

Worldwide market screening of saffron volatile composition

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Abstract

BACKGROUND: Saffron (*Crocus sativus* L.) is one of the most valuable spices and nowadays its main use is as a foodstuff. Numerous papers have been published on saffron aroma and its volatile content, but nothing has been written about the aroma quality of samples available on the market to consumers. The aim of this study was to analyse and compare 418 commercial samples of saffron belonging to different ISO categories. Ultrasound-assisted extraction (USAE) with an organic solvent and dynamic headspace desorption (DHD) followed by gas chromatography/mass spectrometry were used to screen for saffron volatile composition.

RESULTS: For both methods the saffron aromatic profile was characterised by spicy aromatic notes due to safranal, the most abundant volatile component, by a floral contribution attributable to isophorone and 2,2,6-trimethyl-1,4-cyclohexanedione, together with citrus and spicy notes from 4-ketoisophorone and 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one respectively.

CONCLUSION: USAE allowed the detection of a greater number of compounds, whereas DHD was faster and a smaller amount of saffron was required. Compared with the USAE method, the DHD method defined the samples as having a spicier and more floral aromatic contribution, thus corroborating that the extraction method considerably changes the aromatic fingerprint of saffron samples.

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Keywords: aroma; saffron (*Crocus sativus* L.); GC analysis; ISO categories; volatile composition

INTRODUCTION

Saffron spice comes from the dried stigmas of *Crocus sativus* L. and nowadays its main use is as foodstuff. During the drying process the stigmas lose up to 80% of their weight, thus reducing the moisture content of saffron to 70–100 g kg⁻¹. Important modifications have also been observed in terms of colour,^{1,2} taste and aroma.³ There are different drying processes depending on the country of production and therefore different characteristics and qualities of saffron.¹ By far the most important saffron-producing country is Iran (>90%), followed by Greece, Morocco, India, Spain and Italy.⁴ The quality of saffron is determined by ISO 3632,⁵ which classifies it into three categories according to physical and chemical parameters. Of these three parameters, colour has traditionally been the most appreciated, with aroma and taste only recently being noticed.

On the international market, producers know that initially saffron has a wide variety of aromatic notes, some of which are lost and others of which become more intense and piercing over time.⁶

In terms of the aroma chemistry of saffron, 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (safranal) is the major compound together with 3,5,5-trimethyl-2-cyclohexene-1-one (isophorone), 2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-ketoisophorone), 3,5,5-trimethyl-3-cyclohexene-1-one (an isomer of isophorone), 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde

(an isomer of safranal), 2,2,6-trimethyl-1,4-cyclohexanedione, 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC) and 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one.^{7,8} Some authors have reported the identification of more than 160 volatile compounds in saffron spice, though others have asserted that the number of these substances is not so high, since many of them could either be generated as artefacts when an exhaustive isolation procedures are employed.⁹

The main techniques currently used to isolate saffron aroma compounds are solvent extraction,^{10–13} distillation, including steam distillation and simultaneous distillation/extraction (SDE),¹⁴ headspace techniques³ and supercritical fluid extraction (SFE).¹⁵ Some of these techniques seem to facilitate the extraction of several less important compounds (e.g. SFE) or to generate a large number of substances (e.g. SDE), but the best two extraction meth-

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ods appear to be solvent extraction and headspace techniques.⁹ Numerous papers have been published on saffron aroma and its volatile content,^{9,14,16,17} but nothing has been written about the aroma quality of samples available on the market to consumers.

The aim of this study was to analyse and compare 418 commercial samples of saffron belonging to different ISO categories. Ultrasound-assisted extraction (USAE) with an organic solvent and dynamic headspace desorption (DHD) followed by gas chromatography/mass spectrometry (GC/MS) were used to screen for saffron volatile composition.

EXPERIMENTAL

Samples

Four hundred and eighteen saffron samples from Greece, India, Iran, Italy, Morocco and Spain belonging to different harvests (from 2004 to 2006) and commercial categories (according to ISO 3632⁵ specifications) were collected for study (Table 1). The samples were obtained directly from the producers with a guarantee of origin and freedom from adulteration and were kept at 4 °C in the absence of light until their analysis.

All samples were analysed in duplicate using the two proposed methods.

Chemicals and reagents

Safranal of 98% purity was obtained from Sigma-Aldrich (Madrid, Spain) as a standard. The solvents diethyl ether and cyclohexane were purchased from Panreac (Barcelona, Spain). Water was purified through a Milli-Q system (Millipore, Bedford, MA, USA). Series of safranal standard solutions in diethyl ether and cyclohexane were prepared and used to construct calibration curves for USAE/GC/MS and DHD/GC/MS respectively. A filtration membrane made of hydrophilic polytetrafluoroethylene (PTFE) with a porosity of 0.45 µm (Millipore) was used.

