

# Isolation and Identification of the Aroma Components from Saffron (*Crocus sativus*)

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In this work we have studied the volatile components of saffron, the dried, dark-red stigmata of *Crocus sativus* L. flowers. The isolation of the aroma, was achieved using the following techniques: (a) steam distillation (SD), (b) micro-steam distillation extraction (MSDE), and (c) vacuum head space method (VHS). The determination of the volatile components was performed using some different gas chromatography–mass spectrometry (GC–MS) instruments and methods. The characteristic compounds are 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde, namely safranal; 3,5,5-trimethyl-2-cyclohexen-1-one, namely isophorone; 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.

**Keywords:** Saffron; capillary; GC–MS; volatile compounds

## 1. INTRODUCTION

Saffron is the most expensive of spices, it comes from the dried red stigmata of *Crocus sativus* L. flowers. It is used widely as a food additive because of its color and flavor (taste and aroma) (Basker and Negbi, 1983).

Sources of this strong coloring capacity are glycosyl esters of crocetin carrying one up to five glucoses, which are unusual water-soluble carotenoids (Tarantilis et al., 1995). The digentiobiosyl ester of crocetin ( $C_{44}H_{64}O_{24}$ ), known as crocin, also occurs in fruit of *Gardenia jasminoides*, is the most abundant of these components. Picrocrocin ( $C_{16}H_{26}O_7$ ), a monoterpene glycoside, is the principal bitter tasting substance. One of the main components of essential oil of saffron responsible for its aroma is safranal ( $C_{10}H_{14}O$ ). It is a monoterpene aldehyde, formed in saffron during drying and storage by hydrolysis from picrocrocin (Castellar et al., 1993; Himeno and Sano, 1987; Iborra et al., 1992; Sujata et al., 1992; Tarantilis et al., 1994).

Past research on the aroma of this spice has been focused on the composition and identification of the volatile components of saffron using gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC–MS) (Rodel and Petrzika, 1991; Zarghami and Heinz, 1971).

The isolation of volatile fraction is a prior step to GC–MS analysis, which has been carried out in different ways. The composition of the aromas that are obtained is greatly dependent upon the methods that are employed for isolation.

The classical method, steam distillation (SD), only rarely provides samples that reproduce the typical aroma of the spice. The drastic conditions, of this method, produce artifacts and destroy sensitive components. It is most important to avoid or to minimize artifacts of volatile ingredients during isolation. The micro-simultaneous steam distillation–solvent extraction method (MSDE) sometimes allows only low concentrations of the volatile components, and the high boiling point constituents are over-enriched (Godefroot et al., 1981; Nickerson and Likens, 1966; Schmitt, 1984).

The dynamic head space method (DHS) primarily detects low-boiling components; on the other hand, only a minute volume of high boiling point components is found, or none at all (Joulain, 1987; Mookherjee et al., 1989). The vacuum head space method (VHS) detects both low and high boiling point compounds. A major advantage of this method consists of the fact that the aroma concentrate is obtained in the direct form through condensation (Joulain, 1986).

The aim of the present work is the study of the aroma components of saffron using three different isolation methods (SD, MSDE, and VHS) as a prior step in the GC–MS analysis.

## 2. EXPERIMENTAL PROCEDURES

**2.1. Plant Materials.** Stigmata of pure red Greek saffron were kindly supplied by the "Cooperative of Saffron, Krokos Kozanis".

**2.2. Isolation.** *2.2.a. Steam Distillation (SD).* A classic SD apparatus was used. The sample flask (1 L) was charged with 100 g of stigmata of pure red Greek saffron. The steam distillation was carried out for exactly 4 h. The condenser on the head was cooled with water at room temperature (20–25 °C).

*2.2.b. Micro-simultaneous Steam Distillation-Solvent Extraction (MSDE).* The MSDE apparatus (in low-density solvent configuration) was bought from Chrompack (Middelburg, The Netherlands). The extraction solvent was 3 mL of diethyl ether (0.03% BHT). The sample flask (100 mL) was charged with 10 g of stigmata of pure red Greek saffron in 50 mL of deionized water. The steam distillation–extraction was carried out for exactly 2 h. The condenser on the head was cooled (–10 °C) with a water–glycol mixture.

