Characterization and classification of Spanish paprika *(Capsicum annuum* L.*)* by liquid chromatography coupled to electrochemical detection with screen-printed carbon-based nanomaterials electrodes

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ABSTRACT

Screen-printed electrodes based on graphite, carbon nanotubes, carbon nanofibers, and graphene were tested as amperometric detectors for the determination of polyphenols by high performance liquid chromatography (HPLC). The chromatographic performance as well as the obtained sensitivity, detection and quantification limits suggest that carbon

nanofibers modified screen-printed electrode (SPCE-CNF) is the amperometric sensor that provides the best analytical performance. Upon this confirmation, chromatographic data obtained using SPCE-CNF were exploited by means of linear discriminant analysis to successfully characterize and classify 96 Spanish paprika (*Capsicum annuum* L.) samples with protected designation of origin: from La Vera (including sweet, bittersweet and spicy types) and from Murcia (including sweet and spicy types).

Keywords: liquid chromatography, electrochemical detection, carbon-based screen-printed electrodes, polyphenols, paprika (*Capsicum annuum* L.)

1. INTRODUCTION

Paprika is a red powder condiment with a characteristic flavour that comes from drying and grinding certain varieties of red peppers of the genus *Capsicum annum* L. Paprika is one of the most commonly used species due to its double use: in the preparation of cooking dishes for its characteristic aroma and flavour, and in the preparation of sausages due to both its different flavour and its antioxidant power. The two best-known varieties in Spain and the only ones recognized as Protected Designation of Origin (PDO) by the European Commission on Agriculture and Rural Development, come from the region of La Vera in Cáceres (Extremadura) and the region of Murcia [1].

La Vera Paprika is obtained by grinding the totally red fruits of the varieties from the Ocales group (*Jaranda*, *Jariza* and *Jeromín*), and the *Bola* variety [2]. It is characterized by its smoky aroma and taste achieved during the process of drying the peppers using smoke produced with oak and/or holm oak wood. Depending on the paprika taste, they can be classified into three different groups: i) the sweet paprika (*Bola* and *Jaranda*

varieties); ii) the bittersweet paprika (*Jaranda* and *Jariza* varieties); and iii) the spicy paprika (*Jeromín*, *Jariza* and *Jaranda* varieties) [2].

Murcia paprika is obtained by grinding fully red pepper of the *Bola* variety. The first seeds that probably came from America produced elongated and pungent fruits. However, the environmental conditions of this area of Southeast Spain cause that the fruit became more rounded and mostly sweet [3]. As before, those can also be classified depending on its taste.

Paprika is considered as an excellent source of bioactive compounds with beneficial effects on health, such as carotenoids, ascorbic acid and phenolic compounds. Among those, polyphenols are one of the most interesting ones due to their beneficial properties as antioxidant, antidiabetic, antitumor, antimutagenic, and anti-inflammatory [4-6]. In foods, polyphenols contribute to their bitterness, colour, taste, smell and oxidative stability. In the last decade epidemiological studies have shown that diets rich in polyphenols confer a certain protection against the development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [7].

However, these bioactive compounds levels are very variable and can be affected by the red peppers maturity, genotyping and cultivation practices [4,5,8,9]. In this regards, taking into account that polyphenols content seems to be related among others to plant varieties and pre- and post harvests treatments, their distribution in Spanish paprika may be attributed to the different varieties of red pepper [4,5].

A number of analytical methods have been developed for the determination of polyphenols and the characterization of a great variety of plants and fruit-based products, being the use of liquid chromatography (LC) with ultra-violet (UV) detection or coupled to mass spectrometry (LC-MS) the most common one [10-18]. However, electrochemical detection (EC) appears as a very convenient alternative to the former detection modes

taking advantage of its good sensitivity, low cost and the electroactive character of polyphenols [19,20].

In the last years screen-printing microfabrication technology has undergone a great progress allowing the mass production of numerous highly-reproducible single-use screen-printed electrodes (SPEs) with an accessible and low-cost character. SPEs usually include a three electrode configuration (working, counter and reference electrodes) printed on the same strip. SPEs are well-known for their design versatility, low-cost and commercial availability, as well as the possibility of using a great diversity of compositions of printing inks. In addition, SPEs present the advantage that they do not need to be polished as the typical glassy carbon electrodes (GCEs) [21-23].

Thus, the coupling of LC to EC with disposable SPEs represents an attractive option for the determination of polyphenols. Furthermore, the use of SPEs where the working electrode surface has been modified with nanomaterials such as carbon nanotubes (CNTs), carbon nanofibers (CNFs) or graphene (GPH) provides a larger electrodic surface and enhanced electron transfer, with the subsequent improvement in the analytical performance [22].

In the present work, the use of different carbon-based modified SPEs such as carbon (SPCE), multi-walled carbon nanotubes (SPCE-CNT), carbon nanofibers (SPCE-CNF), and graphene (SPCE-GPH) were evaluated for its application as a simple, more sensitive, and less expensive high performance liquid chromatography method with electrochemical detection (HPLC-EC) in the determination of polyphenols. On the other hand, SPCE-CNF as the optimal SPE was applied as amperometric detector for the characterization and classification of 96 Spanish paprika (*Capsicum annuum* L.) samples with PDO by HPLC-EC.

