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FIRST-ORDER DISCRIMINATION OF METHANOLIC EXTRACTS FROM PLUMS ACCORDING TO HARVESTING DATE USING FLUORESCENCE SPECTRA. QUANTIFICATION OF POLYPHENOLS

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Abstract

Fluorescence spectroscopy in combination with chemometric analysis was applied to discriminate between Japanese Angeleno variety of plums, according to the date of harvesting. Emission spectra (obtained from 280 to 500 nm, and from 345 to 500 nm, respectively) of methanolic extracts of plums at two excitation wavelengths (280 and 330 nm, respectively) were obtained. The fluorescence spectral data were firstly processed by Principal Component Analysis (PCA), as an exploratory study, to extract relevant information from the spectral data, and revealed differentiation between plum samples based in the harvested time. In addition, Partial Least-Squares-Discriminant-Analysis (PLS-DA) was used for the development of the classification models, allowing 100% accuracy to differentiate between the date of harvesting, independently that pulp or skin plum extracts were analyzed. Spectral patterns of plums showed significant differences during maturation period, with a special emphasis between the months of May and September. In addition, calibration models were obtained for different individual polyphenols with partial least-squares (PLS) regression, obtaining the best results for epicatechin and neochlorogenic acid determination.

Keywords: Fluorescence; Discriminant analysis; PCA; PLS-DA; PLS; plums; polyphenols

19 **1. Introduction**

20 Fruit consumption is essential for a healthy diet thanks to the great contribution of benefits thereof
21 [1]. As a result, consumption of fruit is increasingly requiring strict quality parameters, also, is
22 important the goal of preserving fresh products on the market for longer periods of time. To have
23 a good acceptation of fruits in the market requires appropriate physico-chemical properties related
24 to fruit maturity stages. Typically, the fruit gatherers use morphological changes such as fruit
25 colour, changes in shape, taste and softness, as indicators for determining the optimal maturity
26 stage for harvesting [2]. One of the objectives of the collection of the fruit before its full maturity
27 is to keep the fresh product on the market for a longer time. In consequence, it is important to
28 dispose the sufficient knowledge to ensure that their products have the highest possible quality
29 and to predict early harvest characteristics and post-harvest behaviour, as well as determining the
30 optimum date of harvest. However, the traditional methods for determining the optimal maturity
31 stage for harvesting are destructive, time consuming, laborious, and costly, and require specific
32 sample preparation steps [3].

33 Stone fruits, including plums, are polyphenol rich. The most important phenolic compounds in
34 plums are hydroxycinnamic acids, mainly caffeoylquinic acid isomers, where neochlorogenic
35 acid is predominant [4]. Together with other phenolic acids, such as flavonoids, the level of this
36 compound varies significantly among the harvest date. These compounds are described in many
37 works as fluorescence compounds, being fluorescence spectroscopy an appropriate technique for
38 their analysis [5,6].

39 The use of classification techniques has made remarkable progress during the last decades in all
40 fields, for example, food, pharmaceuticals, environmental, biomedical matrices, and so forth. In
41 the agronomic-food field, it is becoming a common tool both for controlling production and for
42 studying the influence of storage on the qualities of the final product [7,8]. Actually, the fact of
43 having instruments not excessively sophisticated that are capable of obtaining abundant

44 information on the characteristics of the samples has facilitated the implementation of these
45 techniques.

46 Until now, spectroscopic techniques in combination with chemometrics have been widely
47 employed for discrimination of different food matrices such as food of vegetal origin [9],
48 alcoholic beverages [10], herbs and spices [11] and many others [12]. However, there are few
49 works in the literature about the use of fluorescence for discrimination of foods in spite the
50 presence of compounds with fluorescence properties in fruits and foods in general. Fluorescence
51 signal, as descriptive variable with classification purposes, in the food field, has the advantage
52 that a relatively small number of the compounds present in food samples contributes to native
53 fluorescence, thus increasing the selectivity of the information.

54 For example, fluorescence in combination with different chemometric approaches has been used
55 in the discrimination of apple juices using right angle or front-face fluorescence due to
56 antioxidants properties [13], the discrimination between apple juices belonging to two categories:
57 those produced directly, not from concentrate, and those reconstituted from concentrate apple
58 juices [14] or between commercial berry fruit beverages [15]. Another example included the use
59 of excitation – emission fluorescence spectroscopy coupled with multi-way chemometrics
60 techniques for the classification of large beers [16]. Also, this technique has been used for the
61 discrimination between Argentinean yerba mate from three commercial categories employing
62 first and second-order models and different chemometric approaches (LDA, QDA, PARAFAC
63 and N-PLS) [17].