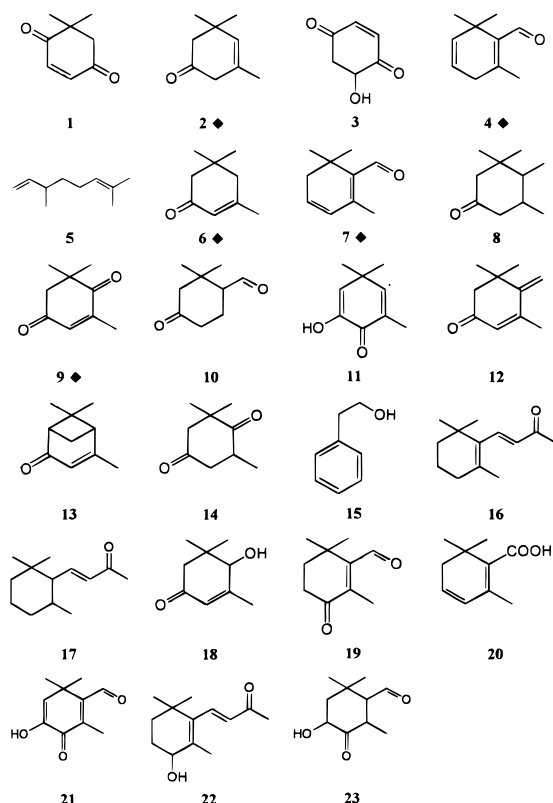
*2.2.c. Vacuum Head Space (VHS) Method.* The VHS system apparatus was very simple, equipped with a glass sample flask, two traps, and a water pump (vacuum 16–20 mbar), Polylabo (Strasbourg, France). The sample flask (1 L) was charged with 100 g of stigmata of pure red Greek saffron and was heated at 30 °C. Isolation was performed for a period of 6 h while the volatile components condensed on the dioxide snow–acetone-bath (–70 °C) traps and removed with 3 × 5 mL of dichloromethane freshly distilled.

**2.3. GC–MS Instrumentation and Conditions.** (a) A GC Perkin-Elmer (Norwalk, CT) Sigma 3B instrument equipped with a heated flame ionization detector (FID) and a capillary FFAP (50 m × 0.32 mm × 0.52 μm) column with helium as

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**Table 1. GC-MS Conditions**

