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Chemical composition of the essential oils and volatile fractions from the aerial parts of *Crocus sativus* L. (Iridaceae) using microwave assisted hydrodistillation (MAHD) and headspace solid phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometric (GC-MS) analysis

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Original Research

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Abstract:

In this report, chemical composition of the essential oils and volatile fractions from the aerial parts of *Crocus sativus* L. (Iridaceae) was investigated using microwave-assisted hydrodistillation (MAHD) and headspace solid-phase microextraction (HS-SPME), followed by gas chromatography-mass spectrometry (GC-MS) analysis on DB-5 and HP-5MS columns. Twenty constituents were identified by matching their mass spectra, retention indices, and co-injection with reference standards. In the MAHD-derived essential oil, seventeen compounds accounted for 97.3% of the total composition, with safranal (37.2%) as the major component, followed by 4-ketoisophorone (11.2%), hexadecane (9.1%), hexadecanoic acid (8.1%), tetradecanoic acid (7.5%), nonacosane (6.2%), and *n*-eicosane (5.1%). The HS-SPME volatile fraction exhibited a higher safranal content (63.5%), accompanied by hexadecanoic acid (10.2%), ethyl hexadecanoate (6.7%), tetradecanoic acid (5.7%), and isophorone (3.0%). These findings reveal pronounced method-dependent variations in the volatile profiles, underscoring the dominance of safranal and highlighting the potential of saffron aerial parts as an alternative source of valuable aroma compounds.

Keywords: *Crocus sativus* L.; Iridaceae; Microwave Assisted Hydrodistillation (MAHD); Headspace Solid Phase Microextraction (HS-SPME); Volatile fractions; Safranal (2,6,6-Trimethyl-1,3-cyclohexadien-l-carboxaldehyde)

1. Introduction

The Iridaceae family, encompassing approximately 260-300 species of rhizomatous or bulbous perennials primarily distributed across temperate regions of the Northern Hemisphere, represents a phytochemically diverse lineage renowned for its rich reservoir of bioactive secondary metabolites, including isoflavonoids (*e.g.*, irigenin, tectorigenin), xanthones, quinones, stilbenes; terpenoids such as iridals and irones, and C-glycosylflavones, which underpin extensive traditional medicinal applications for anti-inflammatory, antimicrobial, antioxidant, cytotoxic, and phytoestrogenic properties (Goldblatt et al., 1998; Rodriguez, 1999; Singab et al., 2016).

Crocus sativus L. (Iridaceae), renowned as saffron, stands as one of the most venerable medicinal plants, with a documented history spanning over 3,500 years across ancient civilizations including Iran, Egypt, Greece, Rome, India and China (Srivastava et al., 2010; Kothari et al., 2021), where its stigmas have been prized not only as a culinary spice but also for therapeutic applications in treating diverse ailments such as cardiovascular disorders, gastrointestinal disturbances, menstrual irregularities, cognitive impairments, inflammation, infections, and more, as evidenced by traditional Persian (*e.g.*, Avicenna's Canon of Medicine), Unani, Ayurvedic, and Greco-Roman texts.

In Iran, the primary producer worldwide, saffron is cultivated on approximately 100,000 to 120,000 hectares (Saeidnia, 2012; Bahmani et al., 2014). Throughout Asia, *C. sativus* has long been valued as a powerful medicinal plant for treating coronary artery disease, hypertension, gastrointestinal disorders, menstrual irregularities as well as memory and cognitive impairments. It is also a popular spice in cooking. Numerous studies suggest that its therapeutic benefits stem from antioxidant and anti-inflammatory actions affecting the nervous, cardiovascular, immune, and respiratory systems. Both animal and human research on saffron extracts demonstrate anticonvulsant and anti-Alzheimer's effects (Khazdair et al., 2015; Boskabady et al., 2016).

C. sativus L., commonly known as the saffron crocus, is a perennial, herbaceous monocotyledon belonging to the Iridaceae family (Guclu et al., 2020). This sterile triploid species (2n = 3x = 24 chromosomes) yields the world's most valuable spice–saffron–derived from the dried red stigmas of its autumn-flowering purple blooms (https://en.wikipedia.org/wiki/Crocus_sativus, Accessed on January, 12 2025).

The exquisite value of saffron arises from its labor-intensive harvest: Each flower produces only three delicate stigmas, which must be hand-picked and dried, requiring approximately 150,000-170,000 flowers to produce just one kilogram of the spice (https://en.wikipedia.org/wiki/Saffron, Accessed on January, 12 2025).

Iran dominates global production, accounting for around 90% of the world's supply, with cultivation spanning approximately 127,000 hectares in recent years, primarily in the northeastern Khorasan regions (Anabat et al., 2020) (see Fig. 1). The main cultivation regions of this valuable medicinal and herbal species are also shown in Fig. 2.

Over the past decades, extensive phytochemical investigations have unveiled its rich reservoir of bioactive constituents–predominantly crocin, crocetin, safranal, and picrocrocin (responsible for color, aroma, and taste) (Drioiche et al., 2023), alongside flavonoids like kaempferol3-(6-acetylglucoside), isorhamnetin3-*O*-neohesperidoside, syringetinhexoside, xanthoangelol (Drioiche et al., 2023) and quercetin (Drioiche et al., 2023), terpenoids (Goupy et al., 2013), and other volatiles–predominantly concentrated in the stigmas, petals, and corms, with rigorous analytical methods such as HPLC-MS/MS (Koşar et al., 2017; Rasmi et al., 2022) confirming their variability across origins and underscoring saffron's quality indices. Contemporary pharmacological research, encompassing *in vitro*, *in vivo*, and preliminary clinical studies (Roshanravan et al., 2022), has substantiated a broad spectrum of bioactivities including potent antioxidant (Wali et al., 2020; Frusciante et al., 2024), anti-inflammatory (Frusciante et al., 2024; Yang et al., 2024), anticancer (via apoptosis induction and cell cycle arrest) (Bajbouj et al., 2012), neuroprotective (F.Abdel-Rahman et al., 2020), cardioprotective (Nader et al., 2016), antidiabetic (Wali et al., 2020; Drioiche et al., 2023), antimicrobial (Wali et al., 2020), antidepressant (Wang et al., 2010; Siddiqui et al., 2018) and hypolipidemic effects (Basheeruddin et al., 2010), mediated through pathways like NF-κB inhibition (Zeinali et al., 2019), caspase activation (Hoshyar et al., 2013), MAPK/ERK modulation (Boozari et al., 2022), and enhanced enzymatic defenses (*e.g.*, SOD, GPx, CAT) (Hamidian et al., 2023), positioning *C. sativus* as a promising candidate for multifaceted therapeutic interventions (Saadat et al., 2024).