2. EXPERIMENTAL

2.1 Chemicals and samples

All reagents were of analytical grade. For the preparation of the mobile phase methanol (MeOH; Ultra-HPLC Supergradient; PanReac AppliChem, Barcelona, Spain), Milli-Q water (Millipore, Milford, MA, USA), and formic acid (98% PanReac AppliChem, Barcelona, Spain) were employed. Polyphenols, including 4-hydroxybenzoic, homogentisic, gallic, chlorogenic, caffeic, *p*-coumaric, vanillic, syringic, and ferulic acids, and tyrosol, arbutin, syringaldehyde, (-)-epicatechin, ethylgallate, umbelliferone, polydatin, and resveratrol, were supplied by Sigma-Aldrich (St. Louis, MO, USA). Stock standard solutions of each polyphenol were prepared at a concentration of 1000 mg L^{-1} in MeOH. Intermediate working solutions were prepared by appropriate dilution with Milli-Q water.

2.2 Instrumentation

HPLC analyses were performed on an Agilent 1200 series chromatographic system, with a quaternary pump, a vacuum degasser, an autoinjector module, and a personal computer with the Agilent ChemStation software to process the data, all from Agilent Technologies (Palo Alto, CA, USA). The separation was done in a Kinetex C_{18} (100 × 4.6 mm id, particle size 2.6 μm) furnished with a SecurityGuard C_{18} cartridge, both from Phenomenex (Torrance, CA, USA). A mobile phase consisting of 0.1 % formic acid in water (v/v) (solvent A) and MeOH (solvent B) was used to establish the gradient elution. The injected volume was 20 μL and the flow rate was 1 mL min^{-1}.

Used SPEs were obtained from DropSens (Oviedo, Spain), comprising a three-electrode configuration printed on the same strip, with a carbon-ink auxiliary and a silver pseudo-reference electrodes. The working electrode was a carbon-based disk of 4 mm diameter

made of carbon (ref. 110, DS SPCE), graphene (ref. 110GPH, DS SPCE), multi-walled carbon nanotubes (ref. 110CNT, DS SPCE) or carbon nanofibers (ref. 110CNF, DS SPCE). Taking into account that all the considered SPEs can be used for a large set of measurements without noticeable signal deterioration, a single and new SPE unit was used for every working session (up to 1 day).

A HPLC electrochemical cell for SPEs supplied by Dropsens (ref. DRP-HPLCELL) was considered for using the above-mentioned SPEs in the detection process at the optimized potential for each working electrode. The electrochemical flow cell was connected to a μAutolab Type III (EcoChemie, The Netherlands) coupled to a personal computer with GPES version 4.9 data acquisition software (EcoChemie).

2.3 Samples and Sample treatment

A total of 96 samples of paprika were considered in this study, and purchased from different Spanish commercial markets or directly from the paprika production company with the aim to have a representative set of samples according to its origin and type. The paprika samples considered were from Murcia and La Vera (Extremadura) regions, and from three different types: sweet, bittersweet and spicy. 72 samples from the geographical region of La Vera (including 26 sweet, 23 bittersweet, and 23 spicy samples) and 24 samples from the geographical region of Murcia (including 12 sweet, and 12 spicy samples) were analysed. La Vera and Murcia regions were selected because they are the main producers of Spanish paprika.

Sample treatment was then carried out as follows: 0.3 g of paprika sample were weighed and dissolved in 3 mL of a water:acetonitrile (20:80 v/v) solution by agitation in vortex for 1 min. Then, the sample was sonicated for 15 min and centrifuged for 30 min at 4500

rpm, the supernatant extracts were filtered through 0.45 μm nylon filters and stored at -4ºC until analyzed.

2.4 Chemometric analysis

Linear discriminant analysis (LDA) was used to attempt the classification of paprika samples by using specific routines written by the authors in Matlab 7.1 (MathWorks, Natick, MA, USA). Prior to this, a compression step using the windowed slicing integral method [24] was required in order to decrease the dimensionality of the registered chromatograms.

3. RESULTS AND DISCUSSION

3.1 HPLC-EC optimization

Four different carbon-based screen-printed electrodes (SPCE, SPCE-CNT, SPCE-CNF and SPCE-GPH) were considered to evaluate the effect of different substrate electrodes in the determination of polyphenols in paprika samples with EC. For this purpose, firstly, 17 polyphenols were selected based on majority polyphenolic compounds already identified in paprika: 4-hydroxybenzoic, homogentisic, gallic, chlorogenic, caffeic, *p*-coumaric, vanillic, syringic, and ferulic acids, and tyrosol, arbutin, syringaldehyde, (-)-epicatechin, ethylgallate, umbelliferone, polydatin, and resveratrol [4, 25].

Afterwards, the chromatographic conditions were optimized to achieve the separation of the considered polyphenols. The best separation was obtained with the following gradient elution between the two solvents: 0 to 2 min, 95% H_2O; 2 to 4 min, 95% → 75% H_2O; 4 to 12 min, 75% H_2O; 12 to 14 min, 75% → 55% H_2O; 14 to 16 min, 55% H_2O; 16 to 18

min 55% → 5% H_2O; 18 to 20 min, 5% H_2O; 20 to 21 min, 5% → 95%; and 21 to 30 min 95% H_2O.