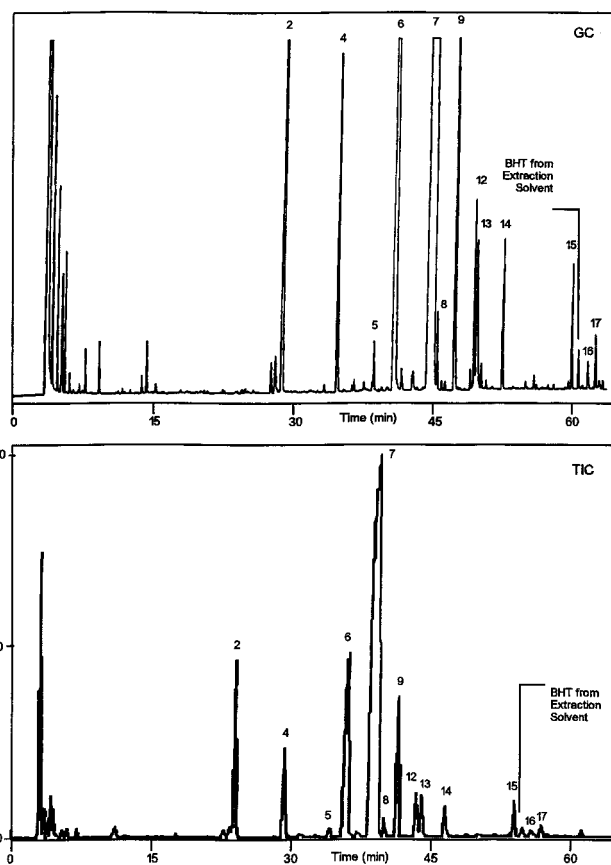
	method A	method B	method C
GC column	Perkin-Elmer Sigma 3B FFAP (50 m × 0.32 mm × 0.52 μm)	Hewlett-Packard Model 5890 II Ultra-1 methyl silicone (25 m × 0.32 mm × 0.50 μm)	Hewlett-Packard Model 5890 II Ultra-1 methyl silicone (25 m × 0.32 mm × 0.50 μm)
carrier oven	helium (3.0 mL/min) 60 °C (8 min) to 240 °C at 2 °C/min (30 min)	helium (3.0 mL/min) 70 °C (3 min) to 150 °C at 2 °C/min (1 min) to 200 °C (10 min)	helium (3.0 mL/min) 60 °C (1 min) to 280 °C at 5 °C/min (10 min)
injection detector	split (100:1), 1 μL, inlet 250 °C FID (290 °C)	split (100:1), 1 μL, inlet 200 °C FID (230 °C)	split (100:1), 1 μL, inlet 250 °C FID (260 °C)
MS	VG analytical MS spectrometer 7070 F	HP MS spectrometer 5989 A	HP MS spectrometer 5989 A
ionization mode source	electron impact (EI)	electron impact (EI)	electron impact (EI)
temperature (°C)	200	175	175
ionization energy (eV)	70	70	70

**Figure 1.** Molecular structure of aroma compounds of saffron (compound numbers as in Table 2; ◆ = the characteristic compounds of saffron).

carrier gas, coupled to an VG Analytical MS spectrometer 7070 F were used. (b) A GC Hewlett-Packard (Palo Alto, CA) Model 5890 II with a FID detector, a capillary Ultra-1 (methyl silicone) (25 m × 0.32 mm × 0.50 μm) column and helium as carrier gas coupled to HP MS spectrometer 5989 A were used. The GC-MS conditions are listed in the Table 1.

### 3. RESULTS AND DISCUSSION

Identification of the essential oil components was achieved by GC-MS. Retention time and mass spectra were compared with data from the literature (Zarghami and Heinz, 1971; Rodel and Petrzika, 1991) and spectra of the National Bureau of Standards mass spectrometric library. Table 2 demonstrates the GC-MS data and the identified compounds of the aroma of saffron isolated by three methods (MSDE, VHS, and SD) and analyzed using both FFAP and methyl silicone columns in three conditions. The structures of identified compounds are shown in Figure 1.

**Figure 2.** Chromatograms of a MSDE extract from stigmata of pure red Greek saffron with (A) FID detection (top); (B) MS detection, TIC signal (bottom), for GC-MS analysis. Chromatographic conditions are described in Table 1 (method A). Peak numbers are as in Table 2.

A typical GC chromatogram on capillary FFAP column and a total ion chromatogram (TIC) of a MSDE extract from stigmata of pure red Greek saffron are shown in Figure 2. The above analysis revealed the main characteristic components to be 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde, namely safranal (ca. 70% of the material in the extract, peak 7), 3,5,5-trimethyl-2-cyclohexen-1-one, namely isophorone (ca. 14%, peak 6), 3,5,5-trimethyl-3-cyclohexen-1-one, isomer of isophorone (ca. 5%, peak 2), 2,6,6-trimethyl-2-cyclohexen-1,4-dione (ca. 4%, peak 9), and 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde, isomer of safranal (ca. 3%, peak 4). Many other volatile compounds (ca. 1-0%) were also present (peaks 5, 8, and 12-17).

