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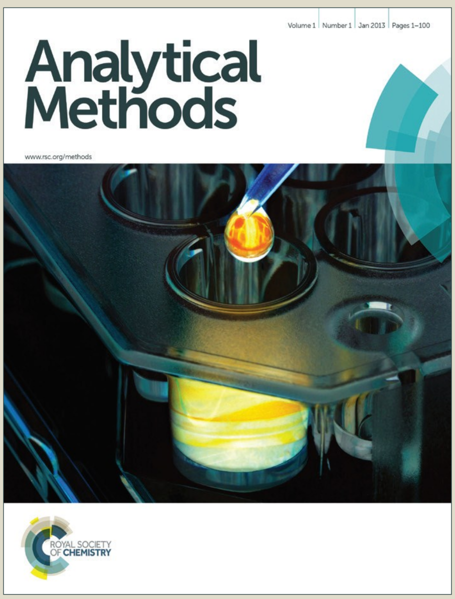

# Characterization and Discrimination of Saffron by Multisensory Systems, SPME-GC-MS and UV-Vis Spectrophotometry

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**Abstract**

Different electronic sensor systems coupled with multivariate data analysis were applied to characterize and classify seven saffron samples and to verify their declared geographical origin. The proposed electronic sensing consists of a low cost electronic nose (E-nose) based on metal oxide semiconductor sensor and a voltammetric electronic tongue (VE-tongue) based on voltammetric sensors. The ability of multivariable analysis methods such as Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Support Vector Machines (SVMs) to classify the saffron samples according to their geographical origin have been investigated. Both PCA and HCA have shown an overlapping of the E-nose responses. Moreover, the SVMs analysis of the E-nose database reached 66.07 % success rate in the recognition of the saffron samples odour. On the other hand, good discrimination has reached using PCA and HCA in the VE-tongue characterization case, beside a 100 % of the accuracy in the saffron flavour recognition was attained. To validate the proposed electronic sensing systems, analytical chemical methods such as SPME-GC-MS and UV-Vis Spectrophotometry were used. This analytical method could be a helpful tool to identify the composition of volatile compounds of the analysed saffron samples. Moreover, the UV-Vis Spectrophotometry was also used to determine the non-volatile profile of the samples from different geographic origins. It is demonstrated that the electronic sensing systems findings are in a satisfactory correlation with the analytical methods. In the light of these results, it might say that the electronic systems offer a fast, simple and efficient tool to recognize the declared geographical origin of the saffron samples.

**Keywords:** Electronic nose; Voltammetric electronic tongue; SPME-GC-MS; UV-Vis Spectrophotometry; Saffron; Geographic differentiation; Multivariable analysis.

## 1. Introduction

Saffron is commonly used as spice and food colorant, and less extensively, as a textile dye, perfume and as cosmetics. It is extracted from the dehydrated stigmas of the saffron flower (*Crocus sativus* L.). For a long time, folk herbal medicines have used saffron for the treatment of numerous illnesses owning to its analgesic and sedative properties. In recent years, Abdullaev[1,2] has demonstrated the possible use of saffron as effective anticancer and chemopreventive agent in clinical trials. Besides, the quality of saffron is an important factor for culinary, medicinal, and commercial purposes. In the international trade, Iran, Greece, Morocco, India, and Spain are among the most producers and exporters countries of saffron in the world. Globally, although 90 % of world's production of saffron comes from Iran, Morocco still remains in the top ten of the world's biggest producers, ranked fourth after India, Greece and just before Spain[3]. In the Southwest of Morocco, the region of Taliouine is considered as the Moroccan main zone for saffron cultivation, altitude 1200 m – 1630 m, latitude 30°31' N and a longitude of 7°55' W[4], where it benefits from optimal conditions of soil altitude and arid-dry climate with harsh winters, calcareous soils, rich in sand and in silt but with low clay concentration[5]. Besides, the practices are traditional with specialized labour (irrigated crop, harvesting of flower and pruning scars by hand).

Saffron's commercial quality is determined by the ISO/TS 3632-2 standard recommandation[6]. Indeed, it is closely associated with aroma[7,8], taste[9] and color[10]. The organoleptic properties of saffron spice are given by the presence of carotenoid derivatives. These compounds are responsible for such attributes: crocin, a group of glycoside derivates from the carotenoid crocetin; terpenic aldehydes known as safranal and the glycoside terpenoid picrocrocin, respectively (picrocrocin for flavour and safranal for aroma)[7]. Like almost all foodstuff products, the price of saffron is directly depending on its quality, which is also related to the geographical origin of the production area. The Protected Designation of Origin (PDO) for agricultural products has been introduced with official European regulations, which allow the labelling of some products with the names of the geographical area of production. Moreover, the problem of geographic identification of food becomes more attractive when it concerns to restricted production areas. To deal with this subject, many methods have been employed for the determination of the geographical origin of saffron. Among these methods, there are Liquid Chromatography- Diode Array Detector Coupled with Mass Spectrometry/ Mass Spectrometry Electron Spray Ionization (LC-DAD/MS/MSESI)[9], Thermal Desorption- Gas Chromatography- Mass Spectrometry (TD-GC-MS)[7], Gas Chromatography equipped with a Flame Ionization Detector (GC-FID)[11], Near-Infrared Spectroscopy[12], $^{13}$C Isotopic Analysis[13] and High-Performance Liquid Chromatography (HPLC)[14].

These standard analytical methods can give very detailed information about the chemical compounds present in saffron. However, they still require time-consuming measurements, sample preparation and a qualified staff for ascertaining the origin of the component. The development of precise and rapid methods for the pattern screening of the saffron samples, according to their geographical origin, could be of help for the assignment of a “designation of origin” trade mark.

Analysis of volatile compounds (headspace) and electrochemical species have been proposed as rapid patterns screening of several products[15,16]. E-nose and E-tongue have widely been suggested for the monitoring of food quality. Both devices consist of arrays of non-selective gas or liquid sensors with a broad and partially overlapping selectivity towards compounds, which are present in a sample[17]. The sensor arrays are coupled to an appropriate pattern recognition model that is capable of retrieving information from complex signals[18]. These electronic sensing systems could represent a convenient alternative for screening due to their rapidity, simplicity and low cost to classify products with a different chemical "fingerprint"[19]. Electronic nose technology has been shown to be able to discriminate saffron samples from different origins[7]. However, to our knowledge, no research on the electronic tongue technology has been published on saffron origin identification. The aim of this study was to test the ability of multisensory system to characterize and differentiate several kinds of saffron picked up from different countries using a multisensory systems combined with SPME-GC-MS and UV-Vis Spectrophotometry analysis and three supervised and unsupervised pattern recognition methods (PCA, SVMs, HCA) for accurate classification.

## 2. Materials and methods

### 2.1. Measurements on saffron samples

In this study, 7 saffron samples from 3 different countries (Morocco, Iran and Syria) were analysed. The samples are distributed as follows: 5 saffron samples of 5 different areas from Taliouine, Morocco (Saffron Taliouine (ST_xx with xx= 16, 70, 117, 148 and 150)) which were obtained directly from a cooperative to avoid possible undeclared mixtures, one saffron samples from Iran (SI) and one saffron samples from Syria (SS), these two samples are bought from the market. Saffron samples have been harvested in the period between October and November 2013. All saffron samples were stored in a dry and dark place in order to minimize any deteriorative changes to the aroma and taste until their processing[20]. All analyses were conducted within three months after sample collection.

