

Geographical differentiation of saffron by GC–MS/FID and chemometrics

E. Anastasaki · C. Kanakis · C. Pappas ·
L. Maggi · C. P. del Campo · M. Carmona ·
G. L. Alonso · M. G. Polissiou

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Abstract The volatile compounds of saffron of different origins were investigated to check their suitability as markers of geographic differentiation. A total of 247 saffron samples from Greece (40 samples), Iran (84 samples), Italy (60 samples) and Spain (63 samples) which were harvested in 2006 were analysed using ultrasound-assisted extraction, gas chromatography followed by mass spectrometry and flame ionisation. All regions were easily differentiated by canonical discriminant analysis. The percentages of correct classification and validation were 96.4 and 94.3%, respectively. These investigations showed the potential of saffron volatiles to discriminate saffron samples with different geographical origins.

Keywords Saffron · Geographic differentiation · Volatile compounds · Gas chromatography · Canonical discriminant analysis

Introduction

Saffron, the dried red stigmata of *Crocus sativus* L. flower, is the most expensive of spices. Its price in retail market is five euros per gram. Saffron adds its faint, delicate aroma, pleasing flavour and magnificent yellow colour to foods.

The main saffron producing countries are Greece, Iran, Italy and Spain. Saffron's commercial quality is determined by specifications recommended by the ISO/TS-3632 standard [1]. The substances responsible for its characteristic quality are crocetin esters, picrocrocin and safranal, which is the main compound of the aroma of saffron [2–4].

The volatile compounds of saffron can be classified into two categories. The first one involves constituents having structures that bear a distinct similarity to that of safranal and are reported also as isophorone related compounds (C₉ and C₁₀ group of compounds) [5, 6]. Such compounds are isophorone, 4-ketoisophorone, 2,2,6-trimethyl-1,4-cyclohexanedione, 2-hydroxy-3,5,5-trimethylcyclohex-2-en-1, 4-dione, 4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one, 4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-diene-1-carboxaldehyde, 4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde. The second category involves C₁₃-norisoprenoids. These compounds are generated from lipophilic carotenoids [6, 7]. Such compounds are *E*-4-(2,6,6-trimethyl-cyclohexyl)-but-3-en-2-one, 3-[(*E*)-but-1-enyl]-2,4,4-trimethyl-cyclohex-2-enol, 3-(but-1-enyl)-2,4,4-trimethylcyclohexan-1-ol, (*E*)-4-(2,6,6-trimethyl-7-oxa-bicyclo-[4.1.0]-heptan-1-yl)-but-3-en-2-one. Additionally, the composition of the volatiles that are obtained is greatly dependent upon the methods that are employed for the isolation [7–9].

Today, there is an increasing interest by producers and consumers for high-quality food products with a clear geographical origin. For this reason a great number of methods have been employed for the determination of the geographical origin of food products. These methods can be divided into: analytical techniques (e.g. mass spectrometry, infrared spectroscopy), separation techniques (e.g. gas chromatography coupled with mass spectrometry) and other techniques (e.g. sensor technology-electronic nose) [10, 11].

E. Anastasaki · C. Kanakis · C. Pappas · M. G. Polissiou (✉)
Laboratory of Chemistry, Department of Science,
Agricultural University of Athens, 75 Iera Odos,
118 55 Athens, Greece
e-mail: mopol@aua.gr

L. Maggi · C. P. del Campo · M. Carmona · G. L. Alonso
Cátedra de Química Agrícola, ETSI Agrónomos,
Universidad Castilla-La Mancha, Campus Universitario,
02071 Albacete, Spain

Chemometric analysis of the data provided by the analytical instruments is needed for such a multifactorial approach [10, 12]. In the case of the determination of the geographical origin of food products using their volatile compounds, gas chromatography is the most appropriate technique [13–20]. The determination of food authenticity and the detection of adulteration are major issues in the food industry and are attracting an increasing amount of attention for saffron producers, researchers and consumers.

Saffron quality, as already been reported, is related to an obvious commercial value [1], hence adulteration is possible which may result in an unfair competition among the saffron producers and harm the rights of consumers. Thus, there is a significant interest in accurate methods for saffron characterization that could be used to prevent adulteration and to classify saffron from different geographical origins or countries. The differentiation of saffron from different countries has been successfully achieved by measuring chemical compounds present in the saffron matrix (e.g. aroma, taste and colour compounds) using various analytical techniques such as high performance liquid chromatography (HPLC) [21], near-infrared (NIR) spectroscopy [22], and by ^{13}C isotopic analysis [23].