64 The application of classification methods, as chemometric strategies for predicting a qualitative
65 response, implies building a model that can assign an individual to a category based on the data
66 that have been collected to describe it. In this context, a category (or class) is a group of objects
67 sharing similar characteristics. In discriminant analysis, spectral data are assigned to definite
68 classes, so that qualitative information complements quantitative spectral data. The purpose of
69 the classification methods is to obtain weighted combinations of data that minimize variances

70 within classes and maximize variances between classes. Then, the classification rules are used to
71 assign new or unknown samples to the most probable subclasses. Prior to discriminant analysis,
72 principal component analysis is often applied to spectral data sets to reduce data set size and
73 minimizing possible co-linearity effects. The validity of a classification method can be verified
74 by a comparison of distances or testing [18].

75 With respect to the classifications of plum samples, UV–Vis, near infrared (NIR) and
76 synchronous fluorescence, in combination with chemometric methods, have been used to
77 discriminate samples of high-quality plum brandies of different varietal origins [19], and front-
78 face fluorescence has been used to discriminate samples from different maturation stages [20].

79 With this background, in this work, we will explore the use of fluorescence, using the classical
80 right-angle technique, from methanolic extracts of plums, in combination with chemometrics
81 (classification and quantification techniques), for the discrimination of plums according to their
82 date of harvesting, and the quantification of the content of the main polyphenol compounds in
83 plums.

84

85 **2. Materials and methods**

86 **2.1. Samples and standards**

87 A total of fifty-six samples were used in this study. Samples were collected on an experimental
88 plot located in the "Vegas Bajas del Guadiana" (Badajoz, Spain) in an altitude of 184 m. Variety
89 of plums was a late-maturation Japanese Angeleno plum variety planted in 2005. Samples were
90 divided in four groups when they were analyzed: extracts from skin of plums collected in May
91 (group 1), extracts from skin of plums collected in September (group 2), extracts from pulp of
92 plums collected in May (group 3) and extracts from pulp of plums collected in September (group
93 4).

94 Standard solutions of catechin, epicatechin, chlorogenic acid, neochlorogenic acid and
95 procyanidin B2 were used to register reference spectra. Catechin, epicatechin and neochlorogenic
96 acid were purchased from Sigma Aldrich Chemie (Steinheim, Germany), chlorogenic acid was
97 obtained from Fisher Scientific, and procyanidin B2 was supplied by Extrasynthése (Genaym,
98 France).

99 **2.2. Preparation of methanolic extracts**

100 Samples were peeled and skin was separated from pulp before lyophilization and extraction to
101 perform the different analysis. Then, 0.5 g of lyophilized samples were used for extraction with
102 10 mL of methanol:water:formic acid (50:49:1, v/v), using an ultrasonic extraction for 14 minutes.
103 After that, extracts were centrifuged for 10 min, at 10000 rpm, at 4ºC. Supernatants were diluted
104 1/100 (v/v) with methanol for further analysis.

105 **2.3. Reference polyphenols analysis**

106 Polyphenols analysis of samples was performed by HPLC following the method described by
107 Cabrera-Bañegil et al. [6]. An Agilent 1260 Infinity High Performance Liquid Chromatograph
108 (Agilent Technologies, CA, USA) and a Teknokroma Tracer Excel 120 ODS-A column (150 mm
109 × 4.6 mm and 5 μm particle size) were used. Mobile phase was composed of 0.5% (v/v) formic
110 acid and water (A), and acetonitrile (B). Analytes were eluted in gradient mode: 90% of 0.5%
111 (v/v) formic acid in water (eluent A) and 10% of acetonitrile (eluent B) was held for 20 min.
112 Between 20 and 45 min the percentage of eluent B increases from 10 up to 30% and, between 45
113 min and 46 min, the percentage of eluent B increases from 30 up to 100% and the formic acid
114 content decreased in correspondence. These conditions were maintained until 53 min and, finally,
115 the eluent B content was decreased to the initial conditions (10% B), and the column was re-
116 equilibrated for 5 min. A flow of 0.5 mL/min was used and a volume of 20 μL was employed as
117 injection volume. A fast-scanning fluorescence detector was used and excitation/emission
118 wavelengths were set at 270/ 350 nm, for catechin, epicatechin and procyanidin B2, and at

119 320/430 nm for chlorogenic and neochlorogenic acids. The quantification of polyphenolic
120 compounds was carried out by standard addition calibration.

121 **2.4. Fluorescence measurement**

122 Fluorescence data were obtained from pulp and skin methanolic extracts, by means of a
123 fluorescence spectrophotometer Varian Model Cary Elipse (Agilent Technologies, Madrid,
124 Spain) in the conventional mode, using a right angle. A quartz cell of 10 mm was used. Emission
125 spectra (280 – 500 nm, each 1 nm) were recorded at an excitation wavelength of 280 nm; and
126 emission spectra (345 – 500 nm, each 1 nm) were also collected at an excitation wavelength of
127 330 nm. Slits of excitation and emission monochromators were set at 5 nm, respectively, with a
128 scan rate of 300 nm/min. To obtain the excitation – emission matrix the excitation range was from
129 240 to 380 nm, each 5 nm, and the emission range was from 280 to 500, each 1 nm.

