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Use of whole electrophoretic profile and chemometric tools for the differentiation of three olive oil qualities

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ABSTRACT

Olive oil is a liquid fat obtained from olives (the fruit of *Olea europea*). It is one of the most important ingredients of the Mediterranean diet, due to its health benefits. Depending on its quality, olive oil can be classified as extra virgin (EVOO), virgin (VOO) and lampante (LOO). Currently, an official method defines the quality parameters of the different categories of olive oil using different analytical techniques and a sensory analysis through a Panel Test. However, the evaluation of olive oil quality by tasting panels has some drawbacks, such as the subjectivity of the analysis and the lack of panels accredited outside Spain. For this reason, fast, simple and reliable analytical methods, which can differentiate the categories of olive oil are needed.

In this work, the potential of a method using capillary electrophoresis (CE) with ultraviolet (UV) detection as an additional method to the ones already included in the official method has been investigated. The separations were performed using a 45 mM sodium tetraborate buffer (pH 9), and the analytes were measured at 200 nm.

For chemometric model construction, the whole electrophoretic profile was processed. It required a correction of migration time shift, which was solved using two internal standards (naphthol and benzoic acid), and a correction of the drift baseline. The results obtained after applying the method to 130 olive oil samples are very promising, achieving

40 success rates above 91%. Finally, the use of all information found in the electropherogram
41 was compared with that based on the selection and integration of only some peaks.
42
43 **Keywords**: Capillary electrophoresis; olive oil classification; migration time shift
44 correction; chemometric models

1. Introduction

During the last few decades, consumers have shown a great interest in knowing the quality of different food products with high added value, among which the extra virgin olive oil (EVOO) stands out. The excellent quality of the EVOO is the result of a careful process that starts in the tree and ends in the bottle. It requires an exhaustive control of each production stages and factors that potentially affect the shelf life of the final product.

The International Olive Council and the European Community have defined the quality of olive oil in terms of physico-chemical parameters including free fatty acids, peroxide values and UV specific extinction coefficients (K_{232} and K_{270}) among others analysis and finally a sensory assessment through a Panel Test. The classification provided by the Panel Test is established based on two criteria: presence or absence of the fruity attribute and the total intensity of defects. This classification establishes three categories known as EVOO (positive fruited, null defect median), virgin, VOO (positive fruity, defect median between 0 and 3.5) and lampante, LOO (fruited null, median defect greater than 3.5). However, this assessment is currently very questionable, since this methodology has some drawbacks, such as the subjectivity of the analysis and the lack of accredited panels in some countries. In addition, fraud alerts in the olive oil market are becoming more frequent. For these reasons, in recent years researchers have moved their attention to the search for alternative or complementary analytical methods to the Panel Test.

To date, most of the analytical methods proposed are based on the study of chemical compounds responsible for odour sensory descriptors, i.e. volatile compounds present in olive oil samples. Here, Gas Chromatography (GC) acquires a great importance and it has been proposed coupled to several detectors. Specifically, solid-phase microextraction followed by GC coupled to Flame Ionization Detector (FID) [1], Atmospheric Pressure Chemical Ionization source in combination with GC coupled to Hybrid Quadrupole Time-of-flight Mass Spectrometry (MS) [2], and Headspace - GC coupled to Ion Mobility Spectrometry (IMS) [3] have been investigated to discriminate the different olive oil categories (EVOO, VOO and LOO).

Moreover, it is important to take into account that sensory analysis is not only based on those compounds responsible for smell, the tasters assign the olive oil category after their perceptions on nose (odour) and mouth (taste). Therefore, the search for analytical

76 methods that allow the determination of non-volatile compounds is also crucial for the
77 establishment of an alternative or complementary method to the Panel Test. For this
78 reason, in this work, an analytical method based on Capillary Electrophoresis (CE) with
79 UV detection for the discrimination of EVOO, VOO and LOO has been proposed, since
80 this analytical technique has shown a great potential to guarantee the authenticity of olive
81 oil and detect its adulteration with other oils [4,5]. CE is a simple, versatile and low-cost
82 technique, providing a short time of analysis and high separation efficiency [5], which
83 makes it an attractive option to solving this food problem.

84 Furthermore, it is important to highlight that most researchers focus on the identification
85 of chemical compounds of olive oil to assign its category. However, this assignment could
86 involve greater fraud, since the olive oil can be easily adulterated by including or
87 eliminating those compounds responsible for the discrimination. In addition, the taster
88 decides based on a set of sensations, i.e. a mixture of compounds at different
89 concentrations. Therefore, in this work, the use of the entire electrophoretic profile for
90 the classification of oils has been proposed.