The objective of the present investigation was to check the possibility of using volatile compounds to discriminate saffron from different countries of the world, especially from Iran, Greece, Spain and Italy, using ultrasound-assisted extraction (USAE) for isolation, GC–MS/FID for analysis and discriminant analysis.

Materials and methods

Plant material

A total of 247 saffron samples (harvested in 2006), from Greece (40 samples), Iran (84 samples), Italy (60 samples) and Spain (63 samples) were obtained directly from the producers in order to avoid the case of adulteration and to guarantee their origin.

Standards

Safranal of 98% purity was obtained from Sigma-Aldrich (Athens, Greece) and used as a standard.

Isolation of the volatile compounds

The isolation of the volatile compounds was performed in an ultrasound water bath Sonorex (Berlin, Germany) Super RK 255H type (300 × 150 × 150 mm internal dimensions), at the fixed frequency of 35 kHz. The temperature of the sonicated water bath was 25 °C. The sample flask

was charged with 5 g of saffron. The solvent system extractant was 50 mL of diethyl ether. Each saffron sample was sonicated two times for 15 min (two fractions per saffron sample). For each sonication a new volume of the solvent extractant was added in the sample flask. After the end of each sonication the whole organic extract (100 mL) was collected. The whole organic extract was concentrated to 1/3 using a rotary evaporator type Heidolph Laborota 4000 Efficient (Schwabach, Germany). The temperature of the water bath was 20 °C. After that the organic extract was placed into a volumetric cylinder and it was further concentrated by a gentle flow of nitrogen up to a 5 mL volume and a minor quantity of anhydrous magnesium sulphate was added [24].

Chromatographic analysis of the volatile compounds

Gas chromatography–mass spectrometry (GC–MS)

In order to tentatively identify the volatile compounds of saffron's organic extract, GC–MS was used. A Hewlett Packard 5890 Series II chromatograph (Palo Alto, CA, USA) equipped with a 5972 Series mass selective detector (MSD) in the electron impact mode (70 eV) and a HP-5 ms capillary column (30 m, 0.25 mm i.d., 0.25- μm film thickness) with helium as carrier gas at 1 mL min $^{-1}$ was used for the analysis of saffron's organic extract. Column temperature was initially kept for 3 min at 50 °C, then gradually increased to 180 °C with a rate of 3 °C min $^{-1}$, and finally increased to 250 °C at a rate of 15 °C min $^{-1}$ and kept for 5 min. The injector and detector temperatures were set at 220 and 290 °C, respectively. One microliter of each saffron organic extract was injected manually in the splitless mode.

Gas chromatography (GC)

The analysis was performed under the same conditions as GC–MS, using a Hewlett Packard 5890 Series II chromatograph equipped with a flame ionisation detector (FID). 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 equation

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

where $\text{area}_{\text{safranal}}$ = safranal's peak area/10 6 in the GC chromatogram [8].

Statistical analysis

Evaluation of the statistical significance of differences between the mean content of each volatile compound

between the countries was performed by analysis of variance (ANOVA) using the SPSS 14.0 for Windows statistical program (SPSS Inc., Chicago, IL, USA). Discriminant analysis was carried out with SPSS 14.0 for Windows to differentiate the saffron samples into the four producing countries.

Results and discussion

Gas chromatographic analysis

The chemical identification of saffron's volatiles was determined by GC–MS. Volatile constituents were tentatively identified by comparing their elution order and mass spectra with data from the NBS75K mass spectra library and published data [5–8, 25, 26]. The quantification of saffron's volatiles was determined by GC-FID. Table 1 demonstrates the volatile constituents present in the 247 saffron samples, their quantity in g kg^{-1} of total volatile content [27] and their minimum and maximum values.

As it can be observed from Table 1, the main volatile compounds in the 247 saffron samples are safranal (C09), followed by 4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1,4-diene-1-carboxaldehyde (C19), 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC) (C21), isophorone (C04), 2,2,6-trimethyl-1,4-cyclohexanedione (C07), 4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (isomer II) (C14), 4-ketoisophorone (C05), 4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde (isomer II) (C18), 4-methylene-3,5,5-trimethylcyclohex-2-enone (C10).