The principal goal of this study is to delineate chemical composition of essential oils and volatile parts derived from the

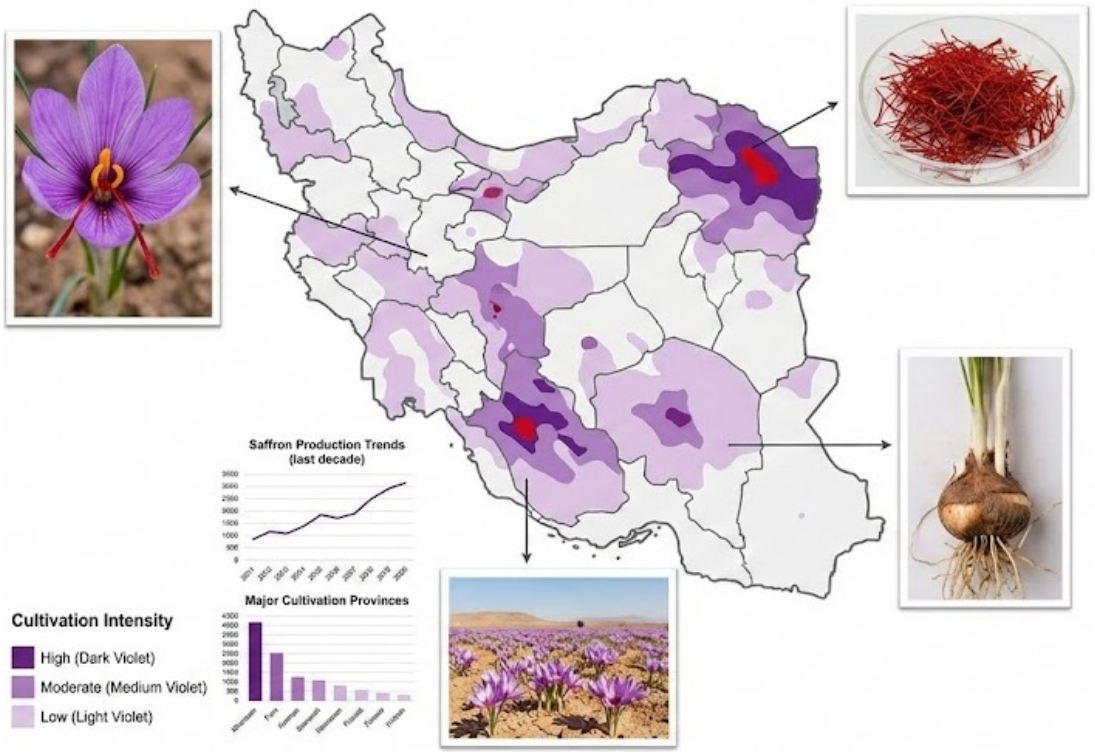

Figure 1. Representation of widespread distribution of *Crocus sativus* L. in different Iranian regions.

aerial parts of *C. sativus* L. utilizing gas chromatography-mass spectrometry (GC-MS). To the best of our knowledge, no previous studies have reported the extraction of essential oils from this species using microwave-assisted techniques, making this investigation the first of its kind. Although headspace solid-phase microextraction (HS-SPME) analyses of the plant's volatile constituents have been documented, the reported profiles exhibit considerable variability to some extent. Therefore, this study examines the volatile composition to ascertain the effects of environmental factors–such as geographic origin, humidity, salinity, and other variables–on the yield and composition of the isolated essential oils.

2. Experimental

2.1 Chemicals and supplies

A certified reference standard mixture containing a homologous series of *n*-alkanes ranging from C_9 (nonane) to C_{40} (pentacosane), each at a concentration of 40 mg/L in hexane, was obtained from Fluka (Buchs, Switzerland). High-purity helium and nitrogen were employed as carrier gases for gas chromatography–mass spectrometry (GC-MS) and gas chromatography (GC) analyses, respectively. The microwave oven utilized for essential oil isolation was a Samsung model CM1089A (South Korea) with a power rating of 1.1 kW and a capacity of 26 L. To capture the volatile constituents from the aerial parts of the plant material, headspace solid-phase microextraction (HS-SPME) was performed using a manual SPME holder equipped with a 1-cm fused-silica fiber coated with a 75 µm polydimethylsiloxane/carboxen (PDMS/CAR) bifunctional stationary phase (Supelco, Bellefonte, PA, USA). This fiber was selected for its effectiveness in adsorbing compounds over a wide range of volatilities.

All extractions were conducted in 10 mL clear glass vials (22 mm × 46 mm) sealed with crimp-top caps (MicroLiter Analytical Supplies Inc., Suwanee, GA, USA). The vials were closed with aluminum seals incorporating 20 mm PTFE/silicone septa (Supelco). This septum composition was chosen to reduce adsorptive losses and avoid contamination during the elevated-temperature incubation step of the HS-SPME procedure.

2.2 Plant material

Due to its significant economic value, *C. sativus* L. is cultivated on a large scale in various regions of Iran (see Fig. 1 and Fig. 2). In the current report, the aerial parts of *C. sativus* L. were harvested at the flowering stage on November 10, 2024, from the Herbal Drugs Raw Materials Research Center (HDRMRC), Islamic Azad University, Shahrood, Iran. Botanical authentication was performed by a local expert, and a voucher specimen was deposited at the Herbarium of the Agricultural Research Center of Semnan Province (accession number: CSA2024.s1). The collected plant material was vacuum-dried at 37 °C until the moisture content stabilized below 5%. The dried samples were subsequently pulverized in a high-speed rotary cutting mill and sieved to obtain particles passing through a 100-mesh screen (particle size $\leq$ 120 µm). The resulting powdered material was stored at 4 °C prior to extraction and analysis.

2.3 Gas chromatography (GC) analysis

GC analyses were conducted using a Shimadzu 15A gas chromatograph fitted with a split/splitless injector (split ratio 1:30) and a flame ionization detector (FID), both maintained at 250 °C. High-purity nitrogen was employed as the carrier gas at a flow rate of 1 mL/min. Separations were performed on a DB-5 capillary column (50 m × 0.2 mm i.d., film thickness 0.32 µm). The oven temperature program was as follows: initial hold at 60 °C for 3 min, followed by a ramp of 5 °C/min to 220 °C, and then isothermal at 220 °C for 5 min. Relative percentages of constituents were determined directly from FID peak areas using a Shimadzu CR5

Figure 2. Large scale cultivation and harvesting of *Crocus sativus* L. In different parts of Iran.

integrator, without applying response factor corrections.