91 A disadvantage of CE is its poor repeatability of migration times, which is caused by the
92 fluctuations of electroosmotic flow, among other factors. It must be corrected to carry out
93 the comparison of electrophoretic profiles. To prevent this problem, relative migration
94 times or various capillary coatings are used [6]. However, the shift of migration times is
95 non-proportional, and the use of various capillary coatings is expensive. Herein, the use
96 of two internal standards (ISs) is proposed to correct the misalignment between analyses.

97 2. Materials and methods

98 *2.1. Reagents*

99 All the reagents used in this work were of analytical grade and solvents were HPLC-
100 grade. Sodium tetraborate, sodium hydroxide (NaOH), hexane and hydrochloride acid
101 (HCl) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The polyphenols
102 sinapinic acid (SIA), p-coumaric acid (p-COU), gentistic acid (GTA) and oleuropein
103 (OLE) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Vanillic acid (VAN),
104 syringic acid (SYA), ferulic acid (FA), were from Fluka (Buchs, Switzerland). Stock
105 solutions of 1 mg mL^{-1} were prepared using a mixture of purified water with HPLC-grade
106 methanol (Scharlau, Gato Perez, Spain) (1:1 v/v). Mix solutions at 10 mg L^{-1} were

107 prepared daily by diluting the stock solutions with methanol/ water (1:1 v/v). The internal
108 standard (IS) solutions 1-naphthol and benzoic acid were also supplied by Sigma-Aldrich.
109 Stock solutions of 1 mg mL^{-1} were prepared using a mixture of purified water/methanol
110 (1:1 v/v) (Scharlau).

111 Solutions from 1 M HCl and 0.1 M NaOH were used to condition and clean the capillary.
112 Water was used from a Milli-Q® apparatus from Millipore (Bedford, MA, USA). All
113 samples were filtered through a Nylon membrane of 0.45 µm pore size (Analysis Vinicos
114 S.L, San Juan, Spain) before analysis.

115 *2.2. Samples*

116 A total of 130 olive oils samples (40 EVOO, 40 VOO and 50 LOO) were supplied by the
117 ''*Interprofesional del Aceite de Oliva Español"* (Spain), the ''*Agencia para el Aceite de*
118 *oliva del Ministerio de Agricultura, Alimentación y Medio Ambiente*" (Spain), and the
119 official control services from the ''*Consejería de Agricultura, Pesca y Desarrollo Rural*
120 *de la Junta de Andalucía*" (Spain). The samples were stored in bottles at - 20 °C until
121 analysis.

122 *2.3. Instrumentation and software*

123 Separations were performed on a Beckmann P/ACE MDQ Capillary Electrophoresis
124 System (Palo Alto, CA, USA), equipped with a diode array detector and using a fused-
125 silica capillary (Beckman Coulter) of 75 µm inner diameter, a total length of 50.2 cm and
126 an effective separation length of 40 cm.

127 In addition, a High-Speed Centrifuge with Microprocessor Control J.P. Selecta, S.A. from
128 Abrera (Barcelona, Spain), and a vortex from Heidolph (Schwabach, Germany) were used
129 during the sample treatment. pH-meter with a resolution of ±0.01 pH unit (Crison model
130 pH 2000, Barcelona, Spain) was also used.

131 Data acquisition was accomplished using a computer equipped with Beckmann 32 Karat
132 software. The raw data were translated into ASCII text files and Microsoft Excel 2016
133 was used for the visualization and alignments of the electropherograms. Data processing
134 was carried out using Matlab (The MathWorks, Natick, MA, USA 2002), PLS Toolbox
135 5.5 (Eigenvector Research, Inc., Manson, WA, USA) and STATGRAPHICS Centurion
136 XV (StatPoint Technologies Inc., Warrenton, VA, USA).

137 *2.4. Sample treatment*

138 Sample treatment was based on a liquid-liquid extraction (LLE) using a mixture of
139 methanol/water (1:1, v/v) as extraction solvent. Before sample treatment, olive oil
140 samples were defrosted at room temperature in the dark for 90 minutes. Then, 1 g of the
141 tempered oil sample was placed into a 5 mL Eppendorf tube. 1-mL of hexane was added
142 to remove fats and 950 µL of a mixture of methanol/water (1:1 v/v) was added for the
143 LLE. In addition, 50 µL of an IS mixture (400 µg mL^{-1}) of naphthol and benzoic acid in
144 methanol/water (1:1 v/v) was also added. The mixture was mixed by hand and vortexed
145 for 30 s on medium strength. After 15 min the two phases were separated without
146 centrifugation and upper oil/hexane phase was removed with a syringe and discarded.
147 After this process, the lower aqueous phase (300 µL aprox.) was transferred to a vial with
148 insert for the CE separation.