Table 2 demonstrates the average values and the total variance of each volatile compound in each country, expressed as g kg^{-1} of total volatile content [26]. In the Greek saffron samples the main volatiles are C09, C04, C21, C07, C19, C05, C14, phenyl ethyl alcohol (C03) and C10. In the Iranian saffron samples the main volatiles are C09, C04, C19, C07, C21, C05, C14, C18 and C10. In the Italian saffron samples the main volatiles are C09, C19, C21, C04, C07, C05, C14, C18, 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one (C06), while in the Spanish saffron samples the main volatiles are C09, C19, C21, C04, C07, C14, C05, C06 and (*E*)-4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]heptan-1-yl)but-3-en-2-one (C24). Comparing the main volatiles between the four countries, it can be observed that only the first five are the same but they have different hierarchical order. Especially in the Italian and Spanish saffron samples the first five volatiles have the same hierarchical order, while in the Iranian samples the hierarchical order is different. The same is observed for the Greek samples where the hierarchical order is different from that in the Iranian, Italian and Spanish samples. The average

content of safranal (C09) ranging from 335.9 g kg^{-1} (Spanish saffron) to 488.6 g kg^{-1} (Greek saffron) showed significant differences between the four countries. In particular, safranal showed significant differences between Greece, Iran, Italy and Spain but it was not significant different between Iran and Spain. Significant differences were also observed for C19 while for C21 there were not any significant differences between Greece and Iran and between Italy and Spain. For compound C04 (isophorone) between Greece and Spain there were not any significant differences instead between Iran and Italy the amount of isophorone showed significant differences. For C07 significant differences were not found between Greece, Italy and Spain but between Iran, Italy and Spain the amount of C07 was significantly different. For the rest of the detected volatile compounds, it can be seen from Table 2 that they present significant differences between the countries of saffron origin. For this reason it was decided to use all the detected volatiles in the discriminant analysis.

Discriminant analysis

In order to differentiate the saffron samples between the four countries discriminant analysis was applied. As it is mentioned in the preceding paragraph, all the detected volatile compounds were used in the discriminant analysis. The selection of the most significant variables was performed by stepwise analysis [28]. The variables were included in the model one by one, choosing at each step the variable that made the most significant additional contribution to the discrimination (i.e. with the largest *F* value). Measured values were excluded from the model if they were shown to be redundant. On the basis of the selected variables, different independent discriminant functions were computed by canonical discriminant analysis. Their maximum number is equal to either the number of variables or the number of groups (in our case geographical origin) minus one. In this case since the number of groups is four (four saffron producing countries), the number of discriminant functions is three. The canonical discriminant characteristics of the testing data from the saffron samples are shown in Table 3.

Eigen values show that the first discriminant function has the highest canonical correlation (0.916) and explain 48.1% of the total variance. According these values, the first two functions are the most discriminating ones and provide a great contribution to the discrimination (81.4%) [29]. The statistical significance of each discriminant function was also evaluated on the basis of the Wilks' Lambda factor. This parameter ranges from 1.0 (no discriminatory power) to 0.0 (perfect discriminatory power) [28]. A total Wilks' Lambda values of the first two discriminant functions 0.011–0.072 show a good discriminant

Table 1 Chemical identification of the volatile compounds of the 247 saffron samples, expressed as g kg⁻¹ of total volatile content

