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# Targeted and untargeted techniques coupled with chemometric tools for the evaluation of sturgeon (*Acipenser gueldenstaedtii*) freshness during storage at 4 °C

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25 **Abstract**

26 The physico-chemical, textural and color parameters and fluorescence spectra of  
27 aromatic amino acids and nucleic acids (AAA + NA), tryptophan residues,  
28 nicotinamide adenine dinucleotide (NADH), and vitamin A were scanned on sturgeon  
29 samples kept at 4 °C up to 12 days. Using the principal component analysis (PCA),  
30 the riboflavin and NADH spectra allowed clear differentiation between sturgeon  
31 samples according to their storage time. The best result was obtained by applying the  
32 common components and specific weights analysis (CCSWA) to the riboflavin and  
33 NADH data tables since better differentiation was achieved between the 4 group  
34 sturgeon samples aged: 2 days; 5, 6 and 7 days; 8 and 9 days and 12 days. The  
35 proposed methodology demonstrates the ability of CCSWA to evaluate sturgeon  
36 freshness levels according to their storage time.

37

38 **Keywords:** sturgeon (*Acipenser gueldenstaedtii*); freshness; fluorescence; texture;  
39 physico-chemical; color.

40

41 **Chemical compounds studied in this article:** Propan-2-ol (PubChem CID: 3776); n-  
42 hexane (PubChem CID: 8058); acetic acid (PubChem CID: 176); chloroform  
43 (PubChem CID: 6212); sodium sulphate anhydrous (PubChem CID: 24436); celite  
44 (PubChem CID: 24261); sodium thio-sulphate 5-hydrate (PubChem CID: 24477);  
45 sodium hydroxide (PubChem CID: 14798); potassium iodide (PubChem CID: 4875);  
46 2-thiobarbituric acid (PubChem CID: 2723628); trichloroacetic (PubChem CID:  
47 20079), perchloric acid (PubChem CID: 24247), boric acid (PubChem CID: 7628);  
48 phenolphthalein (PubChem CID: 4764); ethanol (PubChem CID: 702); chlorohydric  
49 acid (PubChem CID: 313); sulfuric acid (PubChem CID: 1118).

50       **I.       Introduction**

51       Sturgeons are among the most world's precious wildlife resources. In 2013, the  
52       production of the cultured sturgeon, in the world, reached 75,000 tons (Wang et al.,  
53       2016). These northern hemisphere fishes can exist in large river systems, lakes,  
54       coastal waters, and inner sea throughout Eurasia and North America. Sturgeons are  
55       species of biological and economic importance. Sturgeon is the common name for 27  
56       species of fish belonging to the family *Acipenseridae*, but 4 species are extinct  
57       (Birstein, 1993). The 23 remaining species are grouped into 4 genera with 2 species in  
58       *Huso*, 2 species in *Scaphirhynchus*, 3 species in *Pseudoscaphirhynchus* and 16  
59       species in *Acipenser* (Scott & Crossman, 1973). Most of the world's sturgeon  
60       population has submitted significant decline, especially due to over-fishing, habitat  
61       destruction and pollution. At the same time, sturgeon has become a popular species  
62       due to its high level of good-quality proteins and the presence of many vitamins and  
63       minerals.

64       Being a perishable product, sturgeon's freshness is an important indicator of  
65       its commercial success and commodity. After the death of fish, a series of  
66       complicated chemical, biochemical, and microbial processes occurred resulting in a  
67       valuable loss of fish quality.

68       Several classical tools, named biochemical, colorimetric, microbiological,  
69       physical methods as well as the assessment of organoleptic properties have been  
70       developed to evaluate fish freshness state. Sensory evaluation known as 'Quality  
71       Index Method (QIM)' is a complex method since it depends on multiple  
72       characteristics of fish tissues. Moreover, trained panels are generally expensive, time-  
73       consuming and not always available along with all the different steps of the fishery  
74       chain. Other methods named high-performance liquid chromatography (HPLC),

75 electrochemical and electrophoretic approaches have also been used to evaluate the  
76 quality of fish products. Although these techniques have demonstrated their ability to  
77 assess fish freshness level, they need relative expensive reagents and skilled operators  
78 to perform the experiments. Consequently, to satisfy the need for quality control on-  
79 line and/or at-line in the fish industry and to provide fish with high quality, rapid and  
80 non-destructive techniques are needed (Karoui, Cartaud, & Dufour, 2006; Karoui et  
81 al., 2006; Karoui, Nicolaï, & De Baedemaeker, 2008). The near infrared (NIR) and  
82 mid-infrared (MIR) spectroscopies have been demonstrated their usefulness to  
83 quantify seafood composition as well as to assess fish freshness (Hernández-Martínez  
84 et al., 2014). Front-face fluorescence spectroscopy (FFFS) has, recently, been used for  
85 the: i) differentiation between fresh and frozen/thawed belonging to different fish  
86 species (Hassoun & Karoui, 2016; Karoui, Hassoun, & Ethuin, 2017; Karoui,  
87 Thomas, & Dufour, 2006); and ii) monitoring of freshness of different fish species  
88 (*Merlangius merlangus*, *Scomber scombrus*) (Hassoun & Karoui, 2015; Karoui &  
89 Hassoun, 2017).