### 2.2. E-nose set-up measurement and methodology

An electronic nose system based on a 5-sensors array was used. The experimental system is mainly composed of three parts: sensor array, sampling vessel with system of measurement, and a data acquisition system[21]. In Fig. 1, the sensor array comprised of five different tin-dioxide gas sensors: TGS 8xx (with xx=15, 22, 24, 25 and 42) obtained from Figaro

Engineering, Inc. (Osaka, Japan). In the literature, many studies have stated that the temperature of the sample, sensor chamber, and sensors must be kept constant to achieve repeatable performance of the electronic nose system. This is because a modification of the environmental temperature value can induce a variation of the sensor operating temperature, modifying the sensor sensitivity and then the steady-state conductance value[22]. As a direct cause, a temperature sensor (LM335Z) and a relative humidity sensor (HIH4000-01) from National Semiconductor (Santa Clara, CA, USA) were used for constantly monitoring the inner sensor chamber temperature and relative humidity.

The sampling vessel was a necessary arrangement to transport the odorant molecules of a sample from the vial collection device, via pure nitrogen (as carrier gas), to facilitate contact with the sensors. The data acquisition system measured the variation of sensors conductivity using a PIC16F877 microcontroller, (via a serial RS232 communication port). A PC programmed with an in-house-written program using LabVIEW© software (National Instruments Inc., Austin, Texas, USA) controlled the electronics and acquired data from the sensors. The signal output was measured at 2 s intervals for 10 min.

For the E-nose measurements, the saffron samples were measured without any pre-treatment. Six replicates of each sample of 0.4 g of saffron were placed in a 100 mL airtight glass vial with two small holes in their cover to access the headspace to the E-nose equipment.

### 2.3. Voltammetric electronic tongue set-up measurement and methodology

The voltammetric electronic tongue device consists in an array of seven working electrodes, a platinum counter electrode, and an Ag/AgCl reference electrode, which were housed inside a homemade glass backer, used as e-body of the VE-tongue system[23]. The working electrodes were made up of gold, palladium, platinum, silver, glassy carbon, copper and nickel. An overall view of the VE-tongue system is shown in Fig 2. The electrodes were connected via a relay box to a portable potentiostat PalmSens (PalmSens BV, The Netherlands). The advantage of using VE-tongues based on metallic electrodes is that it is quite simple to remove any accumulated unwanted material on the electrode by simply rinsing with distilled water after each reading. Indeed, an electrochemical cleaning step was performed to prevent the accumulative effect of impurities on electrode surface. The electrodes were rinsed with distilled water after each reading[16]. Several tests were carried out on saffron samples for each working electrode, in order to optimize the electrochemical window range. Thus, cyclic voltammetry (CV) was recorded in a range of varying potentials from -300 mV to 1000 mV with a scan rate of 20 $mV \cdot s^{-1}$. Under the terms of these conditions, the various saffron spices showed anodic and cathodic peaks.

For the VE-tongue measurements, 50 mg of saffron stigmas of each sample were dissolved in 0.1 molar potassium chloride aqueous solutions in a glass beaker. The solution was stirred with a magnetic stirrer for one hour in the dark to minimize the effect of light on taste[20].

### 2.4. Feature extraction

Feature extraction is an essential pre-processing step to pattern recognition. The features used for data analysis are directly extracted from the responses of the sensors array in order to fully exploit the maximum information present in the response. Therefore, for every sensor within the array and measurement performed, four representative features from the E-nose response signals and three representative features form the VE-tongue voltammograms were extracted.

In the case of E-nose system, the dataset comprised the following set of features:

- ♣ **$G_0$**: the initial conductance of a sensor calculated as the average value of its conductance during the first minute of a measurement.

- **$G_s$**: the steady-state conductance calculated as the average value of its conductance during the last minute of a measurement.
- **dG/dt**: the dynamic slope of the conductance calculated between 2 and 7 min of a measurement.
- **A**: the area below the conductance curve in a time interval defined between 2 and 8 min of a measurement. This area is estimated by the trapeze method.

The choice of these features was based on our previous works related to food products[24-26]. Since there were five gas sensors, each measurement was described by 20 variables (5 sensors × 4 features).

In the case of VE-tongue system, the dataset comprised the following set of features:

- **$\Delta I = I_{max} - I_{min}$**: the current change calculated as the difference between maximum and minimum values of the current; where, $I_{max}$ represents the maximum value of the current measured in the final potential range and $I_{min}$ represents the minimal value of the current measured in the initial potential.
- **$S_{ox}$**: the maximum slope of the current curve in the oxidation phase.
- **$S_{rd}$**: the maximum slope of the current curve in the reduction phase.

These parameters were also chosen based on the previous works[16,27]. Since there were seven working electrodes within the array, each voltammetric measurement was described by 21 variables (7 electrodes × 3 features).

### 2.5. Solid Phase Micro-Extraction Gas Chromatography-Mass Spectrometry (SPME-GC–MS)

The analysis of volatile organic compounds (VOCs) from saffron headspace was performed in order to identify the main compounds, which are responsible for their odour, by gas chromatography-mass spectrometry. A SPME fibre of 0.75 mm diameter coated by carboxen/polydimethylsiloxane (CAR/PDMS, Sigma-Aldrich) was used for sampling of VOCs from headspace. 50 mg of each saffron sample was put in a 20 mL vial with PTFE-septum. The aroma equilibration time was at least 30 min at (25 ± 0.5) °C. The SPME fibre was pierced through a septum into the vial for absorption of VOC or rather aroma compounds. The absorption time was 15 min at (25 ± 0.5) °C. The absorbed aroma compounds on the SPME fibre were thermally desorbed for 2 min in the closed GC inlet at 250 °C. The GC-MS analysis was initiated by splitless injection. The equilibration was consistent for all measurements. The analyses were carried out with an GC 6890 (Agilent) equipped with a DB 624 capillary column (30 m × 0.32 mm i.d.: 1.8 µm film thickness), helium as carrier gas[28] and a mass selective detector MSD 5972 (Agilent), the ionisation energy was set to 70 eV and the specific ions are analysed in the m/z-range 30 to 150 with 3.7 scans per second. The detector interface was kept at 280°C. The oven was maintained at 40 °C for 0.5 min and then the temperature was increased to 240 °C (6.5 K/min). Finally, this temperature was maintained for 1 min. Compounds identification was carried out using the NIST library and comparison with those reported in the literature[29-31].

### 2.6. Spectrophotometric analysis

Saffron's quality depends on the concentration of secondary metabolites, such as crocin, safranal and picrocrocin[32]. In this context, the seven saffron samples were analysed using ANACHEM instruments UV220 spectrophotometer in the range from 200 nm to 700 nm by using a quartz cell (1 cm path-length). Absorbance readings at 257 nm, 310 nm and 440 nm were related back to the 1 % solution and expressed as $E_{1cm}^{1\%}$ (257 nm), $E_{1cm}^{1\%}$ (310 nm) and $E_{1cm}^{1\%}$ (440 nm), according to the ISO/TS 3632-2 for the standardized measurement of bitterness and colouring strength, respectively. A blank system was prepared for each treatment and used as analytical blank for the corresponding phase. The reference solution (distilled water) was used in this study as a solvent. For the measurement of the spectrophotometric indexes, moisture and volatile content were evaluated by using the saffron in filaments. After weighting, the samples were introduced uncovered in an oven set at (103 ± 2) °C for 16 h[33].

