International Journal of Gastronomy and Food Science 38 (2024) 101067

Contents lists available at ScienceDirect

International Journal of Gastronomy and Food Science

journal homepage: www.elsevier.com/locate/ijgfs

Quality and authenticity of saffron and sensory aspects

Cristina Anamaria Semeniuc [a], Mara Mandrioli [1,b], Maria Jenica Urs [a], Tullia Gallina Toschi [b,*]

[a] Department of Food Engineering, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Calea Mănăştur, 400372 Cluj-Napoca, Romania
[b] Department of Agricultural and Food Sciences, Alma Mater Studiorum - Università di Bologna, Viale Giuseppe Fanin 40, 40127 Bologna, Italy

A R T I C L E I N F O

Keywords:
Saffron
Crocin
Picrocrocin
Safranal
Quality control
Nutraceutical properties

A B S T R A C T

Saffron possesses a valuable composition of chemicals and distinct sensory properties. This article examines the chemical composition, sensory qualities, and the importance of detecting adulteration within saffron. Recent studies have shown that saffron contains several bioactive substances, such as safranal, picrocrocin, and crocin; these constituents offer various health benefits due to their antioxidant, neuroprotective, and anti-inflammatory effects. The spice's colour comes from water-soluble apocarotenoids, namely the crocin and crocetin sugar esters, which confer the food yellow hues. Picrocrocin is a monoterpene glucoside responsible for the bitter taste of this spice. On the other hand, safranal is the volatile component that gives the spice's aroma. The food industry values saffron's flavour and vivid colour, often using it to make high-end products and functional foods.

Based on minimal quality requirements, the International Organization for Standardization (ISO) has developed a system for classifying saffron in merchandise categories. However, there have been concerns about the accuracy of the results obtained through ISO methods. Because of this, research has been conducted to determine saffron's safranal and crocin levels using the ISO methods compared to other methods.

In summary, saffron is a valuable source of bioactive constituents, frequently used to manufacture functional foods. However, it is imperative to establish and employ reliable methods to identify adulteration so that consumers benefit from the quality and authenticity of this precious spice. Implementing these techniques will help maintain high standards in the food industry, thus ensuring public health protection and consumer confidence.

1. Introduction

Saffron, one of the most expensive spices in the world, is frequently called "Red Gold" and originates from the dried stigma of the Iridaceae family member *Crocus sativus* L., which is sterile. Saffron is cultivated in countries like Greece, Italy, Spain, Afghanistan, Iran, Morocco, and India. Every year, over 300 tonnes of saffron are produced globally (Morozzi et al., 2019; Cardone et al., 2020a).

The plant *Crocus sativus* L. grows slowly and does not reproduce from seeds. Instead, it is grown and propagated by corms, which are bulbous tuberous structures. Six purple tepals, one red pistil, and three golden yellow stamens make up the flower of the plant; the pistil also has three red-branched stigmas. Of all these, only the saffron flower stigmas can be used to prepare saffron spice. The high cost of saffron is due to the manual labour required to harvest and trim the stigmas. Because there are three stigmas per flower, each weighing approximately 2 mg, it takes roughly 150,000 pieces to produce 1 kg of saffron spice (Mohtashami et al., 2021; Cerdá-Bernad et al., 2022). Since ancient Mesopotamia, *Crocus sativus* L. has been highly appreciated for its unique biological, aromatic, flavouring properties and distinct colour. Due to its distinctive sensory characteristics and biological properties, various civilisations and cultures have utilised saffron for almost 3000 years in medicine, dye production, cosmetics, and perfumery (Spence, 2023). Apocarotenoids like safranal, crocin, and crocetin, regarded as bioactive substances, are the primary chemical constituents that are responsible for these characteristics (Avila-Sosa et al., 2022). A large spectrum of biological activities has been demonstrated for these compounds, including antigenotoxic, antitumour, anticancer (Mishra and Mishra, 2023), antioxidant (Maestre-Hernández et al., 2023; Mohd et al., 2023; Nid Ahmed et al., 2024), anti-inflammatory, antidiabetic, antiatherosclerotic, hypotensive, hypoglycaemic, antihyperlipidaemic, antidepressant, and antidegenerative effects (Tsui et al., 2018; José Bagur et al., 2018; Mohtashami et al., 2021; Abu-Izneid et al., 2022; Saadat et al., 2024). Because of their high antioxidant potential and ability to

* Corresponding author.
E-mail addresses: cristina.semeniuc@usamvcluj.ro (C.A. Semeniuc), mara.mandrioli@unibo.it (M. Mandrioli), maria-jenica.urs@student.usamvcluj.ro (M.J. Urs), tullia.gallinatoschi@unibo.it (T. Gallina Toschi).
[1] This author contributed equally to this work as co-first author.

https://doi.org/10.1016/j.ijgfs.2024.101067
Received 20 June 2024; Received in revised form 28 October 2024; Accepted 16 November 2024
Available online 17 November 2024

scavenge free radicals, one of the most intriguing aspects of saffron's bioactive components is how they positively affect human health (Cerdá-Bernad et al., 2022).

Three specific compounds—picrocrocin, crocetin, and safranal—are also responsible for the spice's colour, flavour, and aroma. The water-soluble apocarotenoids, such as sugar esters of crocin and crocetin, are responsible for the yellow hues in food and the spice's colour (Naidis et al., 2023). Picrocrocin, a type of monoterpene glucoside, imparts the spice with its bitter taste. On the other hand, the volatile fraction, safranal, gives the enticing aroma of saffron spice. The high-quality saffron has a sweet, spicy, and floral fragrance with a hint of metallic notes and a hay-like scent (Álvarez et al., 2024). Nonetheless, saffron is frequently adulterated due to the high cost of production and the premium price it commands.

Saffron's quality depends on the concentration of compounds that give it its specific flavour, aroma, and colour [the higher the level of crocin, picrocrocin, and safranal, the higher the quality of saffron (Koocheki and Milani, 2020)] and other quality criteria described hereafter. All are considered when classifying saffron (as grade I, II, or III). The ISO 3632–1:2011 standard provides limits for extraneous matter from floral and plant waste (max. 0.5% for grade I, max. 3% for grade II, and max. 5% for grade III for the filaments and cut filaments of saffron), foreign matter from other plants (max. 0.1% for grade I, max. 0.5% for grade II, and max. 1.0% for grade III for the filaments and cut filaments of saffron), moisture and volatile matter (max. 12% for the filaments and cut filaments of saffron and max. 10% for the powder saffron), total ash on dry basis (max. 8%), acid-insoluble ash on dry basis (max. 1%), soluble extract in cold water on dry basis (max. 65%), flavour strength-absorbance of picrocrocin at 257 nm on dry basis (min. 70 for grade I, min. 70 for grade II, and min. 40 for grade III), aroma strength-absorbance of safranal at 330 nm on dry basis (min. 20-max. 50), colouring strength, which is the absorbance of crocin at 440 nm on dry basis (min. 200 for grade I, min. 170 for grade II, and min. 120 for grade III), and artificial colourants (absent). Physical, chemical, spectroscopic and chromatographic methods (Table 1) are applied to determine these parameters, as reported in the ISO 3632–2:2010 standard; the methods, they can be destructive or non-destructive.

The chemical composition of saffron has been examined in detail, and its stigmas have been shown to contain over 150 different constituents. Apart from the three primary compounds (picrocrocin, safranal, and crocetin esters), it also contains some beneficial constituents such as proteins, carbohydrates, lipids, minerals, fibres, carotenoids, flavonoids, anthocyanins, and vitamins (especially riboflavin and thiamine). However, to prevent weight fraud, moisture is the only nutritional parameter utilised to assess the commercial quality of saffron (José Bagur et al., 2018). Examples of adulteration practices are the addition of pigments or various plants with comparable morphology and colour, and the addition of plants that mimic the pigment composition of saffron or mixing other parts from the same plant.

Extensive research has been done to prevent adulteration by investigating the correlations between saffron's chemical profile and geographical origin. The International Standards Organization has recommended quality control methods to detect saffron adulteration (ISO 3632–2:2010). However, recent studies have shown that these need to be more detailed to differentiate between authentic and adulterated saffron.

Various analytical techniques are available in the literature to investigate the biochemistry of saffron and asses its authenticity (Panara et al., 2023). While highly reproducible in measurements and predictions, spectroscopic methods cannot provide spatial information about objects; computer vision techniques can only analyse visible bands and cannot give information about spectral properties. Recently, hyperspectral imaging has gained attention in detecting food fraud by overcoming these limitations (Bergomi et al., 2022; Malavi et al., 2024).

Herein, a search was conducted in the Scopus (http://www.scopus.com/) and PubMed (https://pubmed.ncbi.nlm.nih.gov/) databases to identify articles published on saffron using the additional keywords "quality control", "authenticity", "adulteration", "storage", "nutraceutical

Table 1
Test methods used to evaluate the quality of saffron.