149 *2.5. CE-UV analysis*

150 Separations were carried out at 20 kV and 25 ºC, at these conditions the average current
151 was 50 µA. Samples were injected using hydrodynamic mode at 0.5 psi for 8 s.
152 Electropherograms were recorded at 200 nm, using normal polarity. The running buffer
153 consisted of 45 mM sodium tetraborate at pH 9.0.

154 Prior to first use, the capillary was conditioned by rinsing with 1 M HCl for 5 min, 0.1 M
155 NaOH for 10 min and milli-Q H_2O for 5 min. The capillary was prepared for daily use by
156 rinsing with 0.1 M NaOH for 5 min, milli-Q H_2O for 5 min and separation buffer for 10
157 min. Before each analysis, the capillary was flushed with 0.1 M NaOH (1 min), milli-Q
158 H_2O (1 min) and separation buffer (2 min).

159

160 **3. Results and discussion**

161 *3.1. Optimization of CE-UV parameters*

162 As polar compounds were extracted during the sample treatment, the optimization of CE-
163 UV was made using a phenolic mixture of seven polyphenols at 10 mg L^{-1} dissolved in
164 methanol/water (1:1 v/v) as representative compounds, since they have polar nature and
165 are present in olive oil. This optimization was carried out on the basis of several papers

166 [7-10] which dealt with the separation of polyphenols, and the parameters related to buffer
167 (buffer nature, concentration and pH), voltage, detection wavelength were also examined.

168 Firstly, the electrolyte nature was selected. The most common buffers to separate
169 polyphenols by CE-UV are boric acid [7,8] and sodium tetraborate [9,10], thus these
170 buffers were investigated in this work. The best results for the separation of the
171 polyphenols mixture selected and for the separation of all the compounds detected in the
172 olive oil samples were obtained using the sodium tetraborate buffer. Then, different
173 concentrations of buffer (25, 45 and 65 mM) and different pH values (8.5, 9.0, 9.5, 10,
174 10.5 and 11.5) were tested. Desired pH values were obtained by adding 1 M NaOH
175 solution to the buffer. Higher tetraborate concentrations, lead to longer analysis times and
176 improved the peak separation. Lower buffer concentrations lead to faster analysis times.
177 The 45 mM buffer represented the best compromise between separation quality and
178 analysis time. Regarding pH, the best separation was obtained using pH 9.0. Therefore,
179 the 45 mM sodium tetraborate buffer with pH 9.0 was selected as optimum.

180 The effect of the applied voltage was studied using the optimized buffer. By increasing
181 the applied voltage, shorter migration times and better separation efficiencies. Limited by
182 the device, 20 kV was the maximum voltage used. Fig. 1 shows the electropherogram
183 obtained at optimized conditions for the separation and detection of the seven selected
184 polyphenols. The elution order was OLE, SIA, GTA, SYA, FA, p-COU and VAN and it
185 can be seen they were obtained in less than 15 min.

186 The aim of this work was not the identification by CE-UV of the polar compounds
187 extracted from olive oil samples

188 , but to obtain a good separation and intensity of oil olive detected peaks which could be
189 used as markers to differentiate the EVOO, VOO and LOO samples. Therefore, the
190 optimized method was applied to analyse the real olive oil samples, obtaining an
191 acceptable electrophoretic profile in term of resolution and sensitivity for the subsequent
192 data processing.

193 *3.2. Optimization of sample treatment*

194 The sample treatment was optimised in order to extract most of the polar compounds from
195 the olive oil sample. The effect of extraction time (between 10 s and 2 min) and the shake

196 mode (vortex at middle and strong intensities) were tested. The vortex at middle
197 intensities for 30 s was selected as optimum, as strong intensity caused emulsions in the
198 sample and a longer time did not improve the results.

199 In addition, different modes to obtain the separation between phases were also studied:
200 (a) centrifugation at 9000 rpm varying the time between 30 s and 5 min, and (b) no
201 centrifugation, mixtures formed two layers over 15 min. In this case, centrifugation was
202 not necessary, which simplifies the extraction procedure making its automation easier.

203 *3.3. Characterization of CE-UV method*

204 In order to check the suitability of the proposed method for the olive oil classification,
205 precision was evaluated in terms of repeatability (intraday precision) and intermediate
206 precision (interday precision), expressed as relative standard deviation percent (% RSD).
207 Repeatability was assessed by application of the whole procedure on the same day to nine
208 olive oil samples (experimental replicates). Each sample was injected twice in the CE-
209 UV system (instrumental replicates). Intermediate precision was evaluated with a similar
210 procedure, with nine samples analyzed on different days. For the evaluation of the
211 precision, two of the unknown compounds of olive oil detected by CE-UV were selected
212 (one in the initial part of the electropherogram and another in the final part). In all the
213 cases, acceptable RSD (< 15%) were obtained for area values, although a very high
214 variation of the migration time was observed, which hinders the subsequent data
215 processing. In addition, this variability of the migration time was not maintained
216 throughout the analysis, and the displacement observed in the initial part of the
217 electropherogram was very different from that observed in the final part. In order to
218 correct this variability, the addition of an IS was investigated.