**Table 2. GC-MS Data of the Aroma Compounds of Saffron**

no.	compd	molecular formula	isolation method, condition, column			mass spectral data [ <i>m/z</i> (%)]
			MSDE method A FFAP	VHS method B methyl silicone	SD method C methyl silicone	
1	5,5-dimethyl-2-cyclohexen-1,4-dione	C <sub>8</sub> H <sub>10</sub> O <sub>2</sub> MW: <sup>a</sup> 138	—	—	4.7	70 (100), 95 (74), 39 (71), 41 (45), 67 (42), 55 (25), 82 (19), 110 (18), 138 (9)
2	3,5,5-trimethyl-3-cyclohexen-1-one	C <sub>9</sub> H <sub>14</sub> O MW: 138	28.7	—	5.4	96 (100), 138 (78), 81 (75), 123 (65), 41 (25), 67 (22)
3	2-hydroxy-5-cyclohexen-1,4-dione	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub> MW: 126	—	—	5.9	43 (100), 69 (59), 84 (32), 111 (40), 126 (6)
4	2,6,6-trimethyl-1,4-cyclohexadien-1-carboxaldehyde	C <sub>10</sub> H <sub>14</sub> O MW: 150	34.4	13.4	6.9	121 (100), 107 (38), 91 (38), 79 (29), 135 (25), 150 (16),
5	3,7-dimethyl-1,6-octadiene	C <sub>10</sub> H <sub>18</sub> MW: 138	38.4	—	—	71 (100), 93 (70), 41 (63), 55 (45), 80 (31), 121 (23), 136 (12), 107 (8), 138 (5)
6	3,5,5-trimethyl-2-cyclohexen-1-one (isophorone)	C <sub>9</sub> H <sub>14</sub> O MW: 138	40.8	16.2	7.3	82 (100), 138 (24), 54 (11), 95 (5), 67 (3), 123 (2)
7	2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde (safranal)	C <sub>10</sub> H <sub>14</sub> O MW: 150	44.9	17.3	9.3	107 (100), 91 (69), 121 (66), 150 (59), 79 (21), 65 (13), 135 (12)
8	3,3,4,5-tetramethylcyclohexan-1-one	C <sub>10</sub> H <sub>18</sub> O MW: 154	45.2	—	—	154 (100), 70 (90), 98 (50), 139 (40), 41 (31), 111 (22), 83 (19), 55 (15), 125 (11)
9	2,6,6-trimethyl-2-cyclohexen-1,4-dione	C <sub>9</sub> H <sub>12</sub> O <sub>2</sub> MW: 152	47.1	14.2	7.8	68 (100), 96 (89), 152 (52), 40 (34), 109 (10), 137 (7),
10	2,2-dimethyl-4-oxocyclohexan-1-carboxaldehyde	C <sub>9</sub> H <sub>14</sub> O <sub>2</sub> MW: 154	—	—	7.9	70 (100), 41 (72), 39 (66), 55 (51), 154 (35), 98 (32)
11	2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one	C <sub>9</sub> H <sub>12</sub> O <sub>2</sub> MW: 152	—	—	8.2	109 (100), 39 (90), 124 (42), 79 (35), 137 (23), 91 (21), 152 (20)
12	4-methylene-3,5,5-trimethyl-2-cyclohexen-1-one	C <sub>10</sub> H <sub>14</sub> O MW: 150	49.1	18.0	9.7	150 (100), 107 (99), 135 (41), 91 (41), 66 (35), 128 (34)
13	4,6,6-trimethyldicyclo-[3.1.1]hept-3-en-2-one	C <sub>10</sub> H <sub>14</sub> O MW: 150	49.3	18.1	—	128 (100), 150 (77), 107 (74), 91 (32), 66 (26), 79 (20)
14	2,6,6-trimethylcyclohexan-1,4-dione	C <sub>9</sub> H <sub>14</sub> O <sub>2</sub> MW: 154	52.1	14.9	—	56 (100), 42 (98), 139 (83), 154 (76), 41 (55), 69 (48)
15	2-phenylethanol	C <sub>8</sub> H <sub>10</sub> O MW: 122	59.7	13.2	—	91 (100), 92 (59), 122 (34), 65 (16)
16	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	C <sub>13</sub> H <sub>20</sub> O MW: 192	61.5	—	—	177 (100), 43 (31), 178 (14), 135 (10), 192 (9), 91 (8), 116 (7), 77 (7), 105 (6)
17	4-(2,2,6-trimethyl-cyclohexan-1-yl)-3-buten-2-one	C <sub>13</sub> H <sub>22</sub> O MW: 194	62.4	—	—	121 (100), 161 (60), 43 (56), 179 (40), 93 (36), 136 (32), 105 (32), 194 (21)
18	4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one	C <sub>9</sub> H <sub>14</sub> O <sub>2</sub> MW: 154	—	—	10.4	98 (100), 70 (56), 39 (55), 111 (14), 53 (13), 154 (5)
19	2,6,6-trimethyl-3-oxo-1-cyclohexen-1-carboxaldehyde	C <sub>10</sub> H <sub>14</sub> O <sub>2</sub> MW: 166	—	—	11.9	91 (100), 39 (95), 79 (70), 121 (48), 107 (47), 65 (36), 53 (29), 166 (16)
20	2,6,6-trimethyl-1,3-cyclohexadien-1-carboxylic acid	C <sub>10</sub> H <sub>14</sub> O <sub>2</sub> MW: 166	—	—	12.5	39 (100), 93 (67), 123 (64), 77 (55), 55 (38), 137 (33), 107 (28), 67 (28), 166 (8)
21	4-hydroxy-2,6,6-trimethyl-3-oxo-1,4-cyclohexadien-1-carboxaldehyde	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub> MW: 180	—	—	13.9	39 (100), 107 (53), 52 (49), 79 (44), 137 (36), 91 (31), 180 (22)
22	2,4,4-trimethyl-3-(3-oxo-1-butenyl)-2-cyclohexen-1-ol	C <sub>13</sub> H <sub>20</sub> O <sub>2</sub> MW: 208	—	—	14.2	41 (100), 59 (68), 81 (65), 91 (44), 123 (35), 138 (30), 161 (22), 208 (11)
23	4-hydroxy-2,6,6-trimethyl-3-oxo-cyclohexan-1-carboxaldehyde	C <sub>10</sub> H <sub>16</sub> O <sub>3</sub> MW: 184	—	—	17.9	43 (100), 55 (53), 67 (43), 95 (32), 125 (31), 109 (28), 155 (8), 184 (4)