2.4 Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analyses were carried out on a Hewlett-Packard 5973 system equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The column effluent was transferred directly into the mass spectrometer ion source. Helium was used as the carrier gas at a flow rate of 1 mL/min. The temperature program mirrored that used for GC-FID analysis, with a final temperature of 230 °C. The ion source and detector temperatures were set at 250 °C. Mass spectra were acquired in electron ionization (EI) mode at 70 eV, with an electron multiplier voltage of 1800 V, a mass range of 30-350 amu, and a scan rate of 2 scans/s. Compound identification was achieved by comparing the recorded mass spectral fragmentation patterns and retention indices (RI, relative to C_9-C_{40} *n*-alkanes) with literature values and those in the Wiley 275 mass spectral library. Additional confirmation was obtained from mass spectra and Kovats indices previously established by our research group. Relative component percentages were calculated from peak areas using a Shimadzu C-R4A Chromatopac integrator on the DB-5 column, without correction factors.

2.5 Microwave-Assisted Hydrodistillation (MAHD)

The microwave oven employed for MAHD was a Samsung model (South Korea) operating at a frequency of 2450 MHz, with a maximum output power of 1000 W. A power setting of 800 W was applied directly to the hydrodistillation setup. The internal cavity of the oven measured 29 × 37 × 40 cm. A hole was drilled in the top of the oven to accommodate the apparatus modification. A 1000 mL round-bottom flask was positioned inside the oven and connected directly to a Clevenger-type apparatus via the hole.

For the MAHD extraction, 8 g portions of dried and powdered aerial parts of the plant material (*C. sativus* L.) were immersed in 500 mL of distilled water and hydrodistilled at 800 W for 20 min in the modified microwave system, which comprised the microwave oven linked to an adapted Clevenger apparatus. Throughout the procedure, the generated vapor was continuously directed through a condenser positioned outside the microwave cavity for condensation. The MAHD process was conducted at varying durations until no additional essential oil was recovered. The mean essential oil yield, calculated from three independent MAHD-based experiments and expressed as grams of oil per gram of dried plant material, was 0.21% (*w/w*). The collected essential oil was stored in amber vials, dried over anhydrous sodium sulfate, sealed under nitrogen and maintained at 4 °C until analysis.

3. Results and discussion

3.1 Chemical profiles of the essential oils and volatiles from the aerial parts of *C. sativus* L.

Under the optimal experimental conditions, parallel analyses using gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS), conducted with DB-5 and HP-5MS capillary columns respectively, enabled the identification of twenty constituents. This was achieved through comparison of their mass spectra, retention indices, and co-injection with reference standards. The chemical composition of the essential oil obtained from the aerial parts of *C. sativus* L. by MAHD is presented in Table 1. Seventeen identified constituents accounted for 94.8% of the total composition in the MAHD-derived oil. The major components were safranal (37.2%), 4-ketoisophorone (11.2%), hexadecane (9.1%), hexadecanoic acid (8.1%), tetradecanoic acid (7.5%), nonacosane (6.2%) and *n*-eicosane (5.1%). In terms of general categories, one monoterpene hydrocarbon, three oxygenated monoterpenes and thirteen non-terpene hydrocarbons were characterized in this oil, accounting for 0.1%, 41.2%, and 56% of the total profile, respectively (see Fig. 3).

On the other hand, the volatile fraction extracted from the same aerial parts by headspace solid-phase microextraction (HS-SPME) was primarily characterized by safranal (63.5%), followed by hexadecanoic acid (10.2%), ethyl hexadecanoate (6.7%), tetradecanoic acid (5.7%) and isophorone (3.0%). From the perspective of classes of natural compounds, the volatile profile consisted of one monoterpene hydrocarbon, two oxygenated monoterpenes and sixteen non-terpene hydrocarbons, which accounted for 0.3%, 64.3%, and 34.3% of the total composition, respectively (Fig. 3).

3.2 Chemical profiles of volatiles of *C. sativus* L. in previously reported papers

Characterization of the volatile profiles from different parts of *C. sativus* L has been the subject of a large number of reports in the literature. Accordingly, the main identified compounds have been shown in Table 2. Safranal emerges as the predominant volatile compound in saffron essential oils across global regions, consistently comprising 19.56-84.38% in various samples (see Table 2), with the highest levels in Italian (Sicily, 84.38%) (Condurso et al., 2017) and Moroccan (31,710 µg/kg) stigmas (Karabagias et al., 2017), while lower in irradiated Indian samples (19.56%) (Zareena et al., 2001) and Japanese corms (not dominant) (Masuda et al., 2012); isomers and derivatives like 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (safranal isomer, up to 5.72% in Italy) (Condurso et al., 2017) and 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC, 45.8 µg/g in Iranian Qaen) (Amanpour et al., 2015) frequently appear. Isophorone variants, including α-isophorone (3.17-16.25%) (Zareena et al., 2001; Koşar et al., 2017), β-isophorone (2.2-11,222.2 µg/kg) (Koşar et al., 2017; Sevindik, 2020) and 4-ketoisophorone (1.2-16.11%) (Koşar et al., 2017; Chen et al., 2020), are prominent secondary constituents, peaking in Turkish one-year stored samples (15,983.9 µg/kg for isophorone) (Sevindik, 2020) and Chinese freeze-dried (16.09% for 4-ketoisophorone) (Chen et al., 2020), but reduced post-irradiation in India (from 5.25% to 6.17% for α-isophorone) (Zareena et al., 2001). Fatty acids such as hexadecanoic acid (3.3-25%) (Sereshti et al., 2014; Koşar et al., 2017), linoleic acid (4.13-8.12%) (Shao et al., 2014) and tetradecanoic acid (14.3%) (Sereshti et al., 2014) dominate in Iranian Qaen (25.0% hexade-

Table 1. Chemical profiles of essential oils and volatile fractions from the aerial parts of *Crocus sativus* L. obtained by microwave-assisted hydrodistillation (MAHD) and headspace solid-phase microextraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS)[a].