219 *3.4. Selection of internal standard*

220 In order to improve the precision of method in terms of migration time, the addition of an
221 IS was investigated. Specifically, four of the most common ISs used for polyphenol
222 determination by CE-UV were explored in this work: salicylic acid [11], epicatechin [7],
223 naphthol [12] and benzoic acid [13]. The best results were found using naphthol and
224 benzoic acid, as they do not co-elute with any peak detected in olive oil samples. In
225 addition, these ISs showed very different migration time: naphthol (5.5 min) and benzoic

226 acid (11.5 min) and, they could therefore be used to correct the peak misalignment of the
227 different regions of electropherogram.

228 *3.5. Data processing*

229 *3.5.1. Alignment and baseline correction*

230 Firstly, a pre-processing of data, consisting of two alignments and a baseline correction
231 was carried out.

232 The peak misalignment in CE is due to changes in pressure, temperature or external and
233 uncontrolled conditions and therefore it must be corrected in order to guarantee a certain
234 degree of success in the following chemometric analysis. As above-mentioned, the shift
235 of migration time through the CE separation was not stable, so two different ISs are
236 needed to correct this fluctuation: one appearing at the beginning (naphthol) and benzoic
237 acid appearing at the end of the electropherogram.

238 Fig. 2a shows the peak misalignment between analysis and as can be seen in Fig. 2b,
239 when electropherogram was aligned using the naphthol as IS, the peaks that appeared
240 before 8.5 min were correctly aligned, but the peak misalignment was not corrected after
241 8.5 min. On the contrary, if the benzoic acid was used as IS, the peaks appearing in the
242 second region of the electropherogram (after 8.5 min) were aligned correctly, while the
243 peaks misalignment were not corrected before 8.5 min (Fig. 2c). For this reason, both ISs
244 are necessary to correct the shift of migration time. Naphthol was used to align the region
245 between 0 and 8.5 min and benzoic acid to align the region after 8.5 min (Fig. 2d).

246 Once the data were aligned, it was necessary to correct the baseline by subtracting the
247 mean value of the background (an empty section of peaks, between 2 and 2.5 min). In
248 addition, some of analysis showed a drift baseline problem, i.e. the baseline was deviated
249 from the horizontal line. Drift is a parameter related to the detector signal fluctuation and
250 it is usually associated with the detector heat-up. In order to correct this phenomenon, the
251 slope of deviation was calculated between 2.5 and 13.0 min (end of electropherogram,
252 since after this time no peaks appear). The calculated slope was multiplied for each time
253 data and the result was subtracted from its corresponding signal data (Signal
254 $corrected_i$=signal-slope*$time_i$). Fig. 3 shows an example of drift and baseline correction.

255 *3.5.2. Chemometric models for olive oil classification*

256 The pre-processed electropherograms (from 2.5 min to 13.0 min) were used to obtain
257 chemometric models for olive oil classification. Initially, individual PCA for each
258 category using auto-scaled data and a confidence interval of 95 % was carried out, to
259 detect possible outliers. In this case, six olive oil samples were detected (two EVOO, one
260 VOO and three LOO). These samples showed a different behaviour to the rest of the
261 category, so that they could be either experimental errors or that their category had been
262 erroneously assigned by the sensorial analysis. Notice that olive oil samples analysed by
263 CE were only tested by one official Panel Test, and it is a not error-free procedure. For
264 this reason, these samples were eliminated and not included during the model
265 construction.

266 Then, data were split into two subsets. The chemometric model was built using 80% of
267 the analysed samples (training set) and the remaining 20% was used for their further
268 validation (validation set).

269 For the construction of chemometric models, a non-supervised Principal Component
270 Analysis (PCA) with a variance coefficient (VC) of 99 % was carried out to reduce
271 dimensionality and extract the most relevant information. In this case, the data set was
272 reduced to 61 principal components. Subsequent, Linear Discriminant Analysis (LDA)
273 was used as a supervised linear projection technique to incorporate class information into
274 the model and ascertain if the samples are grouped in separated clusters. Fig. 4 shows the
275 PCA-LDA models for three categories of olive oil and, as can be seen, the separation
276 between them was more than evident.

277 Finally, k-nearest neighbour method (k-NN), using k=3, was applied to the validation set
278 to obtain the percentage of samples correctly classified. The validation rate was 91.3 %.
279 Just one EVOO sample was classified as VOO and one VOO sample as LOO (Table 1).