90 The present work aims, for the first time, to explore the potential use of  
91 textural, colorimetry, physico-chemical and FFFS data tables for monitoring sturgeon  
92 fillets freshness kept at 4 °C up to 12 days. To extract information and to establish the  
93 link between the different data tables, a multi-block statistical analysis named  
94 common components and specific weight analysis (CCSWA) was applied; this  
95 statistical chemometric tool consists in determining a common space of representation  
96 for the different data tables. Previous results showed the effectiveness of CCSWA to:  
97 i) monitor modification in triglycerides and the protein network during cheese  
98 ripening (Karoui, Downey, & Blecker, 2010); and ii) investigate the effect of raw

99 material on the evolution of sea bass fillets quality during storage (Karoui, Hassoun,  
100 & Ethuin, 2017).

## 101 **2. Materials and methods**

### 102 **2.1. Chemicals and reagents**

103 All of the chemicals used in this study were of analytical grade and obtained  
104 from VWR or Merck. Sodium sulfate anhydrous, Celite® 545, hexane, isopropanol,  
105 and boric acid were obtained from VWR International, while 2-thiobarbituric acid  
106 (99%), trichloroacetic (99.5)% and sodium hydroxide (NaOH, 40%) were procured  
107 from Merck, Darmstadt, Germany.

108

### 109 **2.2. Fish samples preparation**

110 Sturgeon samples, caught in winter, came from Aquatrade sea farm located in  
111 Astrakhan, Russia. The 5-year-old sturgeon (*Acipenser gueldenstaedtii*) has a natural  
112 diet composed of wheat flour, wheat, mono-calcium phosphate, sunflower oil, fish oil  
113 and a group of vitamins. Within the Aquatrade sea farm, the sturgeon samples were  
114 slaughtered by asphyxiation/hypothermia in sea farm and kept on ice (0 to 2°C) in  
115 expanded polystyrene boxes during transport. Upon arrival to the French laboratory  
116 (48 hours), the fish samples were first beheaded, eviscerated, processed to obtain  
117 sturgeon slices, and then packaged one by one in plastic bags and sealed. A total of  
118 twenty-one (21) sturgeon samples were kept at 4 °C and randomly analysed on day 2,  
119 5, 6, 7, 8, 9 and 12. All the measurements were determined in triplicate by using  
120 different samples.

121

### 122 **2.3. Physico-chemical analysis**

123 The proximate composition (protein and fat contents) of sturgeon samples was  
124 determined only on day 2, while the moisture and pH values were performed during  
125 the whole storage period. The protein content was determined by the Kjeldahl method  
126 ( $N \times 6.25$ ) using an automatic Kjeldahl system (Kjeldahl™ 8100), while the fat level  
127 was measured according to the Association Française de Normalisation AFNOR  
128 (1991) using a rotary evaporator (Büchi, Rotavap R-3) at speed 7. The moisture  
129 content was determined by drying sturgeon samples in an oven (Air Concept,  
130 FIRLABO, Emerainville, France) at 110 °C for 15 hours (Eymard, 2003). The pH  
131 value was measured directly on sturgeon slices using a digital pH meter (WTW pH  
132 330i Taschen-pH-Meter, WTW GmbH).

133 Lipid oxidation was assessed following the determination of the peroxide  
134 value (PV) and the thiobarbituric acid reactive substances (TBARS) level. The PV,  
135 expressed as milliequivalents of peroxide oxygen per kg of fish (mEq/kg of fish) was  
136 determined by iodometric titration using the procedure developed by Egan, Kirk, &  
137 Sawyer (1981). The TBARS value, expressed as mg malonaldehyde (MA)/kg of fish,  
138 was evaluated according to the procedure of Guizani, Rahman, Al-Ruzeiqi, Al-  
139 Sabahi, & Sureshchandran (2014). The absorbance was measured at 532 nm by using  
140 a spectrophotometer (spectrophotometer Anthelie data, Anthelie 2 light, Secomam,  
141 Toulouse, France).

142

#### 143 **2.4. Total volatile basic nitrogen measurements**

144 The Total volatile basic nitrogen (TVB-N) content, expressed as mg TVB-  
145 N/100g of sturgeon flesh, was determined by the official steam distillation method  
146 according to the Commission Regulation (EC) 2074/2005 using an automatic Kjeldahl  
147 system (Kjeldahl™ 8100).

