflammatory, and antitumor properties.[5]

Saffron (*Crocus sativus*) contains enough amounts of therapeutic agents i.e., crocetin, crocins, picrocrocin, safranal, minerals, essential oils, and a small amount of vitamins B_1 and B_2.[6] The quantity and quality of these bioactive constituents from saffron are greatly influenced by the extraction process. The extraction is a critical process for the isolation and fractionation of bioactive compounds. Several extraction techniques either alone or in combination with other methods have been used for the separation of major bioactive compounds from saffron.[4] So far, extraction and purification techniques both traditional (solvent extraction and maceration) and novel (microwave, sonication, supercritical fluids, emulsion liquid extraction, pulsed electric field) have been used for the separation and purification of bioactive compounds from different portions of saffron.[7]

It is very essential to select suitable extraction parameters like solvent, temperature, and time of extraction, because these variables can affect the quantity and quality of saffron extract as saffron, contains both lipophilic (safranal) and hydrophilic (crocins and picrocrocin) compounds. In this regard alcohol and water are considered proper solvents for picrocrocin and crocins respectively, while for safranal, petroleum ether and diethyl ether are considered as best extraction mediums. Researchers have examined the influence of different parameters on saffron extraction efficiency such as extraction method, temperature, time, solvent, sample to solvent ratio, sample physical state, etc.[8–10]

An effective extraction method should be safe, efficient, economically reasonable, and environment-friendly having fewer impurities.[11] Conventional extraction methods have several drawbacks such as degradation (hydrolytic and thermal) of compounds, long extraction duration, more energy utilization, and less productivity.[7] Due to such limitations, researchers have considered replacing the conventional techniques with novel methods such as supercritical fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction, etc.[12] Novel extraction techniques are based on "green" models having optimum utilization of solvents and energy.[13] Some of the most promising novel techniques of saffron extraction will be briefly highlighted in this review. The examples highlighted provide only a small snapshot of recent advances in saffron extraction technologies along with the advantages, limitations of each technique.

Chemical composition of saffron

Saffron's flowers are principally comprised of anther, corm, stigma, and tepal. Chemical analysis of saffron stigmas has revealed the presence of several components including fats, minerals, sugars, vitamins, and other secondary metabolites such as anthocyanins, carotenoids, flavonoids, and terpenes (Table 1).[2] Among these, stigma is a valuable source of bioactive constituents including anthocyanins, flavonoids, pigments, volatile aromatic essences, and vitamins.[22] Chemical analysis of saffron stigmas has revealed the presence of over 150 volatile and non-volatile compounds.[23] The saffron contains three major compounds including safranal, picrocrocin, and crocetin esters. It also contains carbohydrates, proteins, raw fiber, fats, minerals, anthocyanins, carotenoids, flavonoids, and several other components which are beneficial to human health.[22]

The most abundant bioactive components in saffron are crocins, crocetin, kaempferol, lycopene, picrocrocin, quercetin, safranal, α-carotene, β-carotene, and zeaxanthin. Crocin, crocetin, and picrocrocin present in stigma attribute to sensory characteristics of saffron like aroma, color, and taste.[6] Crocins (mainly α-crocin) have several applications in the food industries due to their ability to produce golden red color i.e. stable water-soluble. Crocins play an important role as anti-Alzheimer, anti-schizophrenia, and memory booster due to their antioxidant properties. Crocetin and its derivatives are also used as a colorant and have different health-promoting therapeutic effects in the field of the pharmaceutical and food industry.[22,24] Picrocrocin is the colorless portion of stigma and responsible for bitter flavor and aroma. The isophorone and safranal are unstable volatile compounds present in the stigma that contributes to the aroma of saffron. Isophorone and safranal are known for their anticonvulsant and antidepressant functions.[23]

Table 1. Nutritional composition of saffron indicating its chemical constituents.

Nutritional components	Concentration	Reference
Moisture	10–14%	Rahimi; Shahi et al.[14,15]
Ash	6–7%	
Crude fat	5–8%	
Crude protein	12–14%	
Crude fiber	4–5%	
Reducing sugars	20%	
Gums and dextrins	9–10%	
Starch	6–7%	
Pentoses	6–7%	
Volatile oil	0.6–0.9%	Christodoulou et al.[16]
Palmitic acid	16.2 g/100 g	Lim[17]
Linoleic acid	28.5 g/100 g	
Linolenic acid	21.0 g/100 g	
Phosphorus	3270 μg/g	D'Archivio and Maggi; Jadouali et al.[18,19]
Magnesium	1300–1350 μg/g	
Calcium	862–1070 μg/g	
Iron	92–110 μg/g	
Potassium	10.70–14.86 μg/g	
Sodium	42–100 μg/g	
Vitamin A	27 μg	Lim; Hashemi et al.; Karimi et al.[17,20,21]
Vitamin B1	0.115 mg	
Vitamin B2	5–13 μg	
Vitamin B6	1.01 mg	
Vitamin C	80 mg	

Hence, all bioactive compounds present in saffron have health-promoting effects in anticancer, antidepression, antioxidant, and antitumor activities; decreased anxiety and insomnia; and enhanced memory.[25] Despite the biological effects and chemical properties of major bioactive compounds present in saffron, in traditional foods, saffron is used for its color, flavor, and aroma while it is not only used as a spice, it has long been known as a medicinal plant due to its therapeutic potential. Though, the introduction of synthetic chemistry-based drugs affected the medicinal and pharmaceutical applications of saffron.[26] The major bioactive ingredients of saffron along with their chemical properties are given in Table 2.

Compounds contributing the color, taste, and aroma

Saffron exhibits deep and strong color due to the compound of carotenoids. Firstly in 1818, Aschoff isolated the key carotenoid of saffron known as crocin. The molecular formula and structure of crocin were recognized by Karrer and Salomon[40] while its glycosidic nature was analyzed by Decker.[41] After the glycosylation process in crocin, a dicarboxylic carotenoid develops that is known as crocetin. In an aqueous extract of saffron, the crocins at high concentrations pool together and go through the aggregation Naess et al.,[42] Pfander and Wittwer[43] separated six crocins, whereas Speranza et al.[44] analyzed the cis- and trans-isomers of crocins by HPLC and UV-V spectrophotometry. Furthermore, Carmona et al.[45] identified four more crocins. In spice, the number of glycosidic esters of crocetin differs according to quality, but it may attain high percentages from 25 to 35% based on the weight in dried saffron. The bioactive properties of saffron are due to the presence of higher levels of carotenoids.[46]