130 **2.5. Data processing and multivariate analysis**

131 Firstly, all spectra were smoothed using the Savitzky Golay method to eliminate some noise
132 signals [21]. In order to explore the main variation among the four groups of samples, Principal
133 Component Analysis (PCA) [22] was applied using all the fifty-six samples mentioned before.
134 As two excitation wavelengths, 280 and 330 nm, were used, two data sets were considered for
135 analysis.

136 After that, to evaluate the possibility of discrimination of samples according to the date of
137 harvesting. Partial least-squares-discriminant analysis (PLS-DA) was used as classification
138 algorithm [23]. PLS-DA involves performing a multivariate regression model to establish class
139 limits and placing a numeric value to each object/sample first, and then classifying new samples
140 into a specific class. Data analysis was done using a graphical interface [24] in Matlab (R2016b,
141 The MathWorks, Inc. Natick, MA, USA).

142 To obtain calibration models for polyphenols quantification, PLS regression was applied [25].
143 Cross-validation was used to determine the number of components to use in the calibration and
144 to evaluate the performance of the models. Number of components were selected according to the
145 explained variance. The Unscrambler version 6.11 (CAMO Software AS, Oslo, Norway) was
146 used for data analysis.

147 **3. Results and discussion**

148 **3.1. Spectral information**

149 For this study, methanolic extracts from the pulp and from the skin of the plums with different
150 maturation stages, were obtained. In first place, and with the object to visualize the emission
151 spectral zones, excitation-emission fluorescence landscapes of methanolic extracts of skin and
152 pulp of plums were obtained and two characteristics spectral regions were observed (Figure 1A).
153 The first region presented a maximum excitation wavelength at 280 nm, and the second region
154 presented an excitation maximum at 330 nm. Figures 1B and 1C show the emission spectra for
155 the methanolic extracts from the pulp and from the skin, obtained at the two different excitation
156 wavelengths, the most characteristic ones. In Figure 1B (excitation at 280 nm), the main
157 differences correspond with intensity of signals when samples harvested in different months were
158 compared. This is, extracts from May exhibited higher fluorescence, about three times more, than
159 extracts from September in both cases: pulp and skin. These high intensity maxima have been
160 also obtained, by synchronous fluorescence, in plum brandies samples with little differences in
161 function of the presence or absence of color in the sample [26]. In addition, when the emission
162 spectra of the skin and pulp samples were compared some differences could also be highlighted.
163 Although intensities in May were similar for both skin (blue) and pulp (violet) groups, a shift was
164 shown in their spectra. In the case of extracts from skin, an emission maximum is located at 321
165 nm. However, in the case of extracts from pulp, the emission maximum shows a small
166 hypochromic effect, and it is located at 315 nm. This might be related with different polyphenol

167 compounds present in both extracts. This region is characteristic for catechin, epicatechin and
168 procyanidin, main polyphenols presented in plums [6].

169 In the case of the second excitation wavelength, 330 nm (Figure 1C), similar trends were
170 observed. In this case, an emission maximum appeared at 424 nm for the skin extracts and at 435
171 nm for the pulp extracts. For both extracts, the emission band presents a wide shape. In this
172 spectral region, and with the samples harvested in May, it is noted that the fluorescence intensity
173 of the skin extracts is significantly higher than the pulp extracts. With respect to the samples
174 harvested in September, the fluorescence of the skin extracts decreases, and the fluorescence of
175 the pulp extracts disappears. In accordance with previous studies, this region is characteristic for
176 chlorogenic and neochlorogenic acids, that are the predominant phenolic acids in plums [6,27,28].
177 Also, this fluorescence region maximum has been observed by synchronous fluorescence in
178 colored and colorless plum brandies samples [26].

179 **3.2. Exploratory analysis: Principal Component Analysis**

180 In order to evaluate the main differences between the four groups, an exploratory analysis was
181 performed with PCA. PCA analysis allowed detecting potential outliers and systematic artifacts
182 in the samples. In this case, when residual x- variance was plotted versus leverage, no outliers
183 were observed in our samples. This is an unsupervised method, and it was used to evaluate
184 whether clustering exists without using class membership information. Samples were divided in
185 two data sets according to the excitation wavelength. For each data set, all groups of samples
186 (skin September, pulp September, skin May and pulp May) were analyzed.