280 *3.5.3. Comparison with the results obtained using peak integration*

281 As it was demonstrated, the chemometric models built using the whole electrophoretic
282 profile are very successful. However, the processing of all the data obtained in the
283 analysis is not the most common strategy, and it is usually decided to quantify certain
284 peaks. This section compares the results obtained with this strategy.

285 Fig. 5 shows the electropherograms obtained for an olive oil sample and the selection of
286 19 individual signals to carry out the chemometric treatment. It should be noted that the
287 manual integration was not a simple task due to the misalignment and overlapping of
288 some peaks.

289 It was not possible to find visual differences between categories, since the variability
290 within the category was very high. After carrying out the peak integration, the statistical
291 Tukey test was performed. The Tukey test is a single-step multiple comparison method
292 to find attributes with significant difference in different groups of samples. The results
293 are shown in Table 2.

294 The Tukey test shows that peaks 2, 3, 4, 15, 16 and 17 would be useful to differentiate
295 EVOO samples from the other two categories; while peaks 6, 7 and 10 could be used to
296 identify LOO samples. In addition, the peak 9 would allow the differentiation between
297 the three types of olive oils. This compound could correspond to a positive attribute, since
298 it showed a higher concentration in EVOO samples and smaller concentration in LOO
299 samples.

300 However, as above-mentioned, a great variability within the category was found. It can
301 be easy observed in the standard deviation values shown in Table 2. Consequently, the
302 establishment of intensity/area limits, that could help to identify olive oil samples, was
303 not possible and a chemometric model was built using all the integrated peaks. It would
304 simulate the sensorial analysis, where the taster decides, based on a set of flavours and
305 not a single marker or compounds.

306 Similar to the previous section, an individual PCA for each category using auto-scaled
307 data and a confidence interval of 95 % was carried out, to detect possible outliers. In this
308 case, five olive oil samples were detected (one EVOO, two VOO and two LOO). After
309 the removal of outliers, data were split into two subsets: training set (80% of samples) for
310 chemometric model construction and validation set (20% of samples).

311 The PCA-LDA model is shown in Fig. 6 and, as can be seen, LOO samples were grouped
312 while the AOVE and VOO appear intermixed.

313 Finally, k-NN (using k=3) was also applied to validate the model and results are shown
314 in Table 3. The success rate was 69.6%, demonstrating that for the classification of olive

315 oil samples using LLE-CE-UV, the data processing and the construction of chemometric
316 models should be carried out using the whole electrophoretic profile.

317 **4. Conclusions**

318 In this work the potential of the CE-UV for the characterization of olive oil according to
319 its quality has been demonstrated. This method involves a simple liquid-liquid extraction
320 of the polar compounds using as an extractant methanol/water and allowing the analysis
321 and characterization of an oil sample in approximately 30 min.

322 It has been shown that the use of only certain markers or peaks present in the
323 electropherogram is not enough to obtain a good classification method, since there is an
324 error factor greater than 30%. However, using all the information collected in the
325 electropherogram, a classification success of 91% was obtained. Therefore, this CE-UV
326 method could be presented as a new potential method to be included in the official method
327 of analysis to classify olive oil categories.

328 In addition, the information of the polar compounds determined by CE-UV (detected in
329 the mouth by the taster) would be united with the information of the volatile compounds
330 determined by GC-IMS or GC-MS (detected in nose of the taster). Both CE and GC could
331 be included in the list of techniques already used to differentiate EVOO, VOO and LOO
332 increasing the percentage of success in the classification.

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337 **Table 1.** Validation matrix by k-NN using the electropherogram profile

		Actual classes			
		EVOO	VOO	LOO	
Predicted classes	EVOO	6	0	0	**Success 91.3%**
	VOO	1	6	0	
	LOO	0	1	9	

338
339

340 **Table 2.** Average and standard deviation of peak area including results of Tukey test