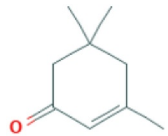
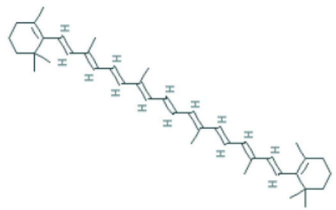
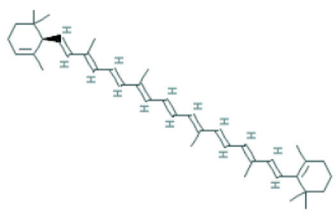
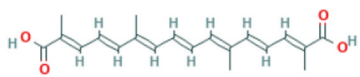
Picrocrocin has a bitter taste, and this chemical constituent is most responsible for the taste of saffron. The dry matter of saffron contains about 26% of the picrocrocin constitutes.[47] It has been analyzed that in the *Crocus genus, C. sativus* is the only edible part of this genus. Picrocrocin is also the precursor for producing safranal, Sánchez et al. determined the degradation kinetics of picrocrocin in saffron extract (aqueous) through thermal processing (5 to 70°C). They also suggested that the picrocrocin was more stable as compared to crocetin esters.[48] In saffron, over 40 compounds have been recognized, which are associated with aroma while safranal is a major compound.[45] The Azafránde LaMancha is the world's most popular saffron because it contains about 65% safranal of the total content of aroma compounds.[26]

Safranal is also produced from picrocrocin through the process of dehydration and hydrolysis during the processing and storage of saffron. The process of dehydration is not only important to preserve the saffron but also responsible for releasing safranal from picrocrocin through enzymatic reactions. According to ISO standard 3236, it was evaluated by determining the absorbance of saffron extract (aqueous) at 330 nm.[26] But this technique is not considered much efficient because at 330 nm it also absorbs the isomers (cis) resulted from crocetin.[49] Other techniques used for analysis include gas chromatography to determine all volatile compounds[50] and HPLC to the quantification of the crocetin esters, picrocrocin, and safranal independently.[46]

Extraction techniques of saffron bioactive constituents

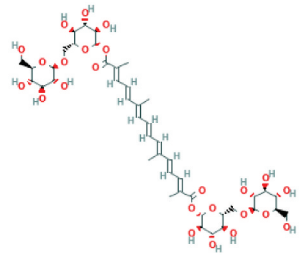
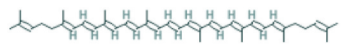
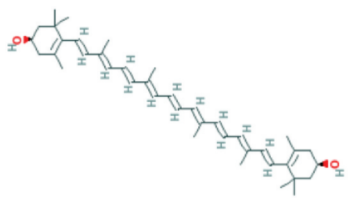
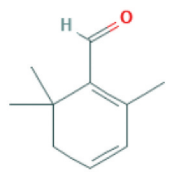
Scientists in the phytochemical field have tracked a variety of components in saffron stigmas. The researchers revealed that three groups of compounds responsible for color, taste, and aroma are present in dried stigma.[7,51] Extraction of various bioactive constituents from different parts of the saffron plant is very essential regarding their further applications in the development of nutraceutical products and other health-promoting foods.[52] Several techniques have been developed to extract saffron bioactive components with the highest quality while obtaining the highest extraction efficiency.[7] As compared to traditional techniques, it is affirmed that by using the proper extraction method the targeted bioactive components can be extracted more efficiently in terms of extraction time and solvent volume.[53]

Table 2. Chemical properties of major saaffron bioactive ingredients .[7].

Family	Bioactive constituent	Chemical formula	Molecular structure	Sensory effect	Biological activity	Reference
Isophorones	Isophorone	$C_9H_{14}O$		Aroma	Antioxidant	Zarghami and Heinz[27]
Carotenoids Carotenoids	β-carotene	$C_{40}H5_6$		Color	Neuroprotective	Ochiai et al.[28]
	α-carotene	$C_{40}H_{56}$		Color	Antioxidant	Bhargava[29]
	Crocetin	$C_{20}H_{24}O_4$		Color	Antifatigue and neuroprotective	Nam et al.; Mizuma et al.[30,31]

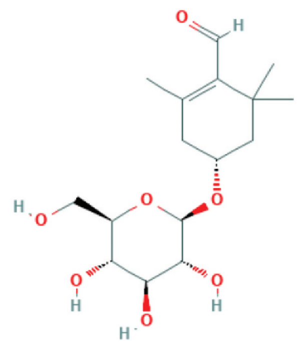
(*Continued*)

Table 2. (Continued).

Family	Bioactive constituent	Chemical formula	Molecular structure	Sensory effect	Biological activity	Reference
	Crocins	$C_{44}H_{64}O_{24}$		Color	Anti-Alzheimer and antischizophrenia.	Mousavi et al.; Finley and Gao[32,33]
	Lycopene	$C_{40}H_{56}$		Color	Antitumor and cardiovascular protection	Kamalipour and Akhondzadeh; Bolhassani et al.[34,35]
	Zeaxanthin	$C_{40}H_{56}O_2$		Color	Antioxidant	Bolhassani et al.[35]
Monoterpene aldehydes	Safranal	$C_{10}H_{14}O$		Aroma	Anticonvulsant and antidepressant	Gout et al.; Hosseinzadeh and Talebzadeh[36,37]

(*Continued*)

Table 2. (Continued).

Family	Bioactive constituent	Chemical formula	Molecular structure	Sensory effect	Biological activity	Reference
	Picrocrocin	$C_{16}H_{26}O_7$		Taste	Anticancer and antiproliferative	Kyriakoudi et al.; Hoshyar et al.[38,39]

According to El Asbahani et al.[52] selection of extraction methods from conventional to advance should be chosen according to the type of targeted bioactive component, heat sensibility, and tissue complexity, etc.

Hence, the successful extraction of bioactive components from plant materials can be achieved by selecting the appropriate extraction technique and considering the relevant tissues and variety of plants.[7] Generally, conventional extraction techniques (maceration, vapor or hydro distillation, soxhlet extraction, and solvent extraction) are not selective, use a large volume of organic solvents, need longer extraction time duration, and in some cases, destroy heat-sensitive bioactive compounds.[54] To overcome such problems, new extraction techniques are introduced and proposed as substitutes for conventional techniques. These modern extraction methods which are considered "green methods" are eco-friendly, safer, fast, efficient, and precise. The pros and cons of different extraction techniques used for saffron extraction are shown in Table 5.

Several extraction techniques are included in green methods such as supercritical fluid extraction, emulsion liquid membrane, ultrasound-assisted extraction, enzyme-associated extraction, microwave-assisted extraction, and pulsed electric field extraction.[7,51] These techniques have the potential to extract saffron bioactive compounds efficiently. Generally, the efficiency of extraction methods mainly depends upon the choice of appropriate solvents, considering solvent-solute affinity and use of co-extraction techniques.[61] As bioactive compounds are sensitive to light, temperature, and humidity, therefore, it's not easy to extract, isolate and characterize these bioactive constituents.[62] For the extraction of plant-based volatile compounds different extraction methods have been used. In this section, some of the extraction methods are reviewed and a summary of extraction studies on saffron bioactive ingredients has been presented in Table 6.