Compound	KI ^a	Volatile compound	AVRG ^b	MIN ^c	MAX ^d
C01	1002	2,2-dimethyl-cyclohexane-1-carboxaldehyde	1.3	0.0	15.1
C02	1039	2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (Isomer of safranal)	5.1	0.0	9.1
C03	1052	Phenyl ethyl alcohol	4.8	0.0	28.7
C04	1055	Isophorone	93.4	2.8	223.2
C05	1077	2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-ketoisophorone)	48.8	5.4	107.7
C06	1093	2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one	8.1	0.0	62.6
C07	1100	2,2,6-trimethyl-1,4-cyclohexanedione	68.7	4.7	110.1
C08	1112	Dihydro-4-hydroxy-2(3H)-furanone	0.5	0.0	16.3
C09	1129	2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (safranal)	380.9	113.6	638.0
C10	1144	4-methylene-3,5,5-trimethylcyclohex-2-enone	9.0	0.0	88.9
C11	1159	2-hydroxy-3,5,5-trimethylcyclohex-2-en-1,4-dione	6.6	0.0	46.2
C12	1172	4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (isomer I)	1.9	0.0	30.4
C13	1221	2,6,6-trimethyl-3-oxo-1-cyclohexen-1-carboxaldehyde	1.7	0.0	35.3
C14	1230	4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (isomer II)	50.1	0.0	117.8
C15	1235	2,3-dihydroxynaphthalene-1,4-dione	1.2	0.0	27.1
C16	1241	2-hydroxy-3,5,5-trimethyl-4-methylen-cyclohex-2-en-1-one	0.1	0.0	2.3
C17	1253	4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde (isomer I)	2.4	0.0	200.7
C18	1255	4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde (isomer II)	10.3	0.0	59.8
C19	1296	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-diene-1-carboxaldehyde	146.1	0.0	340.0
C20	1314	<i>E</i> -4-(2,6,6-trimethyl-cyclohexyl)-but-3-en-2-one	0.1	0.0	1.5
C21	1322	4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde (HTCC)	101.8	31.4	300.1
C22	1331	3-[(<i>E</i>)-but-1-enyl]-2,4,4-trimethyl-cyclohex-2-enol	1.3	0.0	12.8
C23	1337	3-(but-1-enyl)-2,4,4-trimethylcyclohexan-1-ol	0.3	0.0	4.8
C24	1374	(<i>E</i>)-4-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]heptan-1-yl)but-3-en-2-one	7.1	0.0	27.5

^a Kovats indices on nonpolar HP-5 ms column in reference to *n*-alkanes

^b Average value (g kg⁻¹) of 247 saffron samples

^c Minimum value (g kg⁻¹) of 247 saffron samples

^d Maximum value (g kg⁻¹) of 247 saffron samples

power of the model. The significance values less than 0.001 and Chi-square test indicate that there is a highly significant difference between the groups' centroids. Table 4 shows the impact of each variable on the discriminant function after "standardising"; putting each variable on the same platform. According to standardising coefficients for the first function compounds **C19**, **C09**, **C12** and **C01** have the greatest impact and for the second discriminant function compounds **C12**, **C13**, **C01** and **C09**.

The structure matrix coefficients (Table 4) of canonical discrimination show the correlations of each variable in the model with the two main discriminant functions. The first discriminant function was mainly correlated to compounds **C03**, **C19** and **C21** while the second discriminant function was mainly correlated to compounds **C01**, **C09** and **C18**.

The separation among groups in the discriminant space was checked by plotting the first and the second functions (Fig. 1).

The canonical discriminant functions appeared to have a good classification with 96.4% of original grouped cases correctly classified (Table 5). Additionally, to verify the power and the stability of the model a "leave-one-out" cross validation discriminant analysis was performed. From the cross-validation results, it can be seen that 94.3% of cross-validated group cases were correctly classified (Table 5).

Conclusions

The volatile compounds of saffron samples from different regions were investigated by gas chromatography. Each region could be separated from the others using the volatile compounds. The concentration of compounds **C01**, **C09**, **C12**, **C13**, and **C19** made it possible to especially separate Greek, Iranian, Italian and Spanish saffron samples from

Table 2 The total variance of each volatile compound in each country, expressed as g kg⁻¹ of total volatile content