187 In the set of emission spectra with excitation at 280 nm, best discrimination was obtained for
188 scores for PC1 and PC2, explaining 98 and 2% of the variance, respectively. Figures 2A and 2B
189 show the scores and loadings obtained, respectively. Score values for PC1 are higher for samples
190 harvested in May, which means that positive loadings are positively related to these samples. The
191 main variable affecting the separation of groups is observed in the loading for PC1, Figure 2B,

192 and was located at an emission wavelength of 318 nm. This variable might be related with
193 procyanidin and epicatechin that exhibit maxima signal around 314 nm (Figures 3A and 3B). This
194 result is in accordance with the general decrease of total phenolic and total flavonoids in plums
195 of Sanshua variety during fruit maturation [29]. Another group was observed, according to the
196 PC2, which explained only 2% of variance but it was enough for differentiation. In this case, the
197 main variable affecting the separation was at 308 nm (positive) and 340 nm (negative). Score
198 values for PC2 were higher for samples from pulp than from skin. In this case, differentiation
199 might be due to the presence of catechin that presents a maximum signal around 308 nm (Figure
200 3C).

201 In the set of spectra with excitation at 330 nm, best discrimination was obtained for scores of PC1
202 explaining 99% of the variance. Figures 2C and 2D show the scores and loadings obtained,
203 respectively. Along the first component, samples were divided by harvesting date, being the
204 contribution of first component higher for May than for September. In this case, no differentiation
205 was observed according to skin and pulp. In this set, the loadings of the PC1 can be related with
206 the presence of chlorogenic and neoclorogenic acids in plums, showing the main variables
207 affecting the separation of groups at 424 nm for emission wavelength.

208 **3.3. Classificatory analysis: Partial Least-Squares – Discriminant Analysis**

209 After PCA, classificatory analysis was performed with different strategies. In a first step, all
210 samples were considered as training samples and PLS-DA was assayed in both sets of data. With
211 two components, the total variance was explained (100%) in both cases. Results are shown in
212 Table 1. As seen, results confirmed the good classification of the four groups. In this case, a test
213 set was not used as all samples were used as training set. It was observed that better discrimination
214 was obtained for spectra at 280 nm of excitation wavelength (error rate (ER) = 0) than for spectra
215 at 330 nm (ER = 3.5%).

216 A second strategy consisted on dividing the entire sample data set into the training set, comprising
217 the 50% of the samples, and use the rest of samples as test samples. In this case, also two
218 components were enough to explain 100% of the variance. For the training set, acceptable
219 predictions were obtained (Table 2), with ERs of 3.5 and 7% respectively, for the two different
220 excitation wavelengths. However, when the test set (50% of samples) was predicted using these
221 models, all samples were well-attributed to their group, Table 2.

222 Finally, a third strategy, using 30% of samples as training set and 70% of samples as test set was
223 carried out. Total variance was explained by two components in both cases. For training set, 100%
224 of accuracy was obtained in both data sets. In this case, acceptable predictions were obtained for
225 test set, with ERs of 3 and 8%, respectively. These classification studies demonstrated the huge
226 variability between four groups, being possible to create models with only four samples per class
227 and obtain good results for predicted samples. In short, all models could be considered acceptable
228 taking into account the criteria that ER were lower than 10% in all cases [30].

229 **3.4. Quantification of polyphenols**

230 In addition, quantification studies were performed. For that, random samples of plums (twenty-
231 three samples) were analyzed by HPLC to obtain the reference values of polyphenols.

232 Correlation coefficients (Pearson's r) between the different polyphenolic compounds were
233 obtained. The results revealed a high correlation between catechin and epicatechin (r = 0.86),
234 which means, those samples with high content of catechin also present high content of
235 epicatechin. In addition, a high correlation was found between procyanidin and catechin (r = 0.85)
236 and epicatechin (r = 0.90). In the case of chlorogenic and neochlorogenic acids, weak correlation
237 were found with the previous ones, but the correlation between them was 0.58. The high
238 correlations between some polyphenols might influence in the calibration models when individual
239 polyphenols try to be quantified.

240 Using the spectra as X and individual polyphenol content obtained by HPLC as Y, calibration
241 models were obtained by means of cross-validation procedure. Table 3 provides the results
242 obtained for the different models based on the spectra at the two excitation wavelengths.
243 Components were selected according to the explained variance, obtained few components (2 or
244 1) in all cases, which means that overfitting did not occurred.

245 As observed, the best model was obtained for epicatechin, with a low prediction error and a high
246 determination coefficient (R^2). The regression coefficients for this model are shown in Figure 4B,
247 corresponding the main variables affecting the models with the maxima obtained in the spectrum
248 for the pure standard (Figure 3B). Also, acceptable models were obtained for catechin and
249 procyanidin. Regression coefficients for procyanidin model (Figure 4C) offered same information
250 than in the case of epicatechin (Figure 4B), which may be expected due to the similarity of
251 standard spectra for both compounds (Figure 3C). However, in the case of catechin (Figure 4A),
252 the regression coefficients did not show the main variables from catechin (Figure 3A), so this
253 model might be a bit uncertain due to the low concentration of this compound in samples.