The results have been obtained by direct reading of the absorbance (D) as reported in the following equation[32]:

$$\mathrm{E}_{1\mathrm{cm}}^{1\%} = \frac{D \times 10000}{m \times (100 - H)}$$

where D is the absorbance at 257 nm, 310 nm and 440 nm; m is the mass of the saffron sample, in grams; H is the moisture and volatile content of the sample, expressed as a mass fraction by using the following relation:

$$H = \frac{\text{initial mass} - \text{final mass}}{\text{initial mass}} \times 100$$

Our proposal is to express the content of picrocrocin, safranal and crocin as $\mathrm{E}_{1\mathrm{cm}}^{1\%}$ 257 nm, $\mathrm{E}_{1\mathrm{cm}}^{1\%}$ 310 nm and $\mathrm{E}_{1\mathrm{cm}}^{1\%}$ 440 nm, respectively, of the fraction in which it is contained in a dry basis of saffron using the average extinction coefficient for picrocrocin, safranal and crocin ($\varepsilon_{257nm}$ = 103000 $dm^3mol^{-1}cm^{-1}$, $\varepsilon_{310nm}$ = 9280 $dm^3$ $mol^{-1}$ $cm^{-1}$ and $\varepsilon_{440nm}$ = 10515 $dm^3$. $mol^{-1}$ $cm^{-1}$, respectively[33-35] in water and a molecular mass of 977 $gmol^{-1}$, 310 $gmol^{-1}$ and 150 $gmol^{-1}$, consecutively:

$$\% \text{ of secondary metabolites in dry basis} = \frac{(\mathrm{E}_{\lambda_{max}}^{1\%}) \times Mw \times 10}{\varepsilon}$$

where *Mw* is the molecular weight of a secondary metabolite and $\varepsilon$ is the molar extinction coefficient.

### 2.7. Data analysis and chemometric procedures

Multivariate analysis methods play an important role in differentiating different samples of saffron produced in different countries by using the sensory systems. The main objective of using pattern recognition methods in this particular application was to estimate the performance of the E-nose and VE-tongue to discriminate the geographical origin of several saffron types produced in different countries and in a specific region of Morocco by employing both linear and non-linear methods such as Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Support Vector Machines (SVMs).

PCA is a powerful linear and unsupervised pattern recognition technique that has been shown to be effective tool for an easy visualization of the maximum information contained in a dataset [36-39]. It decomposes the primary data matrix by projecting the multidimensional data onto a new coordinate base formed by the orthogonal directions with maximum data variance. The eigenvectors of the data matrix are called Principal Components (PC) and they are uncorrelated among them.

HCA is a linear and unsupervised method for finding the underlying structure of objects through an iterative process that associates or dissociates object by object, and that is halted when all objects have been processed[40]. HCA is known as method for classification in the literature and is a more primitive technique in that no assumptions are made concerning the number of groups. Grouping is done based on similarities or distances[41,42]. The results of hierarchical clustering methods are often displayed as a dendrogram connection.

SVMs approach was used, as a nonlinear and a supervised learning technique, for classification analysis. SVMs are one of the kernel-based pattern recognition methods. SVMs were originally designed for binary classification. Currently there are two types of approaches for multi-class SVMs. One is by constructing and combining several binary classifiers “one-against-one or one-against-all methods”, while the other is by directly considering all data in one optimization formulation. Additional and more detailed information can be found elsewhere[43,44].

## 3. Results and discussion

### 3.1. Electronic nose analysis

Fig. 3(a) shows the typical signals of the conductance (G(t)) generated by the TGS 842 sensor exposed to the seven saffron samples. The sensor signals increase slightly depending on the saffron being measured. The obtained curves for the different saffron samples have a similar shape. This behaviour could be justified by the similarity of the saffron headspace. Furthermore, Fig. 3(b) represents the radar plots of the analysed saffron samples. These plots were constructed by using the dynamic slope value of the each sensor response divided by the maximum slope of the signal of the sensor TGS 842 (in this type of application). As one can see, there is some similarity between fingerprints of the samples from ST_70, ST_148 and ST_150. The same reasoning applies to the saffron samples ST_16 and ST_117 then, it is quite difficult to discriminate between these analysed samples. We can remark that it's not easy to draw significant conclusions by the use of only one variable. Consequently, the use of the multivariable approach is paramount to analyse the overall of the sensors responses.

Indeed, PCA method was used as an exploratory technique to investigate clustering of data points within the multi-dimensional space of features. Fig. 4 shows the projections of the experimental results on a three-dimensional plot (3D) by using the first three new principal components, showing an accumulated variance of 91.35 %. PC1 explained 66.66 % of the total variance in the data set, PC2 explained 18.53 % while PC3 explained 6.16 %; a high value of accumulated variance indicating that nearly all the variance contained in the original information is presented now by only these three new PCs. The PCA has resulted in a primary classification among samples coming from different areas, although this discrimination was not complete. As we can notice, no clear discrimination was been observed between ST_16 and ST_150, on one hand and ST_70 and ST_148 on the other hand. However, clusters corresponding to the samples of the region of Taliouine and those from Iran and Syria are discriminated.

To confirm PCA results, another unsupervised method was used. HCA provided a better alternative for visual representation of high-dimensional data. The results of HCA are presented in Fig. 5 as a dendrogram obtained from the saffron samples applying the Euclidean distance and Ward linkage to define clusters. As shown in the dendrogram, the 56 saffron samples representing the 7 types of saffron (8 replicates per type) were not clustered. This finding revealed the existence of a global similarity among the headspaces of all kinds of saffron, which contribute to the miss discrimination using the HCA method.

On the other hand, SVMs one-against-one classification method was applied to develop a robust classifier model for the E-nose investigations. Second-order of radial basis function (Polynomial) kernel was used to project the training data to a space that maximizes the margin hyper plane. The performance in classification is shown in Table 1 as a confusion matrix. The validation was performed using leave-one-out cross-validation technique. The SVMs reached 66.07 % success rate in the recognition of the seven saffron samples. As it can be noticed in this Table, several mistakes are signalled. Therefore, the results obtained by SVMs indicate that the ST_70 and ST_148 classes are very close to each other, the same thing can be said about SI and SS. For ST_117 and ST_150, these two clusters are well classified. These findings are in good agreement with PCA and HCA results.

### 3.2. Electrochemical fingerprints of saffron by electronic tongue analysis

The voltammetric analysis was carried out in order to determine the electrochemical fingerprint of saffron stigmas by using different electrodes (Ag, Au, Cu, GC, Ni, Pd and Pt). As it is known, the electrochemical response of a given compound depends on the intrinsic chemical nature of both the electrode and the redox behaviour of the product itself. This important cross-selectivity is illustrated in Fig. 6(a) for a glassy carbon electrode immersed in the studied saffron samples. As it is observed, the signals differ slightly between the samples at the extremities of voltammograms. The slight differences between voltammetric responses of the electrode might due to the compounds that have been detected in the saffron solutions. Thus, the radar plots were used in order to see if there are pattern differences among saffron samples of different areas. Fig. 6(b) shows the radar plots of the response of the seven saffron samples by choosing the current change ($\Delta I$) as variable. It can be observed that a clear pattern variation exists between the studied samples.

PCA is frequently employed to generate a reduced set of variables that can be explained the main point of the variance in the original data. PCA analysis was performed on saffron samples in order to evaluate the VE-tongue ability in the geographical classification task of saffron. Fig. 7 illustrates the PCA model effectiveness in classifying the saffron samples. The first three principal components explain an accumulated variance of 71.92 % in the experimental data with relative weight of each one of 36.44 %, 19.06 % and 16.42 %, respectively. It can be seen that a perfect discrimination among all saffron origins have been obtained. Thus, the VE-tongue results seem to be very useful for recognizing correctly the origins of saffron samples even if they were picked up from a very restricted area as is the case of the substrate of Taliouine referenced by ST_xx.

Before performing a supervised technique, HCA was also applied. The HCA dendrogram, with the Euclidean distance and Ward linkage to define clusters for classification of saffron samples of different areas, is shown in Fig. 8. At distance D≈ 12, the samples from each cluster are perfectly grouped in the dendrogram, with each cluster coming off a single branch to the left of the vertical dashed line. At this distance, HCA also has the same results with PCA. There were no samples linked with wrong group, and the clustering classification was completely successful.