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 distillation are placed in this category.[69]

Soxhlet extraction

To isolate components from natural sources, the Soxhlet technique is one of the oldest extraction methods. The technique is used for the separation of components which are thermally stable and have medium to low volatility.[4,54] In this technique, a suitable solvent medium is used to conduct extraction by placing the dried saffron sample in the thimble of the extractor. This process is repeated again and again until the process of extraction has been completed.[61] Saffron picrocrocin was extracted through the exhaustive and successive method of the Soxhlet technique by using different solvents like diethyl ether, methanol, and light petroleum. In this study lipids and picrocrocin, non-glucoside carotenoids and lipids, glucoside carotenoids were extracted in diethyl ether, light petroleum, and methanol, respectively.[70] Picrocrocin and lipids separated in the diethyl ether phase are subjected to drying through evaporation, then defatted through Soxhlet to purify picrocrocin. Further for filtration, it was dissolved in methanol and the resultant filtrate was analyzed by HPLC.[71]

Additionally, an aqueous extract of saffron was prepared through Soxhlet extraction by the addition of 100 mL distilled water with a 15 g powder saffron sample into the extractor, and the extraction process was conducted for 18 hours.[72] To explore the therapeutic and anticancer aspects of saffron, extraction of saffron bioactive compounds was performed by using organic or aqueous solvents in many studies.[72–74] Soxhlet extraction has some disadvantages as compared to other novel extractions methods, as the extract is not pure, consumes more energy and solvent, low safety and efficiency, and needs more extraction time.[75] The Soxhlet method can be modified regarding its working efficiency by using it in combination with other advanced extraction techniques.

Table 3. Summary of medicinal properties of saffron in humans.

Medical issues	Saffron dose	Consequences	Reference
Cardiovascular disease	• 30 mg/day of stigma extract given for 8 weeks.	• ↓ Body mass index, waist circumference, and serum oxidized low-density lipoprotein cholesterol.	Abedimanesh et al.[140,141]
	• 10 mg/day of Crocetin given for 8 weeks.	• ↓ Body mass index, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, monocyte chemoattractant protein-1; ↑ high-density lipoprotein	Abedimanesh et al.[142]
	• 30 mg/day of Crocin given for 8 weeks.	• ↓ Serum oxidized low-density lipoprotein cholesterol	Abedimanesh et al.[141]
Diabetes mellitus (type 2)	• 15 mg/day of Crocin given for 3 months.	• ↓ Fasting blood glucose, and glycated hemoglobin	Sepahi et al.[143]
	• 15–30 mg/day of stigma extract given for 8–12 weeks.	• ↓ Fasting blood glucose; both lipid profile and glycated hemoglobin was affected in an inconsistent manner.	Moravej Aleali et al.; Karimi-Nazari et al.; Shahbazian et al.[144–146]
	• 100–1000 mg/day of stigma powder given for 8–12 weeks.	• Fasting blood glucose, serum lipid profile, and blood pressure showed an inconsistent effect	Ebrahimi et al.; Mobasseri et al.[147–149]
Alzheimer's disease	• Stigma extract was given a dose of 30 mg/day for 4–12 months.	• Cognitive functioning was improved	Akhondzadeh et al.[150]
Depression	• Petal extract was given (30 mg/day) for 6 weeks.	• Improvement in depression symptoms	Moshiri et al.[151]
	• Crocin was given (30 mg/day) for 8 weeks.	• Improvement in depression symptoms	Mazidi et al., Jam et al.[152,153]
	• A dose of stigma extract (28–30 mg/day) was given for 6–12 weeks.	• Improvement in depression symptoms	Akhondzadeh et al., Jalali et al., Kashani et al.[150,154,155]
Metabolic syndrome	• Crocin was given (30–60 mg/day) for 6–8 weeks.	• ↓ Pro-oxidant and antioxidant balance	Nikbakht-Jam et al., Javandoost et al., Kermani et al.[156–158]
	• For 12 weeks, stigma powder was given (100 mg/day)	• Fasting blood glucose and lipid profile were affected inconsistently; ↓ serum heat shock protein antibody, balance of pro-oxidant and antioxidant; ↑ serum leptin	Kermani et al., Zilaee et al.[159–161]
Memory disorders	• Petal extract was given a dose of 30 mg/day for 3 weeks	• Improvement in short-term memory	Ghodrat et al.[162]

Table 4. Major biological activities of saffron and its bioactive compounds.

Effective material	Biological activity	In Vivo/In Vitro	Reference
Saffron extract	Antioxidant, effects on the eye, anticancer activity	Mice, rat	Khorasany et al., Zhang et al.[163,164]
Crocin	Hypolipidemic, antioxidant activity	Quails, rats	Thushara et al.[165]
Crocetin	Hypolipidemic, hypoglycemic, antidiabetic, antibreast cancer, anticervical cancer, anticolorectal cancer, antileukemia, antiliver cancer, antipancreatic cancer, and anti-skin cancer activity	Quails, swiss Wistar rats, male webster mice	Zarkogianni et al., Zhuang et al.[137,166]
Safranal	Apoptosis induction	Humans	Samarghandian et al., Samarghandian et al.[72,167]
Crocin, safranal, saffron extract	Antidiabetic, hypoglycemic, antidepressant	Alloxan-diabetic rats, male BALB/c mice, quails, healthy male rats, healthy volunteers	Khorasany et al., Pitsikas[163,168]
Crocin, saffron extract	Antioxidant,	Rats, humans	Khorasany et al., Mohammad et al.[163,169]
Crocin, crocetin, safranal saffron extract,	Anticonvulsant, antidepressant, anti-Alzheimer effects, antiParkinson, neuroprotective, anxiolytic, and hypnotic, reduce the release of neurotransmitters, improve memory and learning skills, reduce morphine dependency	Rats, mice,	Khorasany et al.[163]
Safranal, saffron extract	Antibacterial, antiseptic, antifungal, antinociceptive, anti-inflammatory effects, analgesic, reduce the discomfort of teething infants	Healthy volunteers, mice	Khorasany et al.[163]
Encapsulated saffron extract/	Antitumor effects, satiety enhancer, weight loss promoter, antidepressant	Adult, outpatients, overweight women, mice	Gout et al., Zhang et al.[36,164]

Table 5. Advantages and limitations of different extraction methods applied for saffron .[7].