Compound	Volatile compound	Greece			Iran			Italy			Spain		
		AVG ± SD	Range	AVG ± SD	Range	AVG ± SD	Range	AVG ± SD	Range	AVG ± SD	Range		
C01	2,2-dimethyl-cyclohexane-1-carboxaldehyde	2.9 a ± 0.8	1.8–4.4	–	–	2.6a ± 4.8	0.0–14.16	0.8b ± 2.06	0.0–15.07				
C02	2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (Isomer of safranal)	6.4a ± 0.8	3.8–8.0	5.1b ± 1.5	0.0–7.6	5.5b ± 1.8	2.5–9.1	4.1c ± 1.5	0.0–7.4				
C03	Phenyl ethyl alcohol	13.6a ± 6.2	6.6–28.7	4.6b ± 2.4	0.0–12.6	–	–	3.5b ± 2.8	0.0–10.8				
C04	Isophorone	97.0a ± 23.9	61.8–135.1	120.0b ± 24.1	40.25–205.8	58.9c ± 17.5	2.8–84.05	88.7a ± 33.6	24.1–223.2				
C05	2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-ketosphorone)	41.2a ± 11.2	21.2–63.2	71.2b ± 19.2	20.1–107.6	45.9a ± 26.7	6.1–87.3	25.7c ± 17.4	5.4–81.3				
C06	2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one	6.0a ± 2.6	0.0–10.7	7.6a ± 7.6	0.0–62.6	6.2a ± 4.8	0.0–18.6	11.8b ± 7.5	3.8–34.4				
C07	2,2,6-trimethyl-1,4-cyclohexanedione	60.2a ± 6.8	47.8–77.4	82.5b ± 14.8	9.7–110.1	56.0a,c ± 11.4	32.6–73.4	67.8a,d ± 20.7	4.7–109.9				
C08	Dihydro-4-hydroxy-2(3H)-furanone	3.1a ± 4.2	0.0–16.3	–	–	–	–	–	–				
C09	2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (safranal)	488.6a ± 37.4	423.3–580.7	346.9b ± 84.2	113.6–469.5	403.9c ± 93.6	212.91–609.1	335.9b ± 108.6	138.0–638.0				
C10	4-methylene-3,5,5-trimethylcyclohex-2-enone	13.4a ± 2.5	8.2–18.1	10.7a ± 2.7	3.4–19.4	5.9b ± 11.1	0.0–88.9	6.8b ± 3.6	1.9–17.0				
C11	2-hydroxy-3,5,5-trimethylcyclohex-2-en-1,4-dione	9.0a ± 6.5	2.4–21.7	6.9a,b ± 5.5	0.0–46.2	6.2a,b ± 8.1	0.0–27.2	4.9b ± 5.3	0.0–26.4				
C12	4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one(isomer I)	4.4a ± 2.3	1.9–9.4	1.9b ± 3.3	0.0–30.4	1.9b ± 3.9	0.0–12.3	0.4c ± 1.1	0.0–4.8				
C13	2,6,6-trimethyl-3-oxo-1-cyclohexen-1-carboxaldehyde	1.5a,b,c,d ± 1.4	0.0–4.3	1.4a,b,d ± 4.0	0.0–35.3	3.3a,c ± 5.2	0.0–18.6	0.7a,b,d ± 1.1	0.0–3.6				
C14	4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (isomer II)	36.4a ± 11.5	13.3–54.8	61.0b ± 15.3	1.0–117.8	31.1a ± 9.5	11.9–49.8	62.5b ± 21.5	0.0–110.5				
C15	2,3-dihydroxynaphthalene-1,4-dione	1.5a ± 4.4	0.0–27.1	0.8a ± 2.4	0.0–11.8	1.2a ± 2.5	0.0–9.2	1.5a ± 2.3	0.0–12.0				
C16	2-hydroxy-3,5,5-trimethyl-4-methylen-cyclohex-2-en-1-one	–	–	–	–	0.2a ± 0.6	0.0–2.3	–	–				
C17	4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde (isomer I)	2.1a ± 0.3	1.5–2.6	3.0a ± 1.2	0.0–6.0	0.3a ± 0.7	0.0–2.5	4.0a ± 25.2	0.0–200.7				
C18	4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde (isomer II)	5.7a ± 3.3	0.0–11.0	14.6b ± 8.6	0.0–38.8	10.1c ± 8.6	0.0–25.4	8.0a,c ± 9.7	0.0–59.8				
C19	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-diene-1-carboxaldehyde	54.8a ± 12.9	33.7–97.0	118.0b ± 40.0	63.6–241.0	207.5c ± 43.5	121.3–278.6	183.1d ± 60.1	0.0–340.0				
C20	E-4-(2,6,6-trimethyl-cyclohexyl)-but-3-en-2-one	–	–	–	–	0.2a ± 0.4	0.0–1.5	–	–				
C21	4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde (HTCC)	87.6a ± 18.0	56.3–126.5	80.4a ± 46.4	31.4–245.7	124.4b ± 48.3	63.7–203.6	118.0b ± 51.7	44.0–300.1				
C22	3-[(E)-but-1-enyl]-2,4,4-trimethyl-cyclohex-2-enol	0.6a ± 0.5	0.0–1.6	0.5a ± 0.7	0.0–2.8	2.8b ± 1.6	0.0–5.7	1.5c ± 2.0	0.0–12.8				
C23	3-(but-1-enyl)-2,4,4-trimethylcyclohexan-1-ol	–	–	0.1a ± 0.5	0.0–3.7	1.0b ± 1.2	0.0–4.8	–	–				
C24	(E)-4-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]heptan-1-yl)but-3-en-2-one	9.4a ± 3.0	4.6–15.4	8.4a ± 4.9	0.0–27.5	1.6b ± 2.4	0.0–8.3	9.1a ± 6.5	0.0–26.1				