To confirm the result of PCA and HCA analysis, the SVMs with the second-order polynomial the secondary kernel and the one-against-one model have been applied for the recognition and classification of the 7 geographical origins of saffron. As in the PCA and HCA, 21 response features from the sensor array are used as inputs to the SVMs. Table 2 shows the confusion matrix of the SVMs classifier. As it can be noticed in this Table, 100 % of the accuracy in the recognition of the saffron samples was achieved. These results indicate that all saffron samples were perfectly classified.

### 3.3. SPME-GC-MS results

The SPME-GC-MS analysis that we have conducted aims to identify VOCs present in the headspace of saffron samples from different countries. The total ion chromatograms (TIC) of the aroma composition of saffron samples are depicted in Fig. 9. The volatile compounds were tentatively identified by comparing their mass spectra with the standard mass spectra library. The most representative VOCs recorded on the TIC, by order of appearance, are as follow: Acetic acid, 2(5H)-furanone, isophorone (2-cyclohexen-1-one, 3,5,5-trimethyl-) and safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde). Comparing the main volatiles between the seven kinds of saffron, it can be observed that they share the same hierarchical magnitude but with different proportions.

In order to analyse the main difference between the seven saffron areas, we have established the aroma composition profile according to the seven classes, as shown in Table 3. Indeed, seven major compounds were identified in saffron (Fig. 10). It should be noted that the amount of the extracted compounds is expressed as a percentage of the obtained peak area relative to the total area of all peaks of the TIC. Most of the compounds detected and tentatively identified have been previously reported in other studies on the aroma composition of saffron[31,45].

Amongst them, safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) is the major characteristic of saffron aroma[7,11]. Safranal showed significant differences between the all samples. Its percentage is high for the ST_148 compared to the other samples. Acetic acid (compound 2), isophorone (compound 4) and 2(5H)-furanone (compound 5) were found at a much higher proportion[7]. On the other hand, Carmona[7] demonstrated that acetic acid is capable to differentiate saffron from its origin by mean of GC-MS. In addition, in his study, the content of acetic acid is different from sample to another. Therefore, the saffron sample released the acetic acid gas; the last one decompose to methane gas and $CO_2$, as a result, the TGS 842 signal increase depends on the amount of methane present in headspace. Hence, it is now easy to realize that these compounds are maybe the main responsible of the slight misclassification in the case of the use of E-nose analysis; they play a part in differentiation between the all saffron samples. Indeed, it can be observed that saffron samples of ST_70 and ST_148 share almost the same percentages of the identified VOCs. This may explain the clear overlap of these two classes in the E-nose findings (Fig. 4). The samples from ST_16, ST_117 and ST_150 have different, although slight, percentages of the identified VOCs. The semi-quantitative determined volatile compounds in saffron as well as the variation in their concentration are responsible for different aromatic odours.

### 3.4. Secondary metabolites analysis results

As a secondary part of liquid analysis, saffron samples were analysed by UV-Vis Spectrophotometry in order to confirm the results obtained by the VE-tongue and also to evaluate the absorbance values due to the presence of their secondary metabolites like crocin, safranal and picrocrocin. Table 4 shows the value of absorbance measured at different wavelengths of solutions, after a dilution 1/10 of a 0.5 $gL^{-1}$ extract. In this table, the crocin contents (g/100 g), the safranal contents (mg/100 g) and the picrocrocin contents (g/100 g) in the considered saffron samples have been reported. The obtained results were comparable with those reported by Cossignani[29], who used GC-FID analysis as an analytical method. Also a broad range of values was generally reported for the secondary metabolites contents[4,46]. Furthermore, all saffron samples were classified according to the ISO specifications (ISO/TS 3632-2) regarding moisture and volatile matter content, as well as the main characteristics using UV–Vis spectrophotometry. Table 5 shows the categories classification of crocin, safranal and picrocrocin of the analysed saffron samples by using the UV-Vis spectrophotometer and the ISO/TS 3632-2 standard recommendations[6], which ranks saffron according to the lowest category. We can notice that the saffron aroma quality of all the analysed samples is good according to the value of safranal observed in the Fig. 10 and the absorbance values of safranal given in the Table 5 which shows that all the saffron samples are belong to category I. However, regarding the bitterness of taste of saffron, which is evaluated by the absorbance value of picrocrocin, we can conclude taking into account the value given in Table 5 that, only ST_117 belongs to category II, ST_16 and ST_70 to category III and the rest are classified into category IV.

## 4. Conclusion

The possibility of classifying saffron samples based on their geographical origin by the combination of E-nose, VE-tongue and pattern recognition techniques has been demonstrated. The analytical methods such as SPME-GC-MS and UV-Vis Spectrophotometry analysis were also used to validate the olfactory and gustatory findings obtained by the E-nose and VE-tongue, respectively. PCA, an unsupervised classificatory technique, built with the VE-tongue sensors appears better than the model built with E-nose. This would be explained by the fact that some chemical parameters contained in the headspace of the electronic nose do not have a relevant rule in the class discrimination, contrarily to the case of VE-tongue, which reveals a good discrimination of the all clusters. The analysis performed by SPME-GC-MS reveals that some difference in the composition of volatile compounds of the seven samples were observed. A total of seven volatile compounds of the analysed saffron were identified. This technique seems to give rich information in the aim to confirm which volatiles are having sensory impact in the saffron samples. Moreover, the samples were analysed by spectrophotometric analysis in order to evaluate the absorbance values due to the presence of their secondary metabolites, crocin, safranal, and picrocrocin. A classification according to the ISO specifications (ISO/TS 3632-2) regarding moisture and volatile matter content saffron was also performed. The obtained results suggest that the electronic systems and its combination with SPME-GC-MS, UV-Vis Spectrophotometry and pattern recognition methods offer a fast, simple and efficient tool to distinguish samples of different composition and declared geographical origin of saffron.

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## Figure captions

**Figure 1:** Electronic nose setup for headspace evaluation of saffron.

**Figure 2:** Voltammetric electronic tongue setup for the evaluation of saffron.

**Figure 3:** **(a)** Electrical conductance of TGS 842 sensor towards exposures to seven saffron samples; **(b)** Radar plots of the E-nose response to the seven saffron samples (expressed as the dynamic slope of the conductance).

**Figure 4:** Scores plot of a PCA analysis for the discrimination of the saffron samples by using an E-nose system.

**Figure 5:** Hierarchical Cluster Analysis (HCA) dendrogram of seven saffron samples measured by E-nose.

**Figure 6:** **(a)** Voltammetric responses of Glassy Carbon electrode immersed in solution of saffron samples; **(b)** Radar plots of the response of the seven saffron samples by VE-tongue (expressed as the current change ΔI).

**Figure 7:** PCA plot performed on the 7-studied saffron samples measurements gathered using the VE-tongue.

**Figure 8:** Hierarchical cluster analysis (HCA) dendrogram of seven saffron samples measured by VE-tongue.

**Figure 9:** Total ion chromatograms (TIC) of seven saffron samples originated from three different countries.

**Figure 10:** Comparison of the importance of saffron volatile components from the different area.

## Table captions

**Table 1:** SVM results for the classification of the saffron samples measured by E-nose.

**Table 2 :** SVM results for the classification of the saffron samples measured by VE-tongue.

**Table 3**: Relative amount of main volatile saffron ingredients from GC-MS headspace analysis.

**Table 4:** Absorbance values (at 440, 310, 257 nm) and crocin, safranal and picrocrocin concentrations.

**Table 5:** Relative quality categories according to the ISO/TS 3632-2 normative of studied saffron samples.

Figure 1 :