Test method	Saffron in filaments form and cut filaments form	Type of test	Saffron in powder form	Type of test	Reference
Identification test	Examination by visualisation using a magnifying glass	Non-destructive	The colorimetric reaction with diphenylamine solution	Destructive	ISO 3632–2:2010
Microscopic examination	Observation in water, observation in an aqueous solution of NaOH, KOH or chloral hydrate, observation in aqueous iodine in iodide solution	Destructive	Idem	Destructive	
Determination of floral waste content	The floral waste is physically separated and then weighed	Non-destructive	–	–	
Determination of foreign matter content	The foreign matter is separated physically and then weighed	Non-destructive	–	–	
Determination of extract soluble in cold water	Extraction with cold water, filtration, drying of the extract obtained and weighed	Destructive	Idem	Destructive	
Determination of moisture and volatile matter content	Oven drying for 16 h at 103 °C	Destructive	Idem	Destructive	
Determination of total ash	Destruction of organic matter by heating at 550 °C until constant mass	Destructive	Idem	Destructive	
Determination of acid-insoluble ash	Treating the total ash with HCl, filtration, incineration, and weighing of the residue	Destructive	Idem	Destructive	
Determination of the main characteristics (flavour strength expressed as picrocrocin, aroma expressed as safranal, and colouring strength expressed as crocin) using a UV–vis spectrometric method	Measuring the specific absorbance of aqueous extract at three wavelengths (257 nm for picrocrocin, 330 nm for safranal, and 440 nm for crocin)	Destructive	Idem	Destructive	
Identification of synthetic water-soluble acidic colourants by thin-layer chromatography (TLC)	Artificial water-soluble acidic colourants are isolated and eluted by chromatography on a polyamide microcolumn, and identified by TLC	Destructive	Idem	Destructive	
Identification of synthetic water-soluble acidic colourants by high-performance liquid chromatography (HPLC)	Artificial water-soluble acidic colourants are isolated and eluted by chromatography on a polyamide microcolumn and identified by HPLC in reverse phase with a diode array detector (DAD)	Destructive	Idem	Destructive	

properties", "sensory characteristics", "crocin", "picrocrocin", and "safranal" without applying any filter; 671 publications were obtained from the Scopus database and 293 from PubMed. An increase in the number of publications over the last 10 years (2014–2024) in both databases was noted (Fig. 1). These were selected based on their relevance to the topic of the publication and the author's expertise (multiple publications), prioritising those that addressed official or new analysis methods; their citation numbers and the international relative importance of the journals where the articles were published were also considered. Those that did not meet these criteria or were not written in English were excluded. Eighty-four papers were cited in this review, being the most relevant among the many identified.

2. Harvesting and storage of saffron

Harvesting, collecting, transporting, drying, packaging, and storing are the stages involved in the manufacture of saffron (Fancello et al., 2018). Several factors can influence the quality of saffron stigmata, both before and after harvesting. Climatic conditions, corm origin, soil properties, water availability, fertilisation, and organic or conventional production methods can all impact its quality. For instance, extreme water stress can raise secondary metabolite concentrations in saffron stigmata, leading to higher levels of safranal, picrocrocin, and crocin. It has been found that the condition of the soil before harvesting can also impact the quality of saffron; the soil type influences the formation of flower tepal and stamen, thus producing by-products (Cardone et al., 2020b).

Research has shown that saffron flowers harvested at full bloom have higher ratios of crocin, picrocrocin, and safranal than those harvested at a later stage; delayed harvests can reduce the quality of saffron (Erden et al., 2016). The cultivation site also influences the quality of saffron; according to a study by Cardone et al. (2019), a cultivation site with higher air temperature and less frequent rain during the flowering phase produced the best stigma production with high-quality features.

A recent study by Aalizadeh et al. (2021) showed that applying organic fertilisers (manure and vermicompost) and bio-fertilisers improved the qualitative characteristics of saffron; under these procedures, the highest percentage of picrocrocin and crocin was achieved. In addition, compared to the control treatment, the combined application of bio-fertilisers increased the amount of safranal by 53%. Conversely, post-harvest practices, including drying techniques and storage times, can impact the quality of saffron (Fallahi et al., 2021). The drying process of stigmas is one of the most critical steps in turning them into spice; this post-harvest step is essential in developing its specific physical and chemical characteristics (Cid-Pérez et al., 2021). It is what gives saffron its unique taste, aroma, and colour. During the drying and storage processes, the characteristic odour of saffron develops. Safranal, picrocrocin, and crocin concentrations, as well as the solubility of the metabolite, define the quality of saffron (Chen et al., 2020). Physical, chemical, and biochemical factors must be regulated during the drying process to achieve the desired properties of saffron. The temperature and humidity should be kept at optimal levels until the stigmas have lost 80% of their weight; a temperature range of 35–45 °C and a maximum relative humidity of less than 50% are recommended. Since crocins are soluble in water and could be lost, washing the stigmas should be avoided. Light exposure can also impact saffron's quality (Chaouqi et al., 2018; Aghaei et al., 2019).

The dehydration conditions can impact the concentration of secondary metabolites. However, several factors, such as temperature, enzymatic activity, light exposure, moisture content, and oxygen level, influence the rate of deterioration. A high drying temperature reduces the microbial load (Fancello et al., 2018).

Saffron can be dried using sun- or ventilated air-drying. The most common industrial method uses hot air with ventilation, but it is energy-intensive and is lengthy, degrading thus the quality characteristics of saffron spice. Higher drying temperatures increase the dye strength compared to treatments at lower temperatures. Conventional drying is recommended for the best taste. Low-temperature drying is less expensive initially, but it takes longer and leads to uncontrolled drying conditions, which deteriorate the compounds and reduce the quality of saffron (Chen et al., 2020; Mollafilabi et al., 2020).

Regardless of the drying method, it affects the spice's taste, aroma, and colour. After drying, saffron should be kept in tightly closed containers and protected from direct sunlight to prevent oxidative or hydrolytic breakdown of its secondary metabolites. The decomposition rate depends on the storage temperature, relative humidity of air, and exposure to light. Proper drying and storage are critical to obtain saffron of suitable quality (Chaouqi et al., 2018; Sereshti et al., 2018).

3. Active compounds in saffron

The *Crocus sativus* L. saffron is a traditional medicinal herb containing bioactive compounds; its quality and value depend on the levels of three characterising chemical markers: crocin, picrocrocin, and safranal. The contents of various active ingredients reported in the literature may be more consistent; significant differences are mainly due

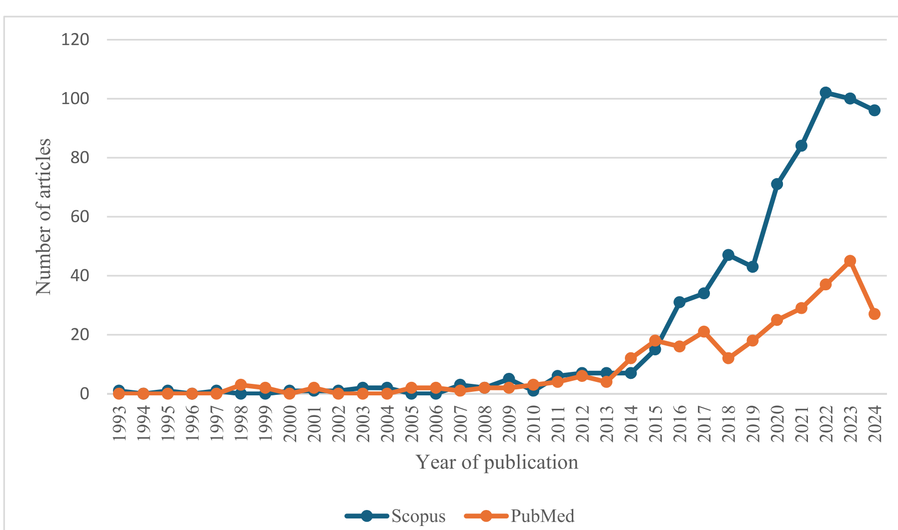

Fig. 1. Number of articles retrieved from Scopus and PubMed using the keywords "saffron", "quality control", "authenticity", "adulteration", "storage", "nutraceutical properties", "sensory characteristics", "crocin", "picrocrocin", and "safranal" without applying any filter.

to the different origin areas of spice, production technologies, or the analytical methods applied.

3.1. Crocetin and crocin

Crocetin is a natural carotenoid compound present in the stigmas; it is glycosylated by glucosyltransferase, allowing the formation of different *cis* and *trans* forms of crocetin-derived glycosides named crocin, such as *trans*-crocetin di-(β-D-gentibiosyl) ester, *trans*-crocetin (β-D-neapolitanosyl)-(β-D-gentibiosyl) ester), *trans*-crocetin (β-D-glucosyl)-(β-D-neapolitanosyl) ester, *cis*-crocetin di-(β-D-gentibiosyl) ester, *cis*-crocetin (β-D-glucosyl)-(β-D-gentibiosyl) ester, and *cis*-crocetin di-(β-D-glucosyl) ester. In contrast to crocetin, crocin is a carotenoid soluble in water because of its glucosidic bond, which gives saffron its characteristic yellow-red colour (Sabatino et al., 2011). This carotenoid's peculiar water-solubility property is essential if it must be dispersed or conveyed in a water-soluble substrate; for this reason, besides being used as a flavouring spice, it is often used to colour foods. Crocin is the most representative compound, with percentages ranging from 17.9% to 54.88% (Lage and Cantrell, 2009; Masi et al., 2016). Moratalla-López et al. (2019) reported that the content of crocetin ester is between 16% and 28%; in some harvesting years, it can reach up to 30%. The content of crocin is a crucial quality parameter to evaluate saffron and the products that it contains. Crocin is a compound with a long chain structure that contains unsaturated hydrocarbons, which makes it highly sensitive to temperature, acidity, light, and oxygen. Studies have shown that the degradation rate of crocin is significantly affected by temperature and pH. A study regarding the impact of culinary processing time on bioactive compounds of saffron indicated that crocin undergoes up to 50% degradation after 30 min of cooking (Rodríguez-Neira et al., 2014). In an experiment where saffron was used in production of craft beer, it was found that heat treatment had affected the product's colour (Buiatti et al., 2024). Therefore, it is recommended to use crocin as a natural colourant in moderately or low acidic foods or in those exposed to mild heat treatment (Karasu et al., 2019). To preserve the integrity of this molecule, in gastronomic preparations it is advisable to add the spice only in the final stages, thus avoiding high temperatures for prolonged periods. To preserve and enhance the peculiar characteristics of saffron, macerating the whole filaments or powder for up to 24 h before culinary use and adding it to the preparation shortly before the end of cooking is also recommended.