Finally, the optimal working potential to carry out the amperometric measurements of the polyphenols at each of the considered SPEs was studied in the range from 0.6 to 1.5 V. For each SPE the selection of the optimal working electrode potential was based on the hydrodynamic voltammograms obtained from chromatograms measured at fix potential values (results not shown), taking into account the potential that gave the highest response combined with the best baseline for most of the considered polyphenols. Optimum working potential were 1.3 V, 1.2 V, 1.1 V and 1.0 V *vs.* Ag/AgCl pseudoreference electrode for the SPCE, SPCE-CNT, SPCE-CNF and SPCE-GPH, respectively. Figure 1A shows a representative chromatogram obtained using a SPCE-CNF after injection of 20 μL of a solution containing 10 mg L^{-1} of each polyphenol.

3.2 Sensitivity, limit of detection (LOD), limit of quantification (LOQ), repeatability and reproducibility

Once the optimal HPLC-EC conditions were established, the response of the four considered carbon-based SPEs (SPCE, SPCE-CNT, SPCE-CNF and SPCE-GPH) was characterized. Figure 1B shows a comparison of the chromatograms obtained by EC detection using the considered SPEs at the previously optimized working electrode potential after injection of 20 μL of a solution containing 10 mg L^{-1} of each polyphenol. In all cases, an acceptable separation with reasonably well-defined peaks was obtained employing the selected gradient. However, EC peaks obtained using SPCE-CNF were the ones that mainly exhibited the highest signals with a better definition combined with the best baseline.

The analytical performance of the proposed HPLC-EC method using SPCE, SPCE-CNT, SPCE-CNF or SPCE-GPH was also evaluated for the 17 considered polyphenols. Table 1 summarizes the sensitivities calculated from the slope of the calibration lines of each polyphenol at the four considered SPEs and the correlation coefficients, as well as the limits of detection (LOD) considered as 3 times the standard deviation of the intercept over the slope of the calibration curve of the target compounds, and the limits of quantification (LOQ) calculated as 10 times the previous ratio for all 17 polyphenols. Very good linear responses of the peak area versus concentration were achieved for most of the studied polyphenols at the four tested SPEs, being SPCE-CNF the only SPE that allows the determination of all the considered polyphenols. Concerning sensitivities, it can be seen how the best sensitivities for most of the analysed polyphenols was achieved on SPCE-CNF. The LODs of the 17 polyphenols in the four used SPEs ranged from 0.1 to 14.6 mg L^{-1} depending on both the considered polyphenol and the used SPE (Table 1), and the LOQ varied from 0.4 to 48.8 mg L^{-1} depending again on both the polyphenol and the used SPE (Table 1). In general terms, the LOD and LOQ values provided by SPEs modified with carbon nanomaterials (CNT, CNF and GPH) are lower than those achieved by the conventional unmodified SPCE, which could be associated with the much larger effective surface area that present these modified carbon- based SPEs in comparison to unmodified SPCE [22]. In particular, the SPCE-CNF and the SPCE-GPH are among the tested SPCE those that present the lower LODs and LOQs.

In comparison with previous works, the LODs and LOQs achieved in this study for the determination of polyphenols by HPLC-EC using SPEs are similar or slightly better than those reported by HPLC-UV [26-28], depending again on both the considered polyphenol and the used SPE. However, these LOD and LOQ values are slightly higher compared to those reported by LC-MS techniques [15-16]. It should be pointed out that, to the best of

our knowledge, for HPLC-EC using carbon-based modified SPEs, no previous LOD and LOQ data for polyphenols are available in the literature.

Thus, based on the observed chromatographic performance and the above-discussed calibration data, SPCE-CNF was chosen as the best amperometric sensor to carry out further analyses.

In order to test the repeatability and reproducibility of the selected SPCE-CNF, a solution containing 15 mg L^{-1} of each polyphenol was measured. Repeatability (intra-day) was calculated using the same SPCE-CNF unit for five repetitive measurements whereas reproducibility (inter-day) was estimated on three different days from three different SPCE-CNF units within a series of five repetitive measurements. In the case of repeatability, RSD % values ranging from 1.3 to 8.1 %, depending on the considered polyphenol, were obtained. Good values of reproducibility were also achieved, with RSD % values ranging from 4.0 to 12.8 %, depending again on the considered polyphenol. Repeatability and reproducibility values provided for SPCE-CNF by HPLC-EC at the optimized conditions are of the same order of those reported for voltammetric sensors based on SPCE-CNF [22, 29].

In addition, it has to be taken into account that SPCE-CNF is also an interesting alternative to other traditional GCEs not only for its good chromatographic and analytical performance, but also for the additional advantages associated to the use of SPE, i.e. its low-cost, miniaturized size, the possibility of connection with portable instrumentation, and its ease of use (the SPEs include a three-electrode configuration printed on the same strip that does require the use of any external electrode or any polishing before being used).