254 In the case of chlorogenic and neochlorogenic acids, the best model was obtained for the last one.
255 Both compounds presented a similar spectrum (Figure 3D and 3E), as a result, similar regression
256 coefficients (Figure 4D and 4E) were obtained for their corresponding models, although a shift in
257 the main variables was observed. The better results obtained for neochlorogenic acid might be
258 related with the fact that the concentration interval found for this compound is wider than for
259 chlorogenic acid. Similar determination coefficient was obtained and a high root-mean-square-
260 error for cross validation. (RMSECV) resulted.

261 Calibration models offered promising results which need to be expanded including more samples
262 with high variability. It would be possible to quantify polyphenols in methanolic extracts using
263 simple fluorescence spectra and avoiding large procedures by HPLC, which requires more time,
264 solvents and higher cost.

265 **4. Conclusions**

266 Emission spectra of methanolic extracts of plums were used as fingerprints for their
267 differentiation. PCA allowed discrimination of samples by date of harvesting (May or September)
268 in both data set used. However, the discrimination between the two parts of plums (skin or pulp)
269 was only obtained with emission spectra at 330 nm of excitation. Samples were better
270 discriminated with PLS-DA obtaining accuracy around 100%. Also, models composed by
271 reduced number of samples offered acceptable prediction results. Classification results were due
272 to polyphenol content. In addition, calibrations models obtained by PLS provided good results
273 about individual quantification of polyphenols with R^2 values of 0.74 and 0.89, for
274 neochlorogenic acid and epicatechin, respectively, which could be interesting to investigate in the
275 future to expand the calibration models with more samples.

276

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Figure captions

Figure 1. A) Excitation-emission matrix and contour plot of a methanolic extract from pulp plum sample. B) Samples emission spectra obtained exciting at 280 nm. C) Samples emission spectra obtained exciting at 330 nm.

Figure 2. Score values (A) and loadings (B) obtained from PCA of emission spectra at 280 nm for excitation. Score values (C) and loadings (D) obtained from PCA of emission spectra at 330 nm for excitation.

Figure 3. Emission spectra for different standards: epicatechin (A), procyanidin (B), catechin (C), chlorogenic acid (D) and neochlorogenic acid (E). A, B and C were obtained at 280 nm excitation wavelength and D and E were obtained at 330 nm excitation wavelength.

Figure 4. Regression coefficients obtained for the different models: catechin (A), epicatechin (B), procyanidin (C), chlorogenic acid (D), neochlorogenic acid (E) and chlorogenic + neochlorogenic acids (F).

Table 1. Confusion matrices for the different training sets studied.

Real/ Predicted	May (skin)	September (skin)	May (pulp)	September (pulp)
All samples (Training set)				
Excitation 280 nm				
Real/ Predicted	**May (skin)**	**September (skin)**	**May (pulp)**	**September (pulp)**
May (skin)	14	-	-	-
September (skin)	-	16	-	-
May (pulp)	-	-	12	-
September (pulp)	-	-	-	14
Excitation 330 nm				
	May (skin)	**September (skin)**	**May (pulp)**	**September (pulp)**
May (skin)	12	-	2	-
September (skin)	-	16	-	-
May (pulp)	-	-	12	-
September (pulp)	-	-	-	14
50% samples (Training set)				
Excitation 280 nm				
Real/ Predicted	**May (skin)**	**September (skin)**	**May (pulp)**	**September (pulp)**
May (skin)	7	-	-	-
September (skin)	-	7	1	-
May (pulp)	-	-	6	-
September (pulp)	-	-	-	7
Excitation 330 nm				
Real/ Predicted	**May (skin)**	**September (skin)**	**May (pulp)**	**September (pulp)**
May (skin)	5	-	2	-
September (skin)	-	8	-	-
May (pulp)	-	-	6	-
September (pulp)	-	-	-	7
30% samples (Training set)				
Excitation 280 nm				
Real/ Predicted	**May (skin)**	**September (skin)**	**May (pulp)**	**September (pulp)**
May (skin)	4	-	-	-
September (skin)	-	5	-	-
May (pulp)	-	-	4	-
September (pulp)	-	-	-	4
Excitation 330 nm				
Real/ Predicted	**May (skin)**	**September (skin)**	**May (pulp)**	**September (pulp)**
May (skin)	4	-	-	-
September (skin)	-	5	-	-
May (pulp)	-	-	4	-
September (pulp)	-	-	-	4

Table 2. Confusion matrices for the different tests set studied.