Extraction Procedure	Advantages	Limitations	Reference
Pulsed electric field extraction	• The ability to scale up • Higher efficiency with less extraction duration • Higher extraction yield and purity • Reduced energy consumption • Environmentally suitable	• Precise control of processing conditions • A requirement of higher maintenance	Pourzaki et al.[55]
Emulsion liquid membrane	• More working efficiency • High extraction speed and selectivity • The ability to scale up • Lesser energy consumption • Less usage of toxic organic solvents • Recovery of membrane components	• Emulsion instability • Emulsion leakage	Garavand and Madadlou[56]
Supercritical fluid extraction	• Reduction of organic solvents and extraction time • Higher mass transfer and environmentally safe • Recyclability of supercritical • fluid • Can be operated at ambient temperature	• Not appropriate for pharma-ceutical samples • Polar molecules are insoluble • Expensive system • Complex thermodynamics	Nerome et al.[57]
Ultrasound-assisted extraction	• Easy to use due to simplicity • Rapid extraction rate • Suitable for heat sensitive compounds • More economic • Ease of cleaning • Less interaction with extracting compounds • Enhance solvent diffusion into sample	• Dilution of extract (dynamic sonication) • Production of free radicals (high sonication powers) • Solvent based method • Scaling up for industries not easy	Ameer et al.[58]
Enzyme-assisted extraction	• Use of water instead of chemical solvents • Suitable for separating bound components • from plant matrix • Significant extraction rate	• High cost and sensitivity of enzymes • Difficulty in scaling up for industries	Sagar et al.[59]
High hydrostatic pressure extraction	• Absence of the heating process • Faster mass transfer • Used for extracting strongly polar, weakly polar and non-polar compounds • Higher extraction yield and purity • Decreased energy usage	• Higher expenses of facilities • Difficult to maintain constant processing pressure	Shinwari and Rao[9]
Microwave-assisted extraction	• Simple to operate and selectivity • More economical to scale-up • High extraction yield with less time • High-quality extracts • Cost-effective technique	• Inappropriate for heat-sensitive compounds • High cost of apparatus and equipment • Difficult to operate • Less environment friendly (organic solvents) • Less extraction yield (nonpolar compounds)	Sagar et al.[169]
Soxhlet, maceration and distillation	• Frequently used extraction methods • Simple to operate • Suitable for small-scale industries	• Environmentally not safe • Large number of solvents required • Time-consuming process and less efficiency • Unsuitable for heat-sensitive compounds • Production of byproducts • Degradation of bioactive constituents	Sarfarazi et al., Sagar et al.[59,60]

Table 6. Different studies regarding the extraction of bioactive ingredients from saffron.

Extraction technique	Extraction parameters	Bioactive ingredients analyzed	Optimum conditions for maximum extraction	Reference
Maceration	• Extraction time 2–7 hours • Extraction temperature 5–85°C • Extraction solvent 0–100% ethanol	Picrocrocin, Crocin, Safranal	The maximum level of Picrocrocin (83.91%), Safranal (86.60%), and Crocin (92.42%) were obtained at 2 h extraction time, 85°C temperature, and 33.33% ethanol	Sarfarazi et al., Khazaei et al.[60,63]
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 extraction efficiency	All bioactive constituents	• Methanol/ water (50/50 v/v) followed by ethanol/water (70/30) • Ultrasound increased the extraction efficiency (detected 4120 molecular features).	Rubert et al.[64]
Solid-phase extraction	• Polymer type: gentiobiose imprinted polymer, blank non-imprinted polymer • Solvent type: water, acetic acid 2% (v/v), acetic acid 5% (v/v), and acetic acid 10% (v/v) • Washing solvent: methanol, THF, water, ACN	Crocins	84% extraction recovery of crocins was attained by gentiobiose imprinted polymer, acetic acid 2% (v/v) binding solvent, ACN washing solvent	Mohajeri et al.[65]
Supercritical CO_2extraction	• Temperature: 40, 60, and 80°C • Pressures: 20, 30, 40 MPa • Entrainer type: water, methanol	Picrocrocin, HTCC, Safranal, α-crocin, Deglycosylated crocin	• For picrocrocin, safranal, HTCC: solvent (ethanol) • For deglycosylated crocin and α-crocin: solvent (water) • Optimum pressure for α-crocin, picrocrocin, deglycosylated crocin (30 MPa); for the rest (40 MPa) • Extraction temperature for all components 80°C	Nerome et al.[57]
Emulsion liquid membrane	• Surfactant type: Span 80, ENJ-3029, polyamine type-surfactants • Membrane type: CCl4, N-decane, CH3Cl, toluene • Surfactant concentration: 0.5, 2.5, 7, 7.5, and 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, and 500 rpm Pourabdollah[66]	Crocins, Safranal, Picrocrocin	The best surfactant, membrane type, surfactant concentration, treat ratio, phase ratio, and stirring rate for obtaining the highest extraction efficiency were Span 80 (surfactant), N-decane (membrane), 2.5% (surfactant concentration), 0.3 (treat ratio), 0.8 (phase ratio), and 300 rpm (stirring rate), respectively.	Mokhtari and
		Pulsed electric field extraction	Voltage, Pulse width, Pulse numbers	Crocins, Safranal, Picrocrocin
The maximum level of crocin (14.1%), safranal (15.5%), and picrocrocin (10.2%) were obtained at 5 Kv voltage, 100 pulses number, 35 ms pulse width	Pourzaki et al.[55]			

(*Continued*)

Table 6. (Continued).

Extraction technique	Extraction parameters	Bioactive ingredients analyzed	Optimum conditions for maximum extraction	Reference
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, 19 min) • Extraction volume (2, 10 mL of solvent)	Crocins 1, Safranal, Sicrocrocin	• For safranal (87%): ethanol/water (50/50 v/v), 1 min extraction time, 40°C, 10 mL extraction volume • For crocin 1 (68%): methanol/water (50/50 v/v), 10 min extraction time, 40°C, 10 mL extraction volume • For picrocrocin (80%): methanol/water (50/50 v/v), 1 min extraction time, 40°C, and 10 mL extraction volume	Nescatelli et al.[67]
Ultrasound-assisted extraction	• Methanol %: 0.4, 10.5, 25.2, 40, and 50 (%, v/v) • Sonication duration: 1.2, 7, 15.5, 24, 29.8 min • Active intervals of sonication: 0.2, 0.3, 0.5, 0.7, and 0.8 sec	Crocins, Picrocrocin	• For crocin 1 (19.6%) and crocin 2 (7.5%): 50% methanol, sonication 30 min, active sonication interval 0.2 sec on /0.8 sec off • For total crocins (20.9%) and picrocrocin (12.2%): 44% methanol, 30 min sonication, active sonication interval 0.6 sec on /0.4 sec off	Kyriakoudi et al.[68]

Maceration or solvent extraction

Maceration is a well-known, common, and practical approach for extraction to obtain fundamental bioactive ingredients and whole saffron extract. Solvent extraction is started from maceration by mixing the proper quantity of saffron sample and extraction solvent, subjected to stirring for a specific time duration and speed. After stirring followed by filtration and resulting extract was analyzed through a UV-Vis spectrophotometer to take its absorbance value.[4] Orfanou[76] prepared saffron extract by maceration process and reported that different parameters have a significant role in affecting the coloring strength of saffron extract like extraction time, type of filter, and extraction solvent along with stage at which the extract was filtrated. Therefore, the coloring intensity was reduced considerably by increasing extraction time (up to 24 h), using the filter paper of large pore size, and by filtering the extract before the last dilution. Also, it is stated that to extract the maximum level of saffron bioactive compounds, the best extraction solvent is methanol (50% v/v) afterwards having ethanol (50% v/v) and water.[76]