Num.	Compound	RI (Lit.)[b]	RI (Cal.)[c]	NCCG[d]	Percentage	
					MAHD	HS-SPME
1	Heptanal	901	900	NH	0.4	-
2	Hexanoic acid	967	965.1	NH	-	0.2
3	1-Octen-3-ol	974	972.3	NH	0.1	0.2
4	α-Phellandrene	1002	1004.1	MH	0.1	0.3
5	Linalool	1095	1097	OM	0.5	-
6	*n*-Nonanal	1100	1100	NH	0.1	0.7
7	Isophorone	1118	116.5	NH	1.5	**3.0**
8	4-ketoisophorone	1140	1142.1	NH	**11.2**	2.5
9	Safranal[e]	1196	1195.7	OM	**37.2**	**63.5**
10	4-Methylene-isophorone	1216	1219.4	OM	3.5	0.8
11	(*E*)-β-Ionone	1487	1486	NH	3.4	0.1
12	Hexadecane	1600	1600	NH	**9.1**	0.2
13	Heptadecane	1700	1700	NH	2.5	0.7
14	Tetradecanoic acid	1765	1764.5	NH	**7.5**	**5.7**
15	Methyl hexadecanoate	1920	1920.4	NH	-	1.5
16	Hexadecanoic acid	1959	1958.4	NH	**8.1**	**10.2**
17	*n*-Eicosane	2000	2000	NH	5.1	0.9
18	Linoleic acid	2132	2130.1	NH	0.8	0.2
19	Ethyl hexadecanoate	2184	2182.7	NH	-	**6.7**
20	Nonacosane	2900	2900	NH	6.2	1.5
	Total				**97.3**	**98.9**

[a] The compounds are listed in order of their elution on an HP-5MS column.
[b] Retention indices in the literature
[c] Calculated retention indices
[d] NCCG: Natural compound constituting group
[e] Safranal: 2,6,6-Trimethyl-1,3-cyclohexadien-l-carboxaldehyde

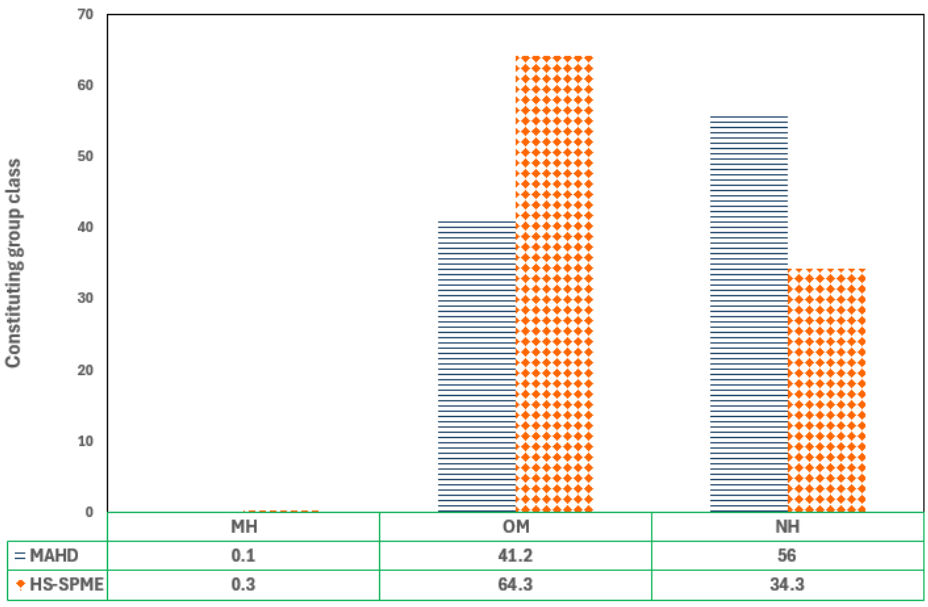

Figure 3. Comparison of the class of natural compound constituting groups of the essential oil and volatile fractions from the aerial parts of *Crocus sativus* L. using microwave assisted hydrodistillation (MAHD) and headspace solid phase microextraction (HS-SPME) techniques.

Table 2. Major constituents of the volatiles and essential oils of *Crocus sativus* L. in different parts of the world.

Sampling area/Country	Major constituent components	Ref.
Greece (Cooperative of Saffron, Krokos Kozanis)	Safranal (2,6,6-Trimethyl-1,3-cyclohexadien-l-carboxaldehyde); isophorone (3,5,5-trimethyl-2-cyclohexen-1-one); 3,5,5-trimethyl-3-cyclohexen-1-one (isomer of isophorone); 2,6,6-trimethyl-2-cyclohexen-1,4-dione; and 2,6,6-trimethyl-1, 4-cyclohexadiene-1-carboxaldehyde (isomer of safranal)	(Tarantilis et al., 1997)
India (Nuclear Research Laboratory, Shrinagar, Kashmir)	**Control:** Safranal (32.93%), α-isophorene (5.25%), dihydro-β-ionene (3.71%), 2,4,4-trimethyl-3-carboxaldehyde-5-hydroxy-1-cyclohexanone 2,5-diene (3.4%) and ketoisophorene (3.17%)	(Zareena et al., 2001)
	Irradiated (5 kGy): Safranal (19.56%), α-isophorene (6.17%), ketoisophorene (3.48%), dihydro-β-ionene (3.43%) and 2,4,4-trimethyl-3-carboxaldehyde-5-hydroxy-1-cyclohexanone 2,5-diene (2.8%)	
Italy and Iran	**Sample 1: Salerno (Southern Italy):** Safranal (59.13%), 2,6,6-trimethyl-4-hydroxy-1,4-cyclohexadien-3-one-1 carboxaldehyde (6.71%), 3,5,5-trimethyl-2-cyclohexen-1-one (6.37%) and 3,5,5-trimethyl-2-cyclohexen-1,4-dione (3.78%)	(D'Auria et al., 2004)
	Sample 2: Sardinia, Italy (San Gavino, Cagliari): Safranal (41.13%),2,6,6-trimethyl-4-hydroxy-1,4-cyclohexadien-3-one-1-carboxaldehyde (7.56%), 2-isopropyliden-3-methyl 3,5-hexadienal (7.07%), 3,5,5-trimethyl-2-cyclohexen-1-one (5.79%) and 3,5,5-trimethyl-2-cyclohexen-1,4-dione (4.57%)	
	Sample 3: Abruzzo (Central Italy, Altopiano di Navelli): Safranal (72.49%) and 2-isopropyliden-3-methyl 3,5-hexadienal (3.37%)	
	Sample 4: Iranian sample: Safranal (63.24%), 3,5,5-trimethyl-2-cyclohexen-1-one (8.13%) and 3,5,5-trimethyl-2-cyclohexen-1,4-dione (5.30%)	
Italy (Navelli (L'Aquila), central Italy)	**Ethanolic extracts of tepals:** 2-Phenylethyl alcohol (15.0%), tetracosane (10.5%), ethyl hexadecanoate (10.0%) and heptadecane (9.6%)	(Tirillini et al., 2006)
	Anthers of flowers: 2-Phenylethyl alcohol (50.4%) and 2-phenethyl acetate (15.4%)	
Iran (Khorasan Province, Northeast of Iran)	Safranal (26.29%), Bicyclo[3,2,0]hept-2-ene-4-ethoxy-endo (5.6%), linoleic acid (4.77%) and 4-hydroxy-2,6,6-trimethyl-1cyclohexene-1-carboxalde-hyde (β-Homocyclocitral) (4.44%)	(Jalali-Heravi et al., 2009)
Japan	2(5H)-Furanone (59.80%), hexadecanoic acid (15.65%), (*E*)-2-methyl-2-butenal (8.44%) and (*Z*,*Z*)-9,12-octadecadienoic acid (4.74%)	(Masuda et al., 2012)
Iran (Qaen, South Khorasan Province, Northeast of Iran)	Hexadecanoic acid (25.0%), safranal (16.8%), tetradecanoic acid (14.3%), 5,5-dimethyl-2-methylene-3-cyclohexene-1-carboxaldehyde (7.5%), α-isophorone (4.9%) and 4,4-dimethyl-2-cyclopenten-1-one (4.9%)	(Sereshti et al., 2014)