Procedure and instrumentation

USAE/GC/MS

USAE was performed in a Super RK 255H ultrasound water bath (Sonorex, Berlin, Germany) at a fixed frequency of 35 kHz. The temperature of the sonicated water bath was 25 °C. The sample flask was charged with 4 g of saffron stigmas. The solvent system extractant was 40 mL of diethyl ether. Each saffron sample was sonicated twice for 15 min (two fractions per saffron sample). For each sonication a new volume of the solvent system extractant was added to the sample flask. The organic extract (80 mL) was concentrated using a Laborata 4000 Efficient rotary evaporator (Heidolph Instruments GmbH & Co.KG, Schwabach, Germany). The

volume of the final aromatic extract in diethyl ether was 4 mL. The collected diethyl ether was analysed by GC to check if there was a loss of saffron volatiles during the evaporation procedure, but no saffron volatiles were detected.

An HP 5890 Series II chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with an HP 5972 mass-selective detector in electron impact mode (70 eV) and an HP-5MS capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) with helium as carrier gas at a flow rate of 1 mL min⁻¹ was used for the analysis of saffron aromatic extracts. The column temperature was initially held for 3 min at 50 °C, then increased to 180 °C at a rate of 3 °C min⁻¹ and finally increased to 250 °C at a rate of 15 °C min⁻¹ and held for 5 min. The injector and detector temperatures were set at 220 and 290 °C respectively. Samples (1 µL) were injected manually in splitless mode.

For quantification of safranal the external standard method was applied. The calibration curve was established for the series of safranal standard solutions in diethyl ether, with the equation

$$\text{mg safranal kg}^{-1} \text{ saffron} = 86.6 \times \text{Area}_{\text{safranal}} \quad (R^2 = 0.999)$$

where $\text{Area}_{\text{safranal}} = \text{safranal peak area}/10^6$ in the GC chromatogram.

DHD/GC/MS

Ground saffron (10 mg) was placed in a stainless steel tube and DHD was carried out using a Perkin-Elmer TurboMatrix ATD thermal desorption system (Norwalk, CT, USA). The headspace isolation conditions were as follows: oven temperature, 50 °C; desorption time, 5 min; cold trap temperature, -30 °C; helium inlet flow rate, 45 mL min⁻¹.

The desorption unit was coupled to a Varian CP-3800 gas chromatograph (Palo Alto, CA, USA) equipped with a Saturn 2200 ion trap mass spectrometer provided with a VF-5MS Factor Four fused silica capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) from Varian (Palo Alto, CA, USA). The column temperature was initially held for 2 min at 80 °C, then increased to 200 °C at 10 °C min⁻¹ and held for 5 min and finally increased to 250 °C at 20 °C min⁻¹ and held for 5 min. The transfer line and detector temperatures were set at 230 and 300 °C respectively. Helium was used as carrier gas at a flow rate 1 mL min⁻¹. In the mass spectrometer the electron impact mode was set up at 70 eV. The mass range varied from 40 to 500 u.

A calibration curve was constructed by injecting 10 µL aliquots of safranal standard solutions into stainless steel thermal tubes containing glass wool, previously conditioned for 1 min at 300 °C under a nitrogen flow of 100 mL min⁻¹.

The quantification of safranal was again carried out using the external standard method. The calibration curve was established for the series of safranal standard solutions in cyclohexane, with the equation

$$\text{mg safranal kg}^{-1} \text{ saffron} = 10.88 + 36.76 \times \text{Area}_{\text{safranal}} \quad (R^2 = 0.998)$$

where $\text{Area}_{\text{safranal}} = \text{safranal peak area}/10^6$ in the GC chromatogram.

Not all volatile compounds present in saffron are commercially available as standards, so safranal has been used as reference and other volatile substances have been compared against it.

Table 1. Numbers of saffron samples from each country belonging to categories specified in ISO 3632⁵

Country	Category I	Category II	Category III
Greece	112	10	0
India	2	0	0
Iran	101	48	8
Italy	60	0	0
Morocco	4	0	0
Spain	60	5	8
Total samples	339	63	16

Statistical analysis

Evaluation of the statistical significance of differences was performed by analysis of variance (ANOVA) using the SPSS 15.0 for Windows statistical program (SPSS Inc., Chicago, IL, USA). Discriminant analysis was carried out with SPSS 15.0 for Windows to differentiate the saffron samples into the three ISO categories.