148

## 149 **2.5. Instrumental techniques**

### 150 **2.5.1. Color measurements**

151 The color of sturgeon samples was determined using the Minolta Chroma  
152 Meter version CR-300 (Konica Minolta Sensing Europe, Roissy Charles De Gaulle,  
153 France) according to the method described by Botosoa, Chénè, & Karoui (2013).  
154 Measurements were performed directly on sturgeon [samples](#) following the system of  
155 CIE (1976):  $L^*$  describing lightness ( $L^* = 0$  for black,  $L^*=100$  for white),  $a^*$   
156 describing intensity in red (+60 = red, -60 = green),  $b^*$  describing intensity in yellow  
157 (+60 = yellow, -60 = blue). The total color difference ( $\Delta E^*$ ) between the 2-day-old  
158 sturgeon samples (considered as the reference) and the other samples was calculated  
159 as follows:

$$160 \quad \Delta E^* = \sqrt{(L^*)^2 + (a^*)^2 + (b^*)^2}$$

161

### 162 **2.5.2. Texture profile analysis measurements**

163 The texture profile analysis (TPA) was used to evaluate the texture based on  
164 the compression of the sample with a TA.XTPlus texture analyzer (Micro Stable  
165 System, Goldamin, UK) equipped with a cylindrical probe ( $\emptyset = 1$  cm). The  
166 measurement was composed of two consecutive 40% compressions of sturgeon  
167 perpendicularly to the muscle fibers' orientation with a crosshead speed of 1 mm/s.  
168 The TPA settings were as follows: pre-test speed: 1mm/s; post-test speed: 10 mm/s;  
169 target mode distance: 10 mm; trigger force: 5 g. The delay between the first and the  
170 second compression was fixed to 5 s.



171 The force-time curve was analysed to determine seven texture parameters:  
172 gumminess, adhesiveness, cohesiveness, chewiness and springiness. All  
173 measurements were performed at room temperature (20 °C).

174

### 175 **2.5.3. Fluorescence measurements**

176 Fluorescence spectra were recorded using a Fluoromax-4 spectrofluorometer  
177 (Jobin Yvon, Horiba, NJ, USA). Sturgeon samples of 2 cm length, 1 cm width and 0.5  
178 cm thickness were cut from the middle of the sturgeon slices. Sturgeon slices were  
179 mounted between two quartz and emission spectra of aromatic amino acids and  
180 nucleic acids (AAA + NA), tryptophan residues, nicotinamide adenine dinucleotide  
181 (NADH) and riboflavin were acquired at 20 °C with the excitation wavelength set at  
182 250, 290, 340 and 380 nm, respectively. The excitation spectra of vitamin A were  
183 scanned with the emission wavelength set at 410 nm.

184

### 185 **2.6. Statistical analysis**

186 In order to compare the evolution of the sturgeon freshness state throughout  
187 storage, Fisher's least significant difference (LSD) method, used as part of the one-  
188 way analysis of variance (ANOVA) at a level of 5%, was applied to the physico-  
189 chemical, textural and color parameters. The correlation between some specific  
190 parameters and storage time was determined.

191 Regarding fluorescence spectra, normalisation was applied to all the spectra by  
192 reducing the area under each spectrum to a value of 1 (Karoui, Dufour, & De  
193 Baerdemaeker, 2006a). Specifically, the shift of the peak maximum and the peak  
194 width changes in the spectra were considered.

195 In order to obtain the maximum of information contained in each data table,  
196 the PCA was applied, separately, to the physico-chemical, colorimetric, TPA, and  
197 fluorescence measurements. This statistical multivariate treatment was, earlier, used  
198 to observe similarities among samples, reducing the dimension to two PCs, while  
199 keeping most of the original information found in the data tables.

200 In a second step, the CCSWA was applied to the riboflavin and NADH spectra  
201 allowing the best discrimination of sturgeon samples as a function of their storage  
202 time. The objective of this technique is to describe several data tables observed for the  
203 same sample. The CCSWA takes into account the maximum inertia (total variance) of  
204 the data tables. It consists of determining a common space of representation for all the  
205 data tables. The CCSWA deals with the analysis of inertia that is the total variance in  
206 data tables and enables the overall data collected to be described by taking into  
207 account the relation between the different data tables (Karoui et al. 2006a). Similarity  
208 maps of the samples can be drawn by projection on the planes defined by each couple  
209 of the  $CC1$ ,  $CC2$ ...,  $CCn$  dimensions. Orthogonal spectral patterns related to the  $CCn$   
210 dimension can be calculated. The PCA and CCSWA were performed using MATLAB  
211 software (Matlab, Version 6.5, Release 12, The MathWorks).

212

### 213 **3. Results and discussion**

#### 214 **3.1. Proximate analysis**

215 Proximate analysis of fresh sturgeon aged 2 days showed that protein and lipid  
216 contents were 14.79% and 12.28%, respectively, in agreement, with the findings of  
217 Badiani et al. (1996) who pointed out levels of protein and lipid in the 17.60 -  
218 21.01% and 2.66 - 15.31% range, respectively and those of Pyz-Łukasik and

219 Paszkiewicz (2018) who observed for Siberian sturgeon values of 15.69 and 12.57%  
220 for protein and lipid content, respectively.