Regarding the extracts, attention must also be paid to the extracting solvent and pH conditions, and it is also crucial to accurately measure its concentration and consider its stability during storage, especially when using it in pharmaceutical or nutraceutical preparations. Another property that crocetin exhibits, beyond various biological effects, is the ability to improve oxygen diffusivity. This feature is highly relevant not only in the therapeutic field, but also for industrial applications that require a high level of oxygen diffusivity, such as the production of enzymes by fermentation, oxidation-reduction reactions in sewage treatment, and the production of plasticisers for the plastic and rubber industry (Giaccio, 2004).

3.2. Picrocrocin

Picrocrocin, with chemical name 4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cy-clohexene-1-carboxaldehyde, is a glycoside, the precursor of the monoterpene glycoside of safranal; it is a colourless, water-soluble substance that gives saffron its typical bitter taste. Picrocrocin is the second most present active ingredient after crocin, with quantities varying from 0.05% to 28.78% (Alonso et al., 2001; Caballero-Ortega et al., 2007; Lage and Cantrell, 2009; Masi et al., 2016; Predieri et al., 2021). Picrocrocin is a relatively stable compound and, unlike crocin, does not show differences in concentration at different cooking times (Rodríguez-Neira et al., 2014). Because of its bitter taste, certain limitations apply to the use of saffron and its extracts in the food industry; therefore, the correct quantification of picrocrocin, the main compound responsible for this, is of fundamental importance. Research on picrocrocin's bitterness has determined threshold concentrations of 5.34 mg/L for detection and 7.26 mg/L for recognition (Chrysanthou et al., 2015). Formulation choices must refer to these concentrations to develop products and extracts that consumers widely accept. In any case, other chemical components also contribute to the gustatory definition. Predieri et al. (2021) reported that crocin can contribute to the bitter intensity of saffron and key sensory characteristics such as astringency and pungency. The natural presence of crocetin esters masks the perception of saffron's bitterness; in contrast, the presence of aromatic compounds improves it. Other variables that influence and increase the perception of bitterness are higher serving temperature and presence of ethanol (Chrysanthou et al., 2015). A particular experiment on crocin revealed that, at comparatively low concentrations, this substance produces both bitter and astringent effects when dissolved in solution (Predieri et al., 2021).

3.3. Safranal

Safranal, with the chemical name 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde, a cyclic monoterpene, is not present in the fresh plant; it is formed due to the action of β-glucosidase on picrocrocin, with the liberation of aglycone 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, and then because of dehydration in the drying stage. Safranal is the constituent responsible for saffron's characteristic aroma; it appears as a pale yellowish oily liquid, is soluble in water, and is very volatile (D'Auria et al., 2003; Caballero-Ortega et al., 2007). The literature reports safranal concentrations between 0.02% and 0.48% (Caballero-Ortega et al., 2007; Lage and Cantrell, 2009; Masi et al., 2016; Predieri et al., 2021). Apart from safranal, more than 160 volatile constituents have been found; of these, 4-ketoisoforone and dihydroxy-oforone are those that primarily contribute to saffron's aroma (Amanpour et al., 2015; Cardone et al., 2020b). Many parameters influence the concentrations of these active ingredients, such as production area, cultivation method, processing technology, and storage conditions (Lage and Cantrell, 2009; Sarfraz et al., 2024). The ISO 21983:2019 standard indicates guidelines for the harvesting, transportation, separation of stigma, drying, and storage of saffron before packing.

4. Quality control of saffron

Given its high cost, saffron is susceptible to counterfeiting, mainly by mixing it with parts from other plants; the most frequent adulterants used are safflower, marigold, turmeric, and pepper (Koocheki and Milani, 2020; Maquet et al., 2021). The traders sell the spice in the form of pistils or powder, and the risks of adulteration are significantly higher for the latter; the plant's anatomical part is no longer recognisable in the ground spice. Other forms of adulteration include the addition of foreign substances (condensed or older saffron), various parts of the saffron plant (stamens or cut and coloured perigone), substances that increase weight, animal substances (salted and dried meat fibres), artificial substances (coloured gelatine fibres), and organic dye substances (Koocheki and Milani, 2020). Corradi and Micheli (1979) reported that powdered saffron is rarely genuine and is often mixed with foreign substances. Therefore, when purchasing, which is a warning for consumers and chefs, it is best to choose saffron in stigmas. In addition, the volatile substances are lost when crushing the saffron stigmas to powder, decreasing the intensity of its aroma. Thus, the official control bodies must adopt procedures to verify the quality of saffron to prevent fraud and classify it correctly; the analytical methods to determine quality parameters must be standardised.

Standards ISO 3632–1:2011 and ISO 3632–2:2010 specify the requirements and associated test methods (Table 1) for dried saffron derived from the pistils of *Crocus sativus* L. flowers. The ISO

3632–1:2011 standard mentions physical criteria and chemical properties, with their limits, based on which saffron (in filaments, cut filaments, and powder) is classified in a quality category (I, II or III). The test methods laid down in the ISO 3632–2:2010 standard (Table 1) include an identification test (visual examination of saffron with a magnifying glass) followed by a microscopic examination to establish whether the sample is exclusively composed of *Crocus sativus* L. stigmas or contains fragments of pistils and grains of pollen; it also highlights any floral waste and foreign bodies. In the case of saffron in powder form, the identity test involves mixing it with a diphenylamine solution; if the sample is pure, a blue colour is formed immediately that quickly turns reddish-brown.

The content of floral waste and foreign matter is determined only for saffron in filaments and cut filaments. Other testing methods are mentioned in the specialised literature, which, although advanced, cannot be used officially to classify the quality of saffron as they still need to be standardised. A case in point is the method based on sequence-characterised amplified regions (SCARs) developed by Marieschi et al. (2012) to detect plant adulterants in saffron. The technique enables the detection of low amounts (up to 1%) of several bulking agents (*Arnica montana* L., *Bixa orellana* L., *Calendula officinalis* L., *Carthamus tinctorius* L., *Crocus vernus* L. (Hill), *Curcuma longa* L., and *Hemerocallis* sp.) based on their random amplified polymorphic DNA markers (RAPDs).

Measuring the water content in saffron is very important to establish its microbiological stability and commercial value (Naviglio et al., 2010). The method indicated by the ISO 3632–2:2010 standard to determine saffron's moisture and content of volatile matter is based on its gravimetric weight loss after heating at 103 °C for 16 h. The Karl Fischer titration method suggested by Naviglio et al. (2010) to estimate the moisture content in saffron powder is faster, interference-free, and requires a smaller amount of saffron than the gravimetric procedure; however, the volatile matter content is not determined in this way. Economic adulteration by mixing saffron with other plants or foreign materials, which increases its weight, can also be detected by determining the cold-water-soluble extract, total ash, and acid-insoluble ash (FAO/OMS, 2021).

The determination of synthetic water-soluble acidic colourants in saffron consists of their extraction, isolation and elution, as well as detection and quantification using thin-layer chromatography (TLC) or reversed-phase high-performance liquid chromatography coupled with diode array detection (RP-HPLC-DAD); during extraction, successive washes or acidic treatment eliminates the natural saffron pigments, particularly crocin. The HPLC method only applies to detect and quantify the following colourants at levels above the minimum required performance limits (MRPLs): amaranth (1 mg/kg), azorubine (1 mg/kg), orange II (2 mg/kg), ponceau 4R (1 mg/kg), roccelline (2 mg/kg), quinoline yellow (1 mg/kg), sunset yellow (1 mg/kg), tartrazine (1 mg/kg), and yellow 2G (1 mg/kg). Using artificial colourants to enhance the colour of counterfeit saffron (mixed with non-saffron plant material) is probably the largest food safety issue associated with saffron fraud, as many adulterants have been reported to have adverse effects on human health (Bhooma et al., 2020). By using mid-level data fusion of TLC imaging and Raman spectral data, Dai et al. (2023) distinguished with excellent accuracy both artificial colourants (red 40, 96% and yellow 5, 100%) and adulterants like extraneous natural plant materials (safflower, 100% and turmeric, 100%). Fattahi et al. (2023) recommended an easy-to-use method to assess saffron adulteration with synthetic colourants like tartrazine, sunset yellow, ponceau 4-R, and erythrosine; it involves applying a spectral fingerprint provided by an ion mobility spectrometer (IMS) coupled with multivariate data analysis.