RESULTS AND DISCUSSION

According to the specifications of ISO 3632,⁵ 81% of the commercial saffron samples belonged to category I, 15% to category II and only 4% to category III.

In Table 2 the main volatile compounds (expressed as g kg⁻¹ total volatile content) found in the samples are reported, separated according to ISO category and analysis method.

With respect to comparison of the two methods at first sight, USAE allowed for the determination of a higher number of volatile constituents, up to 22, while DHD only allowed for six, although other compounds were detected at trace levels.

Safranal (compound 1), isophorone (compound 4), 2,2,6-trimethyl-1,4-cyclohexanedione (compound 5), 4-ketoisophorone (compound 7), 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one (compound 9) and 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (compound 13) were the six main volatile compounds detected in all three categories using both methods. The total content of these compounds ranged between 540 and 630 g kg⁻¹ for USAE, whereas it was almost 1000 g kg⁻¹ for DHD. For both methods, analysis of the samples indicates that the major constituent of the aromatic composition of saffron is safranal, as reported previously.^{7,18} In terms of saffron flavour chemistry, compound 1 has typical saffron spicy aromatic notes.¹⁹

In terms of volatile composition screening of samples available on the market, with the USAE method the safranal content varied significantly between categories I, II and III, ranging from 336 to 429 g kg⁻¹, whereas with the DHD method no significant differences were observed between the three categories, with levels similar to those (~600–720 g kg⁻¹ volatile fraction) reported by others.^{7,14,20} The content of compound 4 was 87 g kg⁻¹ for category I and decreased to 76 and 78 g kg⁻¹ for categories II and III respectively with USAE, whereas DHD gave 188 g kg⁻¹ for category I and 165 g kg⁻¹ for categories II and III, confirming data (~140 g kg⁻¹) reported by Cadwallader.¹⁹ For both methods, ANOVA did not allow us to separate the content of compound 4 between the three categories. Also, for compound 5 obtained by both USAE and DHD, there were no significant differences in its content between the three categories. With regard to saffron flavour chemistry, compounds 4 and 5 contribute to the aromatic profile of this spice with floral notes.^{19,21} For USAE the content of compound 7 varied from 41 g kg⁻¹ (category I) to 42 g kg⁻¹ (category II) and 40 g kg⁻¹ (category III), whereas for DHD it varied from 39 g kg⁻¹ (category I) to 34 g kg⁻¹ (category II) and 35 g kg⁻¹ (category III). For both methods the levels obtained were in agreement with data (~40 g kg⁻¹) reported by Cadwallader¹⁹ and there were no significant differences in the content of compound 7 between the three categories. Citrus aromatic notes have been attributed to compound 7 by Rödel and Petrzika.²¹ The content of compound 9, characterised by spicy aromatic notes,¹⁹ increased from 10 g kg⁻¹ (category I) to 22 g kg⁻¹ (category III) for USAE and from 21 g kg⁻¹ (category I) to 28 g kg⁻¹ (category III) for DHD. Also in this case there were no significant differences in the content of compound 9 between the three categories. The content of compound 13 decreased slightly from 6 g kg⁻¹ (category I) to

3 g kg⁻¹ (category III) for USAE, while it increased from 31 g kg⁻¹ (category I) to 41 g kg⁻¹ (category III) for DHD. Again, for both methods there were no significant differences in the content of compound 13 between the three categories.

With USAE the content of compound 2 was 153 g kg⁻¹ for category I, 122 g kg⁻¹ for category II and 163 g kg⁻¹ for category III and it was possible to distinguish category II from categories I and III. The content of compound 3 decreased from 116 g kg⁻¹ for category I to 66 g kg⁻¹ for category III, showing significant differences between categories I and II and category III. The content of compound 6 increased from 51 g kg⁻¹ for category I to 71 g kg⁻¹ for category III, showing significant differences between the three categories. The content of compound 8 was 13 g kg⁻¹ for category I, 7 g kg⁻¹ for category II and 16 g kg⁻¹ for category III, while that of compound 10 was 9 g kg⁻¹ for category I, 10 g kg⁻¹ for category II and 8 g kg⁻¹ for category III. The content of compound 11 was 9 g kg⁻¹ for all three categories, whereas the content of compound 12 decreased from 7 g kg⁻¹ for category I to 4 g kg⁻¹ for category III. The contents of compounds 14 and 15 increased from 6 g kg⁻¹ for category I to 44 g kg⁻¹ for category III and from 5 g kg⁻¹ for category I to 36 g kg⁻¹ for category III respectively, so for both compounds there were significant differences between the three categories. The contents of compounds 16 and 17 ranged from 5 g kg⁻¹ for category I to 19 g kg⁻¹ for category III and from 4 g kg⁻¹ for category I to 13 g kg⁻¹ for category III respectively. In both cases it was possible to distinguish categories I and II from category III. The contents of compounds 18, 19 and 20 did not show significant differences between the three categories. Finally, compounds 21 and 22 were detected only for category I at 1 g kg⁻¹. Furthermore, the 22 volatile components were used in a discriminant analysis to differentiate the saffron samples according to the three ISO categories (Fig. 1). The samples were clearly separated by two canonical discriminant functions, the first explaining 99.5% of the variance and the second explaining 100% of the accumulated variance. Compounds 2, 16 and 6 were observed as being the variables that contributed most to the differentiation in function 1, while compounds 6 and 16 contributed greatly to the differentiation in function 2.