A wide variety of solvents like organic solvents, water, and their combination have been used to obtain saffron bioactive compounds including crocin, picrocrocin, and safranal.[60] For example, using aqueous-based extraction strategies, water-soluble carotenoids (crocin), which are found in large quantities in saffron can be separated from stigma. To get saffron bioactive compounds, solvents like methanol, water, and petroleum ether are frequently used.[77,78] To extract different bioactive compounds and polyphenols from the saffron plant, the use of ethanol, water solvent mixture at different ratios was discovered as the best media.[79–81] A trace amount of contaminant and residues may be present in bioactive extracts that are obtained by organic solvents, this made them hazardous for nutraceutical and pharmaceutical uses. So, to overcome this problem, water or organic solvents having low boiling points (n-pentane) should be used, and after this steam distillation is recommended.[52]

Steam or hydro-distillation extraction

In the steam or hydro-distillation extraction process, water vapors act as a carrier of volatile compounds. So, these water vapors penetrate in herbal cells thus in result isolate the volatile components from the sample. A dried saffron sample is put for soaking in a mixture of alcohol and water or water only and then the mixture is subjected to heating up to the boiling point. Steam carried the volatile components to the condenser for cooling and liquefying to collect the compounds.[4,61] Tarantilis and Polissiou[82] conducted research to extract saffron volatile components by using steam distillation, vacuum headspace distillation, and micro-steam distillation, the GCMS output confirmed that vacuum headspace and micro-steam distillation techniques are successful for the extraction of isophorone, safranal, and their isomers. Zareena et al.[83] also examined the alternation by steam distillation, after γ-irradiation in coloring agents and volatile compounds of saffron. In the result, excluding the 5 kGy doses, there are no changes in saffron bioactive compounds as affected by γ-irradiation.[83]

In another study, micro-simultaneous and hydro-distillation extraction was used to quantify the safranal. Diethyl ether was used as the extraction solvent and a mixture of glycol and water was used for cooling the condenser (−10°C).[84] Volatiles were isolated from a dried stigma sample of saffron cultivated in two different regions of Turkey through the micro distillation process. Further, the extract was analyzed via GC-MS and GC techniques, and 26 compounds were categorized. According to results saffron volatiles can be safely isolated and characterized by micro distillation following GC-MS. Because in comparison to conventional water or steam distillation process, micro distillation is a milder method.[85] There are different demerits of this technique for example hydrolysis and cyclization, long processing time, loss of some polar molecules due to overheating. Steam distillation has an updated version which is turbo distillation, and this turbo distillation allows to achieve high efficiency by recycling water source in a short period of extraction time. Steam distillation up till now is a suitable method for extraction of semi-industrial and industrial uses due to ease of methodology, affordable instrumentation, and high selectivity.[52]

Novel extraction techniques

To eliminate the drawbacks of traditional extraction methods such as long extraction periods, the necessity of using solvents with high purity, low extraction selectivity, solvent consumption in huge quantities, and degradation of heat-labile components, new methods have been implied.[69]

Supercritical fluid extraction

In the supercritical fluid extraction method, supercritical fluids are used as extraction solvents. The most used supercritical solvent is carbon dioxide also known as a green solvent without having any side effects or toxic influences. Carbon dioxide is considered a selective solvent due to having low viscosity, density similar to liquids, and maximum diffusivity.[54] Generally, fewer polar compounds (safranal) are extracted with carbon dioxide, while initially polar compounds (crocin, crocetin) are removed with organic or aqueous solvents (e.g. ethanol).[86] Lozano and colleagues (2000) used a 200 mg dry saffron sample to extract safranal through the supercritical fluid extraction technique. In the extraction chamber, supercritical fluid was held for 5 min under pressure before the circulation of supercritical fluid through the extraction unit. In methanol (3 ml) safranal was collected for further HPLC analysis. For safranal isolation, the supercritical fluid extraction technique was reported as a non-destructive technique.[87]

Ahmadian-Kouchaksaraie and Niazmand[88] conducted the CO_2-mediated extraction of antioxidant compounds from saffron. The highest amounts of anthocyanins, flavonoids, and phenolic contents were determined through 16.4 MPa pressure at 62°C for 47 min. Also, the main bioactive compounds of saffron including safranal and crocin were extracted using CO_2-mediated extraction alone and in combination with methanol, respectively.[86] The obtained results showed that 33% crocin was obtained at a 1.0 cm^3/min flow rate by using 19.3 MPa pressure, 44°C for 110 min. While above 90% safranal was obtained at 0.9 cm^3/min flow rate by using 21.3 MPa pressure, 92°C for 122 min. As compared to crocin, safranal was more efficiently recovered due to the high affinity of supercritical CO_2 for non-polar compounds.[86]

As compared to the conventional extraction method, supercritical fluid extraction (CO_2-based) was used to extract saffron bioactive compounds (crocin derivatives, picrocrocin, safranal, HTCC) using aqueous solvent and methanol as separating medium. Methanol was best for the extraction of picrocrocin, safranal, and HTCC, while crocin derivatives were extracted more efficiently in an aqueous solvent. HPLC analysis also established that the supercritical fluid extraction method gave maximum productivity of saffron bioactive components.[57] Major advantages of the supercritical fluid extraction technique include less solvent requirement, reduced extraction time, high selectivity, facilitated automation, highly pure extracts, and the ability to be used with other methods.[54]

Ultrasound-assisted extraction

Ultrasound is mainly the rotational process of compression and expansion of sound waves within the frequency range of 20 kHz–100 MHz. Ultrasound-assisted extraction includes the use of sound waves having high-frequency and intensity, and these sound waves interact with sample material. Sonication involves the mechanism of generating, amplifying, and collapsing the micro-bubbles, which occurs through the cavitation phenomenon.[89] Using this theory ultrasound extraction has been used to release the organic and inorganic compounds from the plant-based materials by intensifying mass transfer and enhancing the interaction between solvent and target compounds.[90]

Kadkhodaee and co-workers used high-power ultrasound to extract the bioactive constituents from saffron at a frequency of 30 kHz. The effect of different parameters on the productivity of extracted compounds was also examined such as mode of sonication extraction time, and frequency of sound waves. The results revealed that ultrasound remarkably decreased the extraction time and also significantly improved the extraction efficiency.[91] Jalali-Heravi at el.[92] used ultrasonic extraction to extract volatile compounds from Iranian saffron and later isolated and detected them through the GC-MS technique. GC–MS represented 90% of the total peak area and different forty compounds were detected through GC-MS which represented. Kyriakoudi[68] recovered crocins and picrocrocin from dried saffron stigma by using aqueous methanol as extracting solvent. Extraction parameters were optimized by response surface methodology. So, the recovery of apocarotenoids was also speeded up using ultrasonic extraction.

