DEVELOPMENT OF ANALYTICAL METHODOLOGIES TO DETERMINE THE ELEMENTAL PROFILE OF SAFFRON FOR GEOGRAPHICAL ORIGIN ASSESSMENT STUDIES

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Abstract – In the present study, analytical methodologies based on inductively coupled plasma – mass spectrometry (ICP-MS) were developed for the determination of the elemental profile of saffron as a tool for its geographical traceability. Due to the high commercial price of the spice, special focus was paid in using only a minute amount of sample (30 mg).

Keywords: saffron, geographical origin, elemental profile, ICP-MS

1. INTRODUCTION

Saffron is comprised of the dried red stigmas of the plant *Crocus sativus* L. (Fig. 1), a perennial, triploid and genetically sterile plant that belongs to the *Iridaceae* family [1]. It is the most expensive spice in the world [2] and is highly valued in the food industry for exerting color and flavor to certain dishes and beverages [3]. Its coloring properties are attributed to a group of water-soluble apocarotenoids, the sugar esters of crocetin (8,8′-diapocarotene-8,8′-dioic acid), known as crocins. The bitter taste is mainly assigned to the colorless monoterpene glucoside picrocrocin (4-(*β*-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) whereas the aroma is principally attributed to safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) [4]. Polar extracts of saffron or its individual apocarotenoids have been also associated with a variety of pharmacological actions (e.g. anti-inflammatory, anticancer, antioxidant etc.) provided that "the plant material used is authentic and of high quality" as highlighted by Kyriakoudi, Ordoudi, Roldán-Medina and Tsimidou [5] in a recent review.

Saffron is currently produced in Asia (Iran, India, Afganistan), Europe (Greece, Spain, Italy, France) and North Africa (Morocco). The ~90% of the world's total annual saffron production originates from Iran whereas Greece is the major producer of saffron in Europe.

Fig. 1. The flower of the plant *Crocus sativus* L. (left) and the red dried stigmas that constitute the spice saffron (right) (photo gallery of LFCT, AUTH, Greece).

Nowadays, there is an increasing concern from both producers and consumers for high-quality food products with a certified geographical origin. This need has led to the establishment of "PDO" (Protected Designation of Origin) labels in Europe [6]. PDOs are agricultural products and foodstuffs which are produced, processed and prepared in a given geographical area using recognized know-how. The main goals of such legislation are to support diversity in agricultural production, to protect consumers and product names against fraud. In the case of saffron, several PDOs are currently established in Europe but in fact these represent tiny amounts of saffron at the time being (few kilos). Being the most expensive spice worldwide [2], fraudulent practises are very usual phenomena in the international market. In particular, mislabeling of geographical origin of saffron is a real problem widely encountered. Discrimination of saffron of different origins based on their physicochemical or organoleptic properties is rather difficult. Admixtures of expensive European saffron with cheap one mainly from Iran are very common practices in the spice market. The solution in detection and prevention of fraud in terms of misleading geographical origin is the application of robust analytical techniques.

So far, particular emphasis has been given on the use of the water-soluble and volatile saffron compounds as markers for its geographical origin using various techniques such as high performance liquid chromatography (HPLC) [7], gas chromatography – mass spectrometry/flame ionization detector (GC-MS/FID) [8], near-infrared (NIR) spectroscopy [9,10], proton transfer reaction time-of-flight mass spectrometry (PTR-TOF-MS) [11] as well as stable isotope analysis [12]. However, the elemental composition of foods could be also used as an indicator for their geographical origin, reflecting soil characteristics, area of production and environmental growing conditions [13]. Atomic spectroscopy (e.g. ICP-AES) and mass spectrometry (e.g. ICP-MS) are employed for the determination of essential, trace and ultra-trace elements. They allow the quantitative determination of various elements in a great variety of samples at trace (ppb-ppm) and ultra-trace (ppq-ppb) levels and can identify and classify a specific food commodity according to its geographical origin. Of particular interest is the study of Rare Earth Elements (REE). In such studies, chemometrics are usually used for the statistical treatment of obtained data for basic pattern recognition. To the best of our knowledge information about the elemental profile of saffron is extremely limited [14,15].

The main objective of the present study was the development of analytical methodologies based on ICP-MS for the determination of trace and ultra-trace elements (including REE) present in saffron as a tool for its geographical origin assessment.

2. MATERIALS AND METHODS

2.1. Reagents and standards

All of the chemicals were of the highest purity required. In particular, all reagents used were of analytical grade. The elemental standard solutions were prepared by diluting stock solutions (TraceCERT® Fluka Standards) of 1000 mg/L. High-purity water (resistivity > 18 MΩ · cm) was used for all dilutions. All vessels and glassware were cleaned with an automatic acid steam cleaning system TraceClean® (Milestone) and then rinsed with high-purity water before use.