With regard to DHD, the mean contents of volatile compounds were not significantly different between ISO categories, which could be explained by the absence of sample handling. This confirms that saffron aroma has the same volatile fingerprint independently of its category,²⁰ which may be useful to detect adulteration of saffron aroma.

Using USAE, significant differences in the volatile composition between ISO categories were observed, which could be explained by the ultrasound action that allows the isolation and detection of less volatile compounds of higher molecular weight. Also, the ultrasound water bath was operated at 35 kHz, at which frequency no artefacts are produced.¹⁴ Farmers produce saffron belonging to category I; subsequently, according to its postharvest treatment, saffron maintains its category or evolves to category II or III. As is known, saffron of the best quality belongs to category I not only for colour and flavour but also for aroma. USAE could be a good tool to distinguish ISO categories by means of the different contents of compounds 6, 14 and 15. As their contents increase, saffron aroma evolves negatively and its category worsens. Compounds 21 and 22 are quality markers for category I and therefore saffron containing these compounds is of higher quality.

With respect to the sensory flavour profile, the data obtained corroborate that the extraction method considerably changes the aromatic fingerprint of saffron samples.

Table 2. Main volatile compounds (expressed as g kg⁻¹ total volatile content) of saffron samples belonging to categories I, II and III specified in ISO 3632²