221 The water content level of sturgeon samples aged 2 days was 75.75% ( $\pm 0.28$ ),  
222 in agreement, with the findings of Badiani et al. (1996) who observed water content in  
223 the 65.93-77.59% range. The water content value showed a significant decrease  
224 ( $P < 0.05$ ) between the second and the eighth days of storage since it passed from  
225 75.75% to 71.54%. This decrease could be explained by the changes in the water-  
226 protein interactions and the reduction of the water-holding capacity of fish muscle  
227 since muscle proteins are denatured during storage, in agreement with the  
228 investigation of Ayala et al. (2011).

229 The mean pH values of sturgeon samples varied from 6.11 to 6.23 during 12  
230 days of storage at 4°C (Fig. 1a), in agreement with the findings of Izquierdo-Pulido,  
231 Hatae, & Haard (1992) who observed pH values of struggled sturgeons in the 6.10-  
232 6.20 range during 7 days of iced storage. During storage, the pH values increased and  
233 then decreased gradually with significant difference ( $P < 0.05$ ) between day 2 and day  
234 12. The increase of pH might be ascribed to the formation of amines and ammonia  
235 derived from microbial activity and degradation of proteins and non-protein  
236 nitrogenous compounds (Chaijan, Benjakul, Visessanguan, & Faustman, 2005;  
237 Hassoun & Karoui, 2015). The ultimate post-mortem pH is dependent on the  
238 physiological state and the type of muscle (Hassoun & Karoui, 2015; Izquierdo-  
239 Pulido et al., 1992). Additionally, the pH value is a function of the post-mortem  
240 evolution of the flesh that is influenced by, diet, seasons, level of activity, and/or  
241 stress during the catch (Periago et al., 2005).

242

243 **3.2. Evolution of peroxide values during storage**

244 In the present study, the PV was employed for determining the primary lipid  
245 oxidation products during the storage period of sturgeon samples. Results indicated  
246 that the PV of samples increased significantly ( $P < 0.05$ ) during storage and reached a  
247 mean value of 2.50 mEq/kg of sturgeon on day 7 (Fig. 1b). These results suggested  
248 the formation of hydroperoxides, which are the primary lipid oxidation products in  
249 fish, in agreement with the findings of Karoui & Hassoun (2017) who observed the  
250 same trend on Atlantic Mackerel (*Scomber Scombrus*) and Bahram et al. (2016) who  
251 noted similar evolution on Beluga sturgeon. Due to the instability of the  
252 hydroperoxide, a sharp decrease in the PV was observed after 7 days of storage, in  
253 agreement with our previous findings (Karoui & Hassoun, 2017). Indeed, the breaking  
254 of the hydroperoxides into a wide variety of secondary oxidation products such as  
255 aldehydes and other decomposition products would be achieved on day 7.

256 In order to determine the freshness state of sturgeon samples, a conversion of the PV  
257 expressed in mEq/kg of sturgeon (Fig. 1b) to mEq/kg of fat was performed. The  
258 results showed that the levels of PV of sturgeon samples varied from 4.07 to 20.33  
259 mEq/kg of fat. Taking into account the findings of Huss (1995), the PV of fish and  
260 fish products should not be above 10-20 mEq/kg of fat to be used for human  
261 consumption. Therefore, it could be concluded that sturgeon samples are acceptable  
262 up to 6 days of storage at 4°C since PV of 20.33 mEq/kg of fat was observed on day  
263 7.

264

### 265 3.3. Evolution of thiobarbituric acid reactive substances during storage

266 Malondialdehyde is a second breakdown product of lipid oxidation and its  
267 level is directly related to off-odours in fish. Once the lipid oxidation reaction started,  
268 it becomes self-propagating and difficult to be controlled. The TBARS values

269 increased significantly ( $P < 0.05$ ) during the storage time and reached 1.94 mg  
270 malonaldehyde/kg fish on day 6 and thereafter showed a decrease towards the end of  
271 the storage period (Fig. 1c). The TBARS values did not exceed the 2 mg  
272 malonaldehyde/kg fish during the considered storage period indicating the  
273 acceptability of sturgeon samples. The decrease of the level of TBARS after day 6  
274 could be explained by the interaction between: i) malonaldehyde and amines,  
275 nucleoside and nucleic acid, proteins, amino acids of phospholipids and other  
276 aldehydes that are known as the end-products of lipid oxidation; and/or ii) unstable  
277 low molecular compounds as well as its break down with organic acid, alcohols, and  
278 so on (Maqsood & Benjakul, 2010).