Continued of Table 2.

Sampling area/Country	Major constituent components	Ref.
China (Baicaoyuan test base, Zhejiang Province,)	**Hydrodistillation (HD):** Safranal (30.72%), *trans*-β-ionol (8.68%), linalool (5.36%), linoleic acid (4.13%) and 6,10,14-trimethylpentadecan-2one (3.16%)	(Shao et al., 2014)
	Soxhlet extraction (SE): Safranal (38.17%), linoleic acid (8.12%) and 6,10,14-trimethylpentadecan-2one (3.02%)	
	Supercritical Fluid Extraction (SFE): Safranal (40.31%), linoleic acid (6.69%) and 6,10,14-trimethylpentadecan-2one (3.28%)	
Iran (Qaen, South Khorasan Province, Northeast of Iran)	Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) (2168 μg/g), 2-(1,1-dimethylethyl)phenol (1432 μg/g), 2-ethoxy-5-methoxybenzaldehyde (1147 μg/g), isophorone (3,5,5-trimethyl 2-cyclohexen-1-one) (845 μg/g), 4-ketoisophorone (2,6,6-trimethyl-2-cyclohexene-1,4-dione) (625 μg/g), 4-hydroxy-3,5,5-trimethylcyclohex-2-enone (353 μg/g), 2-methyl-2-butanol (291 μg/g), nonanoic acid (141 μg/g), 2-hexanol (88.4 μg/g), octanoic acid (63.5 μg/g), 2-hydroxyisophorone(2-hydroxy-3,5,5-trimethyl-2-cyclohexenone) (57.4 μg/g), 5,5-dimethyl-2-methylene-3-cyclohexene-1-carboxaldehyde- (51.5 μg/g) and 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC) (45.8 μg/g)	(Amanpour et al., 2015)
Italy (Sicily)	Safranal (84.38%), 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (Safranal isomer) (5.72%), α-isophorone (3.96%), furfural (1.91%) and 2,4-dimethylbenzenecarboxaldehyde (1.2%)	(Condurso et al., 2017)
Iran	Safranal (2,2,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldeyde) (7650 μg/kg), 1,5,5-trimethyl-6-(2-butenylidene)-cyclohexene, (megastigma-4,6(*E*),8(*E*)-triene) (1113 μg/kg), 5,5-dimethyl-2-methylene-3-cyclohexene-1-carboxaldehyde (689.5 μg/kg), 3,7-dimethyl-octa-1,6-dien-3-ol (linalool) (587.5 μg/kg) and 2,4-dimethylbenzenecarboxaldehyde (130.5 μg/kg)	(Karabagias et al., 2017)
Spain	Safranal (4510 μg/kg), 3,5,5-trimethyl-3-cyclohexen-1-one (β-isophorone) (924.5 μg/kg), 5,5-dimethyl-2-methylene-3-cyclohexene-1-carboxaldehyde (357.5 μg/kg), linalool (120.8 μg/kg), 2,6,6-trimethyl-2-cyclohexene-1,4-dione(4-ketoisophorone) (113.8 μg/kg) and 5-hydroxy-2,5-cyclohexadien-1-one-2,4,4-trimethyl-3 carboxaldehyde (110.2 μg/kg)	
Greece	Safranal (6450 μg/kg), 2,4,5-trimethyl-benzaldehyde (1380 μg/kg), β-isophorone (747 μg/kg), megastigma-4,6(*E*),8(*E*)-triene (715.5 μg/kg), linalool (286 μg/kg), 5,5-dimethyl-2-methylene-3-cyclohexene-1-carboxaldehyde (285.5 μg/kg) and 2,6,6-trimethyl-2,4-cycloheptadien-1-one (Eucarvone) (243 μg/kg)	
Morocco	Safranal (31,710 μg/kg), 5,5-dimethyl-2-methylene-3-cyclohexene-1-carboxaldehyde (2890 μg/kg), 1-methyl-3-(1-methylethyl)-benzene (*m*-cymene) (1960 μg/kg), β-isophorone (779 μg/kg), dodecene (492 μg/kg) and isophorol (3,5,5-trimethyl-2-cyclohexen-1-ol) (452 μg/kg)	

Continued of Table 2.