Different letters between rows indicate significant differences at the 0.05% level
AVG Average, SD standard deviation

Table 3 Canonical discriminant characteristics

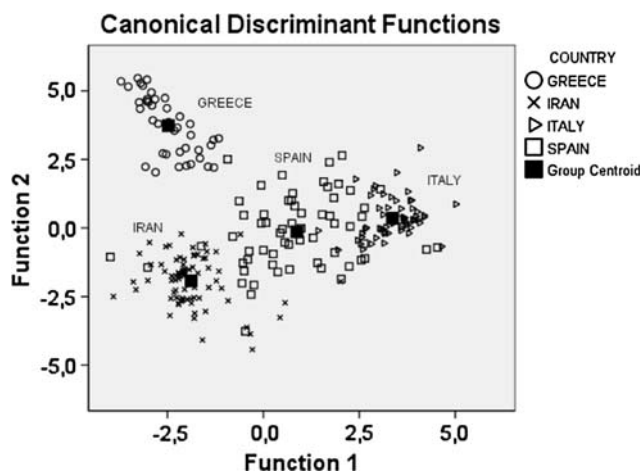
Function	Eigen value	Variance (%)	Cumulative (%)	Canonical correlation	Wilks' Lambda	Chi-square	Deg. Fre.	Significance
1	5.233	48.1	48.1	0.916	0.011	1060.780	39	0.000
2	3.623	33.3	81.4	0.885	0.072	626.189	24	0.000
3	2.021	18.6	100.0	0.818	0.331	262.581	11	0.000

Table 4 Canonical discriminant standardised coefficients and structure matrix of volatile compounds detected in saffron's organic extracts

Variables	Standardised coefficients		Structure matrix ^a	
	Function 1	Function 2	Function 1	Function 2
C01	0.612	1.181	0.087	0.211 ^b
C03	-0.108	0.687	-0.477 ^b	0.441
C05	0.200	-0.589	-0.188	-0.270
C06	0.318	-0.033	0.014	-0.057
C09	1.233	1.005	-0.028	0.289 ^b
C11	0.103	-0.560	-0.057	0.059
C12	-0.666	1.333	-0.088	0.153
C13	0.170	-1.145	0.075	0.020
C17	0.358	0.039	-0.024	-0.018
C18	-0.198	0.214	-0.037	-0.191 ^b
C19	1.551	0.563	0.497 ^b	-0.208
C21	0.285	0.102	0.179 ^b	0.043
C23	0.333	0.110	0.232	0.018

^a Pooled within-groups correlations between discriminant variables and standardised canonical discriminant functions

^b Largest absolute correlation between each variable and any discriminant function

**Fig. 1** A plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis

each other. The percentages of correct classification and validation were 96.4 and 94.3%, respectively. These investigations showed the potential of saffron volatiles to

Table 5 Classification and cross-validation results

Geographical origin	Country	Predicted group membership					Total
			Greece	Iran	Italy	Spain	
Original	Count	Greece	40	0	0	0	40
		Iran	0	82	1	1	84
		Italy	0	0	60	0	60
		Spain	1	3	3	56	63
	%	Greece	100.0	0.0	0.0	0.0	100.0
		Iran	0.0	97.6	1.2	1.2	100.0
		Italy	0.0	0.0	100.0	0.0	100.0
		Spain	1.6	4.8	4.8	88.9	100.0
Cross-validated	Count	Greece	40	0	0	0	40
		Iran	0	81	1	2	84
		Italy	0	0	59	1	60
		Spain	2	6	2	53	63
	%	Greece	100.0	0.0	0.0	0.0	100.0
		Iran	0.0	96.4	1.2	2.4	100.0
		Italy	0.0	0.0	98.3	1.7	100.0
		Spain	3.2	9.5	3.2	84.1	100.0

discriminate saffron samples with different geographical origin.

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