279

#### 280 **3.4 Evolution of total volatile basic nitrogen during storage**

281 Fish decomposition is mainly caused by the action of spoilage bacteria which  
282 is responsible for the apparition of off-odours and off-flavours (Lyhs, Lahtinen, &  
283 Schelvis-Smit, 2007). A level of 25 mg TVB-N/100 g of fish has been considered as  
284 the upper limit above which the seafood product is considered spoiled and unfit for  
285 human consumption (Luten, Jacobsen, Bekaert, Saebo, & Oehlenschlager, 2006).  
286 After 5 days of storage at 4 °C, sturgeons presented low values of TVB-N (~ 3.50  
287 TVB-N/100 g of sturgeon), indicating that samples were fit for human consumption  
288 (Fig. 1d). After this storage time, an increase in the level of TVB-N was observed  
289 since it reached on day 9 the level of 41.07 TVB-N/100 g of sturgeon. This increase  
290 could be ascribed to the accumulation of protein breakdown products such as  
291 ammonia, indole, putrescine, trimethylamine and other off odour compounds, as a  
292 result of microbial growth and endogenous enzymes, in agreement with the findings  
293 of Song, Liu, Shen, You, & Luo (2011) and Hassoun & Karoui (2016).

294 From the obtained results, it could be concluded that PV, TBARS and TVB-N gave  
295 different results regarding the acceptability of sturgeon samples for human  
296 consumption (6, 12, and 8 days according to the PV, TBARS and TVB-N methods,  
297 respectively) and thus at least two of them could not be considered an effective tool to  
298 determine the freshness state of sturgeon, in agreement with the finding of Hassoun &  
299 Karoui (2016).

300

### 301 3.5. Instrumental techniques

#### 302 3.5.1. Evolution of color parameters during storage of sturgeon samples at 4°C

303 Color is one of the major attributes that affect the consumer perception quality  
304 of a fish product. Appearance is an all-inclusive term involving muscle structure  
305 characteristic, pigment concentration, spoilage level (Karoui, Hassoun, & Ethuin,  
306 2017). Regarding the  $L^*$  values, no significant difference ( $P>0.05$ ) was observed  
307 between the investigated sturgeon samples indicating that this parameter was not  
308 affected by the considered storage time (Table 1a). The slight changes of  $L^*$  values  
309 according to the storage time could be due to a greater water deposit on the sturgeon  
310 surface (Hernández et al., 2009). Regarding  $a^*$  values, and according to Table 1a, a  
311 significant difference ( $P<0.05$ ) was observed between samples aged 9 days or less  
312 from those aged 12 days. A high correlation ( $R= 0.83$ ) was obtained between  $a^*$  and  
313 storage time. The obtained results were in line with those of Karoui et al. (2017) who  
314 found a similar trend for refrigerated and frozen-thawed sea bass (*Dicentrarchus*  
315 *labrax*) fillets.

316 A different trend was observed for  $b^*$  values (Table 1a). The intensity of  $b^*$  values  
317 (blue to yellow color) increased progressively during the storage time from  
318  $5.20 \pm 1.20$  to  $9.95 \pm 0.38$ , indicating a movement of flesh color toward more

319 yellowish color (Zhao, Li, Wang, & Lv, 2012; Karoui et al., 2017). The alteration of  
320 yellowness color could be ascribed to the lipid oxidation as indicated by the PV and  
321 TBARS values.

322 To get more information regarding the evolution of color parameters during  
323 storage, total color difference ( $\Delta E$ ) has been determined. The ANOVA did not show a  
324 significant difference ( $P > 0.05$ ) between the investigated samples during the whole  
325 storage time (Table 1a).

326

### 327 3.5.2. Evolution of texture parameters during storage of sturgeon samples at 4°C

328 The texture of fish muscle is an important quality attributes that depend on  
329 several parameters such as hardness, cohesiveness, springiness, chewiness, resilience,  
330 and adhesiveness, as well as the internal cross-linking of connective tissue (Cheng,  
331 Sun, Han, & Zeng, 2014). From Table 1b, textural parameters showed a significant  
332 difference ( $P < 0.05$ ) between sturgeon samples aged 2 days from the others, except for  
333 the springiness. The hardness is related to the strength of muscle structure, which in  
334 turn is dependent on amino acid composition. During the considered storage period,  
335 the fish muscle may undergo a series of changes such as hydrolyse of muscle proteins  
336 and loss of water holding capacity inducing a decrease in the hardness values, in  
337 agreement with the findings of others (Zhao et al., 2012; Hassoun & Karoui, 2016;  
338 Caballero et al., 2009). The extent of these changes depends on many factors  
339 including the level of activity, stress during catching, intra-variability of fish and  
340 storage time.

341

### 342 3.5.3 Global analysis of the physico-chemical and instrumental data tables

343 The process of data gathering from the textural, color, and physico-chemical  
344 data tables was applied using multivariate statistical analyses. **Fig. 2a** showed the  
345 PCA similarity map performed jointly on the physico-chemical, color, and textural  
346 data tables. The map defined by the PCs 1 and 2 (41.04 and 19.89% of the total  
347 variance, respectively) showed the presence of 3 groups of sturgeon: those aged 2  
348 days (group 1); 5, 6 and 7 days (group 2) and 8, 9 and 12 days (group 3). The PC1  
349 separated mainly the sturgeon samples aged 2 days which had positive score values  
350 from the others. The PC2 allowed clear differentiation between samples aged 5, 6 and  
351 7 days from those kept during 8 days and more.