Sampling area/Country		Major constituent components	Ref.
Turkey	**Eskisehir 1**	Safranal (64.1%), α-isophorone (10.38%), β-isophorone (6.4%), hexadecanoic acid (3.3%), 4-(2,2,6-trimethyl-cyclohexan-1-yl)-3-buten-2-one (β-Ionene) (1.25%) and safranal isomer (1.13%)	(Koşar et al., 2017)
	Safranbolou	Safranal (49.33%), α-isophorone (16.25%), β-isophorone (8.38%), hexadecanoic acid (4.05%), 4-(2,2,6-trimethyl-cyclohexan-1-yl)-3-buten-2-one (β-ionene) (2.8%), 4-ketoisophorone (2.5%) and 3,3,4,5-tetramethylcyclohexan-1one (2.28%)	
	Eskisehir 2	Safranal (77.9%), α-isophorone (13.5%), β-isophorone (2.2%), safranal isomer (1.9%) and 4-ketoisophorone (1.2%)	
Iran	**Sargol-I**	Safranal (49.64%), acetic acid (9.49%), 4-ketoisophorone (8.72%), isophorone (8.20%) and 2,6,6-trimethyl-1,4-cyclohexanedione (4.9%)	(Azarabadi et al., 2018)
	Sargol-II	Safranal (50.29%), isophorone (10.22%), 4-ketoisophorone (9.19%), acetic acid (7.05%) and 2,6,6-trimethyl-1,4-cyclohexanedione (4.92%)	
	Pushal-I	Safranal (50.42%), isophorone (12.32%), 4-ketoisophorone (10.39%), acetic acid (6.82%) and 2,6,6-trimethyl-1,4-cyclohexanedione (4.99%)	
	Pushal-II	Safranal (57.02%), isophorone (12.51%), 4-ketoisophorone (10.53%), acetic acid (7.70%), and 2,6,6-trimethyl-1,4-cyclohexanedione (5.19%)	
	Bunch	Safranal (61.31%), 4-ketoisophorone (15.98%), isophorone (13.52%), 2,6,6-trimethyl-1,4-cyclohexanedione (7.23%) and acetic acid (5.63%)	
Iran (Kerman)		2(5H)-Furanone (691.8 ppb), safranal (610.1 ppb), acetic acid (566.3 ppb), isobutanal (451.8 ppb), biogenicaldehyde (272.6 ppb), 4-ketoisophorone (161.2 ppb), acetaldehyde (130.4 ppb) and α-isophorone (106.6 ppb)	(Ghanbari et al., 2019)
Twenty-six samples from 9 countries: Afghanistan, Algeria, China, France, Greece, India, Iran, Morocco, and Spain		Safranal (dominant, 22.1 – 62.4%), 4-ketoisophorone (2,6,6 trimethyl-2-cyclohexene-1,4 dione)(5.0 – 13.0%), acetic acid (1.0-6.2%), 2(5H)-furanone (0.7 – 6.8%) and 1,4-cyclohexanedione-2,2,6- trimethyl (1.8 – 6.8%)	(Zwane et al., 2020)
China (Hangzhou)		**Microwave Drying:** Safranal (20.54%), dihydrooxophorone (18.98%), 4-ketoisophorone (16.11%), α-isophorone (14.57%) and 2,3-dimethoxytoluene (11.68%)	(Chen et al., 2020)
		Oven Drying: Safranal (15.7%), 2,3-dimethoxytoluene (13.94%), dihydrooxophorone (13.18%) and 4-ketoisophorone (11.73%)	
		Infrared Drying: Safranal (22.18%), dihydrooxophorone (16.88%), 4-ketoisophorone (16.09%), α-isophorone (15.68) and 2,3-dimethoxytoluene (12.83%)	
		Freeze Drying: 4-Ketoisophorone (16.09%), safranal (10.31%), 2,3-dimethoxytoluene (7.19%), 3-methyl-2-cyclohexen-1-one (5.37%) and α-isophorone (5.17%)	
Turkey (Mersin province)		**Freshly dried:** Safranal (14093.9 μg/kg), β-isophorone (11222.2 μg/kg), isophorone (8761.9 μg/kg), β-ionone (6863.6 μg/kg), 4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde (5777.2 μg/kg), 4-oxoisophorone (4675.4 μg/kg), 2,2,6-trimethyl-1,4-cyclohexanedione (3764.2 μg/kg) and 2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde (894.5 μg/kg)	(Sevindik, 2020)
		One-year stored: Isophorone (15983.9 μg/kg), 4-oxoisophorone (8946.5 μg/kg), safranal (80349.4 μg/kg), 2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde (5687.1 μg/kg), 2,2,6-trimethyl-1,4-cyclohexanedione (4878.0 μg/kg), 4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde (2333.1 μg/kg), β-isophorone (245.3 μg/kg) and β-ionone (160.7 μg/kg)	

Continued of Table 2.

Sampling area/Country	Major constituent components	Ref.
Spain (Castilla-La Mancha region)	**Spanish Stigmas:** Safranal (7443 μg/kg) and isophorone (1477 μg/kg)	(Cerdá-Bernad et al., 2022)
	Iranian Stigmas: Safranal (7656 μg/kg) and isophorone (1531 μg/kg)	
	Greek Stigmas: Safranal (7429 μg/kg) and isophorone (1936 μg/kg)	
Morocco (Boulemane region)	Phorone (12.90%), (*R*)-(-)-2,2-dimethyl-1,3-dioxolane-4-methanol (11.65%), isopropyl palmitate (9.68%), dihydro-β-ionone (8.62%), safranal (6.39%), *trans*-β-ionone (4.81%), 4-ketoisophorone (4.72%) and 1-eicosanol (4.55%)	(Drioiche et al., 2023)
Algerian cultivars	Non-polar extracts of petals: **Chloroform extract:** Valeric acid (20.8%), linoleic acid (11.4%) and nonadecane (9.0%)	(Djenhi et al., 2025)
	Non-polar extracts of petals: **Petroleum ether extract:** 1,3-Bis(trimethylsilyl) benzene (10.8%), nonacosane (9.9%) and eicosane (9.5%)	
	Non-polar extracts of petals: **Hexane extract:** Heptacosane (14.3%), eicosane (10.2%) and hexamethylcyclotrisiloxane (10.0%)	
	Non-polar extracts of stigmas: **Chloroform extract:** *E* and *Z* isomers 1-(26,6-trimethyl-1-cyclohexen-1-yl)-3-methyl-2-heptane (17.1%), safranal (7.1%) and 1,9-tetradecadiene (7.1%)	
	Non-polar extracts of stigmas: **Petroleum ether extract:** 9-Tetradecadiene (33.7%), *Z*-14-nonacosane (13.9%) and vitamin E (7.8%)	
	Non-polar extracts of stigmas: **Hexane extract:** 14b-Pregnan (21.7%), hexadecyloxirane (19.3%) and cyclotrisiloxane hexamethyl (10.4%)	