The main characteristics of saffron (flavour strength expressed as picrocrocin, aroma strength expressed as safranal and colouring strength expressed as crocin) are determined using an ultraviolet–visible (UV–Vis) method. Specifically, it involves measuring the variation in optical density of an aqueous extract of saffron between 200 nm and 700 nm at ambient temperature. The data are obtained by directly reading the specific absorbance of picrocrocin at 257 nm, crocin at 440 nm, and safranal at 330 nm; the results are expressed in $A_{1\,cm}^{1\%}(\lambda_{max})$ on dry basis. The advantages of this method are that it is fast, inexpensive, and easy to carry out. However, this technique has some quantification errors and, therefore, limitations. *Cis*-crocin isomers interfere with picrocin, while with safranal, both *cis*- and *trans*-crocin isomers, lead to the overestimation of these two compounds in samples with large amounts of crocin. *Trans*-crocin isomers display a first band at 260 nm (glycosidic ester bond) and a second between 400 and 470 nm (typical of carotenoids); the *cis*-crocin isomers also have a third band at 328 nm. Kaempferol derivatives in the sample, which absorb UV–Vis light at 264 and 344 nm, are other compounds that can interfere with these measurements. Another limitation of this method is the low solubility of safranal in water, which is the solvent used to prepare the saffron extract before UV–Vis analysis (Avila-Sosa et al., 2022). The Uv–Vis method is also vulnerable to any yellow colourant used as an adulterant, natural plant materials (safflower, marigold, and turmeric), or artificial substances, as it increases the extract's absorbance value at 440 nm, thus giving a false reading (Dai et al., 2020; Sabatino et al., 2011). As the ISO 3632 UV–Vis spectrophotometric method cannot separate and quantify individual components of saffron (Zalacain et al., 2005), the three main characteristic compounds of saffron (picrocrocin, crocin, and safranal, responsible for its organoleptic properties) were also studied using chromatographic techniques; some are detailed below in chronological order. Despite their great sensitivity and accuracy, they are time-consuming and expensive, requiring complicated sample preparation, specialised laboratory settings, and well-trained operators (Dai et al., 2020, 2023).

A study that used HPLC-PDA/ESI-MS (high-performance liquid chromatography combined with a photodiode array and electrospray ionisation mass spectrometry) to evaluate saffron adulteration has shown that UV–Vis spectrophotometric analysis is not a specific method and cannot detect the addition of up to 20% (w/w) adulterants (like safflower, marigold, and turmeric) (Sabatino et al., 2011). The gas chromatographic (GC) method developed by Bononi et al. (2015), using ethyl alcohol as a solvent and ultrasonic-assisted extraction, is more specific than the UV–Vis method and more useful for commercial comparisons of saffron quality. D'Archivio et al. (2016) classified Italian saffron geographically based on the relative abundance of crocins, safranal, picrocrocin, and flavonoids quantified by HPC-DAD and applying linear discriminant analysis. The comparative evaluation of the ISO 3632 UV–Vis method with an HPLC-DAD method by García-Rodríguez et al. (2017) to quantify safranal in saffron revealed that the latter avoids the overestimation due to overlapping with other compounds that absorb at 330 nm; therefore, it is preferable to the UV–Vis method when the safranal content is used to classify saffron into commercial categories. The study by Liu et al. (2018), an integrated approach combining HPLC/DAD, GC/MS, near-infrared (NIR) spectroscopy, and chemometrics, was used to discriminate saffron samples from Iran and China geographically; they also studied the correlation between the safranal content determined by the ISO 3632 UV–Vis method and HPLC and GC. Their findings suggested that the ISO standard needs to be revised, particularly for the analysis of safranal. Suchareau et al. (2021) proposed an improved method to quantify crocins in saffron by HPLC-DAD; they used an internal standard quantification method that applies a response factor corrected with the molecular weight of each crocin. In a recent study, Filatova et al. (2024) used HS-SPME headspace solid-phase microextraction (HS-SPME) for sample extraction, followed by gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) for screening of non-targeted volatiles in saffron in 38 authentic saffron samples and 25 samples of plant materials (potential saffron adulterants such as safflower, calendula, capsicum, and turmeric). The principal component analysis (PCA) and partial least squares discriminative analysis

(PLS-DA) were used in the chemometric approach of the data generated, which resulted in a good separation of authentic saffron from plant materials. Safranal was one of the 11 volatile markers identified for saffron (2-methyl furan, 2(5H)-furanone, 6-methyl-2-heptanone, 6-methyl-5-hepten-2-one, dihydroisophorone, isophorone, 4-oxoisophorone, 2-hydroxy-4-oxoisophorone, safranal, mint furanone, and 4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde).
Using saffron volatiles as a case study, Parastar and Weller (2024) proposed a hybrid strategy that applies the design of experiment (DOE) and machine learning (ML) methodologies to optimise the gas chromatography-ion mobility spectrometry (GC-IMS) conditions in a non-targeted volatilomic/flavoromic analysis. They also identified safranal among the seven main volatile compounds that are characteristic of saffron (phenylethyl alcohol, safranal, α-isophorone, 4-oxoisophorone, 2(3H)-furanone dihydro-5-propyl-, (5H)-furanone, and 2,6,6-trimethyl-4-hydroxy-1-cyclohexene-1-carboxaldehyde).

Research on choices made by restaurants or consumers when purchasing saffron indicates that preferences are oriented towards lower prices, local origin, quality certifications, and organic farming techniques (Sanjuán-López and Resano-Ezcaray, 2020). In commercially-available saffron packs, the product category usually needs to be reported; this would help the consumer to be aware of the quality when purchasing the product. Sarfraz et al. (2024) suggest that high-quality saffron should include roughly 30% crocin, 5–20% picrocrocin, and 0.5–2.5% safranal (a percentage of total stigma weight).

For the valorisation and protection of the quality of agri-food production relating to saffron, consortia have been established which led to the recognition of some PDO products (Protected Designation of Origin): Krokos Kozanis (Greece), Zafferano dell'Aquila (Italy), Azafrán de la Mancha (Spain), Zafferano di San Gimignano (Italy), Safranbolu Safrani (Turkey), and Zafferano di Sardegna (Italy). The chemical compositional characteristics that each must satisfy are described in the products' specifications (single documents) admitted for protection. These characteristics refer to the concentrations of the three chemical molecules that define the product's quality: crocin, picrocrocin, and safranal. In all registered PDOs, the compliant contents of these molecules refer to the specifications indicated in the ISO 3632–1:2011 standard and correspond to product category I, except for Zafferano dell'Aquila. The specification does not directly refer to the ISO standard table. However, it indicates, for the colour, a crocin number greater than 7.5% for the powder and 6.0% for the filaments, while for the aroma, a safranal content greater than 3% for the powder and 4% for the filaments. Zafferano dell'Aquila's specification indicates the picrocrocin evaluation and the parameter $\Delta E_{Picrocrocin} > 0.400$ for both the powder and the filaments. The introduction of the $\Delta E_{Picrocrocin}$ parameter allows a correct assessment of picrocrocin. It is known that the spectrophotometric reading at 257 nm overestimates its actual content due to constantly occurring interferences with crocetin esters (Del Campo et al., 2010). Considering this evident gap, other authors have proposed the calculation of $\Delta E_{Picrocrocin}$ (Corradi and Micheli, 1979). All these determinations are carried out using spectrophotometric analytical methods with all the analytical limits highlighted by the literature related to an overall quantification without any previous chromatographic separation.

The purchase of a PDO product or a non-pulverised product certainly helps to protect the consumer from counterfeits and guarantees a superior category product. In the case of PDO production in Zafferano San Giminiano, Zafferano di Sardegna, Safranbolu Safrani, and Azafrán de la Mancha, the producers rightly decided to market it only in stigmas and not ground to reduce the possibility of adulteration. On the other hand, in the past, even the famous gastronome Artusi Luciano and Artusi and Artusi (2011), considering these aspects, recommended in the recipe for Risotto alla Milanese: "Saffron, if you have a bronze mortar at home, buy it in kind, crush it finely and dissolve it in a drop of hot broth before throwing it into the rice." Fig. 2 shows the method to correctly use saffron in the gastronomic preparations.