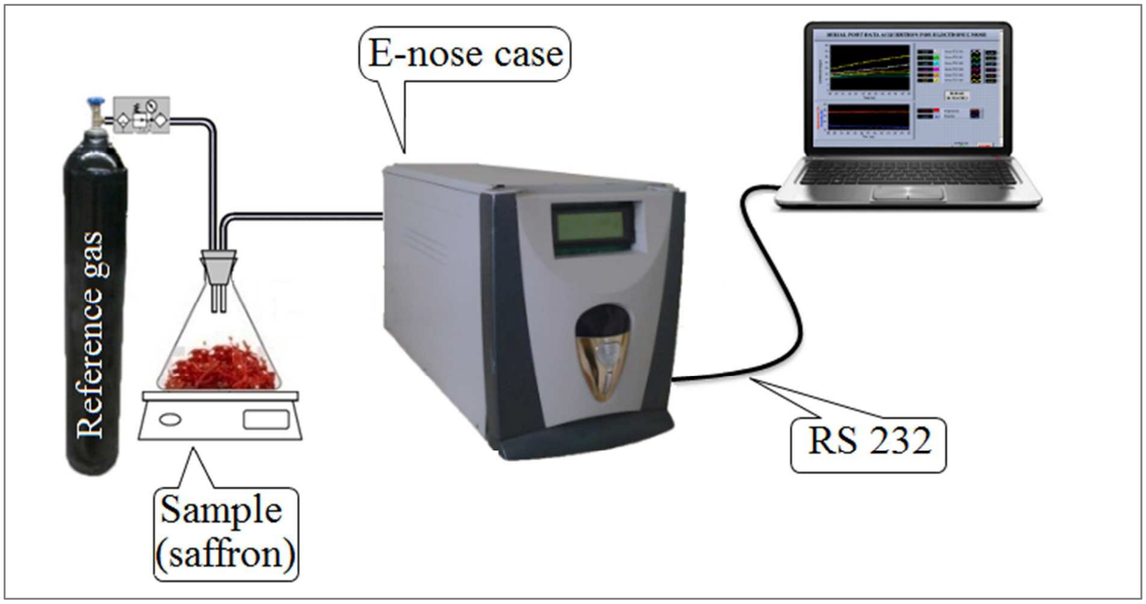


**Figure 2 :**

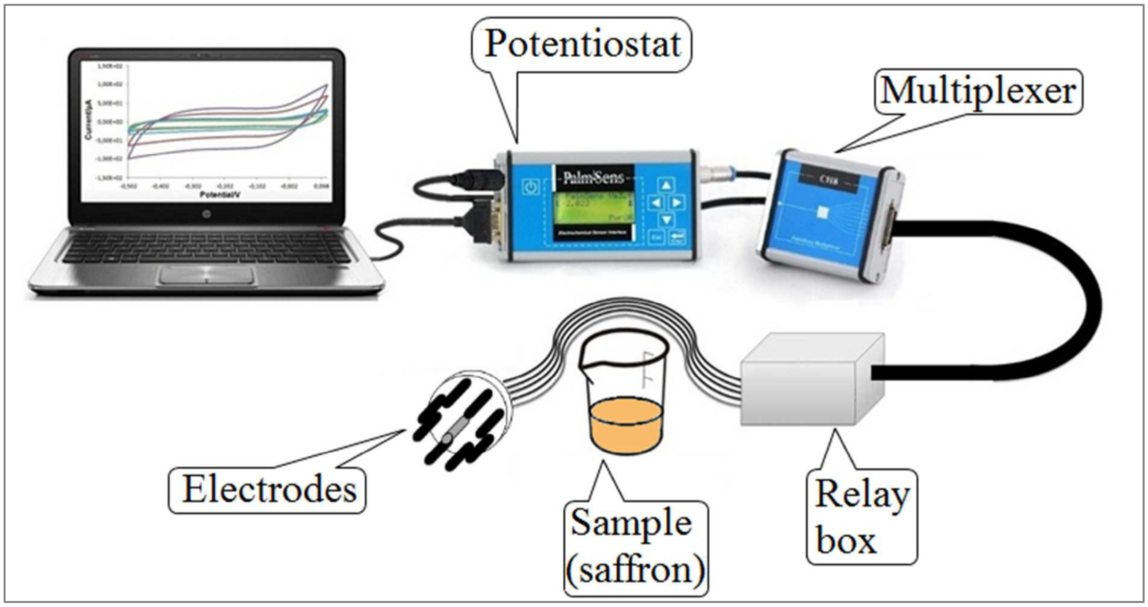

Potentiostat
Multiplexer
Electrodes
Sample
(saffron)
Relay
box

**Figure 3 :**

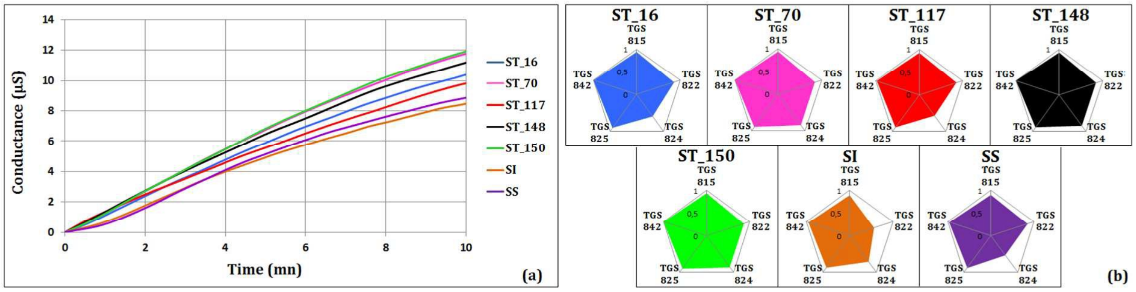

Conductance (µS)
Time (mn)
ST_16
ST_70
ST_117
ST_148
ST_150
SI
SS
(a)
TGS 815
TGS 822
TGS 824
TGS 825
TGS 842
(b)

Figure 4 :