<sup>a</sup> MW = molecular weight.

The results presented in Table 2 show some qualitative differences among the three isolation methods. The samples from MSDE, VHS, and SD isolation methods revealed 13, 8, and 16 compounds, respectively.

The drastic conditions of the SD method produced significantly more higher boiling point components than in the original aroma of saffron. Many of these constituents are probably artifacts, such as 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxylic acid. This compound

might have been caused by oxidation occurring, of safranal, during the isolation. Moreover, this means that safranal as all aldehydes is easily oxidized, particularly when exposed to the higher temperatures encountered during steam distillation. It should be noted that the SD method is extremely unsuitable for identifying the volatile constituents of saffron because of the present of caretenoids. Several volatile compounds present in the concentrate were presumably

degradation components of saffron carotenoids resulting from the action of heat and oxygen on these ingredients (Crouzet and Kanasawud, 1992; Kanasawud and Crouzet, 1990).

The MSDE method, produced both low and high boiling point components. The conditions of this technique were less drastic than in case of SD. Nevertheless fragments of thermal degradation of carotenoids such as 3,7-dimethyl-1,6-octadiene were present.

The eight components that were found and identified in the VHS concentrate were also found in the essential oil that was produced by the two others methods. Many of these components were derivatives of safranal and isophorone. On the other hand, only the 3,5,5-trimethyl-3-cyclohexen-1-one, isomer of isophorone, did not separate and was not identified. Presumption, the VHS concentrate was more similar to MSDE than to SD.

In summary, it can be said that the true nature of saffron aroma probably involves contributions from many of the isolated, separated, and identified components in this study. The characteristic compounds are safranal, isophorone, 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). The VHS and MSDE techniques produced concentrates whose fragrances are more similar to the aroma of saffron than in case of the SD method.

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