5. Nutraceutical properties of saffron

The main components of a saffron plant are the corm, foliar structure, and floral organs (Khan et al., 2020). The saffron spice is obtained by drying the *Crocus sativus* L. plant stigmas; it is commonly used in

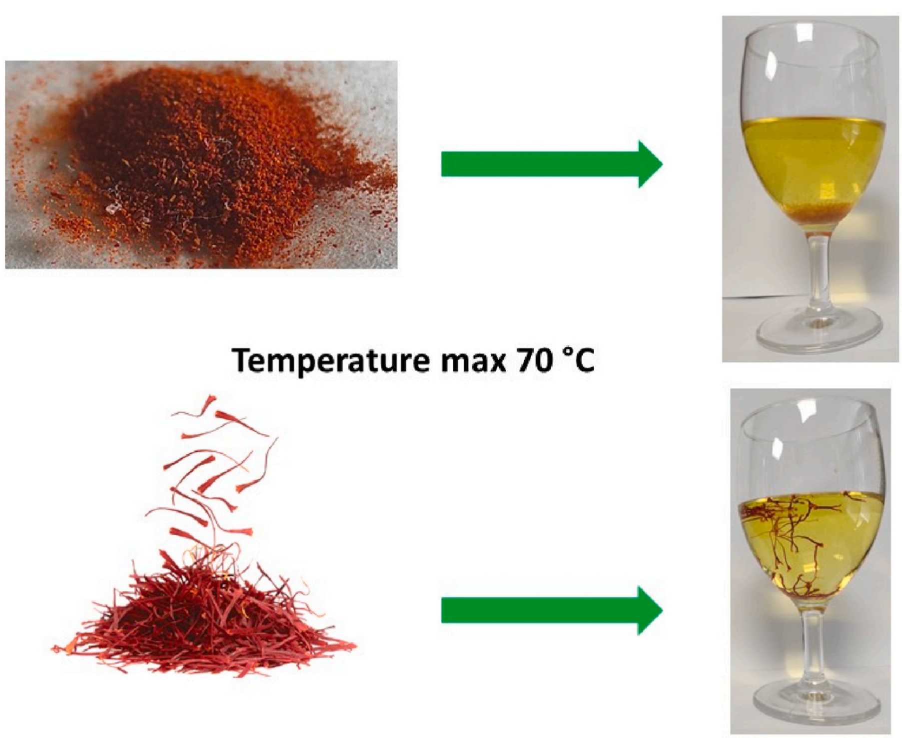

Fig. 2. The method to correctly use saffron in gastronomic preparations.

cooking to impart aroma, flavour, and colour to food and beverages (José Bagur et al., 2018).

Because of its therapeutic properties, saffron has long been considered a medicinal plant (Fujii et al., 2022). The spice's popularity has been attributed to apocarotenoids like safranal, which is responsible for the unique scent of saffron, crocin for the intense colour, and picrocrocin for the bitter taste (Bukhari et al., 2018). Folk medicine has utilised saffron as an antispasmodic and eupeptic to treat colds, coughs, bronchial spasms, asthma, lumbar pain, menstrual cramps, heart disease, smallpox, and scarlet fever (Husaini et al., 2021).

In a placebo-controlled, double-blind, randomised trial by Gout et al. (2010), the hypothesis was tested that oral supplementation with "Satiereal" (an extract of saffron stigma) may enhance satiety. Sixty healthy, slightly overweight women participated in this study for eight weeks. Thirty-one were given a "Satiereal" capsule twice daily, and 29 were given a matching placebo. The results showed decreased snacking frequency in the treated group and a more significant reduction in body weight, thus demonstrating a satiating effect of "Satiereal".

Mashmoul et al. (2014) assessed the anti-obesity properties of saffron ethanolic extracts (40 and 80 mg/kg) in rats. For 12 weeks, animals were fed a diet heavy in fat to cause obesity. In the following eight weeks, rats were subjected to a high-fat diet and saffron extracts. At 80 mg/kg, the saffron extract significantly reduced the food consumed by obese rats; the atherogenic index, which measures the ratio of low-density lipoprotein (LDL) cholesterol to high-density lipoprotein (HDL) cholesterol, improved at 40 mg/kg. These results thus demonstrated the anti-obesity effects of saffron extract in a preclinical study.

The meticulous research by Gismondi et al., in 2012 delved into the *in vitro* antioxidant properties of saffron extract and its antineoplastic effects against the highly metastatic murine B16-F10 melanoma cell line. The study revealed the highly reducing and chemo-preventive activity of the extract with the scientific rigour and reliability.

Beiranvand et al. (2016) conducted a study to determine whether saffron affects the severity of premenstrual syndrome in females. The control group was given placebo capsules once daily for two menstrual cycles, while the tested group was given capsules containing 30 mg of stigma extract from dried saffron. The results indicated that saffron lessens the severity of premenstrual syndrome; however, additional research is required to validate its efficacy in treating the condition.

In another study, Ebrahimi et al. (2019) evaluated the effects of saffron supplementation as an adjunct therapy in type 2 diabetes. Participants were randomized to either saffron tablets or placebo for 12 weeks. Saffron supplementation significantly reduced waist circumference and malondialdehyde in fasting blood compared with placebo. However, it did not affect cardiometabolic risk markers in people with diabetes.

Khan et al. (2020) explored the potential nutraceutical efficacy of some ethanolic extracts prepared from leaf, corm, petal, and stigma of saffron as anti-inflammatories, analgesics, anticoagulants, and antidepressants in mice. The stigma ethanolic extract and petal ethanolic extract were shown to be safe, natural remedies for inflammation, pain, depression, and blood coagulation. These properties were attributed to carotenoids (crocin, crocetin, picrocrocin, and safranal) and flavonoids (kaempferol), the bioactive compounds with the highest concentration in these parts of saffron.

In another study, Sun et al. (2020) investigated the pharmacological potential of saffron wastes (tepal and stamen) in terms of bioactive flavonoids. Kaempferol-3-*O*-sophoroside and quercetin-3-*O*-sophoroside were extracted from tepal and then evaluated for cytogenetic and antioxidant effects. Quercetin-3-*O*-sophoroside showed the best antioxidant effect; it inhibited H_2O_2-induced cell apoptosis by diminishing the level of cellular reactive oxygen species. Moreover, quercetin-3-*O*-sophoroside did not exhibit a cytogenic effect on meristem cells in onion root; nevertheless, a chromosomal abnormality was noticed at the highest concentration (200 ppm) tested.

Marzabadi et al. (2022) evaluated the impact of a 12-week administration of Saffrotin® capsules (an ethanolic extract prepared from saffron) on oxidative stress, cognitive outcomes, serum antioxidant levels, and anti-inflammatory markers in patients with mild-to-moderate Alzheimer's disease who were treated with donepezil. Patients in the saffron group exhibited improved antioxidant, inflammatory, and oxidative stress profiles than those in the placebo group. This finding suggests that it has additive, beneficial effects on circulatory markers in patients with Alzheimer's disease. Nevertheless, the study did not reveal any impact of saffron intake on cognitive outcomes.

Najafabadi et al. (2022) examined the ability of saffron to treat erectile dysfunction in men. Sixty-two individuals with at least mild erectile dysfunction were randomly divided into two groups that received two daily doses of either 15 mg placebo capsules or saffron for six weeks. Saffron was seen to improve erectile function more effectively than the placebo. It was concluded that saffron may be an effective and safe alternative to alleviate erectile dysfunction, particularly in patients who refuse or cannot take phosphodiesterase type 5 inhibitors.

A recent *in vitro* study by Khan et al. (2024) investigated if saffron affected key intermediates in prostate cancer development. Notable apoptotic pathway-mediated suppression of cell proliferation was seen in androgen-sensitive prostate cancer cell lines. According to these findings, man with prostate cancer may benefit from taking saffron supplements in addition to their regular course of therapy.

6. Conclusions

In conclusion, saffron, derived from *Crocus sativus* L. stigmas, is one of the most expensive spices; cultivation, harvesting, processing, and storage practices significantly impact its quality and value. The spice's distinctive colour, flavour, aroma, and medicinal properties are attributed to its complex biochemical composition, especially crocin, crocetin, picrocrocin, and safranal.

The frequent adulteration practices of saffron, mainly by mixing with other plant materials and artificial colourants, pose a challenge for quality control. Although there are ISO-standardised analysis methods, some may not always effectively classify saffron quality, like the ISO 3632 UV–Vis spectrophotometric method for quantification of picrocrocin, safranal, and crocin. It does not include any preliminary separation of the three compounds of interest, and due to interferences, it leads to an approximate estimation of them. Moreover, more effective quality control measures may mitigate adulteration risks by adopting more selective and sensitive analytical methods, so that consumers receive authentic, high-quality saffron for culinary and medicinal purposes. Advanced analytical techniques, such as liquid or gas chromatography, provide more precise and sensitive authentication and quality assessment procedures. It would be desirable to validate and harmonise new techniques to be used as a reference to quantify the specific markers of authenticity and quality and achieve correct and objective classification of saffron. An update of the ISO3632-2 standard by introducing an HPLC-DAD method could simultaneously and precisely determine the three main characteristic molecules: crocin, picrococin, and safranal.

Saffron's nutraceutical properties have been extensively studied, showcasing its potential therapeutic benefits in various conditions. From its role in enhancing satiety to its anti-obesity, anti-inflammatory, and antidepressant effects, saffron has promise as an extractive and vegetal remedy. Several studies suggest its potential in managing conditions like premenstrual syndrome, Alzheimer's disease, type 2 diabetes, erectile dysfunction, and even a possible inhibiting effect on the development of prostate cancer.

It is advised that saffron be bought in filaments instead of powder form to avoid the risk of fraud. Considering the expanding market as both a spice and a supplement, the specification of saffron quality (grade I, II, and III) on the label would allow the consumer to make an informed decision when purchasing the product. When used as an ingredient in a gastronomic preparation, it is important to not use high temperatures and prolonged cooking times. Knowledge of this spice and awareness regarding price and quality is not even among technicians and chefs; by contrast, the less expensive powdered product is more common, while the presence of the spice in stigmas in small shops and large-scale retail trade is rare.