2.2. Samples

Authentic Greek saffron (harvest year 2015) donated by the Saffron Cooperative of Kozani (Greece) was used to set up the ICP-MS methods. Saffron sample was stored at ambient temperature in absence of light.

2.3. Preparation and UV-Vis characterization of saffron aqueous extract

Saffron was carefully ground with a pestle and mortar just prior to analysis. The finely ground plant material (0.05 g) was extracted with high-purity water (100 mL) by rigorous agitation (~500 rpm) at ambient temperature for 1 h. All manipulations were performed under subdued (yellow) light to minimize photodecomposition of saffron apocarotenoids. The characterization was achieved using UV–vis spectrophotometry. The UV–Vis spectrum after appropriate dilution (1:10, v/v) was recorded in the region 200–600 nm with a spectrophotometer (Analytik Jena Specord 50 plus, Germany) equipped with quartz cells (1 × 1 × 4 cm). Absorption measurements in triplicate were obtained. The results were expressed as $E^{1\%}_{\lambda max}$ according to the equation (1):

$$E^{1\%}_{\lambda max} = D \times 10000/m\,(100 - H) \qquad (1)$$

where D is the absorbance value; m is mass (g); H is the moisture and volatile content of the sample (%, w/w); λ_{max} for crocetin sugar esters 440 nm, λ_{max} for picrocrocin 257 nm and λ_{max} for safranal 330 nm.

2.4. Dissolution procedure

After preliminary studies, 30 mg of ground saffron were digested with 1.7 mL H_2O and 0.3 mL HNO_3. The dissolution procedure was carried out using a Microwave Digestion System with Single Reaction Chamber (MILESTONE UltraWAVE®). Following pre-pressurization of the chamber with N_2 to 40 bar, the samples were digested using the following time–temperature microwave method: Phase 1 – Ramp t = 15 min, power = 1500 W, T1 = 230 °C, T2 = 70 °C, P = 150 bar; Phase 2 – isothermal t = 10 min, power = 1500 W, T1 = 230 °C, T2 = 70 °C, P = 150 bar. The obtained solutions were transferred into glass volumetric flasks, filled up to a final volume of 5 ml with ultrapure water and then submitted to ICP-MS analysis. Dissolutions were performed in triplicate and process blank solutions were prepared and submitted to ICP-MS analysis under identical conditions.

2.5. ICP-MS analysis

ICP-MS analysis was carried out using a Bruker Aurora M90 instrument [90 degree ion mirror ion optics, Collision Reaction Interface (CRI)] equipped with the Quantum (v.3.0, Bruker) software facilities. The instrument was tuned with a 5 µg/L solution of Be, Mg, Co, In, Ba, Ce, Ti, Pb and Th for sensitivity, resolution optimization and mass calibration. The levels of oxide and double charged ions in the plasma were monitored by the $^{140}Ce^{16}O^+/^{140}Ce^+$ (< 2%) and $^{137}Ba^{++}/^{137}Ba^+$ (< 3%), respectively. Three different methods were set up optimizing the instrumental conditions for the quantitative analysis of different elements:

- a method for the determination of selected trace and ultra-trace elements (contaminants) (As, Cd, Co, Cr, Cu, Ni, Pb, Rb, Sb, Se, Sn, Sr, Ti, V, Zn, Zr),
- a method for the determination of REE (Ce, Er, Eu, Gd, La, Nd, Pr, Sc, Sm, Th, Y, Yb
- a method for the determination of As and Se using the CRI.

3. RESULTS AND DISCUSSION

The ISO 3632-1 [16] quality parameters $E^{1\%}_{440}$, $E^{1\%}_{257}$ and $E^{1\%}_{330}$ for the aqueous saffron extract were found to be 293 ± 2.4, 96 ± 1.5 and 40 ± 0.5, respectively, indicating that the sample was of the highest quality (Category I).

Concerning the dissolution procedure, preliminary studies were carried out in order to optimize the conditions for the complete solubilisation of saffron. In particular, efforts were made to minimize the sample amount used taking into consideration the high commercial price of the spice. Tests were carried out in the range 20 – 200 mg using mixtures of various reagents such as HNO_3, H_2O and H_2O_2 at different ratios. Finally, the optimum amount of saffron was found to be 30 mg. This value is much lower than that used by Jia et al. [14] (i.e. 200 mg) for the ICP-AES and ICP-MS analysis of 2 Chinese saffron samples and comparable to that used by D'Archivio et al. [15] (i.e. 20 mg) for the ICP-MS analysis of 27 Italian saffron samples. The most efficient mixture of reagents was found to be H_2O (1.7 mL) and HNO_3 (0.3 mL) whereas D'Archivio et al. [15] used HNO_3 (0.5 mL) for the dissolution procedure and Jia et al. [14] used HNO_3 and $HClO_4$ (20:1, v/v). These authors did not use any apparatus for the dissolution procedure. However, in the present study, the dissolution was carried out in an advanced microwave digestion system that permits the operation at high temperatures and pressures, the control of both the pressure and temperature of each individual sample, the simultaneous digestion of samples of different matrices and the prevention of cross-contaminations. The low volume of HNO_3 is of particular importance since it allows the reduction of the detection limits (DL).