Peak	EVOO	VOO	LOO
1	310 ± 309^{a}	493 ± 362^{b}	$336 \pm 359^{a,b}$
2	1960 ± 1529^{a}	700 ± 362^{b}	539 ± 371^{b}
3	645 ± 400^{a}	454 ± 253^{b}	397 ± 202^{b}
4	13 ± 46^{a}	180 ± 249^{b}	104 ± 120^{b}
5	6337 ± 3740^{a}	$5346 \pm 4770^{a,b}$	3307 ± 3211^{b}
6	14189 ± 8189^{a}	12027 ± 9481^{a}	2349 ± 2746^{b}
7	382 ± 308^{a}	339 ± 206^{a}	124 ± 159^{b}
8	353 ± 224^{a}	289 ± 221^{a}	275 ± 291^{a}
9	1550 ± 1082^{a}	1025 ± 615^{b}	553 ± 541^{c}
10	10245 ± 6520^{a}	10526 ± 5106^{a}	6514 ± 5283^{b}
11	210 ± 214^{a}	191 ± 179^{a}	266 ± 288^{a}
12	1252 ± 767^{a}	964 ± 910^{a}	1018 ± 885^{a}
13	305 ± 269^{a}	489 ± 449^{b}	$378 \pm 319^{a,b}$
14	517 ± 414^{a}	577 ± 375^{a}	527 ± 495^{a}
15	737 ± 321^{a}	445 ± 355^{b}	345 ± 449^{b}
16	224 ± 236^{a}	21 ± 70^{b}	10 ± 50^{b}
17	2239 ± 2151^{a}	790 ± 411^{b}	781 ± 507^{b}
18	138 ± 151^{a}	108 ± 155^{a}	113 ± 210^{a}
19	0 ± 0^{a}	3 ± 16^{a}	27 ± 146^{a}

341 a, b, c superscripts represent different groups of classification for a specific compound
342

343

344 **Table 3.** Validation matrix by k-NN using peak integration

		Actual classes			
		EVOO	VOO	LOO	
Predicted classes	EVOO	6	1	0	**Success 69.6%**
	VOO	0	3	2	
	LOO	1	3	7	

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348 **Figures**

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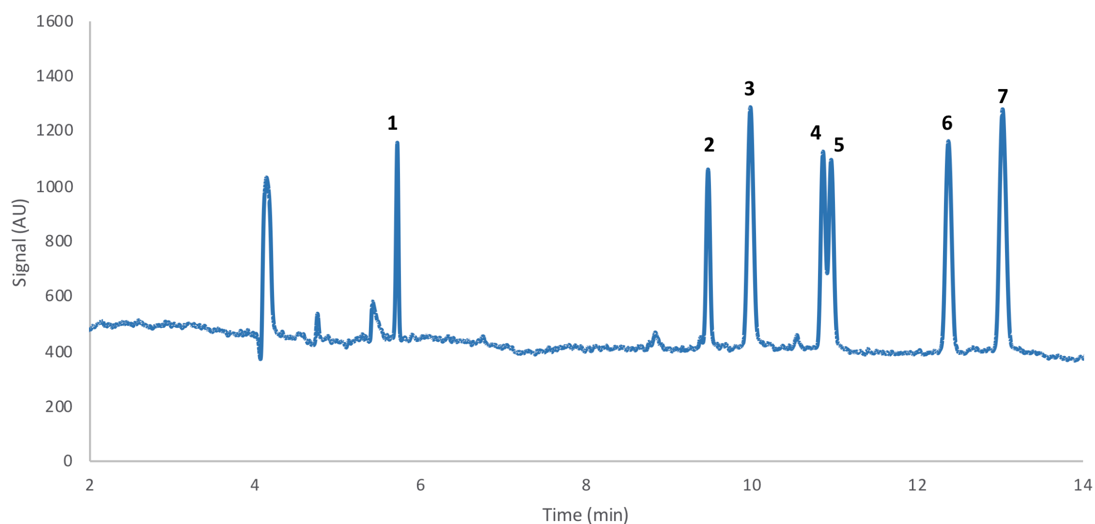
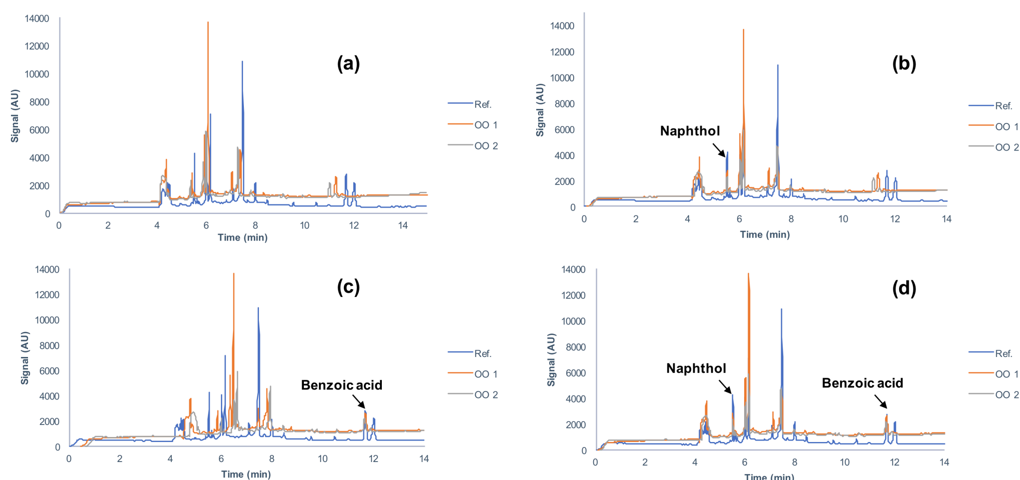