7. Implication of the research on saffron for the gastronomy field

Saffron is a spice that is widely used in the gastronomic field in both traditional and innovative preparations. The peculiarity of the molecules that mainly characterise it, crocin, picrocrocin and safranal, can impart flavour, aroma, and colour to dishes. The product's quality and consistent price are directly related to the presence of these active components. For this reason, it is relevant to discuss the actual classification and the official methods applied today for quality control, often out to date, to prevent fraud and propose feasible and effective alternatives. By being aware of the physicochemical properties of the characterising molecules, such as the peculiar solubility of crocin that, despite being a carotenoid, is completely water-soluble, it will be possible to apply all the necessary instructions in practice, especially during the recipe creation. Thus, the spice can be consciously used with "compatible" ingredients, avoiding prolonged cooking of the final dish at high temperatures (above 70 °C) to prevent degradation of the spice's active constituents. Knowledge of the usual concentrations of the active molecules in traditional dishes and servings, especially in the case of picrocrocin and safranal, is essential to balance its bitterness, the typical saffron olfactory note, and the possibility that the dish can still be considered functional.

8. Implications for gastronomy

The paper focuses on the composition of saffron from *Crocus sativus* L., with particular attention to the characterising active ingredients and their taste. Aspects relating to product classification and quality evaluation are also discussed. The document aims to represent a guide for the conscious choice of raw materials for gastronomic uses and, generally, for the food sector.

CRediT authorship contribution statement

Cristina Anamaria Semeniuc: Writing – review & editing. **Mara Mandrioli:** Writing – review & editing, Conceptualization. **Maria Jenica Urs:** Writing – review & editing. **Tullia Gallina Toschi:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

While preparing and writing this work, the authors did not use artificial intelligence-based applications.

Funding

This research was supported and funded by the Department of Agricultural and Food Sciences, Alma Mater Studiorum - Università di Bologna Gallina Toschi RFO-2022.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

No data was used for the research described in the article.

References

Aalizadeh, M.B., Shafaroodi, A., Ebadi, A., 2021. Evaluation of quantitative and qualitative traits of saffron (*Crocus sativus* L.) in response to organic, chemical and biological fertilizers in climatic conditions of Ardabil province. Crop Physiol. J. 13 (49), 129–147. http://cpj.ahvaz.iau.ir/article-1-1432-en.html.

Abu-Izneid, T., Rauf, A., Khalil, A.A., Olatunde, A., Khalid, A., Alhumaydhi, F.A., Aljohani, A.S.M., Sahab Uddin, M., Heydari, M., Khayrullin, M., Shariati, M.A., Aremu, A.O., Alafnan, A., Rengasamy, K.R.R., 2022. Nutritional and health beneficial properties of saffron (*Crocus Sativus* L): a comprehensive review. Crit. Rev. Food Sci. Nutr. 62 (10), 2683–2706. https://doi.org/10.1080/10408398.2020.1857682.

Aghaei, Z., Jafari, S.M., Dehnad, D., 2019. Effect of different drying methods on the physicochemical properties and bioactive components of saffron powder. Plant Foods Hum. Nutr. 74 (2), 171–178. https://doi.org/10.1007/S11130-019-00729-7.

Alonso, G.L., Salinas, M.R., Garijo, J., Sánchez-Fernández, M.A., 2001. Composition of crocins and picrocrocin from Spanish saffron (*Crocus sativus* L.). J. Food Qual. 24 (3), 219–233. https://doi.org/10.1111/j.1745-4557.2001.tb00604.x.

Álvarez, E., González-Hedström, D., Morán-Valero, M.I., SanJuan, R.M., Trapero, V., Díez-Municio, M., 2024. Evaluation of the organoleptic perception of a new saffron (*Crocus sativus* L.) powder as a flavoring substitute for saffron stigmas in culinary applications. Int. J. Gastron. Food Sci. 35 (March), 100834. https://doi.org/10.1016/j.ijgfs.2023.100834.

Amanpour, A., Sonmezdag, A.S., Kelebek, H., Selli, S., 2015. GC–MS–olfactometric characterization of the most aroma-active components in a representative aromatic extract from Iranian saffron (*Crocus sativus* L.). Food Chem. 182 (September), 251–258. https://doi.org/10.1016/j.foodchem.2015.03.005.

Artusi, L., Artusi, R., 2011. A tavola con gli Artusi: 120 anni dopo. La Scienza in Cucina E L'Arte Di Mangiar Bene. I Migliori Piatti Abbinati a Divagazioni E Aneddoti Di Vita Fiorentina, first ed. Firenze, Italy.

Avila-Sosa, R., Nevárez-Moorillón, G.V., Ochoa-Velasco, C.E., Navarro-Cruz, A.R., Hernández-Carranza, P., Cid-Pérez, T.S., 2022. Detection of saffron's main bioactive compounds and their relationship with commercial quality. Foods 11 (20), 3245. https://doi.org/10.3390/foods11203245.

Beiranvand, S.P., Beiranvand, N.S., Moghadam, Z.B., Birjandi, M., Azhari, S., Rezaei, E., Salehnia, A.N., Beiranvand, S., 2016. The effect of *Crocus sativus* (saffron) on the severity of premenstrual syndrome. Eur. J. Integr. Med. 8 (1), 55–61. https://doi.org/10.1016/j.eujim.2015.06.003.

Bergomi, A., Comite, V., Santagostini, L., Guglielmi, V., Fermo, P., 2022. Determination of saffron quality through a multi-analytical approach. Foods 11 (20), 3227. https://doi.org/10.3390/FOODS11203227.

Bhooma, V., Nagasathiya, K., Vairamani, M., Parani, M., 2020. Identification of synthetic dyes magenta III (new fuchsin) and rhodamine B as common adulterants in commercial saffron. Food Chem. 309 (March), 125793. https://doi.org/10.1016/j.foodchem.2019.125793.

Bononi, M., Milella, P., Tateo, F., 2015. Gas chromatography of safranal as preferable method for the commercial grading of saffron (*Crocus sativus* L.). Food Chem. 176 (June), 17–21. https://doi.org/10.1016/j.foodchem.2014.12.047.

Buiatti, S., Guglielmotti, M., Bertin, F., Bertoli, S., Passaghe, P., 2024. Use of Friulan saffron in the production of craft beer. Eur. Food Res. Technol. 250 (1), 325–335. https://doi.org/10.1007/s00217-023-04389-5.

Bukhari, S.I., Manzoor, M., Dhar, M.K., 2018. A comprehensive review of the pharmacological potential of *Crocus sativus* and its bioactive apocarotenoids. Biomed. Pharmacother. 98 (February), 733–745. https://doi.org/10.1016/j.biopha.2017.12.090.

Caballero-Ortega, H., Pereda-Miranda, R., Abdullaev, F.I., 2007. HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. Food Chem. 100 (3), 1126–1131.

Cardone, L., Castronuovo, D., Perniola, M., Cicco, N., Candido, V., 2020a. Saffron (*Crocus sativus* L.), the king of spices: an overview. Sci. Hortic. 272 (October), 109560. https://doi.org/10.1016/J.SCIENTA.2020.109560.

Cardone, L., Castronuovo, D., Perniola, M., Cicco, N., Candido, V., 2019. Evaluation of corm origin and climatic conditions on saffron (*Crocus sativus* L.) yield and quality. JSFA 99 (13), 5858–5869. https://doi.org/10.1002/JSFA.9860.

Cardone, L., Castronuovo, D., Perniola, M., Scrano, L., Cicco, N., Candido, V., 2020b. The influence of soil physical and chemical properties on saffron (*Crocus sativus* L.) growth, yield and quality. Agronomy 10 (8), 1154. https://doi.org/10.3390/AGRONOMY10081154.

Cerdá-Bernad, D., Valero-Cases, E., Pastor, J.J., Frutos, M.J., 2022. Saffron bioactives crocin, crocetin and safranal: effect on oxidative stress and mechanisms of action. Crit. Rev. Food Sci. Nutr. 62 (12), 3232–3249. https://doi.org/10.1080/10408398.2020.1864279.

Chaouqi, S., Moratalla-López, N., Lage, M., Lorenzo, C., Alonso, G.L., Guedira, T., 2018. Effect of drying and storage process on Moroccan saffron quality. Food Biosci. 22 (April), 146–153. https://doi.org/10.1016/J.FBIO.2018.02.003.

Chen, D., Xing, B., Yi, H., Li, Y., Zheng, B., Wang, Y., Shao, Q., 2020. Effects of different drying methods on appearance, microstructure, bioactive compounds and aroma compounds of saffron (*Crocus sativus* L.). Lebensm. Wiss. Technol. 120 (February), 108913. https://doi.org/10.1016/J.LWT.2019.108913.

Chrysanthou, A., Pouliou, E., Kyriakoudi, A., Tsimidou, M.Z., 2015. Sensory threshold studies of picrocrocin, the major bitter compound of saffron. J. Food Sci. 81 (1), S189–S198. https://doi.org/10.1111/1750-3841.13152.

Cid-Pérez, T.S., Nevárez-Moorillón, G.V., Ochoa-Velasco, C.E., Navarro-Cruz, A.R., Hernández-Carranza, P., Avila-Sosa, R., 2021. The relation between drying conditions and the development of volatile compounds in saffron (*Crocus sativus*). Molecules 26 (22), 6954. https://doi.org/10.3390/MOLECULES26226954.

Corradi, C., Micheli, G., 1979. Caratteristiche generali dello zafferano. Boll. Chim. Farm. 118, 553–562.