Real/ Predicted	May (skin)	September (skin)	May (pulp)	September (pulp)
50% samples (test set)				
Excitation 280 nm				
May (skin)	7	-	-	-
September (skin)	-	8	-	-
May (pulp)	-	-	6	-
September (pulp)	-	-	-	7
Excitation 330 nm				
Real/ Predicted	**May (skin)**	**September (skin)**	**May (pulp)**	**September (pulp)**
May (skin)	7	-	-	-
September (skin)	-	8	-	-
May (pulp)	-	-	6	-
September (pulp)	-	-	-	7
70% samples (test set)				
Excitation 280 nm				
Real/ Predicted	**May (skin)**	**September (skin)**	**May (pulp)**	**September (pulp)**
May (skin)	9	1	-	-
September (skin)	-	11	-	-
May (pulp)	-	-	8	-
September (pulp)	-	-	-	10
Excitation 330 nm				
Real/ Predicted	**May (skin)**	**September (skin)**	**May (pulp)**	**September (pulp)**
May (skin)	8	-	2	-
September (skin)	-	11	-	-
May (pulp)	-	-	9	-
September (pulp)	-	-	-	9

Table 3. Summary of PLS regression models obtained for predicting different polyphenols.

	Range (µg/mL)	Nº components	R^2 (CV)	RMSECV (µg/mL)
Excitation 280 nm				
Catechin	0 – 0.32	2	0.73	0.05
Epicatechin	0.1 – 2.6	1	0.89	0.23
Procyanidin B	0 – 1.7	1	0.67	0.29
Excitation 330 nm				
	Range (µg/mL)	**Nº components**	**R^2 (CV)**	**RMSECV (µg/mL)**
Chlorogenic acid	0 – 0.52	1	0.54	0.09
Neochlorogenic acid	0 – 1.8	1	0.74	0.18
Chlorogenic acid + Neochlorogenic acid	0 – 1.8	1	0.73	0.24