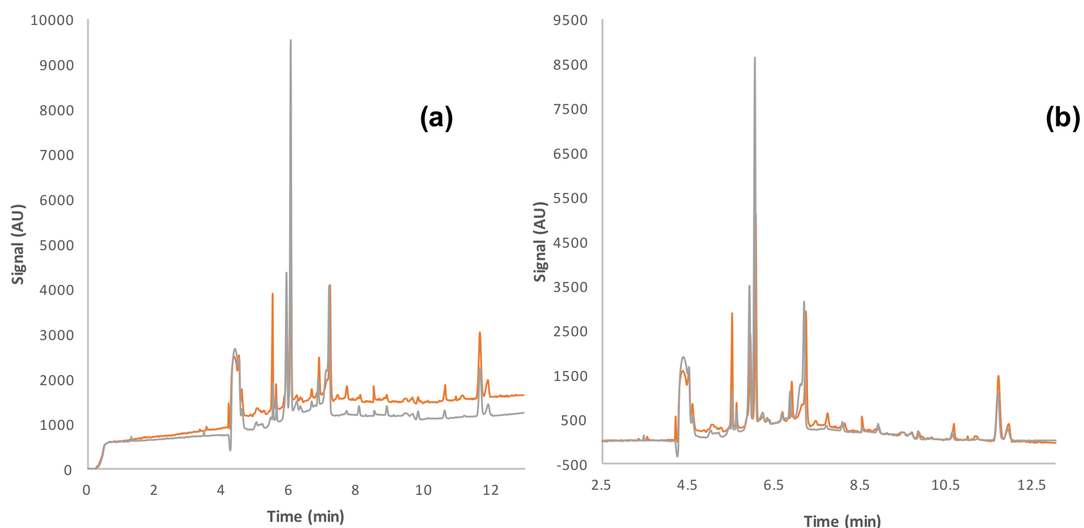
350

351 **Fig. 1.** Separation of seven polyphenolic compounds by CZE under optimized conditions.
352 1. OLE; 2. SIA; 3. GTA; 4. SYA; 5. FA, 6. p-COU; 7. VAN.



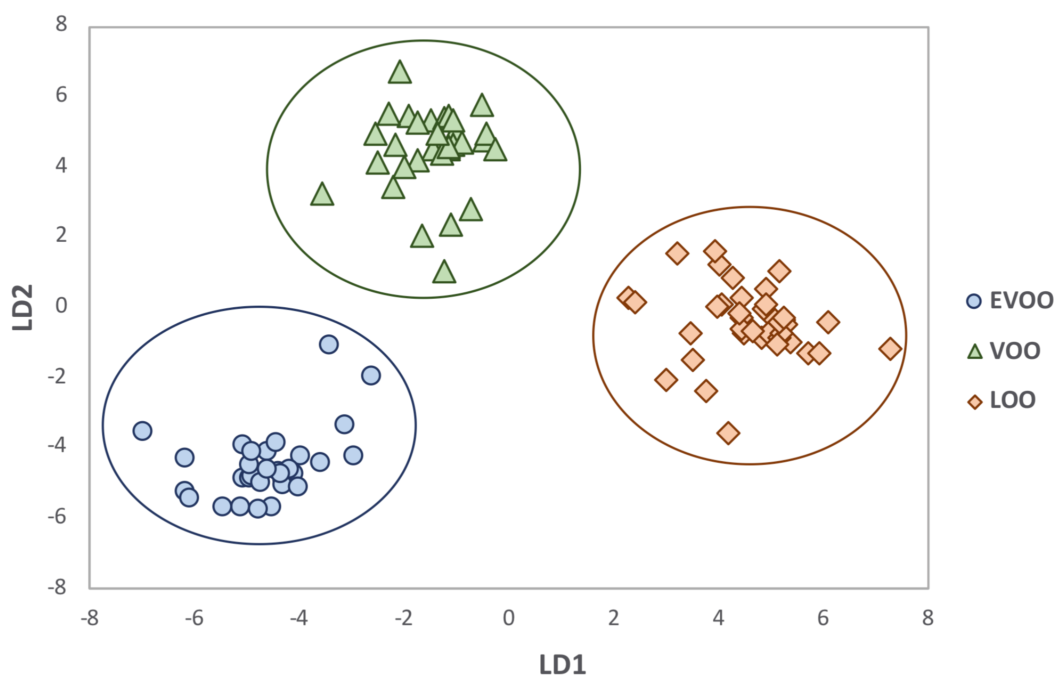
353

354 **Fig. 2.** Correction of peak misalignment: (a) misaligned electropherograms, (b) corrected
355 electropherograms using naphthol as IS, (c) corrected electropherograms using benzoic
356 acid as IS and (d) corrected electropherograms using both ISs.