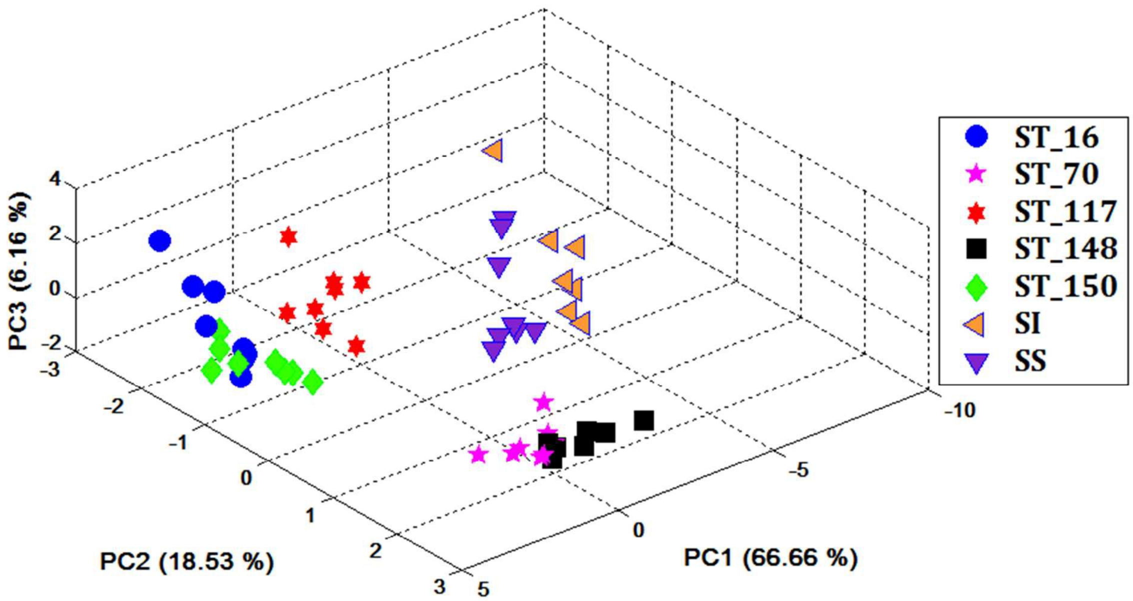

ST_16
ST_70
ST_117
ST_148
ST_150
SI
SS
PC3 (6.16 %)
PC2 (18.53 %)
PC1 (66.66 %)
4
2
0
-2
-3
-2
-1
0
1
2
3
5
0
-5
-10

**Figure 5 :**

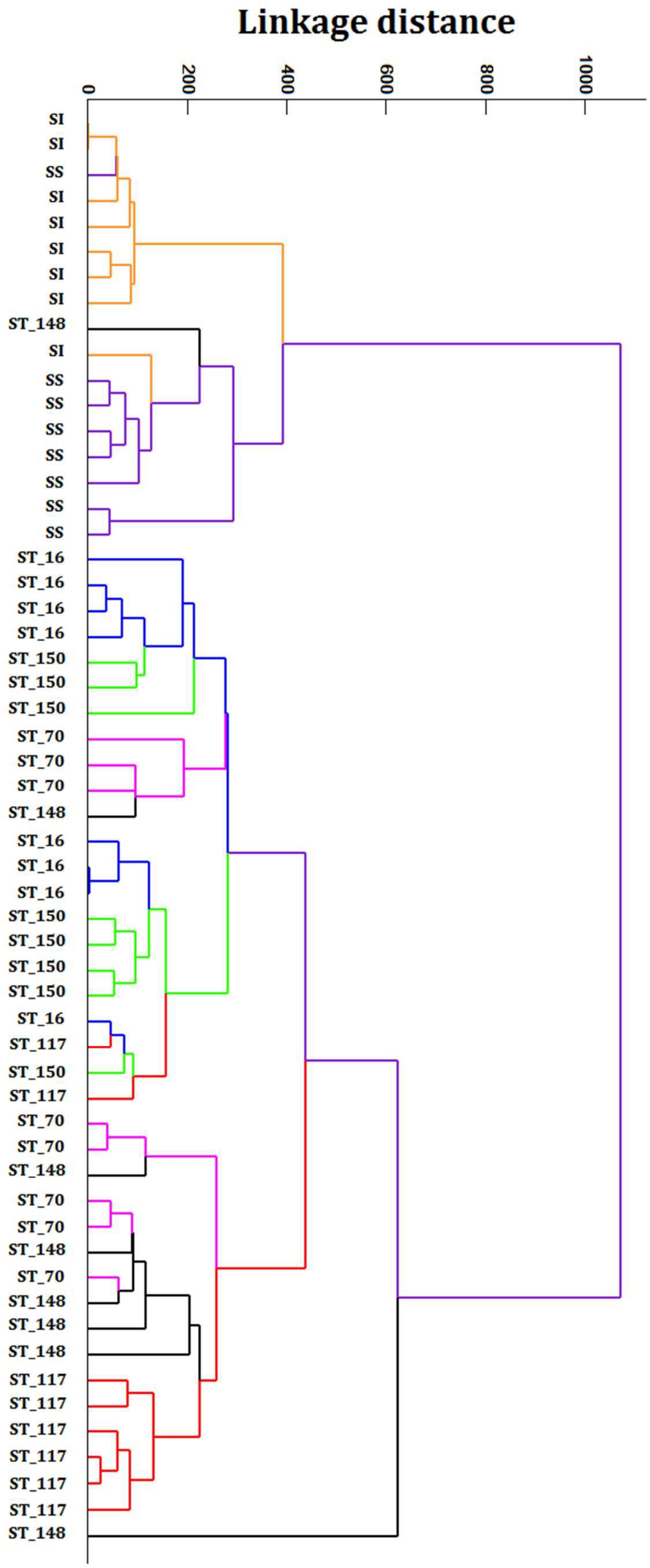

Linkage distance
0
200
400
600
800
1000
SI
SI
SS
SI
SI
SI
SI
SI
ST_148
SI
SS
SS
SS
SS
SS
SS
SS
ST_16
ST_16
ST_16
ST_16
ST_150
ST_150
ST_150
ST_70
ST_70
ST_70
ST_148
ST_16
ST_16
ST_16
ST_150
ST_150
ST_150
ST_150
ST_16
ST_117
ST_150
ST_117
ST_70
ST_70
ST_148
ST_70
ST_70
ST_148
ST_70
ST_148
ST_148
ST_148
ST_117
ST_117
ST_117
ST_117
ST_117
ST_117
ST_148

Figure 6 :

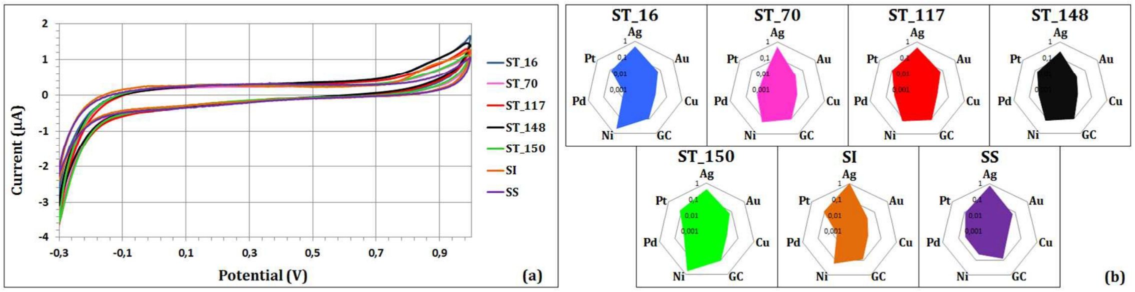

Current (µA)
Potential (V)
ST_16
ST_70
ST_117
ST_148
ST_150
SI
SS
(a)
Ag
Au
Cu
GC
Ni
Pd
Pt
(b)

**Figure 7 :**

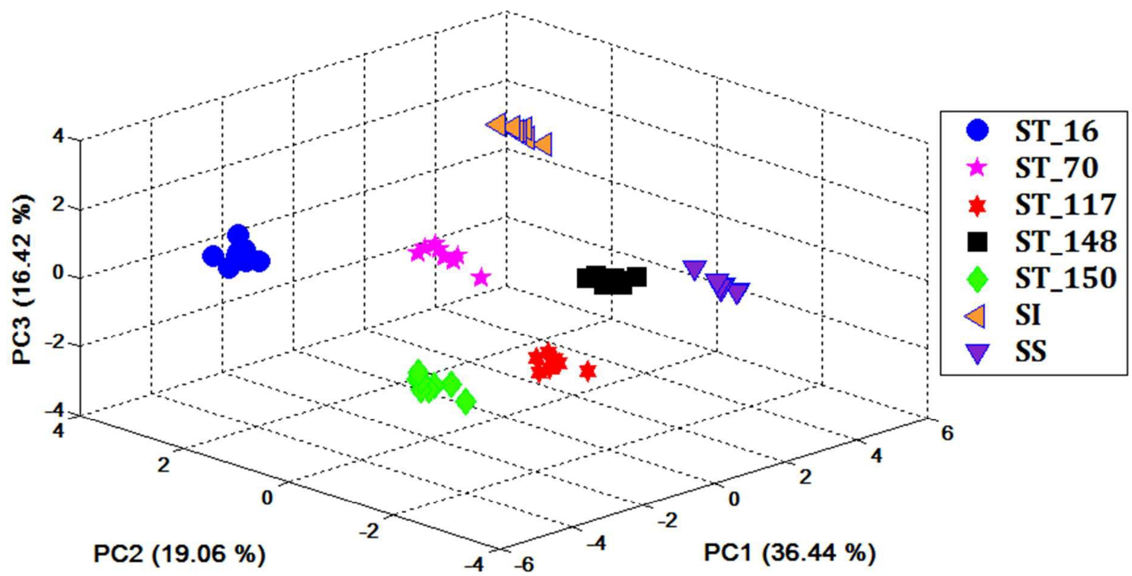


Figure 8 :