canoic) (Sereshti et al., 2014) and Chinese extractions (up to 8.12% linoleic in SE) (Shao et al., 2014), while absent or minor in European samples; other acids like acetic acid (5.63-9.49%) (Azarabadi et al., 2018) are notable in Iranian qualities (*e.g.*, 9.49% in Sargol-I). Unique compounds include 2-phenylethyl alcohol (15-50.4%) in Italian tepals (Tirillini et al., 2006) and anthers, 2(5H)-furanone ($0.7-59.80\%$) (Masuda et al., 2012; Zwane et al., 2020) highest in Japan (59.80%) (Masuda et al., 2012), and dihydro-β-ionone (3.43-8.62%) in Indian (Zareena et al., 2001) and Moroccan (Drioiche et al., 2023) samples. Extraction methods influence profiles: Hydrodistillation yields safranal (30.72%) and *trans*-β-ionol (8.68%) in China (Chen et al., 2020), while SFE boosts safranal to 40.31% (Chen et al., 2020); drying techniques in China show microwave yielding highest safranal (20.54%) versus freeze-drying (10.31%) (Chen et al., 2020); storage in Turkey reduces β-isophorone (from 11,222.2 to 245.3 μg/kg) but elevates safranal (from 14,093.9 to 80,349.4 μg/kg) (Sevindik, 2020). Regional variations highlight Iranian dominance in diversity (*e.g.*, bicyclo[3,2,0]hept-2-ene-4-ethoxy-endo at 5.6% (Jalali-Heravi et al., 2009), 2-ethoxy-5-methoxybenzaldehyde at 1,147 μg/g in Qaen (Amanpour et al., 2015)), Spanish in safranal (4,510-7,656 μg/kg) (Karabagias et al., 2017; Cerdá-Bernad et al., 2022), Greek in megastigma-4,6(*E*),8(*E*)-triene (715.5 μg/kg), and multi-country study (22.1-62.4% safranal (Chen et al., 2020; Zwane et al., 2020), 5.0-13.0% 4-ketoisophorone (Zwane et al., 2020)), underscoring geographical, processing and irradiation impacts on volatile composition as detailed in references from Tarantilis et al. (1997) to Drioiche et al. (2023).

In Moroccan saffron from 11 regions (2023), safranal ranges 22.96-66.07% (highest in Ain Leuh), isophorone 11.58-45.70% (highest in Taliouine-Dar Zaefaran), ketoisophorone 5.46-22.39% (highest in Azilal), and β-isophorone up to 16.49%, with terpenes dominating over 90% of volatiles (Naim et al., 2023). A 2024 study on saffron infusion in oil/water systems shows safranal extraction peaks at 12.3 μg/mg in 1:2 water/oil at 80 °C for 20 min, decreasing at 100 °C in mixed phases but increasing in pure oil, highlighting oil's role in enhancing volatile yields at higher temperatures. These findings underscore ongoing geographical diversification and methodological influences on saffron's volatile composition (Criado-Navarro et al., 2024). More recently reports reveal additional regional and extraction-dependent variations in saffron volatiles; in Algerian cultivars (2025), non-polar extracts of *C. sativus* L petals show dominance of valeric acid (20.80%), linoleic acid (11.4%) and nonadecane (9.0%) in chloroform; 1,3-bis(trimethylsilyl) benzene (10.8%), nonacosane (9.9%) and eicosane (9.5%) in petroleum ether; heptacosane (14.3%), eicosane (10.23%) and hexamethylcyclotrisiloxane (10.0%) in hexane, while stigmas feature 1,9-tetradecadiene (33.7%), *Z*-14-nonacosane (13.9%) and vitamin E (7.8%) in petroleum ether, with safranal at only 7.13% in chloroform alongside isomers of 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-methyl-2-heptane (17.1%), indicating lower safranal in Algerian stigmas compared to traditional regions (Djenhi et al., 2025). Moreover, the hexane extract from the stigmas of *C. sativus* L. consisted of 14b-pregnan (21.7%), hexadecyloxirane (19.3%) and cyclotrisiloxane hexamethyl (10.4%) (Djenhi et al., 2025).

3.3 Comparison of the chemical profiles of this study with those available in the literature

The chemical profile of *C. sativus* L. volatiles presented in Table 1, derived from MAHD and HS-SPME of Iranian aerial parts, serves as a reference dataset against which the geographically and methodologically diverse reports compiled in Table 2 must be systematically compared. An integrated comparison between the results presented in Table 1 and the collective data reported in Table 2 reveals a strong overall coherence in the volatile and essential oil profiles of *C. sativus* L., while simultaneously highlighting the pronounced influence of extraction methodology, geographical origin, plant part, post-harvest processing, and storage conditions on quantitative composition. This exhaustive comparison reveals fundamental concordances, pronounced quantitative disparities along with critical methodological and chemotaxonomic divergences.

In Table 1, both MAHD and HS-SPME analyses confirm that saffron volatiles are overwhelmingly dominated by apocarotenoid-derived compounds, with safranal having relative abundances of 37.2% (MAHD) and 63.5% (HS-SPME) unequivocally identified as the major constituent, a finding that is consistently corroborated by the vast majority of studies summarized in Table 2 across different countries and analytical approaches. The exceptionally high proportion of safranal observed by HS-SPME in Table 1 aligns closely with reports from Italy, Iran, Greece, Spain, Morocco, Turkey, and multi-country surveys, where safranal frequently ranges from approximately 20% to over 80% of the total volatile fraction, or appears as the most abundant compound in quantitative determinations expressed in µg/kg. This convergence strongly supports the role of safranal as the universal chemical hallmark of saffron aroma and quality, irrespective of origin. Safranal is consistently reported as the dominant compound in Greek saffron (Tarantilis et al., 1997), Italian samples from Salerno (59.13%), Sardinia (41.13%), Abruzzo (72.49%), and Sicily (84.38%), Iranian specimens (26.29-63.24%), Spanish saffron (4510 µg/kg; 7443 µg/kg), Turkish accessions from Eskisehir and Safranbolou (49.33-77.9%), Moroccan samples (6.39% and 31,710 µg/kg) and Chinese dried stigmas (10.31-22.18%). The Iranian Sargol and Pushal cultivars (Azarabadi et al., 2018; Ghanbari et al., 2019) exhibit safranal concentrations (49.64-61.31%) that align closely with the HS-SPME value (63.5%) from Table 1, suggesting that Iranian germplasm is characterized by exceptionally high safranal accumulation. Beyond safranal, Table 1 shows substantial levels of isophorone, 4-ketoisophorone, 4-methylene-isophorone, and related cyclohexenone derivatives, which are also repeatedly reported in Table 2 as major or secondary constituents in saffron samples worldwide. The relative abundance of these compounds in MAHD extracts mirrors findings from studies employing hydrodistillation, Soxhlet extraction, supercritical fluid extraction, microwave drying, oven drying, and infrared drying, where thermal or semi-thermal processes promote the formation and accumulation of isophorone-type apocarotenoids through carotenoid and picrocrocin degradation pathways. In contrast, the HS-SPME profile in Table 1, characterized by an enhanced recovery of low-boiling, aroma-active compounds and reduced representation of heavier constituents, closely resembles headspace-based and aroma-focused studies in Table 2, particularly those emphasizing fresh, gently processed, or analytically non-invasive sampling conditions.