No.	Compound	Compound ions ^a (<i>m/z</i>)	USAE (g kg ⁻¹)			DHD (g kg ⁻¹)		
			I	II	III	I	II	III
1	2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (safranal)	107 (100), 91 (86), 121 (62), 150 (47)	405 ± 32b	429 ± 36b	336 ± 27a	654 ± 66a	679 ± 61a	665 ± 64a
2	4-Hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-diene-1-carboxaldehyde	109 (100), 137 (88), 123 (55), 180 (43)	153 ± 14b	122 ± 8a	163 ± 21b	–	–	–
3	4-Hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde (HTCC)	107 (100), 135 (85), 79 (70), 168 (37)	116 ± 17b	112 ± 19b	66 ± 16a	–	–	–
4	3,5,5-Trimethyl-2-cyclohexene-1-one (isophorone)	82 (100), 138 (28) , 54 (12), 95 (9), 41 (7)	87a ± 11a	76 ± 13a	78 ± 14a	188 ± 21b	165 ± 18a	165 ± 19a
5	2,2,6-Trimethyl-1,4-cyclohexanedione	139 (100) , 56 (98), 42 (75), 154 (60)	61 ± 7a	61 ± 8a	57 ± 10a	67 ± 6a	63 ± 7a	66 ± 5a
6	Isomer of 4-hydroxy-3,5,5-trimethyl-2-cyclohex-1-one	98 (100), 70 (48), 69 (42), 154 (3)	51 ± 5a	61 ± 4b	71 ± 5c	–	–	–
7	2,6,6-Trimethyl-2-cyclohexene-1,4-dione (4-ketoisophorone)	68 (100), 96 (86), 152 (46) , 109 (12)	41 ± 16a	42 ± 11a	40 ± 13a	39 ± 10a	34 ± 7a	35 ± 3a
8	Isomer of 4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde	43 (100), 41 (83), 153 (87), 182 (15)	13 ± 2b	7 ± 1a	16 ± 3b	–	–	–
9	2-Hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one	109 (100), 124 (61), 152 (52) , 137 (40)	10 ± 2a	17 ± 4b	22 ± 2b	21 ± 5a	23 ± 3a	28 ± 2a
10	(<i>E</i>)-4-(2,2,6-Trimethyl-7-oxabicyclo[4,1,0]heptan-1-yl)but-3-en-2-one	123 (100), 111 (48), 168 (36), 208 (0.3)	9 ± 1a	10 ± 2a	8 ± 1a	–	–	–
11	Isophorone-4-methylene	107 (100), 91 (50), 108 (50), 150 (46)	9 ± 1a	9 ± 1a	9 ± 2a	–	–	–
12	Phenyl ethyl alcohol	91 (100), 92 (56), 122 (27) , 65 (24)	7 ± 2a	6 ± 1a	4 ± 2a	–	–	–
13	2,6,6-Trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (an isomer of safranal)	121 (100), 91 (50), 107 (40), 150 (8)	6 ± 2a	5 ± 1a	3 ± 2a	30 ± 9a	35 ± 7a	41 ± 6a
14	2-Hydroxy-3,5,5-trimethylcyclohex-2-en-1,4-dione	84 (100), 56 (80), 55 (59), 168 (44)	6 ± 1a	16 ± 2b	44 ± 12c	–	–	–
15	4-Hydroxy-3,5,5-trimethyl-2-cyclohex-1-one	98 (100), 70 (58), 69 (39), 154 (7)	5 ± 1a	13 ± 2b	36 ± 10c	–	–	–
16	4-Hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde	43 (100), 153 (87), 125 (87), 182 (15)	5 ± 2a	4 ± 1a	19 ± 4b	–	–	–
17	2,6,6-Trimethyl-3-oxo-1-cyclohexen-1-carboxaldehyde	91 (100), 93 (83), 121 (77), 166 (28)	4 ± 1a	3 ± 1a	13 ± 3b	–	–	–
18	2,3-Dihydroxynaphthalene-1,4-dione	116 (100), 53 (69), 41 (46), 190 (7)	4 ± 1a	3 ± 1a	4 ± 2a	–	–	–
19	2,2-Dimethyl-cyclohexane-1-carboxaldehyde	107 (100), 41 (40), 43 (36), 140 (12)	4 ± 1a	3 ± 2a	6 ± 2a	–	–	–
20	3-[(<i>E</i>)-But-1-enyl]-2,4,4-trimethyl-cyclohex-2-enol	43 (100), 41 (69), 121 (62), 194 (11)	2 ± 1a	1 ± 1a	4 ± 2a	–	–	–
21	3,5,5-Trimethyl-3-cyclohexen-1-one (an isomer of isophorone)	96 (100), 138 (78) , 81 (75), 123 (65)	1	–	–	–	–	–
22	2(3 <i>H</i>)Furanone-dihydro-4-hydroxy	44 (100), 43 (68), 72 (24), 102 (9)	1	–	–	–	–	–

Different letters within a row indicate significant differences at the 0.05% level. Numbers in parenthesis denote mass abundance in the peak.

^a Ions used for compound identification. Bold type denotes ion selected for compound quantification.

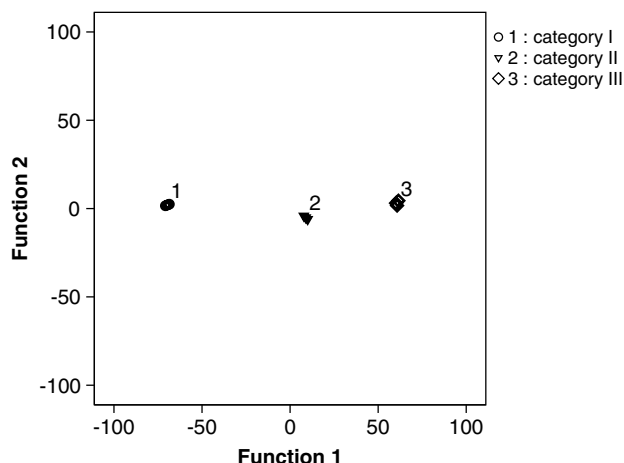


Figure 1. Plot of canonical discriminant functions: according to ISO categories.

CONCLUSION

Four hundred and eighteen samples of saffron belonging to the three ISO categories were analysed using USAE with an organic solvent and DHD followed by GC/MS. The results were then compared to determine the screening of their volatile profile and to give an overview of world production. USAE allowed the detection of a greater number of compounds and differentiation of ISO categories, whereas DHD was faster, required a smaller amount of saffron and was able to characterise the saffron volatile fingerprint. Hence the aromatic profile of saffron available on the market to consumers was characterised by spicy aromatic notes due to compound 1, the most abundant volatile component, by a floral contribution attributable to compounds 4 and 5, together with citrus and spicy notes from compounds 7 and 9 respectively.

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