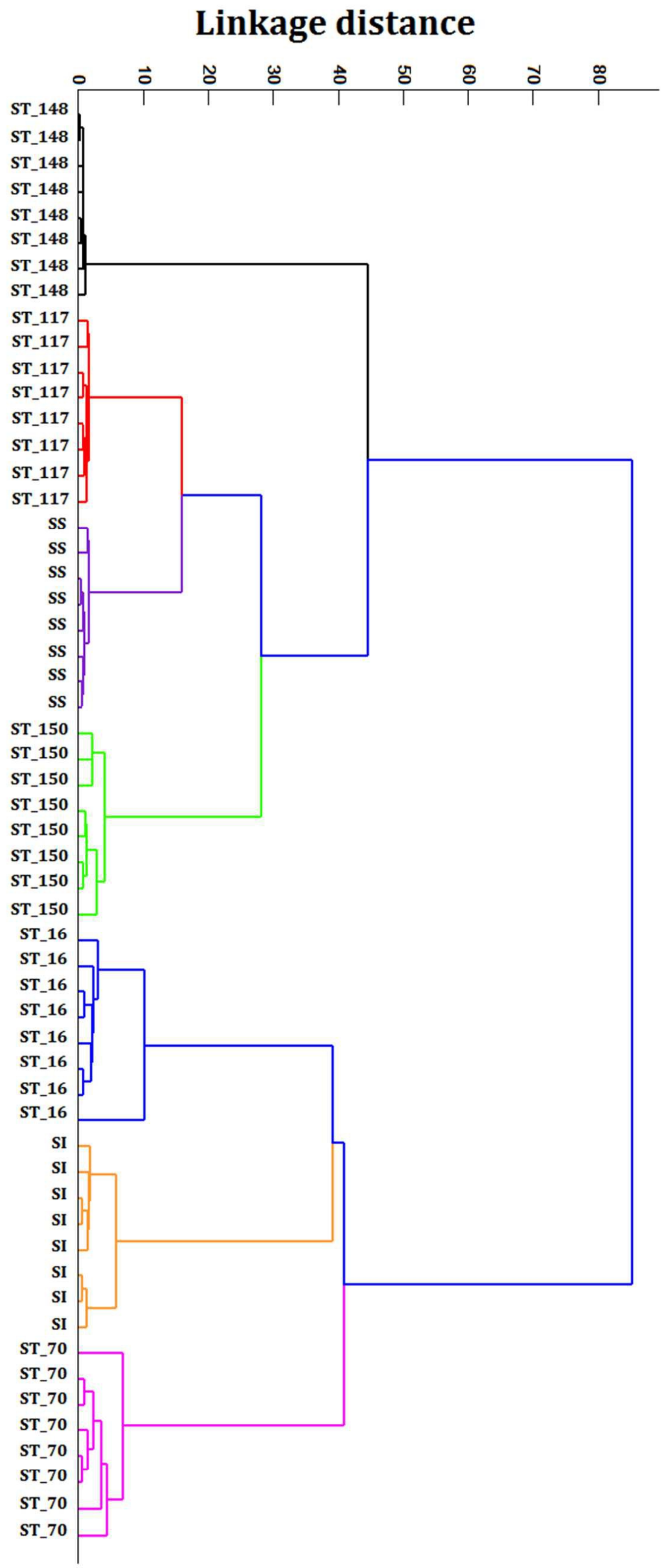

Linkage distance
0
10
20
30
40
50
60
70
80
ST_148
ST_148
ST_148
ST_148
ST_148
ST_148
ST_148
ST_148
ST_117
ST_117
ST_117
ST_117
ST_117
ST_117
ST_117
ST_117
SS
SS
SS
SS
SS
SS
SS
SS
ST_150
ST_150
ST_150
ST_150
ST_150
ST_150
ST_150
ST_150
ST_16
ST_16
ST_16
ST_16
ST_16
ST_16
ST_16
ST_16
SI
SI
SI
SI
SI
SI
SI
SI
ST_70
ST_70
ST_70
ST_70
ST_70
ST_70
ST_70
ST_70

**Figure 9 :**

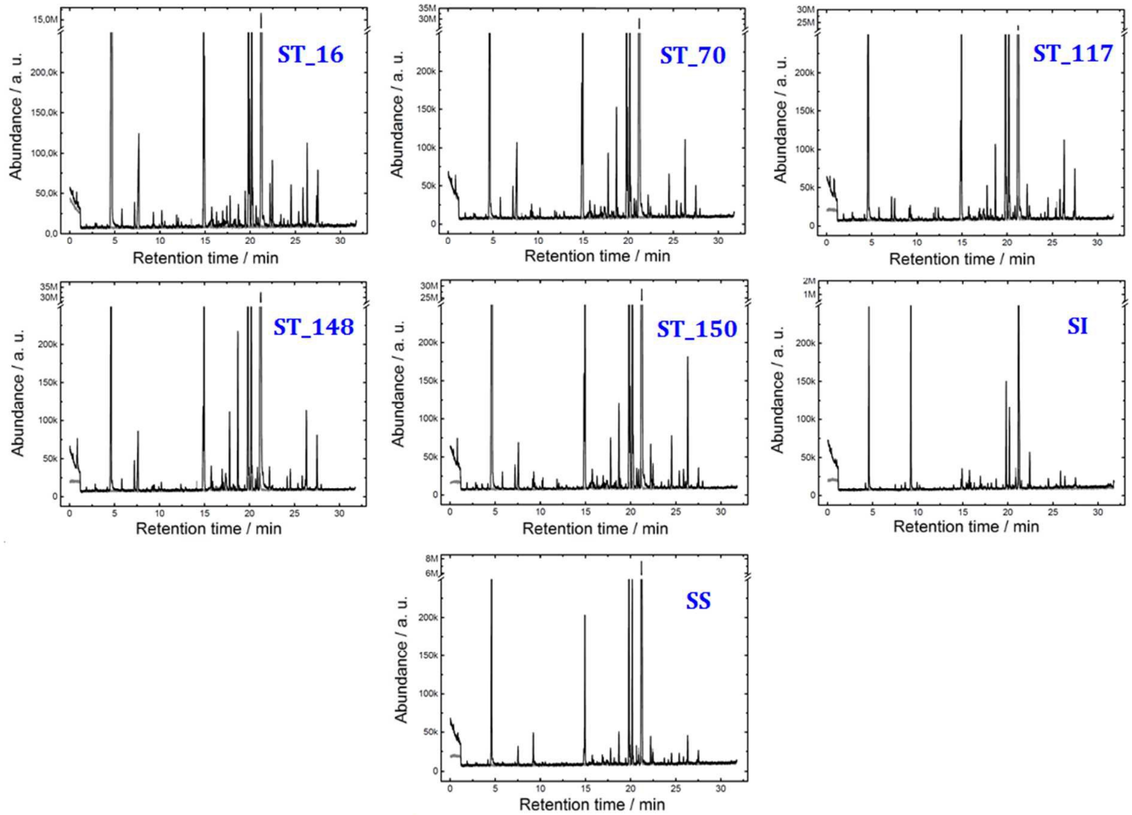
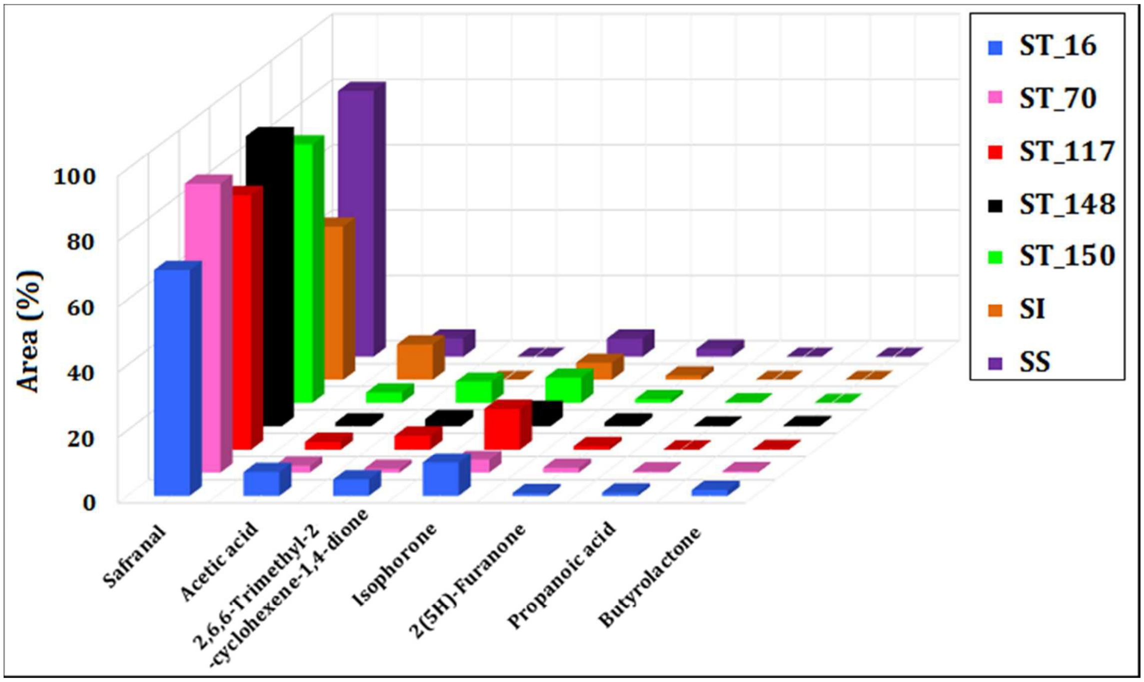