352 To investigate the basis of this discrimination between sturgeon samples  
353 according to their storage time, the variables were studied (**Fig. 2b**). According to the  
354 PC1, sturgeon samples aged 2 days were characterised by the highest values of  
355 hardness, gumminess and cohesiveness, while the aged ones presented the lowest  
356 values. The PC2 indicated that the highest values of TVB-N were observed for  
357 samples aged 8, 9 and 12 days, in agreement with the findings of Hassoun & Karoui  
358 (2016) who pointed out an increase in the TVB-N values of whiting (*Merlangius*  
359 *merlangus*) fillets during 15 days of storage at 4 °C.

360

#### 361 **3.5.4. Evaluation of fluorescence measurements during storage of sturgeon** 362 **samples at 4°C**

363 An example of the normalised AAA+NA emission spectra recorded between  
364 290 and 400 nm after excitation set at 250 nm on sturgeon samples as a function of  
365 storage time is shown in **Fig. 3a**. The maximum emission was observed ~ 375 nm and  
366 the shape of the spectra varied as a function of the storage time. This difference could  
367 be explained by the difference in the molecular structure of the proteins. This was



368 confirmed by the tryptophan emission spectra (**Fig. 1S**) that presented similar trend as  
369 AAA+NA since fresh and aged sturgeon samples exhibited different shapes indicating  
370 that the tryptophan residues **are sensitive** to the changes of their molecular  
371 environment affecting the protein-protein, protein-lipid and/or protein-water  
372 interactions, in agreement with previous findings of **Hassoun & Karoui (2016)**.

373 The normalised riboflavin spectra acquired on sturgeon samples (**Fig. 3b**)  
374 allowed **better clear** differentiation between samples as a function of **the** storage time.  
375 The spectra exhibited two maxima located ~ 463 and 488 nm and a weak one ~ 636  
376 nm. The observed maxima could be attributed to the dissected perimysial sheets and  
377 collagen. In addition, **the** emission spectra in the 405 – 480 nm have been used in  
378 several studies to determine the degree **of fish** (Karoui et al., 2017), cheese (Karoui &  
379 Dufour, 2006) and meat (Sahar, Rahman, Kondjoyan, Portanguen, & Dufour, 2016)  
380 **oxidation**. Indeed, it was assumed that the fluorophores at this spectral range arise  
381 from different stable fluorescent oxidation products; among them, **the** products  
382 formed by the reaction of unsaturated aldehydes with proteins and/or riboflavin photo  
383 breakdown product. In addition, **the**  $\beta$ -carotene absorbs in the 400–500 nm region  
384 and can undergo some photo-degradation (Hansen & Skibsted, 2000), which may  
385 influence the shape of **the** riboflavin spectra. It should be kept in mind that the  
386 fluorophores responsible for the signals in this region are not known yet.

387 Regarding the peak observed ~ 636 nm, an increase in the fluorescence  
388 intensity with the storage time was observed. These findings are in agreement with  
389 those of Durek, Bolling, Knorr, Schwägele, & Schlüter (2012) who attributed these  
390 narrow peaks to **the** protoporphyrin IX, an indicator of food spoilage. In fact, **the**  
391 storage process accelerates the growth of porphyrin- producing microorganisms and  
392 activates the involved enzymes because the extracted fish juice is an excellent

393 medium for them and damage the competing microorganisms which are not  
394 psychrophilic (Durek, Bolling, Knorr, Schwägele, & Schlüter 2012)

395 **Fig. 3c** illustrates an example of the normalised NADH emission spectra  
396 recorded between 360 and 600 nm of sturgeon samples. The spectra exhibited three  
397 peaks located ~ 390, 470 and 487 nm. It appeared that the shape of NADH emission  
398 spectra was correlated with the freshness state of sturgeon samples. Indeed, a decrease  
399 (~ 470 and 487 nm) and an increase (~ 390 nm) in the fluorescence intensity were  
400 observed. The sharp decrease in the fluorescence intensity ~ 470 and 487 nm, which  
401 could be attributed to NADH and flavin adenine dinucleotide (FADH), was ascribed  
402 to the cytoplasm oxidation of the cells throughout storage, leading to the  
403 transformation of NADH to NAD<sup>+</sup>, that modify significantly the shape of the NADH  
404 fluorescence spectra. The obtained results are in agreement with those of Karoui et al.  
405 (2017), who found a high fluorescence intensity ~ 468 nm for fresh sea bass samples  
406 compared to the frozen–thawed ones. On the contrary, aged sturgeon samples  
407 exhibited higher fluorescence intensity ~ 390 nm than the freshest one aged 2 days,  
408 which may be ascribed to the Maillard reaction products. The obtained results were in  
409 accordance with those of Karoui et al. (2006) who pointed out a higher fluorescence  
410 intensity ~ 380 nm for frozen–thawed whiting fillets (*Merlangius merlangus*) than the  
411 fresh ones. The results obtained in the present study strengthen the hypothesis that the  
412 NADH fluorescence spectra can be used as a fingerprint for freshness identification of  
413 sturgeon samples.