Table 1 further reports the presence of aliphatic aldehydes, alcohols, fatty acids, esters, and long-chain hydrocarbons, especially in MAHD extracts, a pattern that is consistent with numerous reports in Table 2 describing significant levels of hexadecanoic acid, tetradecanoic acid, linoleic acid, ethyl hexadecanoate, long-chain alkanes, and related lipid-derived compounds. These constituents are especially prominent in studies focusing on non-polar extracts, tepals, anthers, or whole floral matrices, as well as in samples subjected to prolonged storage or harsher extraction conditions. Their comparatively lower contribution in HS-SPME data from Table 1 reflects methodological selectivity rather than true absence, and parallels observations in Table 2 where extraction selectivity strongly dictates the apparent chemical profile.

Geographical variability documented in Table 2, with notable differences in the relative proportions of safranal, isophorones, ionones, acids, and minor aromatics, is not contradictory to the findings of Table 1 but rather complementary. The compositional pattern observed in Table 1 falls well within the global chemical spectrum reported for saffron, reinforcing the concept that while the qualitative core profile of saffron volatiles is highly conserved, quantitative variations are driven by cultivar, terroir, climate, drying technique, and storage duration. In particular, the elevated safranal levels seen in HS-SPME from Table 1 are consistent with reports of increased safranal formation during storage and aging noted in several studies in Table 2, whereas the richer matrix of fatty acids and hydrocarbons in MAHD aligns with reports emphasizing whole-oil characterization rather than aroma specificity.

Taken as a unified whole, the comparison demonstrates that Table 1 provides a methodologically robust snapshot of saffron volatile chemistry, fully supported by and analytically consistent with decades of international research summarized in Table 2. Together, these data reinforce a comprehensive chemical framework in which safranal and structurally related apocarotenoids define saffron aroma, while secondary lipid-derived and aliphatic compounds modulate chemical complexity according to extraction, processing, and origin. This offers a comprehensive and globally validated understanding of saffron volatile composition.

4. Concluding remarks

This study clearly demonstrates that the chemical profile of *C. sativus* L. is strongly influenced by the extraction technique employed. Both microwave-assisted hydrodistillation (MAHD) and headspace solid-phase microextraction (HS-SPME), when coupled with gas chromatography-mass spectrometry (GC-MS), yield qualitatively similar but quantitatively distinct chemical fingerprints. In both methods, oxygenated monoterpenes and non terpene-derived compounds dominate the relevant profiles, confirming safranal as the principal aroma-active constituent and the key marker

compound of saffron quality. Safranal (2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde) occurs at markedly higher levels in HS-SPME compared with MAHD, reflecting the superior suitability of headspace techniques for capturing highly volatile, thermolabile aroma compounds without solvent or thermal degradation. Conversely, MAHD favors the extraction of less volatile and higher-molecular-weight constituents, including fatty acids, long-chain hydrocarbons, and esters, which contribute marginally to aroma but are relevant to the overall chemical matrix of the essential oil. Safranal is confirmed as the predominant compound in both extracts, yet its relative abundance is markedly higher in the HS-SPME analysis (63.5%) compared to MAHD (37.2%). This substantial discrepancy is analytically significant and is attributable to the fundamental differences in extraction principles: HS-SPME is a solvent-free, equilibrium-based technique that preferentially samples headspace volatiles, thereby enriching for highly volatile and aromatic aldehydes such as safranal. On the other hand, MAHD involves exhaustive hydrodistillation coupled with microwave energy, yielding a more comprehensive essential oil profile that includes higher relative proportions of high-molecular-weight compounds. Notably, 4-ketoisophorone, a key degradation product of crocins and picrocrocin, is present at 11.2% in MAHD but only 2.5% in HS-SPME, suggesting that thermal and hydrolytic processes during hydrodistillation may promote the formation of this norisoprenoid. Furthermore, fatty acids and long-chain hydrocarbons-namely hexadecanoic acid (8.1%), tetradecanoic acid (7.5%), and nonacosane (6.2%)-are substantially enriched in the MAHD extract, whereas they are either absent or present only in trace amounts in the HS-SPME profile. This confirms that HS-SPME is inherently biased toward low-molecular-weight, highly volatile analytes and is not suitable for quantitative assessment of semi-volatile or non-volatile lipophilic constituents.

The significant presence of isophorone, 4-ketoisophorone, 4-methylene-isophorone, and related apocarotenoids in both extracts highlights the central role of carotenoid degradation pathways in shaping saffron aroma. The relative enrichment of these compounds in MAHD extracts is consistent with literature reports indicating that thermal processes promote the conversion of crocetin esters and picrocrocin into secondary norisoprenoids. Aliphatic aldehydes, alcohols, and fatty acids, although present at lower concentrations, further reflect oxidative and enzymatic processes occurring during drying and processing of stigmas.

Overall, the findings of this report confirm that HS-SPME is particularly effective for qualitative aroma profiling and authenticity assessment, while MAHD provides a more comprehensive representation of the essential oil composition, including semi-volatile constituents. These results are consistent with previous scientific studies on saffron volatiles and reinforce the importance of selecting extraction methods based on the analytical objective. Future research should focus on:

(i) Integrating complementary extraction techniques with advanced quantitative and chemometric approaches (*e.g.*, principal component analysis, hierarchical clustering) to better correlate volatile fingerprints with geographical origin, processing conditions, storage stability, and sensory quality.

(ii) The systematic investigation of post-harvest processing-drying, storage, irradiation-on volatile compound evolution, particularly the interconversion of picrocrocin degradation products;

(iii) The valorization of saffron floral bio-residues (tepals, anthers, petals) as sources of bioactive volatiles such as 2-phenylethyl alcohol and its esters;

(iv) Improving the application of green extraction technologies, including MAHD and solvent-free microwave extraction, as sustainable alternatives to conventional hydrodistillation for essential oil extraction; and

(v) Targeted studies on the kinetics of apocarotenoid formation and degradation under different processing regimes to optimize saffron aroma and preserve its high commercial and pharmacological value.

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Authors contributions

Conceptualization and literature search were performed by MM. The first draft of the manuscript was prepared by MM. MM also critically analyzed and gave suggestions to finalize the manuscript. The author read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests

The authors declare that there is no conflict of interest.

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