D'Archivio, A.A., Giannitto, A., Maggi, M.A., Ruggieri, F., 2016. Geographical classification of Italian saffron (*Crocus sativus* L.) based on chemical constituents determined by high-performance liquid-chromatography and by using linear discriminant analysis. Food Chem. 212 (December), 110–116. https://doi.org/10.1016/j.foodchem.2016.05.149.

Dai, H., Gao, Q., He, L., 2020. Rapid determination of saffron grade and adulteration by thin-layer chromatography coupled with Raman spectroscopy. Food Anal. Methods 13 (11), 2128–2137. https://doi.org/10.1007/s12161-020-01828-x.

Dai, H., Gao, Q., Lu, J., He, L., 2023. Improving the accuracy of saffron adulteration classification and quantification through data fusion of thin-layer chromatography imaging and Raman spectral analysis. Foods 12 (2), 2322. https://doi.org/10.3390/foods12122322.

D'Auria, M., Mauriello, G., Rana, G.L., 2003. Volatile organic compounds from saffron. Flavour Fragrance J. 19 (1), 17–23. https://doi.org/10.1002/ffj.1266.

Del Campo, C.P., Carmona, M., Maggi, L., Kanakis, C.D., Anastasaki, E.G., Tarantilis, P. A., Polissiou, M.G., Alonso, G.L., 2010. Picrocrocin content and quality categories in different (345) worldwide samples of saffron (*Crocus sativus* L.). J. Agric. Food Chem. 58 (2), 1305–1312. https://doi.org/10.1021/jf903336t.

Ebrahimi, F., Sahebkar, A., Aryaeian, N., Pahlavani, N., Fallah, S., Moradi, N., Abbasi, D., Hosseini, A.F., 2019. Effects of saffron supplementation on inflammation and metabolic responses in type 2 diabetic patients: a randomized, double-blind, placebo-controlled trial. Diabetes Metab. Syndr. Obes. 12 (October), 2107–2115. https://doi.org/10.2147/DMSO.S216666.

Erden, K., Ozel, A., Özel, A., 2016. Influence of delayed harvest on yield and some quality parameters of saffron (*Crocus sativus* L.). JABS 11 (8), 313–316.

Fallahi, H.-R., Aghhavani-Shajari, M., Sahabi, H., Behdani, M.A., Sayyari-Zohan, M.H., Vatandoost, S., 2021. Influence of some pre and post-harvest practices on quality of saffron stigmata. Sci. Hortic. 278 (February), 109846. https://doi.org/10.1016/j.scienta.2020.109846.

Fancello, F., Petretto, G., Sanna, M.L., Pintore, G., Lage, M., Zara, S., 2018. Isolation and characterization of microorganisms and volatiles associated with Moroccan saffron during different processing treatments. Int. J. Food Microbiol. 273 (May), 43–49. https://doi.org/10.1016/J.IJFOODMICRO.2018.03.014.

FAO/OMS, 2021. CX/SCH 21/5/6-Joint FAO/WHO Food Standards Programme Codex Committee on Spices and Culinary Herbs. Draft Standard for Saffron, Fifth Session. virtual. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/jp/?lnk=1&url=https/253A%252F/252Fworkspace.fao.org/252Fsites/252Fcodex/252FMeetings/252FCX-736-05/252FWorking/2BDocuments/252Fsc05_06e. (Accessed 18 October 2024).

Fattahi, R., Mani-Varnosfaderani, A., Barzegar, M., Sahari, M.A., 2023. An ion mobility spectrometry-chemometrics combination approach for assessing adulteration in saffron (*Crocus sativus* L.) with synthetic colorants. Ind. Crop. Prod. 193 (March), 116161. https://doi.org/10.1016/j.indcrop.2022.116161.

Filatova, M., Hajslová, J., Stupak, M., 2024. Detection of saffron adulteration by other plant species using SPME-GC-HRMS. Eur. Food Res. Technol. 250 (3), 911–922. https://doi.org/10.1007/s00217-023-04443-2.

Fujii, S., Morita, Y., Ohta, T., Uto, T., Shoyama, Y., 2022. Saffron (*Crocus sativus* L.) as a valuable spice and food product: a narrative review. Liverp. Class. Mon. LCM 5 (30), 18. https://doi.org/10.21037/lcm-22-1.

García-Rodríguez, M.V., López-Córcoles, H., Alonso, G.L., Pappas, C.S., Polissiou, M.G., Tarantilis, P.A., 2017. Comparative evaluation of an ISO 3632 method and an HPLC-DAD method for safranal quantity determination in saffron. Food Chem. 221 (April), 838–843. https://doi.org/10.1016/j.foodchem.2016.11.089.

Giaccio, M., 2004. Crocetin from saffron: an active component of an ancient spice. Crit. Rev. Food Sci. Nutr. 44 (3), 155–172. https://doi.org/10.1080/10408690490441433.

Gismondi, A., Serio, M., Canuti, L., Canini, A., 2012. Biochemical, antioxidant and antineoplastic properties of Italian saffron (*Crocus sativus* L.). Am. J. Plant Sci. 3 (11), 1573–1580. https://doi.org/10.4236/ajps.2012.311190.

Gout, B., Bourges, C., Paineau-Dubreuil, S., 2010. Satiereal, a *Crocus sativus* L extract, reduces snacking and increases satiety in a randomized placebo-controlled study of mildly overweight, healthy women. Nutr. Res. 30 (5), 305–313. https://doi.org/10.1016/j.nutres.2010.04.008.

Husaini, A.M., Jan, K.N., Wani, G.A., 2021. Saffron: a potential drug-supplement for severe acute respiratory syndrome coronavirus (COVID) management. Heliyon 7 (5), e07068. https://doi.org/10.1016/j.heliyon.2021.e07068.

International Organization for Standardization, 2010. Spices — saffron (*Crocus sativus* L.). Part 2: Test Methods (ISO Standard No. 3632-2:2010).

International Organization for Standardization, 2011. Spices — saffron (*Crocus sativus* L.). Part 1: Specification (ISO Standard No. 3632-1:2011).

International Organization for Standardization, 2018. Guidelines for the harvesting, transportation, separation of stigma, drying and storage of saffron before packing. (ISO Standard No. 21983:2019).

José Bagur, M., Alonso Salinas, G.L., Jiménez-Monreal, A.M., Chaouqi, S., Llorens, S., Martínez-Tomé, M., Alonso, G.L., 2018. Saffron: an old medicinal plant and a potential novel functional food. Molecules 23 (1), 30. https://doi.org/10.3390/molecules23010030.

Karasu, S., Bayram, Y., Ozkan, K., Sagdic, S., 2019. Extraction optimization crocin pigments of saffron (*Crocus sativus*) using response surface methodology and determination stability of crocin microcapsules. J. Food Meas. Char. 13 (2), 1515–1523. https://doi.org/10.1007/s11694-019-00067-x.

Khan, A., Muhamad, N.A., Ismail, H., Nasir, A., Khalil, A.A.K., Anwar, Y., Khan, Z., Ali, A., Taha, R.M., Al-Shara, B., Latif, S., Mirza, B., Fadladdin, Y.A.J., Zeid, I.M.A., Al-Thobaiti, S.A., 2020. Potential nutraceutical benefits of *in vivo* grown saffron (*Crocus sativus* L.) as analgesic, anti-inflammatory, anticoagulant, and antidepressant in mice. Plants 9 (11), 1414. https://doi.org/10.3390/plants9111414.

Khan, M., Hearn, K., Parry, C., Rasid, M., Brim, H., Ashktorab, H., Kwabi-Addo, B., 2024. Mechanism of antitumor effects of saffron in human prostate cancer cells. Nutrients 16 (1), 114. https://doi.org/10.3390/nu16010114.

Koocheki, A., Milani, E., 2020. Saffronadulteration. In: Koocheki, A., Khajeh-Hosseini, M. (Eds.), Saffron: Science, Technology and Health. Woodhead Publishing, Sawston, pp. 321–334.

Lage, M., Cantrell, C.L., 2009. Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. Sci. Hortic. 121 (3), 366–373. https://doi.org/10.1016/j.scienta.2009.02.017.

Liu, J., Chen, N., Yang, J., Yang, B., Ouyang, Z., Wu, C., Yuan, Y., Wang, W., Chen, M., 2018. An integrated approach combining HPLC, GC/MS, NIRS, and chemometrics for the geographical discrimination and commercial categorization of saffron. Food Chem. 253 (July), 284–292. https://doi.org/10.1016/j.foodchem.2018.01.140.

Maestre-Hernández, A.B., Vicente-López, J.J., Pérez-Llamas, F., Candela-Castillo, M.E., García-Conesa, M.T., Frutos, M.J., Cano, A., Hernández-Ruiz, J., Arnao, M.B., 2023. Antioxidant activity, total phenolic and flavonoid contents in floral saffron bio-residues. Processes 11 (5), 1400. https://doi.org/10.3390/pr11051400.

Malavi, D., Nikkhah, A., Alighaleh, P., Einafshar, S., Raes, K., Van Haute, S., 2024. Detection of saffron adulteration with *Crocus sativus* style using NIR-hyperspectral imaging and chemometrics. Food Control 157 (March), 110189. https://doi.org/10.1016/j.foodcont.2023.110189.