RMSECV: root-mean-square-error for cross validation

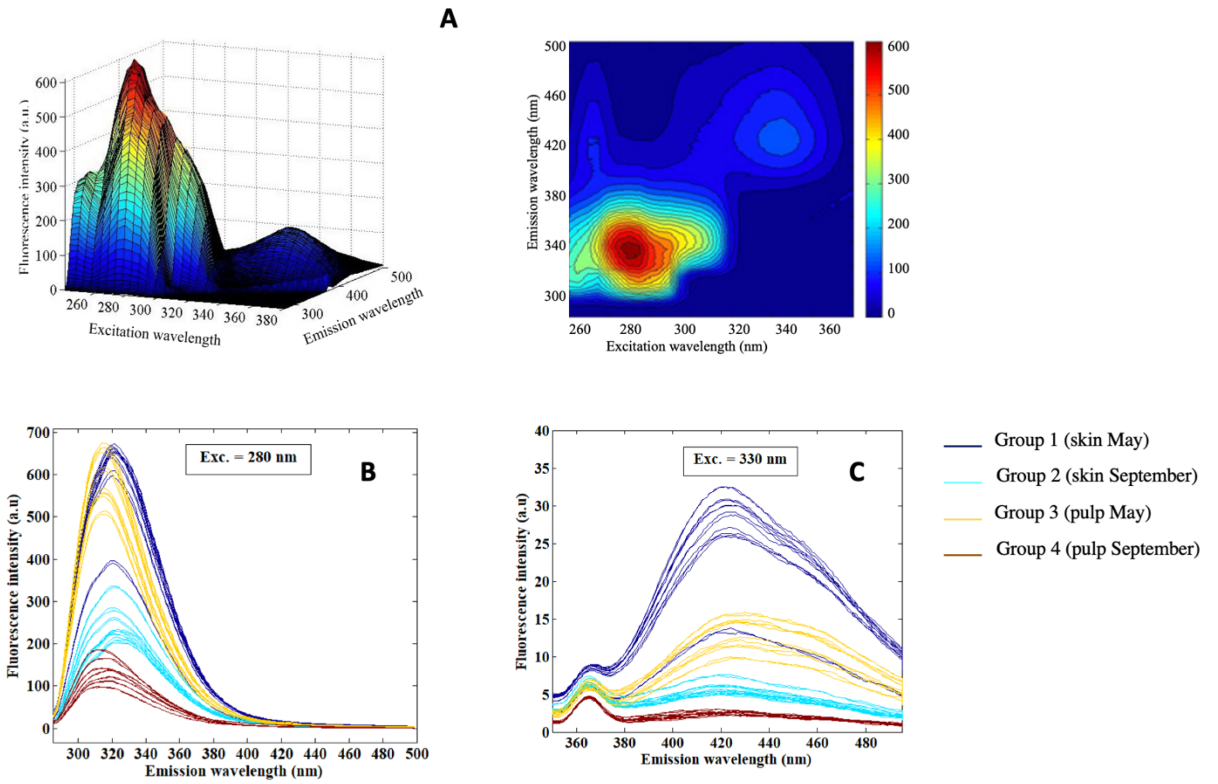

Figure 1

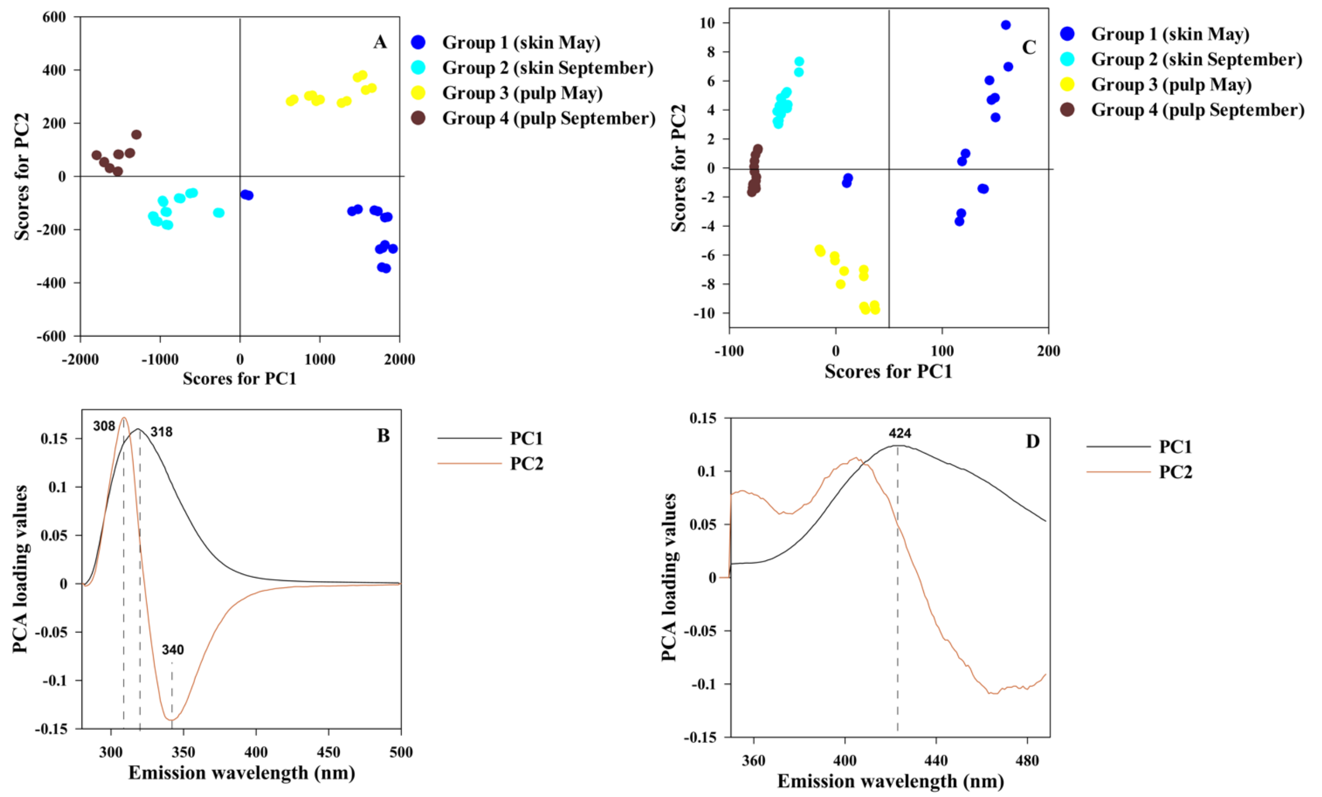