Concerning ICP-MS analysis, in all cases the isotopes to be investigated were selected based on potential interferences and relative abundances. In order to exclude the presence of interferences, for some elements the methods were optimized by analyzing more isotopes (e.g. Rb85 and Rb87; Sr88 and Sr87; Nd142, Nd146 and Nd144; Sm147, Sm148 and Sm152; Gd157 and Gd154; Er166 and Er168; Yb170 and Yb172) or even by applying correction equations with respect to isotopes of other elements potentially interfering. Considering the great variability of the relative abundances of Pb isotopes in nature, the results obtained for its three stable isotopes (Pb206, Pb207 and Pb208) were treated mathematically using the software facilities. The dwell time was optimized for each element in order to obtain the best sensitivity (10 ÷ 60 ms for contaminants, 40 ÷ 60 ms for REEs). Further instrumental details and analyzed isotopes are given in Table 1 for the ICP-MS analysis of trace and ultra-trace elements and in Table 2 for the analysis of REE. For As and Se, a specific method was developed to reduce interferences using the CRI (Skimmer gas: H_2; Skimmer flow = 70 ml/min). The potentials of ion optics, the Ar flows, as well as the dwell time (100 ms) were also optimized.

Table 1. Instrumental conditions for ICP-MS analysis of contaminants (without CRI).

Instrumental conditions		
Gas flow parameters (Argon)	Plasma flow	18.00 L/min
	Auxiliary flow	1.80 L/min
	Nebulizer flow	1.00 L/min
	Sheat flow	0.16 L/min
Plasma power	RF power	1.40 kW
Sample introduction	Sampling depth	7.50 mm
	Pump rate	2 rpm
Ion optics	First extraction lens	- 1.00 V
	Second extraction lens	- 169.00 V
	Third extraction lens	- 201.00 V
	Corner lens	- 215.00 V
	Mirror lens left	41.00 V
	Mirror lens right	20.00 V
	Mirror lens bottom	30.00 V
	Fringe bias	- 2.40 V
Quadrupole scan	Scan mode	Peak hopping
	Dwell time	10 ÷ 60 ms
	Points per peak	1
	Scans/Replicate	15
	Replicates/Sample	5

Analyzed isotopes					
As75	Cr52	Pb207	Sb121	Sr88	Zn66
Cd111	Cu65	Pb208	Se78	Ti49	Zr90
Cd114	Ni60	Rb85	Sn118	V51	
Co59	Pb206	Rb87	Sr87	Zn64	

Table 2. Instrumental conditions for ICP-MS analysis of REEs.

Instrumental conditions		
Gas flow parameters (Argon)	Plasma flow	17.00 L/min
	Auxiliary flow	1.80 L/min
	Nebulizer flow	0.94 L/min
	Sheath flow	0.25 L/min
Plasma power	RF power	1.40 kW
Sample introduction	Sampling depth	7.50 mm
	Pump rate	2 rpm
Ion optics	First extraction lens	- 1.00 V
	Second extraction lens	- 169.00 V
	Third extraction lens	- 203.00 V
	Corner lens	- 210.00 V
	Mirror lens left	46.00 V
	Mirror lens right	22.00 V
	Mirror lens bottom	38.00 V
	Fringe bias	- 2.30 V
Quadrupole scan	Scan mode	Peak hopping
	Dwell time	40 ÷ 60 ms
	Points per peak	1
	Scans/Replicate	20
	Replicates/Sample	5

Analyzed isotopes				
Ce138	Eu153	Nd142	Sc45	Th232
Ce140	Gd154	Nd144	Sm147	Y89
Er166	Gd157	Nd146	Sm148	Yb170
Er168	La39	Pr141	Sm152	Yb172

FURTHER DEVELOPMENTS

Further studies are ongoing in order to verify the applicability of the new developed methods and in general of the elemental profiles for studying/demonstrating the geographical origin of saffron. In particular, the developed ICP-MS methods are applied on saffron samples from Greece and Italy obtained from producers to guarantee their origin and authentication, as well as on commercial saffron samples from various harvest years purchased from the market.

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