3.2 Qualitative analysis

After characterizing the chromatographic profile of the different phenolic compounds and carrying out the full electrochemical characterization, the next step was to attempt the classification of paprika samples employing the same chromatographic conditions. To this aim, the 96 paprika samples extracts were injected at the optimized conditions using a SPCE-CNF and the amperometric responses were registered. Figure 2A shows, as an example, some arbitrary chromatograms obtained for paprika sample extracts from La Vera (including sweet, bittersweet and spicy types) and from Murcia (including sweet and spicy types).

The first consideration to be highlighted is that, as could be expected, a more complex chromatogram, with a higher number of peaks, can be now observed in comparison to the ones obtained for the polyphenol stocks mixtures (Figure 1A *vs.* Figure 2A). The second thing to be said is that, although we would still be able to identify and quantify separately most of them, this was not the aim of the present work, as what we wanted to evaluate is whether or not we could observe a different profile (fingerprint) between the different paprika samples. Thus, from this point on, we focused on the chromatographic fingerprints rather than the individual peaks in order to generate a richer data set to be used for the classification of the paprika samples.

To this aim, the baseline of the registered chromatograms was first corrected and then compressed employing the windowed slicing integral method [24]. This method was chosen as it allows a quicker and more automated way to somehow evaluate the areas of the different peaks, with the advantage that the coefficients vector extracted would be always consistent. In this way, the 1600 data points were compressed down to 184 coefficients. Even though this also meant a larger number of variables for the modelling, this was compensated with the use of a stepwise inclusion method which allowed the removal of the variables that had a lower contribution to the prediction success [30]. From

those, only 41 variables were finally considered by the model to achieve the classification task, which correspond with the vertical grey lines included in Figure 2A. A closer look of these selected sections of the chromatograms (Figures 2B and 2C) confirms that significant differences between the chromatographic profiles obtained from the different paprika sample extracts can be observed. For example, paprika samples from La Vera (including sweet, bittersweet and spicy types) are much more richer in extracted bioactive compounds than those paprika sample from Murcia; whereas spicy paprika samples from both La Vera and Murcia show two characteristic signals at retention times close to 19.5 minutes that could be attributed to some capsaicinoids (confirmed through spike analysis, data not shown), which are the responsible of the characteristic hot taste (pungency) of vegetables [5].

LDA was the chosen pattern recognition method to attempt the classification of paprika samples as it is a supervised method that will specifically seek differences among the different classes, and actually build a qualitative model that later on can be used to classify other samples. The generated 2D and 3D score plots are shown in Figure 3. As can be seen, in Figure 3A, patterns in the figure evidence grouping of the samples that almost separate all the considered classes. Despite the apparent overlapping of some clusters, if we have a look at the 3D plot (Figure 3B), we can see how the overlapping disappears and distinguished clusters were obtained for all the classes.

Analysing more deeply the plot in Figure 3A, we can see how on one side, a clear discrimination between the two regions can be observed; that is, samples belonging to La Vera appearing on the left side of the plot (clusters I-II-III) and samples from Murcia appearing on the right side (clusters IV-V). On the other side, we can see how we were also able to distinguish the different types of paprika; with the spicy ones (clusters I and

IV) appearing on the top side of the plot. Thus, it seems like discriminant function 1 (DF1) mainly relates to samples' origin, whereas DF2 seems to be more related to its type.

Performance of the built model was also numerically assessed in terms of classification rate, sensitivity (the percentage of objects of each class correctly identified) and specificity (the percentage of objects from different classes correctly rejected) [31]. To this aim, the set of samples was divided into two different subsets (train/test) in the ratio 75:25, and the confusion matrix was built (Table 2). As expected from the plot, almost all the samples were correctly classified, with a classification rate of 95.8%. Sensitivity and specificity values, averaged for the considered classes, were 96.7% and 99.0%, respectively.

4. Conclusions

In this work, analytical features of SPCE, SPCE-CNT, SPCE-CNF and SPCE-GPH were compared to each other as amperometric detectors in HPLC-EC. Firstly, the HPLC-EC conditions were optimized for the determination of a mixture of 17 selected polyphenols. At the optimized conditions, all the SPEs gave rise to an acceptable separation being the SPCE-CNF the amperometric sensor that provided the highest and most well-defined signals combined with the best baseline. The LODs and LOQs achieved for the determination of polyphenols ranged from 0.1 to 14.6 mg L^{-1} and 0.4 to 48.8 mg L^{-1}, respectively, depending on both the polyphenol and the used SPE. The best results were obtained with the SPCE-CNF, not only in terms of detection limits, but also in terms of sensitivity. Moreover, SPCE-CNF provided very good values of repeatability and reproducibility, and was successfully used for large sets of measurements without signs of degradation or loss of sensitivity, with the additional advantage that SPEs do not need to be polished nor require any external electrode.

SPCE-CNF was successfully applied as amperometric sensor for the characterization and classification of Spanish paprikas PDOs by HPLC-EC. The discrimination of the different paprika samples has been achieved thanks to the combination of chemometric tools such as linear discriminant analysis with chromatographic techniques coupled to EC detection. This combination allowed proceeding even though no specific compounds (or concentration levels) can be associated to each of the classes, focusing in this way in the overall profile rather than specific compounds. More specifically, in the work presented herein we were able to correctly classify the samples based on its origin and type.