Figure 2

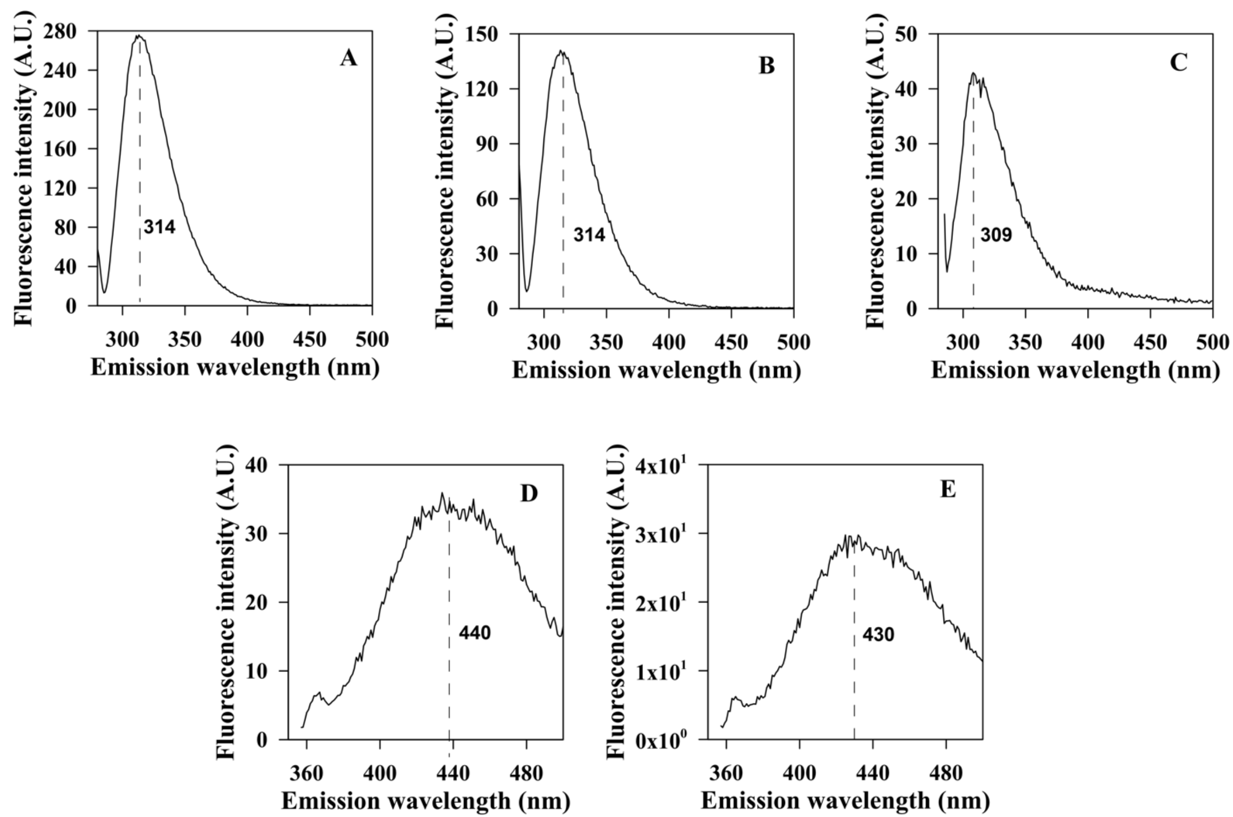

Figure 3

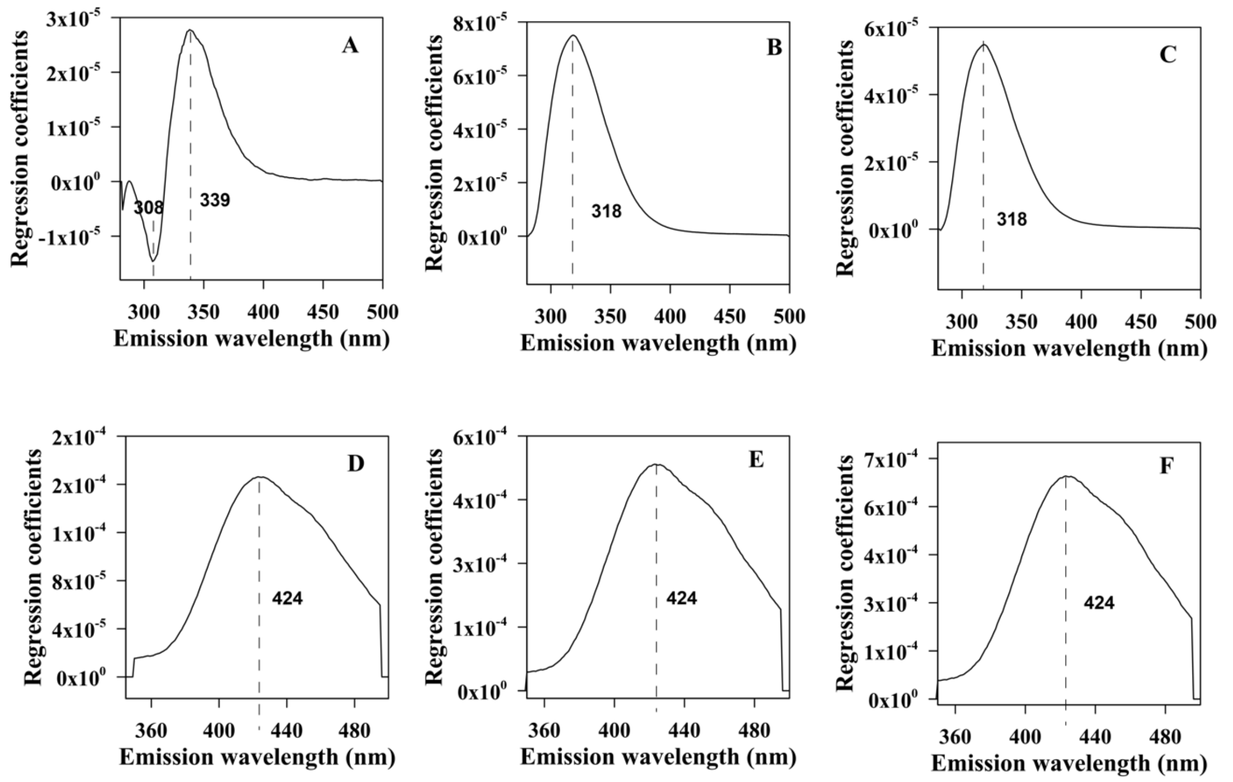

Figure 4