Maquet, A., Lievens, A., Paracchini, V., Kaklamanos, G., De La Calle Guntinas, M.B., Garlant, L., Papoci, S., Pietretti, D., Ždiniaková, T., Breidbach, A., Omar Onaindia, J., Boix Sanfeliu, A., Dimitrova, T., Ulberth, F., 2021. Results of an EU Wide Coordinated Control Plan to Establish the Prevalence of Fraudulent Practices in the Marketing of Herbs and Spices, 30877 EN. Publications Office of the European Union EUR, pp. 1–33. https://doi.org/10.2760/309557.

Marieschi, M., Torelli, A., Bruni, R., 2012. Quality control of saffron (*Crocus sativus* L.): development of SCAR markers for the detection of plant adulterants used as bulking agents. J. Agric. Food Chem. 60 (44), 10998–11004. https://doi.org/10.1021/jf303106r.

Marzabadi, L.R., Fazljou, S.M.B., Araj-Khodaei, M., Sadigh-Eteghad, S., Naseri, A., Talebi, M., 2022. Saffron reduces some inflammation and oxidative stress markers in donepezil-treated mild-to-moderate Alzheimer's Disease patients: a randomized double-blind placebo-control trial. J. Herb. Med. 34 (July), 100574. https://doi.org/10.1016/j.hermed.2022.100574.

Mashmoul, M., Azlan, A., Yusof, B.N.M., Khaza'ai, H., Mohtarrudin, N., Boroushaki, M. T., 2014. Effects of saffron extract and crocin on anthropometrical, nutritional and lipid profile parameters of rats fed a high fat diet. JFF 8 (May), 180–187. https://doi.org/10.1016/j.jff.2014.03.017.

Masi, E., Taiti, C., Heimler, D., Vignolini, P., Romani, A., Mancuso, S., 2016. PTR-TOF-MS and HPLC analysis in the characterization of saffron (*Crocus sativus* L.) from Italy and Iran. Food Chem. 192 (February), 75–81. https://doi.org/10.1016/j.foodchem.2015.06.090.

Mishra, Y., Mishra, V., 2023. Multifaceted roles of crocin, phytoconstituent of *Crocus sativus* L. in cancer treatment: an expanding horizon. South Afr. J. Bot. 160 (September), 456–468. https://doi.org/10.1016/j.sajb.2023.07.038.

Mohd, H.F.A., Othman, R., Mat, A.Q.A., Mohd, H.N., Ramya, R., Wan, S.W.S., Mohd, L.N. H., Mohd, K.M.I.A., 2023. Carotenoids composition, antioxidant and antimicrobial capacities of *Crocus sativus* L. stigma. Food Res. 7 (4), 337–343. https://doi.org/10.26656/fr.2017.

Mohtashami, L., Amiri, M.S., Ramezani, M., Emami, S.A., Simal-Gandara, J., 2021. The genus *Crocus* L.: a review of ethnobotanical uses, phytochemistry and pharmacology. Ind. Crops Prod. 171 (Neovember), 113923. https://doi.org/10.1016/j.indcrop.2021.113923.

Mollafilabi, A., Khorramdel, S., Shabahang, J., 2020. Effects of different drying methods on moisture content, drying time and qualitative criteria of saffron stigma. J. Saffron Res. 7 (2), 177–188. https://doi.org/10.22077/JSR.2018.1872.1072.

Moratalla-López, N., Bagur, M.J., Lorenzo, C., Martínez-Navarro, M.E., Salinas, M.R., Alonso, G.L., 2019. Bioactivity and bioavailability of the major metabolites of *Crocus sativus* L. flower. Molecules 24 (15), 2827. https://doi.org/10.3390/molecules24152827.

Morozzi, P., Zappi, A., Gottardi, F., Locatelli, M., Melucci, D., 2019. A quick and efficient non-targeted screening test for saffron authentication: application of chemometrics to gas-chromatographic data. Molecules 24 (14), 2602. https://doi.org/10.3390/molecules24142602.

Naidis, P., Lykidou, S., Mischopoulou, M., Vouvoudi, E., Nikolaidis, N.F., 2023. Study of the dyeing properties of saffron and ultrafiltrated saffron powders, as colourants for natural and synthetic fibres. Color. Technol. 139 (5), 565–577. https://doi.org/10.1111/cote.12670.

Najafabadi, B.T., Farsinejad, M., Shokraee, K., Momtazmanesh, S., Violette, P.D., Esalatmanesh, S., Kashani, L., Jafarinia, M., Akhondzadeh, S., 2022. Possible effects of Saffron (*Crocus sativus*) in the treatment of erectile dysfunction: a randomized, double-blind, placebo-controlled trial. J. Herb. Med. 32 (March), 100551. https://doi.org/10.1016/j.hermed.2022.100551.

Naviglio, D., Conti, S., Ferrara, L., Santini, A., 2010. Determination of moisture in powder and lyophilised saffron (*Crocus sativus* L.) by Karl Fischer method. Open Access J. Sci. 4 (February), 1–6. https://doi.org/10.2174/1874256401004010001.

Nid Ahmed, M., Abourat, K., Gagour, J., Sakar, E.H., Majourhat, K., Koubachi, J., Gharby, S., 2024. Valorization of saffron (*Crocus sativus* L.) stigma as a potential natural antioxidant for soybean (*Glycine max* L.) oil stabilization. Heliyon 10 (4), E25875. https://doi.org/10.1016/j.heliyon.2024.e25875.

Panara, A., Gikas, E., Thomaidis, N.S., 2023. Complete chemical characterization of *Crocus sativus* via LC-HRMS: does trimming affect the chemical content of saffron? Food Chem. 424 (October), 136452. https://doi.org/10.1016/j.foodchem.2023.136452.

Parastar, H., Weller, P., 2024. Benchtop volatilomics supercharged: how machine learning based design of experiment helps optimizing untargeted GC-IMS gas phase metabolomics. Talanta 272 (May), 125788. https://doi.org/10.1016/j.talanta.2024.125788.

Predieri, S., Magli, M., Gatti, E., Camilli, F., Vignolini, P., Romani, A., 2021. Chemical composition and sensory evaluation of saffron. Foods 10 (11), 2604. https://doi.org/10.3390/foods10112604.

Rodríguez-Neira, L., Lage-Yusty, M.A., López-Hernández, J., 2014. Influence of culinary processing time on saffron's bioactive compounds (*Crocus sativus* L.). Plant Foods Hum. Nutr. 69 (4), 291–296. https://doi.org/10.1007/s11130-014-0447-4.

Saadat, S., Ghasemi, Z., Memarzia, A., Behrouz, S., Aslani, M.R., Boskabady, M.H., 2024. An overview of pharmacological effects of *Crocus sativus* and its constituents. Iran. J. Basic Med. Sci. 27 (4), 391–417. https://doi.org/10.22038/IJBMS.2023.73410.15950.

Sabatino, L., Scordino, M., Gargano, M., Belligno, A., Traulo, P., Gagliano, G., 2011. HPLC/PDA/ESI-MS evaluation of saffron (*Crocus sativus* L.) adulteration. Nat. Prod. Commun. 6 (12), 1873–1876. https://doi.org/10.1177/1934578X1100601220.

Sanjuán-López, A.I., Resano-Ezcaray, H., 2020. Labels for a local food speciality product: the case of saffron. JAE (J. Agric. Entomol.) 71 (3), 778–797. https://doi.org/10.1111/1477-9552.12376.

Sarfraz, M., Khaliq, A., Tahir, M.M., Iqbal, M.A., Rehman, Z. ur, Shan, Q., 2024. Saffron spice quality in response to integrated nutrient management under lesser Himalayan conditions of Rawalakot Azad Jammu & Kashmir. J. Plant Nutr. 47 (1), 18–29. https://doi.org/10.1080/01904167.2023.2254322.

Sereshti, H., Ataolahi, S., Aliakbarzadeh, G., Zarre, S., Poursorkh, Z., 2018. Evaluation of storage time effect on saffron chemical profile using gas chromatography and spectrophotometry techniques coupled with chemometrics. J. Food Sci. Technol. 55 (4), 1350–1359. https://doi.org/10.1007/s13197-018-3046-9.

Spence, C., 2023. Saffron: the colourful spice. Int. J. Gastron. Food Sci. 34 (December), 100821. https://doi.org/10.1016/j.ijgfs.2023.100821.

Suchareau, M., Bordes, A., Lemée, L., 2021. Improved quantification method of crocins in saffron extract using HPLC-DAD after qualification by HPLC-DAD-MS. Food Chem. 362 (November), 130199. https://doi.org/10.1016/j.foodchem.2021.130199.

Sun, C., Nile, S.H., Zhang, Y., Qin, L., El-Seedi, H.R., Daglia, M., Kai, G., 2020. Novel insight into utilization of flavonoid glycosides and biological properties of saffron (*Crocus sativus* L.) flower byproducts. J. Agric. Food Chem. 68 (39), 10685–10696. https://doi.org/10.1021/acs.jafc.0c04076.

Tsui, P.F., Lin, C.S., Ho, L.J., Lai, J.H., 2018. Spices and atherosclerosis. Nutrients 10 (11), 1724. https://doi.org/10.3390/nu10111724.

Zalacain, A., Ordoudi, S.A., Díaz-Plaza, E.M., Carmona, M., Blázquez, I., Tsimidou, M.Z., Alonso, G.L., 2005. Near-infrared spectroscopy in saffron quality control: determination of chemical composition and geographical origin. J. Agric. Food Chem. 53 (24), 838–843. https://doi.org/10.1016/j.foodchem.2016.11.089.