Overall, these features revealed the valuable contribution of the easy, versatile and low-cost HPLC-EC method coupled to disposable nanomaterials modified SPEs not only for the determination of polyphenols but also for characterization, classification and authentication of natural food products.

Acknowledgments

The authors gratefully acknowledge the financial support received from the Spanish Ministry of Economy and Competitiveness under the project CTQ2015-63968-C2-1-P and from the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) under the projects 2017SGR-311 and 2017SGR-310. X. Cetó thanks the support of the Beatriu de Pinós postdoctoral programme of the Government of Catalonia's Secretariat for Universities and Research of the Ministry of Economy and Knowledge.

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Figure captions

Figure 1. (A) Representative HPLC-EC chromatogram of a 10 mg L^{-1} standard solution of the 17 targeted polyphenols under optimal gradient elution conditions. Peak identification as in Table 1; and (B) HPLC-EC chromatogram obtained using SPCE at 1.3 V (a), SPCE-CNT at 1.2 V (b), SPCE-CNF at 1.1 V (c) and SPCE-GPH at 1.0 V (d).

Figure 2. (A) Representative raw chromatograms obtained for certain arbitrary paprika samples extracts: (blue) La Vera spicy, (black) La Vera sweet, (green) La Vera bittersweet, (cyan) Murcia spicy and (red) Murcia sweet. Vertical grey lines correspond to the selected sections of the chromatogram that are included in the final LDA model. (B) and (C) are the enlargements of (A) between 4 to 11min and 15 to 20min, respectively.

Figure 3. (A) 2D and (B) 3D score plots obtained after LDA analysis of the paprika samples chromatograms: (blue ■) La Vera spicy, (white ▼) La Vera sweet, (green ♦) La Vera bittersweet, (cyan ●) Murcia spicy and (red +) Murcia sweet. Additionally, the centroid for each of the classes is also plotted (★). Filled symbols correspond to the samples of the training subset, empty ones to the testing subset.

Table 1. Calibration data of considered mixture of polyphenols on SPCE, SPCE-CNT, SPCE-CNF and SPCE-GPH by using the proposed method. The standard deviations are denoted by parenthesis.

	SPCE				SPCE-CNT				SPCE-CNF				SPCE-GPH			
Peak[a]	**Sensitivity (μA min mg^{-1} L)**	**R^2**	**LOD (mg L^{-1})**	**LOQ (mg L^{-1})**	**Sensitivity (μA min mg^{-1} L)**	**R^2**	**LOD (mg L^{-1})**	**LOQ (mg L^{-1})**	**Sensitivity (μA min mg^{-1} L)**	**R^2**	**LOD (mg L^{-1})**	**LOQ (mg L^{-1})**	**Sensitivity (μA min mg^{-1} L)**	**R^2**	**LOD (mg L^{-1})**	**LOQ (mg L^{-1})**
1	-	-	-	-	-	-	-	-	1.9 (0.3)	0.952	4.5	14.9	2.7 (0.4)	0.958	2.5	8.4
2	9.2 (0.2)	0.999	0.8	2.5	10 (2)	0.923	5.7	19.1	16 (1)	0.993	1.7	5.7	12.2 (0.8)	0.995	1.3	4.4
3	3.0 (0.8)	0.937	8.4	28.1	2.7 (0.2)	0.991	1.9	6.4	3.8 (0.2)	0.994	1.6	5.2	3.1 (0.4)	0.984	2.5	8.3
4	6.1 (0.5)	0.987	2.3	7.7	9.6 (0.4)	0.995	1.0	3.5	7.6 (0.4)	0.993	1.2	4.0	7 (1)	0.947	2.1	6.9
5	6.0 (0.4)	0.992	1.8	6.1	8.2 (0.4)	0.991	1.4	4.6	6.8 (0.3)	0.994	1.1	3.8	5.7 (0.4)	0.988	1.0	3.2
6	0.8 (0.2)	0.929	5.5	18.3	1.1 (0.1)	0.970	3.5	11.6	2.0 (0.5)	0.819	6.8	22.7	0.6 (0.1)	0.970	3.4	11.3
7	2.2 (0.1)	0.995	1.4	4.7	2.8 (0.2)	0.990	2.0	6.7	3.3 (0.2)	0.989	1.5	5.0	2.9 (0.3)	0.971	2.0	6.8
8	2.3 (0.7)	0.845	8.5	28.4	2.6 (0.9)	0.807	9.7	32.4	4.1 (0.6)	0.963	3.9	12.9	2.5 (0.3)	0.989	2.0	6.7
9	3.6 (0.2)	0.992	1.8	6.0	3.4 (0.6)	0.938	5.1	17.1	6.9 (0.4)	0.993	1.7	5.8	9 (1)	0.976	3.1	10.2
10	3.3 (0.2)	0.991	1.9	6.4	2.6 (0.4)	0.944	4.8	16.1	4.6 (0.3)	0.991	1.4	4.7	-	-	-	-
11-12	7.1 (0.3)	0.996	1.3	4.3	8.0 (0.7)	0.986	2.3	7.8	9.7 (0.4)	0.995	1.0	3.4	7.3 (0.6)	0.986	1.4	4.8
13	5.9 (0.8)	0.969	3.6	11.9	8 (2)	0.919	5.9	19.7	12 (1)	0.981	1.7	5.5	3.3 (0.1)	0.998	0.5	1.5
14	7.0 (0.1)	0.999	0.5	1.8	9.0 (0.8)	0.984	2.6	8.6	9.6 (0.2)	0.999	0.4	1.5	0.7 (0.2)	0.869	4.6	15.4
15	1.4 (0.6)	0.832	14.5	48.5	2.1 (0.5)	0.939	8.3	27.5	3.7 (0.6)	0.926	4.1	13.6	5.37 (0.04)	1.000	0.1	0.4
16	2 (1)	0.830	14.6	48.8	-	-	-	-	3.5 (0.3)	0.990	3.2	10.6	2.7 (0.4)	0.979	2.9	9.6
17	4.1 (0.2)	0.993	1.7	5.6	5.5 (0.3)	0.992	1.7	5.8	8.8 (1.4)	0.952	4.5	14.9	0.92 (0.03)	0.997	0.6	1.9