Maggi et al.[93] conducted the ultrasound-assisted extraction of volatile compounds from several commercial varieties of saffron by using the cyclohexane and diethyl ether as a solvent, the findings revealed that ultrasound-assisted extraction can produce a greater extent of volatile constituents. In some other studies of saffron extraction, ultrasound-assisted extraction has been used in combination with other techniques for reducing the extraction time and improving the recovery, these complementary techniques include pressurized solid-liquid extraction,[79] micro-simultaneous hydro distillation extraction,[84] rapid solid-liquid dynamic extraction,[79] and dispersive liquid-liquid micro-extraction.[94] Ultrasound-assisted extraction has several advantages over conventional techniques such as a high extraction rate, more purity of finish product, highly selective, advanced energy and mass transfer, cost-effectiveness, and ability to scale up.[68]

Microwave-assisted extraction

Microwave heating can be produced through the dispersion of electromagnetic waves within a frequency range of 300 MHz to 300 GHz. However, the commonly used frequencies for food, nutraceutical, medical, and pharmaceutical are within the range of 0.915 to 2.45 GHz.[95] In contrast to conventional solvent extractions methods, in microwave-assisted extraction transfer of mass and heat happens in the same direction (from the inner side of plant material to the outer side solvent medium). In a short time, maximum bioactive compounds are collected through accelerated solute transfer resulted from one pot heat-mass transfer. The rise of temperature and heat transfer will be different based on the microwave power and properties of plant material (moisture level, texture, and loss factor).[96] Solvent-based microwave-assisted extraction is established according to solvent polarity, and this solvent-assisted microwave-assisted extraction greatly affects the releasing of bioactive compounds trapped within the complex texture of the plant matrix. The solvents of polar nature are heated up to their boiling point so that they can penetrate properly into the plant matrix for dissolving the bioactive constituents.[97]

Extraction of crocin, picrocrocin, and safranal was performed through microwave extraction technique and optimized by response surface methodology. Process parameters that were optimized include solvent concentration (ethanol 0–100% v\v), extraction time (10–30 min), and temperature (45–125°C). The content of crocin, picrocrocin, and safranal was analyzed through a spectrophotometer at 440, 257, and 330 nm absorbance values, respectively. According to response surface methodology, the optimum level of ethanol concentration, extraction time, and temperature were 59.59%, 30 min, and 95.91°C, respectively.[98] Anthocyanins from tepals of a saffron flower were extracted by microwave extraction at 360 W. Maximum productivity of anthocyanins (101 mg/g) was obtained at optimized levels of extraction time (9.3 min), temperature (48°C), and solvent to sample ratio (77.5 ml/g).[3]

A comparative study was conducted between the microwave-assisted and conventional extraction techniques to analyse the effect of temperature on extracted product stability and recovery. In this work saffron samples of different geographical areas (Greece, Hungary, Italy, Spain, and Turkey) were analyzed. Best outcomes were obtained by using a 10 mg saffron sample, 0.8 mL of methanol, and the extraction process was continued for 30 min. Further nuclear magnetic resonance was used for the characterization of the saffron sample.[99] In contrast to conventional methods, microwave-assisted extraction avoids the presence of undesirable by-products, saves both energy and time, requires minimum solvent, maintains the integrity of extracted bioactive constituents, and thus influences the appearance, purity, and quality of extracted solutes.[97,99]

Pulsed electric field assisted extraction

A pulsed electric field can be generated by transient electrical pulses which remain for micro and nanoseconds at electric power ranged 20 to 80 kV/cm. In this method, electric fields at low electric powers cause the perforation of membranes in plant tissue. Further perforation in result causes temporary or permanent permeabilization of plant membrane and encourages the release of bound bioactive constituents.[100] Pulsed electric field extraction prevents any degradation, hydrolysis, and polymerization of the target compounds, hence, leading to fewer changes in appearance, nutritional profile, and bioactive compounds of a product.[101]

In a study, major bioactive compounds were extracted from saffron. The aqueous extraction was conducted at room temperature, later pulsed electric field treatment was performed at a different width, intervals, powers of pulse, and voltages. According to results pulses within a range of 1–5 kV/cm were enough for generating proper permeation routes within the saffron tissue. As compared to solvent extraction, application of pulsed electric field method at given conditions including 5 kV of electric power, 35 of pulse number, and 100 μs of pulse width showed a significant increase in crocin, picrocrocin and safranal levels from pomace (5.76, 5.9 and 7.5%) and stigma (14, 10.2 and 15.5%).[55]

In numerous plant materials, pulsed electric field extraction method alone or coupled with supercritical fluids or ultrasound has been widely used for extraction of several bioactive constituents like dietary fibers, pigments, phenolic compounds, etc.[102] The main advantages of the pulsed electric field method are improved extraction efficiency and mass transfer, less extraction temperature and time, supports of sensitive compounds, saves energy, and reduces environmental threats.[100] The pulsed electric field method also helps to prevent the growth of some pathogenic and spoilage microbial organisms.[102]

Enzyme-assisted extraction

Enzymes can be used as pre-treatment to assist and enhance the extraction of volatile compounds, phenolics, pigments, and other bioactive constituents.[103] Enzymes destroy cell walls and hydrolyze the bound bioactive constituents (attached with lipids and carbohydrates). In contrast to conventional methods, the enzyme-assisted extraction method reduces the consumption of energy and time in further purification and filtration steps. It has been proved that the application of some natural enzymes like α-amylase, cellulase, cellobiase, fructosyltransferase, hemicellulase, pectinase, pectinesterase, and protease have been utilized in pressurized hot water extraction and solvent extraction methods to produce the extract with improved quality and yield.[104,105]

Lotfi et al.[106] used a commercially available blend of cellulose, hemicellulose, and pectin at several levels for extraction of saffron anthocyanin from plant tepal. Firstly, the enzyme blend was dissolved in distilled water at levels of 1–10% for the separation of anthocyanins at 40°C at different time intervals. According to results above 40% anthocyanin was recovered by using a 5% enzyme level for 1 hour extraction time, as compared to solvent extraction (ethanol).