414 The excitation spectra of the vitamin A scanned on sturgeon samples at  
415 different storage times between 252 and 390 nm with emission wavelength set at  
416 410 nm showed some difference as a function of the storage time (**Fig. 2S**). Indeed, a  
417 red shift of the maximum excitation of vitamin A spectra (i.e., from 296 nm for fresh

418 sturgeon samples to 310 for aged ones) was observed. This shift could be ascribed to  
419 different: i) physical states of the triglycerides in the fat globules; and/or ii)  
420 interactions of the fat globule membrane with protein network and/or lipid-lipid  
421 interactions as supported by Karoui, Dufour, & De Baerdemaeker (2006b).

422 Due to the complexity of the spectra, univariate analysis is not an appropriate  
423 tool to analyse the fluorescence spectra. Thus, chemometric tools allowed to extract  
424 information related to the environment of the intrinsic probes.

425

### 426 **3.5.5. Evaluation of the discriminant ability of fluorescence spectroscopy to** 427 **discriminate between sturgeon samples according to their freshness**

428 The PCA applied, separately, to each fluorescence data table indicated that the  
429 riboflavin and NADH emission spectra enabled the most efficient discrimination  
430 between sturgeon samples as a function of their storage time. Indeed, the similarity  
431 map of the PCA performed on the riboflavin fluorescence spectra defined by the PCs  
432 1 and 2, accounting for 80.80% of the total variance, allowed clear differentiation of  
433 samples according to their storage time (Fig. 4a). According to the PC1, explaining  
434 47.40% of the total variance, sturgeon samples aged 2, 5, 6 and 7 days exhibited  
435 negative score values, whereas those aged 8, 9 and 12 days presented positive scores.  
436 The PC2 accounting for 33.40% of the total variance differentiate between samples  
437 aged: i) 2 days from those aged 5, 6 and 7 days; and ii) 8 and 9 days from those aged  
438 12 days. Quite mostly similar results were obtained regarding the PCA applied on the  
439 NADH fluorescence spectra (Fig. 4b).

440 As the spectra of NADH and riboflavin showed their ability to discriminate  
441 sturgeon samples according to their storage time, a joint analysis of these two data  
442 tables was performed by using the CCSWA. The similarity map defined by the CC1

443 and CC3 allowed clear discrimination between sturgeon samples as a function of their  
444 storage time (**Fig. 5a**). Indeed, samples aged 2 days presented negative score values  
445 according to the CC1 and CC3; those aged 5, 6, and 7 days exhibited positive scores  
446 according to CC1 and negative values according to CC3; samples aged 8 and 9 days  
447 showed mostly positive scores according to CC1 and CC3; finally, samples aged 12  
448 days exhibited negative values according to CC1 and positive ones according to CC3.

449 The map defined by the first and the third common components gave different  
450 weights for the two fluorophores: the CC1 expressed 54.70 and 25.10% of the inertia  
451 of the NADH and riboflavin fluorescence, respectively (**Fig. 5b**). On the contrary, the  
452 CC3 expressed 46.50% of the inertia of riboflavin and a tiny part of NADH (4.70%).  
453 From the obtained results, it could be concluded that the information obtained in the  
454 similarity map 1 and 3 using the NADH and riboflavin spectra were complementary  
455 and that the first and the third common components were related to similar  
456 phenomena observed by each fluorophore.

457 The spectral patterns associated with the common components provide the  
458 characteristic wavelengths that might be used to differentiate between spectra  
459 recorded on sturgeon samples. The spectral patterns are similar to spectra and may be  
460 used to derive structural information at the molecular level. The spectral patterns of  
461 NADH associated with the CC1 and CC3 are presented in **Fig. 5c**. The spectral  
462 pattern 1 associated with the CC1 exhibited a strong positive band ~ 390 nm reporting  
463 that samples aged 8, 9 and 12 days were more oxidised than the others. The results  
464 obtained in the present study confirmed those of Karoui et al. (2017) reporting that the  
465 emission spectra of NADH could be used for the evaluation of fish freshness state.  
466 Moreover, the spectral pattern 3 exhibited four negative bands ~406, 436, 452 and  
467 537 nm and two other positive bands ~375 and 470 nm indicating that during storage

468 time, some modifications occurred at the molecular level of sturgeon samples  
469 inducing changes in the environment of NADH.

470 The spectral patterns of riboflavin associated with the CC1 and CC3 are  
471 shown in **Fig. 5d**. The examination of the spectral pattern 1 associated with the CC1  
472 (**Fig. 5d**) presented an opposition between peaks located ~ 472 nm and 538 nm. The  
473 spectral pattern 3 illustrated an opposition between a positive band ~ 447 nm and a  
474 negative one ~493 nm.