[a] Peak identification: 1) arbutin; 2) gallic acid; 3) homogentisic acid; 4) tyrosol; 5) 4-hydroxybenzoic acid; 6) chlorogenic acid; 7) vanillic acid; 8) caffeic acid; 9) (-)-epicatechin; 10) syringic acid; 11-12) ethylgallate / syringaldehyde; 13) umbelliferone; 14) *p*-coumaric acid; 15) ferulic acid; 16) polydatin; and 17) resveratrol.

Table 2. Confusion matrix built according to the classes assigned by the LDA model to the samples of the testing subset.

	V Sp [b]	*V Sw* [b]	*V Bs* [b]	*M Sp* [b]	*M Sw* [b]
V Sp [a]	5	1	0	0	0
V Sw [a]	0	4	0	0	0
V Bs [a]	0	0	6	0	0
M Sp [a]	0	0	0	4	0
M Sw [a]	0	0	0	0	4

[a] Expected; [b] Found. M: *Murcia*; V: *La Vera*; Sw: *sweet*; Bs: *bittersweet*; Sp: *spicy*.

Figure 1

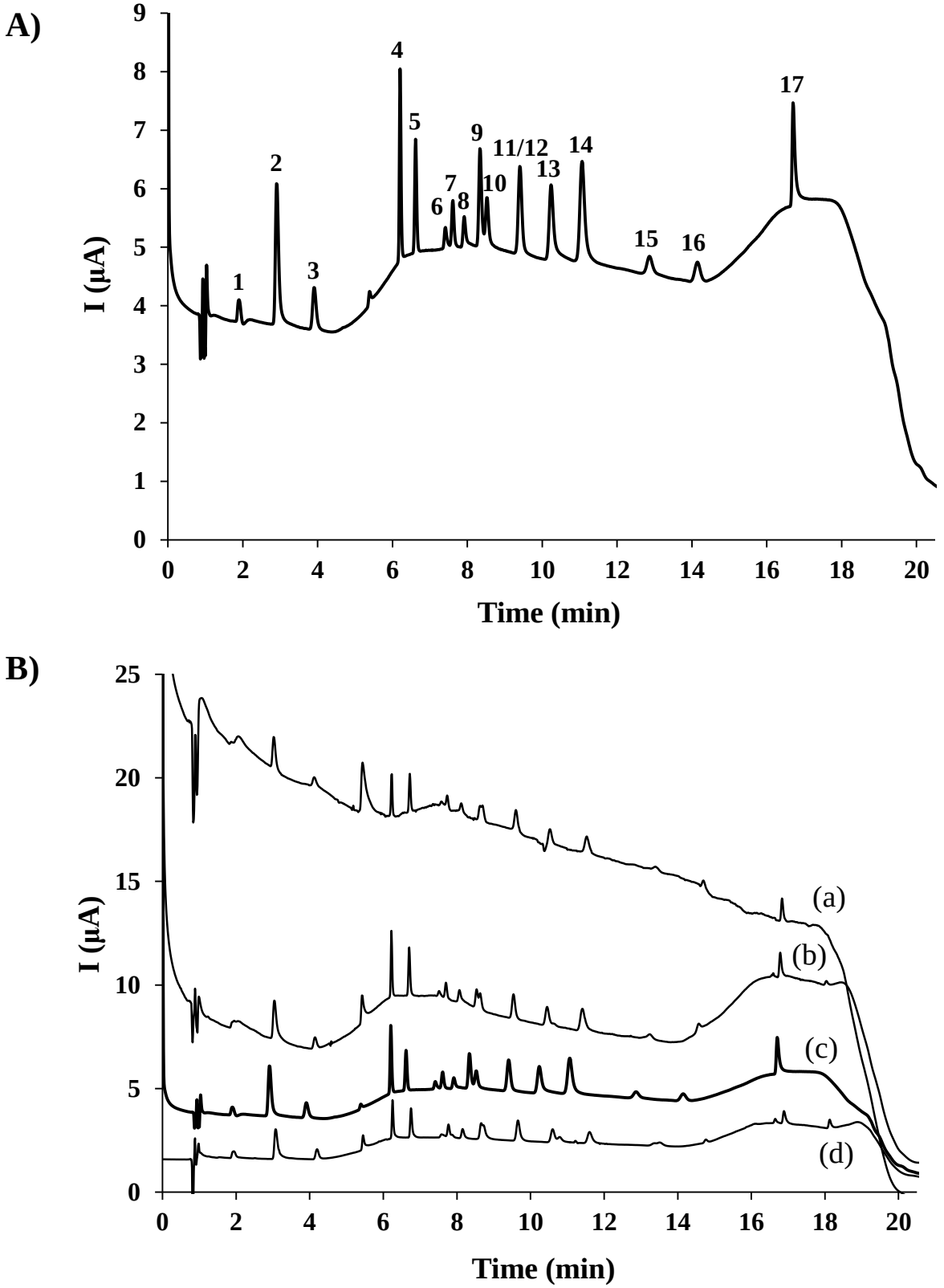

A)
I (µA)
Time (min)
1
2
3
4
5
6
7
8
9
10
11/12
13
14
15
16
17
B)
I (µA)
Time (min)
(a)
(b)
(c)
(d)