The enzyme-assisted extraction produced a more stable color of extract as compared to the solvent extracted sample which was pale and prone to degradation. Additionally, enzyme-assisted extraction can be used in combination with other extraction techniques like supercritical fluid extraction and enzyme-assisted extraction. Because of the natural origin of enzymes, this method is known as a green extraction technique. Different factors should be examined during enzyme-assisted extraction including the type of plant sample, composition, moisture level, particle size, dose and type of enzyme, time duration, temperature, solvent to the solid ratio in the extraction process, and uncomplicated scaling-up.[61,104]

High hydrostatic pressure extraction

The highly hydrostatic pressure is a non-thermal extraction technique operating at ultra-high hydraulic pressure (1000–8000 bars). This method has been suggested for enhancing the amount and quality of bioactive substances from plant-based materials. Application of high pressure inserts a force which as a result induces the mass transfer thus increase the transfer of biologically active compounds from the cell wall of the plants. Covalent bonds are affected by the thermal extraction method, while non-covalent bonds (hydrophobic bonds, hydrogen bonds, and ionic bonds) are also influenced by high hydrostatic pressure extraction. Therefore, covalently bonded bioactive compounds are kept protected by highly hydrostatic pressure extraction.[9] Plant compounds having high molecular weight like proteins and carbohydrates comprise non-covalent bonds. Therefore, these compounds get denatured through ultra-high pressure and do not release into the solvent, resulting in a pure solute. As compared to other extraction methods, highly hydrostatic pressure extraction has rapid extraction times and more solute permeability due to the deprotonation of charged groups. This deprotonation upsets the hydrophobic bonds and salt bridges in cell membranes thus stimulate the solutes permeability.[107]

The highly hydrostatic pressure extraction of saffron components was conducted by Shinwari and Rao[9] by using high pressure of about 1000–6000 bar at the temperature of 30 to 70°C (ascended extraction). Results obtained by the HPLC analysis showed that the application of high-pressure results in a significant increase in crocin (52–63%), picrocrocin (54–85%), and safranal (55–62%). On the contrary increased temperature results in the decreased crocin content up to 25–36%. The optimum conditions of highly hydrostatic pressure extraction used in saffron extraction were the pressure of 5800 bar at the temperature of 50°C for 5 minutes. Bioactive compounds extracted by the highly hydrostatic pressure technique seemed to be more effective in extinguishing cancer cells (28%) in contrast to the components obtained by conventional techniques.[9] This extraction method has several advantages such as requires less amount of solvent, saving both energy and time, safe for the environment and producing different types of components along with maximum extraction purity.[108]

Emulsion liquid membrane extraction

The emulsion liquid membrane extraction method is mostly used in the case of wastewater treatment and hazardous effluents to recover the bioactive compounds and to extract the metal ions respectively. The emulsion liquid membrane technique consists of two emulsions (W_1/O/W_2). Where W_1 represents the internal aqueous phase (stripping agents), W_2 represents the external aqueous phase (feed phase) having the target constituents (organic acids, phenolic compounds, metal ions, etc.), and O denotes the organic membrane phase which contains the carrier, surfactant, diluent, and co-surfactant.[7]

As compared to conventional methods, emulsion liquid membrane has several advantages like highly selective, maximum extraction efficiency and recyclability, faster mass transfer, use of green solvents, less investment costs, and have the ability of extraction and purification at the same time.[56] Mokhtari and Pourabdollah[66] extracted bioactive compounds from saffron stigmas through an emulsion liquid membrane system. This method was optimized by using one factor at a time, and the experimental conditions were 2.5% of span 80 (surfactant), n-decane (diluent), phase ratio of 0.8, treat ratio of 0.3, and 300 rpm as agitating speed. According to results above 90%, saffron bioactive constituents (crocins, picrocrocin, and safranal) were obtained into the aqueous phase of emulsion globules.[66]

Green analytical chemistry regarding saffron extraction

A new concept of green analytical chemistry has been introduced to reduce the consumption and number of different solvents and reagents during sample preparation, extraction, and purification. Green analytical chemistry supports the idea of having no significant environmental effects such as non-solvent, non-destructive and remote.[109] Therefore, this idea is based on different concepts including preventing waste production, use of non-toxic solvents, increase energy efficiency, avoid derivative products, high working safety, small sample size, utilization of green solvents, using solvents and extraction approaches in combination, remote control, and use of integrated eco-friendly analytical approaches.[110]

As green analytical chemistry emphasis on rising fast and economic methods, therefore during last year's demand for green analytical chemistry-based processes has increased as a substitute for the conventional analytical methods.[111] Because of that, highly mechanized, environmentally safe, non-destructive, and sensor-based, strategies meeting the requirements of green analytical chemistry are recommended. Moreover, for monitoring the quality of samples in a chemical-free, fast and safe way, other smartphone applications, image processing systems, and easy-access indirect methods are engaged.[112]

To track some trace elements like bioactive compounds in plant materials, it is compulsory to use direct analytical methodologies like electrochemical and spectrometric methods. For monitoring the adulterations and bioactive constituents in saffron some advanced analytical techniques are used like X-ray, tandem mass spectrometry, NMR metabolite fingerprinting, Raman, and near or middle infrared (NIR or MIR) spectrophotometry and proton transfer reaction mass spectrometry.[64,113–115] Using a small amount of sample can minimize the waste by-product, increase energy efficiency and reduce the solvent amount, for example, the conventional solvent extraction methods could be replaced by solid-phase extraction or micro-extraction systems.[64,116] For extracting the bioactive compounds from saffron, enzyme, microwave, supercritical fluid, and ultrasound-assisted extraction methods are highly recommended because these techniques are under the Green analytical chemistry principle.

Applications of saffron bioactive ingredients

Throughout history, saffron has been utilized in different ways by different nations i.e., dye, medicine, perfume, and spice. Since ancient times, the application of saffron in cosmetics has been reported. It has also been used as a dye for expensive fabrics like cotton, silk, and wool.[117] Nowadays, saffron has great importance in the food sector due to its distinctive aroma, color, and taste. Generally saffron has a hay-like pungent smell and bitter taste due to the presence of safranal and picrocrocin, respectively. While golden yellow color is attributed to the presence of crocin. Saffron is used in the preparation of different foods and is more expensive as a spice as compared to use for medical purposes.[118,119] In different traditional dishes, it is used as a spice either in powder form or as filaments and it is also used to prepare unique bakery items. Saffron is used in different bakery items to give aroma and golden colors like cookies, cakes, and pastries.[120–122] In the next section applications of saffron in different sectors are discussed.

Application of saffron in the food industry

In the dairy industry, saffron has been used in different dairy products, especially in different varieties of cheese due to its sensorial characteristics. The major type of cheese includes Luneberg cheese made from Austrian cow's milk, Piacentinu Ennese cheese made from sheep's milk (from Sicily), and Pecorino allo Zafferano made from pasteurized sheep's milk (from Italy).[4] Licón et al.[123] studied the influence of saffron concentration in sheep's milk cheese regarding different characteristics (chemical, microbiological, sensorial, and textural properties). It was observed that cheeses having saffron were more elastic, firmer, yellow, and microbiologically stable as compared to those having no saffron.