ST_16
ST_70
ST_117
ST_148
ST_150
SI
SS
Abundance / a. u.
Retention time / min

**Figure 10 :**



ST_16
ST_70
ST_117
ST_148
ST_150
SI
SS
Area (%)
100
80
60
40
20
0
Safranal
Acetic acid
2,6,6-Trimethyl-2
-cyclohexene-1,4-dione
Isophorone
2(5H)-Furanone
Propanoic acid
Butyrolactone

**Table 1 :**

| Actual | Predicted | | | | | | |
|---|---|---|---|---|---|---|---|
| | ST_16 | ST_70 | ST_117 | ST_148 | ST_150 | SI | SS |
| ST_16 | 3 | | 1 | | 2 | 1 | 1 |
| ST_70 | | 2 | | 6 | | | |
| ST_117 | | | 8 | | | | |
| ST_148 | | 2 | | 6 | | | |
| ST_150 | | | | | 8 | | |
| SI | 1 | 1 | | | | 3 | 3 |
| SS | | | | | | 1 | 7 |

**Table 2 :**

| Actual | Predicted | | | | | | |
|---|---|---|---|---|---|---|---|
| | ST_16 | ST_70 | ST_117 | ST_148 | ST_150 | SI | SS |
| ST_16 | 8 | | | | | | |
| ST_70 | | 8 | | | | | |
| ST_117 | | | 8 | | | | |
| ST_148 | | | | 8 | | | |
| ST_150 | | | | | 8 | | |
| SI | | | | | | 8 | |
| SS | | | | | | | 8 |

**Table 3 :**

| Name | Area / % | | | | | | |
|---|---|---|---|---|---|---|---|
| | **ST_16** | **ST_70** | **ST_117** | **ST_148** | **ST_150** | **SI** | **SS** |
| Compound 1 | 69.2 | 88.5 | 77.9 | 88.9 | 79.3 | 47 | 81.4 |
| Compound 2 | 7.3 | 2.1 | 2.4 | 1.2 | 3.2 | 10.9 | 5.6 |
| Compound 3 | 5.0 | 1.1 | 4.2 | 2.2 | 6.6 | 0 | 0 |
| Compound 4 | 10.2 | 4.0 | 12.4 | 5.0 | 7.8 | 5.3 | 5.5 |
| Compound 5 | 0.8 | 1.5 | 1.1 | 1.2 | 1.1 | 1.4 | 2.4 |
| Compound 6 | 1.0 | 0.4 | 0.1 | 0.2 | 0.2 | 0 | 0 |
| Compound 7 | 1.8 | 0.4 | 0.2 | 0.3 | 0 | 0 | 0 |

**Compounds: (1)** safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde); **(2)** acetic acid; **(3)** 2,6,6-trimethyl-2-cyclohexene-1,4-dione; **(4)** isophorone (2-cyclohexen-1-one, 3,5,5-trimethyl-); **(5)** 2(5H)-furanone; **(6)** propanoic acid; **(7)** butyrolactone.

Table 4 :

| Sample | % H | $E^{1\%}$ (440nm) | $E^{1\%}$ (310nm) | $E^{1\%}$ (257nm) | ISO category | Crocin g/100g | Safranal mg/100g | Picrocrocin g/100g |
|---|---|---|---|---|---|---|---|---|
| **ST_16** | 9.7 | 147 | 33 | 51 | III | 13,9 | 5,3 | 16,1 |
| **ST_70** | 10.4 | 124 | 36 | 46 | III | 11,7 | 5,8 | 14,5 |
| **ST_117** | 7.3 | 156 | 38 | 57 | II | 14,8 | 6,1 | 17,9 |
| **ST_148** | 10.5 | 166 | 35 | 34 | IV | 15,7 | 5,6 | 10,7 |
| **ST_150** | 8.0 | 113 | 30 | 39 | IV | 10,7 | 4,8 | 12,4 |
| **SI** | 7.3 | 157 | 22 | 34 | IV | 14,9 | 3,5 | 10,6 |
| **SS** | 13.2 | 153 | 23 | 30 | IV | 14,5 | 3,8 | 9,6 |

**Table 5 :**

| Saffron Sample | Categories according to ISO/TS 3632-2 | | | |
|---|---|---|---|---|
| | $E_{1cm}^{1\%}$ (440 nm) absorption value of crocin | $E_{1cm}^{1\%}$(310 nm) absorption value of safranal | $E_{1cm}^{1\%}$(257nm) absorption value of picrocrocin | Results of the category |
| ST_16 | 150 ≥ 147 ≥ 110 (Category III) | 50 ≥ 33 ≥20 (Category I) | 55 ≥ 51 ≥ 40 (Category III) | Category III |
| ST_70 | 150 ≥ 124 ≥ 110 (Category III) | 50 ≥ 36 ≥20 (Category I) | 55 ≥ 46 ≥ 40 (Category III) | Category III |
| ST_117 | 190 ≥ 156 ≥ 150 (Category II) | 50 ≥ 38 ≥20 (Category I) | 70 ≥ 57 ≥ 55 (Category II) | Category II |
| ST_148 | 190 ≥ 166 ≥ 150 (Category II) | 50 ≥ 35 ≥20 (Category I) | 40 ≥ 34 ≥ 30 (Category IV) | Category IV |
| ST_150 | 150 ≥ 113 ≥ 110 (Category III) | 50 ≥ 30 ≥20 (Category I) | 40 ≥ 39 ≥ 30 (Category IV) | Category IV |
| SI | 190 ≥ 157 ≥ 150 (Category II) | 50 ≥ 34 ≥20 (Category I) | 40 ≥ 34 ≥ 30 (Category IV) | Category IV |
| SS | 190 ≥ 153 ≥ 150 (Category II) | 50 ≥ 30 ≥20 (Category I) | 40 ≥ 30 ≥ 30 (Category IV) | Category IV |