Figure 2

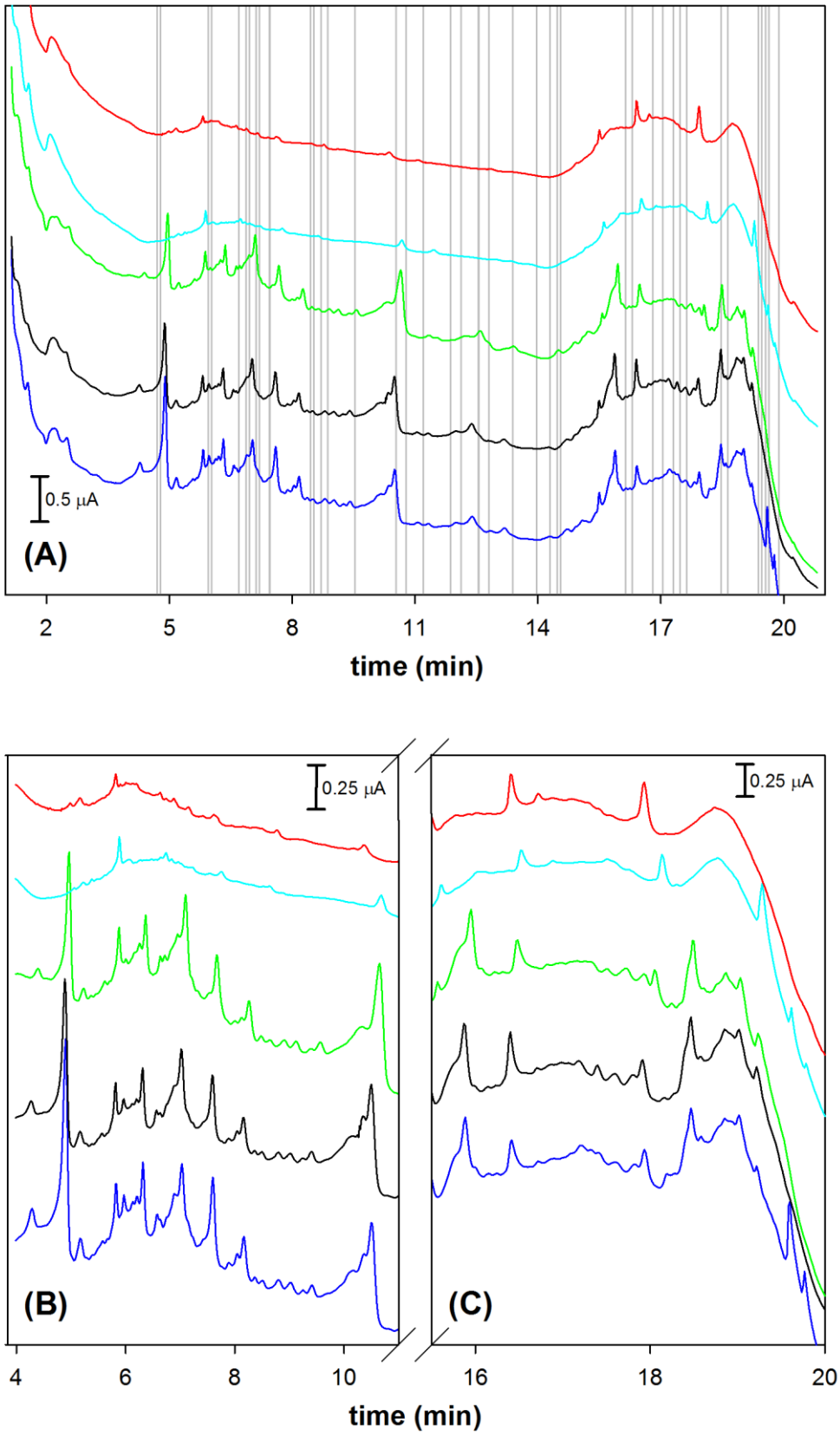
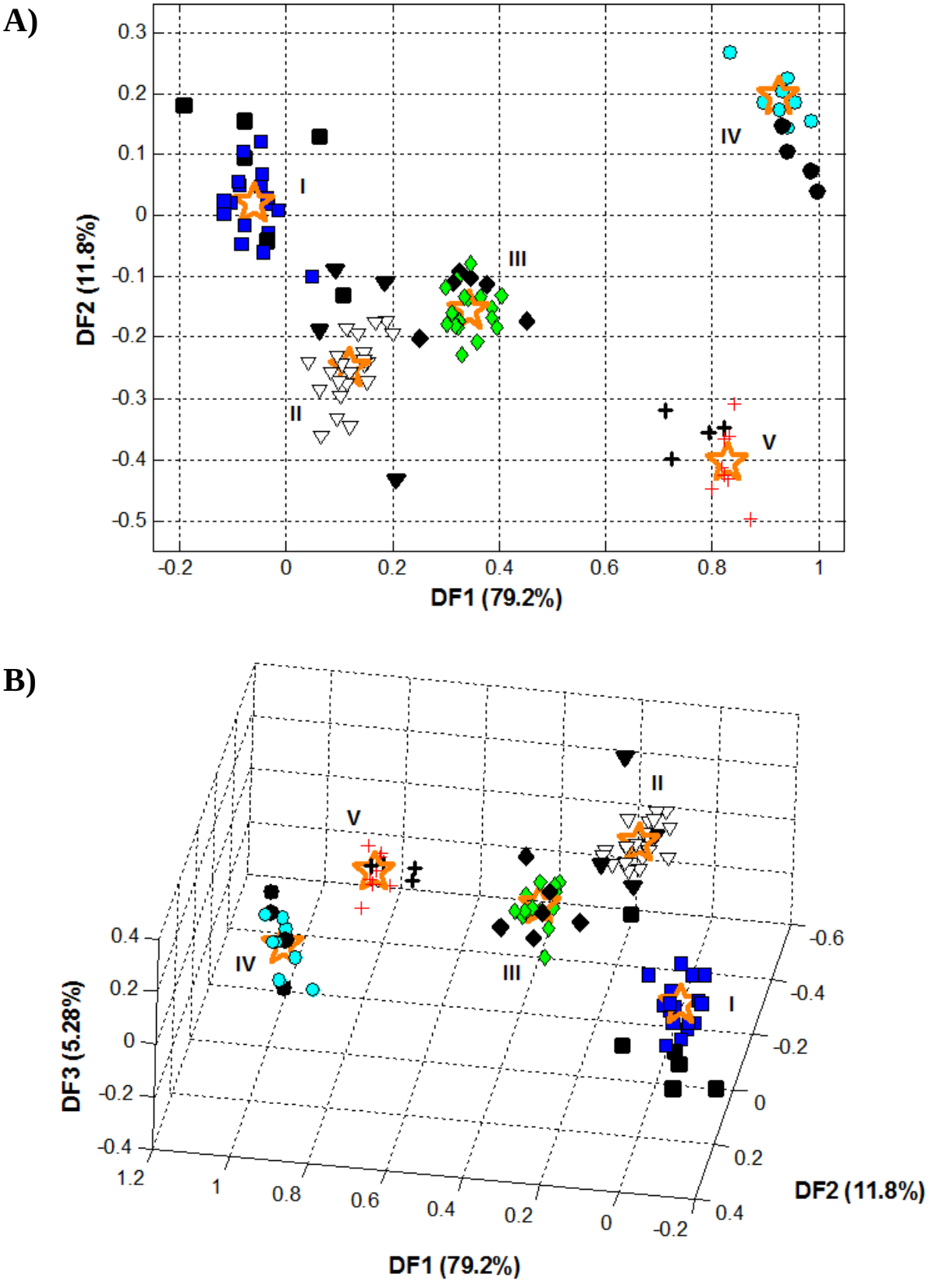
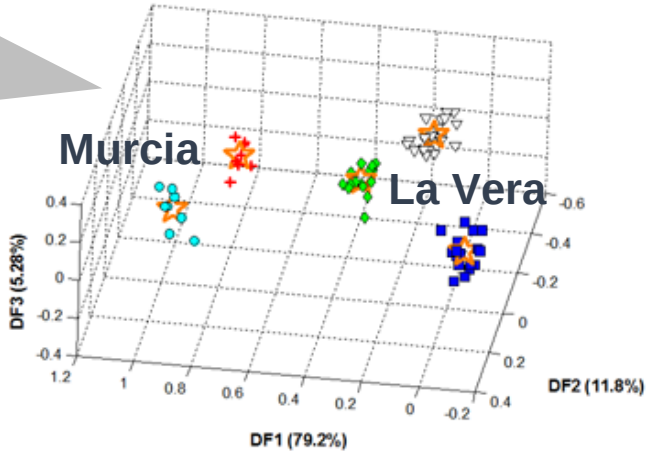

0.5 µA
(A)
2
5
8
11
14
17
20
time (min)
0.25 µA
(B)
0.25 µA
(C)
4
6
8
10
16
18
20
time (min)

Figure 3


A)
DF2 (11.8%)
DF1 (79.2%)
I
II
III
IV
V
B)
DF3 (5.28%)
DF1 (79.2%)
DF2 (11.8%)
I
II
III
IV
V


Murcia
La Vera
DF3 (5.28%)
DF1 (79.2%)
DF2 (11.8%)