Saffron color develops due to crocetin esters, giving the yellowish red color. Colour change might be due to interactions of fat globules with crocetin esters and carotenoids (minor amount) facilitated by phospholipids. Saffron cheese also showed fewer total and lactic acid bacteria counts. This was due to the ability of saffron to decrease the pH to slow down bacterial growth, which in result increased the dry matter content and pressing time. Thus saffron cheese was more elastic, firm having less salt level.[123,124] In another study, fresh ovine cheese was supplemented with saffron regarding color, sensory and microbiological properties along with the antioxidant and physicochemical profile.[125] It was examined that the presence of saffron increased the antioxidant and antimicrobial activity of cheese while no significant change was observed in the physicochemical properties.[125] The antioxidant properties of plants are related to the bioactivity of phytochemicals thus are helpful to prevent oxidative stress. In saffron polyphenols and crocins are strong antioxidative compounds with having bioactive influences.[126]

While in cereal products, saffron has also been used in the development of pasta and was evaluated for physicochemical, sensorial, and textural characteristics.[127] Saffron enhanced the acceptability of enriched pasta for its aroma, color, taste, visual feature, chewiness, gumminess, hardness, and overall acceptability. Furthermore, saffron also improved the antioxidant activity of pasta based on the ABTS and DPPH assays. Firmness in cooked pasta is somehow linked with the hydration of starch granules and following the embedded gelatinized starch particles in the protein matrix of pasta. The high levels of saffron in pasta can cause a reduction of water uptake in the cooking process. This is because non-soluble saffron constituents inhibit the diffusion of water in the gluten matrix.[128] The antioxidant potential of saffron stigma is normally related to its phenolic level along with other active constituents including carotene, crocetin, crocins, and safranal.[129]

While in desserts, Almodóvar et al.[130] prepared two saffron-based desserts including cheesecake with orange jam and saffron, while the other one was white chocolate soup with yogurt and saffron. In both desserts, saffron extract having a standardized level of crocins was used. Results showed that as compared to saffron stigma, the use of saffron extract permitted more accurate dose control and increased the consumer acceptability for the product. During food preparation, various parameters affect the intensity of the desired color of saffron stigmas such as whole raw stigma or grounded, maceration with alcohol or water, time of maceration, heating, etc. All these parameters do not allow uniform control coloring, therefore, for cold dishes (<50°C) saffron extract is considered more suitable, because saffron extract does not require any preheating treatment and gives uniform color intensity throughout the product. Further saffron powder also has a particle size of less than 240 μm, free from undesirable fibers, and easily water-soluble, all these factors do not influence the properties of the final product.[130]

In beverages, saffron extracts are also used in the formulations of different alcoholic and non-alcoholic beverages as well as herbal teas. In beverages, the bitterness of saffron is a limiting feature for consumer's acceptance.[131] Ordoudi et al.[132] investigated the chemical characterization and bio-accessibility of crocins in various infusions developed from herbal tea blends with saffron. According to results, presence of phenolic antioxidants from herbal tea improved the bio-accessibility of crocins, which was evaluated through an in vitro gastrointestinal digestion model. The results showed that crocins are protected from oxidation through the high radical scavenging ability of herbal tea during the digestion process, which protect the crocins and improved their bio-accessibility.

Application of saffron in the cosmetics industry

An interest in saffron applications in different types of cosmetics has taken place. Ethanolic extract of saffron has been used in body lotions, hair care products, moisturizing creams, liquid soaps, shampoos, and sun protection creams.[133] In coming time, concentrated dry saffron extract was added as an ingredient in the cream, face powder, and lotion.[134] The saffron-based formulated cosmetic products were analyzed in different subjects (between 18 to 28 years). As compared to control products, saffron-containing products resulted in more lightening and shining of skin. All these observed characteristics were due to the presence of crocins.[135] Saffron could be a potentially effective antiaging ingredient, therefore researchers from L'Ore´al Company studied the anti-inflammatory and antioxidant activities of crocin. The experiment was conducted on fibroblasts in vitro and normal human keratinocytes.[136]

It was observed that crocin enhanced the antioxidant defense system while restricted the production of pro-inflammatory mediators. Based on their outcomes, the authors suggested that crocin could be used as an effective cosmetic agent to prevent skin aging.[136] The ultraviolet protective effects of saffron have been also emphasized, but Zarkogianni and Nikolaidis[137] have developed an oil-in-water emulsion having saffron. The emulsions were examined regarding anti-solar activity by assessing their sun protection factor. The authors proposed the use of saffron in sunscreen cosmetics as a natural ultraviolet absorbing ingredient.[138]

Application of saffron in the pharmaceutical industry

Despite having a role as an important spice in the food industry, saffron also has a range of pharmacological functions like anticarcinogenic, antidiabetic, anti-inflammatory, antioxidant, cardioprotective, and neuroprotective, etc. Due to this reason, saffron has been regarded as a functional spice.[139] The summary of the medicinal properties of saffron analyzed in humans is given in Table 3. Several research works have analyzed the biological functions of saffron either through summarized the in *vivo, ex vivo* or *in vitro* studies (Table 4).[25,32,168,170–172]

Conclusion and future needs

Saffron has several benefits due to the presence of bioactive compounds. It has wide applications in various industries like food, cosmetics, and the pharmaceutical industries. All the bioactive compounds of saffron are somehow susceptible to environmental factors including enzymes, light, heat, oxygen, pH, and metallic ions. In addition, it is essential to develop and utilize novel extraction routes to efficiently produce the saffron extract bioactive compounds. Therefore, to maintain the quality of target constituents and to enhance the extraction efficiency, it is highly recommended to choose the appropriate extraction method based on green analytical chemistry. These techniques are environmentally friendly and could be used on a high throughput scale. To have more value-added products in different areas of the saffron industry (food, cosmetic, pharmaceutical sector), it is essential to develop and utilize novel methods for the extraction of highly efficient saffron bioactive compounds. Moreover, new research is required regarding the encapsulation of saffron compounds and to study the physiological mechanism of saffron ingredients after ingestion (absorption, distribution, metabolism, and excretion). Another related question to be lectured is to what extent the complexing between saffron components and other compounds affects its' bioaccessibility. To do that, the synergic effect between polyphenols and other substances in different liquid and solids models should be monitored. In a related point, the clinical trials should be performed to gain more authentic facts on saffron components metabolism. Further, for determining the pharmacological and toxicological aspects of saffron compounds it is necessary to study the bioaccessibility and bioavailability of these compounds. The prospective mechanisms of the health-promising effects of saffron compounds are still ambiguous. Meanwhile, additional studies should focus on the binding between saffron compounds and responsible genes to gain a better sympathetic at the molecular level. A major question to be addressed is whether a single purified component in saffron or multiple constituents produced synergic effects on human health. However, the saffron components are technologically instable, and therefore, enhancing the stability of these components should be considered, industrially. Besides, the understanding of interaction between the core and wall materials during encapsulation are required to optimize the procedures. These efforts will be the platform for the development of saffron components and their based products as functional foods for different health-promoting effects.

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ORCID

Xin-An Zeng http://orcid.org/0000-0002-8792-2336
Ibrahim Khalifa http://orcid.org/0000-0002-7648-2961
Rana Muhammad Aadil http://orcid.org/0000-0002-0185-0096
Muhammad Farhan Jahangir Chughtai http://orcid.org/0000-0003-1527-0794
Adnan Khaliq http://orcid.org/0000-0003-4572-8129

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