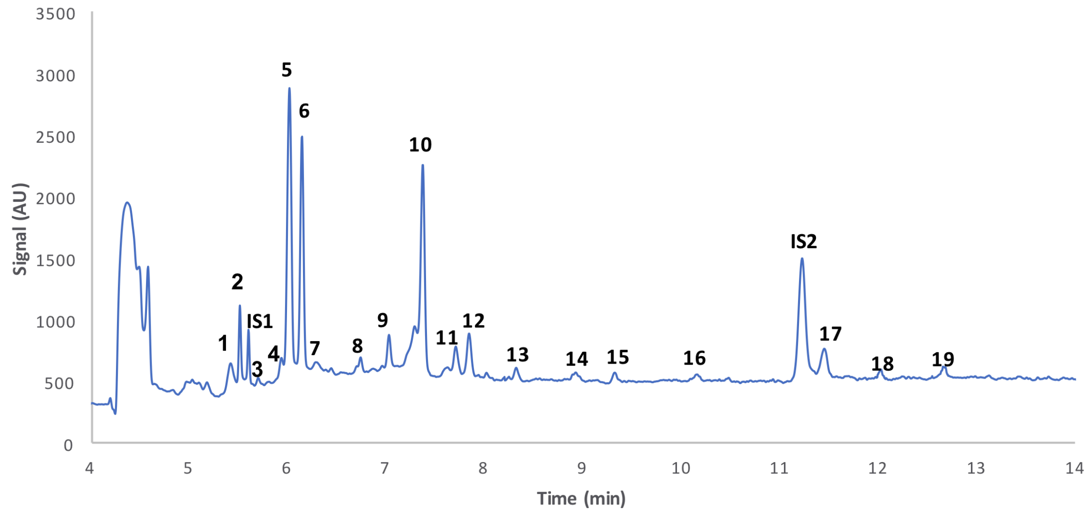
357

358 **Fig. 3.** Correction of baseline and drift. Electropherograms (a) before and (b) after
359 correction.



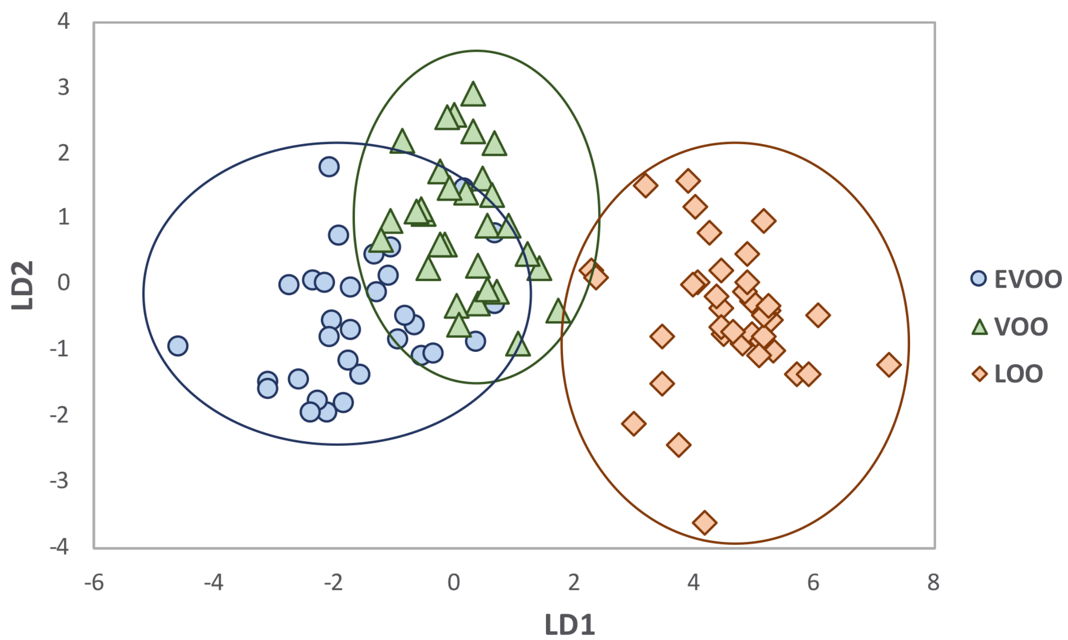
360

361 **Fig. 4**. PCA-LDA model constructed using the whole electropherogram profile.



362

363 **Fig. 5.** Electropherogram of a LOO sample showing the integrated peaks. IS1 and IS2
364 correspond to naphthol and benzoic acid respectively.



365

366 **Fig. 6.** PCA-LDA model constructed using the peak integration.