475

#### 476 **4. Conclusion**

477 In order to meet customers' demands to have fresh products, physico-chemical,  
478 textural, colorimetric and fluorescence measurements were performed on sturgeon  
479 samples during storage at 4 °C up to 12 days. The present study demonstrates the  
480 potential of CCSWA as an unsupervised technique to give information related to the  
481 evolution of sturgeon freshness at the molecular level during storage. The weights and  
482 the spectral patterns issued from the CCSWA showed that the information's contained  
483 in the NADH and riboflavin are complementary. One of the main conclusions of this  
484 study was that the methodology consisting in using FFFS combined to CCSWA,  
485 could be used as a useful and rapid untargeted tool to evaluate the freshness state of  
486 sturgeon samples since four groups were illustrated: 2 days, 5, 6 and 7 days; 8 and 9  
487 days and 12 days.

488

#### 489 **Acknowledgments:**

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494

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650  
651

652 **List of table:**

653 **Table 1:** Color (a) and textural (b) measurements performed on sturgeon samples

654 stored at 4 °C up to 12 days

655

656 **Table 1a:**

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Storage day							
Color Parameters	2	5	6	7	8	9	12
<i>L</i> *	51.17±1.72 <sup>A</sup>	50.65±3.41 <sup>A</sup>	51.8±3.20 <sup>A</sup>	54.95±0.55 <sup>A</sup>	48.1±0.7 <sup>A</sup>	52.02±4.06 <sup>A</sup>	49.68±2.00 <sup>A</sup>
<i>a</i> *	4.14±1.18 <sup>A</sup>	5.07±1.25 <sup>A</sup>	5.15±0.38 <sup>A</sup>	6.52±0.55 <sup>A</sup>	6.68±1.12 <sup>A</sup>	6.45±0.88 <sup>A</sup>	11.13±1.87 <sup>B</sup>
<i>b</i> *	5.2±1.20 <sup>A</sup>	6.13±0.69 <sup>A,B</sup>	8.54±1.01 <sup>B,C</sup>	7.61±1.39 <sup>A,B,C</sup>	9.4±0.93 <sup>C</sup>	9.95±0.38 <sup>C</sup>	8.46±0.90 <sup>B,C</sup>
$\Delta E$	51.60±1.83 <sup>A</sup>	51.27±3.33 <sup>A</sup>	52.75±3.27 <sup>A</sup>	55.86±0.46 <sup>A</sup>	49.46±0.98 <sup>A</sup>	53.35±3.87 <sup>A</sup>	51.61±2.49 <sup>A</sup>

Mean values and standard deviations from three replicates are presented.

Different capital letters (A, B, C) represent statistical *difference between different* storage days ( $P < 0.05$ )

672 **Table 1b:**  
673

Textural parameters	Storage day						
	2	5	6	7	8	9	12
Hardness (g)	603.47±35.97 <sup>A</sup>	204.93±16.62 <sup>C,D</sup>	219.12±50.44 <sup>C,D</sup>	208.97±61.88 <sup>C,D</sup>	167.96±30.44 <sup>D</sup>	240.19±39.90 <sup>B,C</sup>	306.11±25.65 <sup>B</sup>
Adhesiveness (g.s)	-10.51±2.75 <sup>A,B</sup>	-19.46±4.41 <sup>C,D</sup>	-6.50±2.26 <sup>A</sup>	-15.35±3.35 <sup>B,C</sup>	-9.00±2.67 <sup>A</sup>	-11.29±4.96 <sup>A,B</sup>	-25.03±1.89 <sup>D</sup>
Springiness	0.90±0.051 <sup>A</sup>	0.86±0.06 <sup>A</sup>	0.70±0.01 <sup>B</sup>	0.86±0.08 <sup>A</sup>	0.81±0.04 <sup>A</sup>	0.88±0.07 <sup>A</sup>	0.88±0.02 <sup>A</sup>
Cohesiveness	0.65±0.03 <sup>A</sup>	0.49±0.04 <sup>C,D</sup>	0.50±0.03 <sup>C,D</sup>	0.48±0.02 <sup>D</sup>	0.56±0.02 <sup>B</sup>	0.54±0.03 <sup>B,C</sup>	0.52±0.02 <sup>B,C,D</sup>
Gumminess (g)	392.10±32.65 <sup>A</sup>	100.97±10.40 <sup>C</sup>	111.51±31.65 <sup>C</sup>	100.91±32.27 <sup>C</sup>	92.94±13.30 <sup>C</sup>	129.88±19.31 <sup>B,C</sup>	158.74±12.86 <sup>B</sup>
Chewiness (g)	351.46±27.13 <sup>A</sup>	86.13±2.57 <sup>C,D</sup>	77.49±21.32 <sup>C,D</sup>	88.01±36.35 <sup>C,D</sup>	75.52±12.37 <sup>D</sup>	114.46±21.67 <sup>B,C</sup>	140.24±11.29 <sup>B</sup>

674 Mean values and standard deviations from three replicates are presented.

675 Different capital letters (A, B, C, D) represent statistical *differences between different* storage days ( $P < 0.05$ )



676 **List of supplementary figures:**

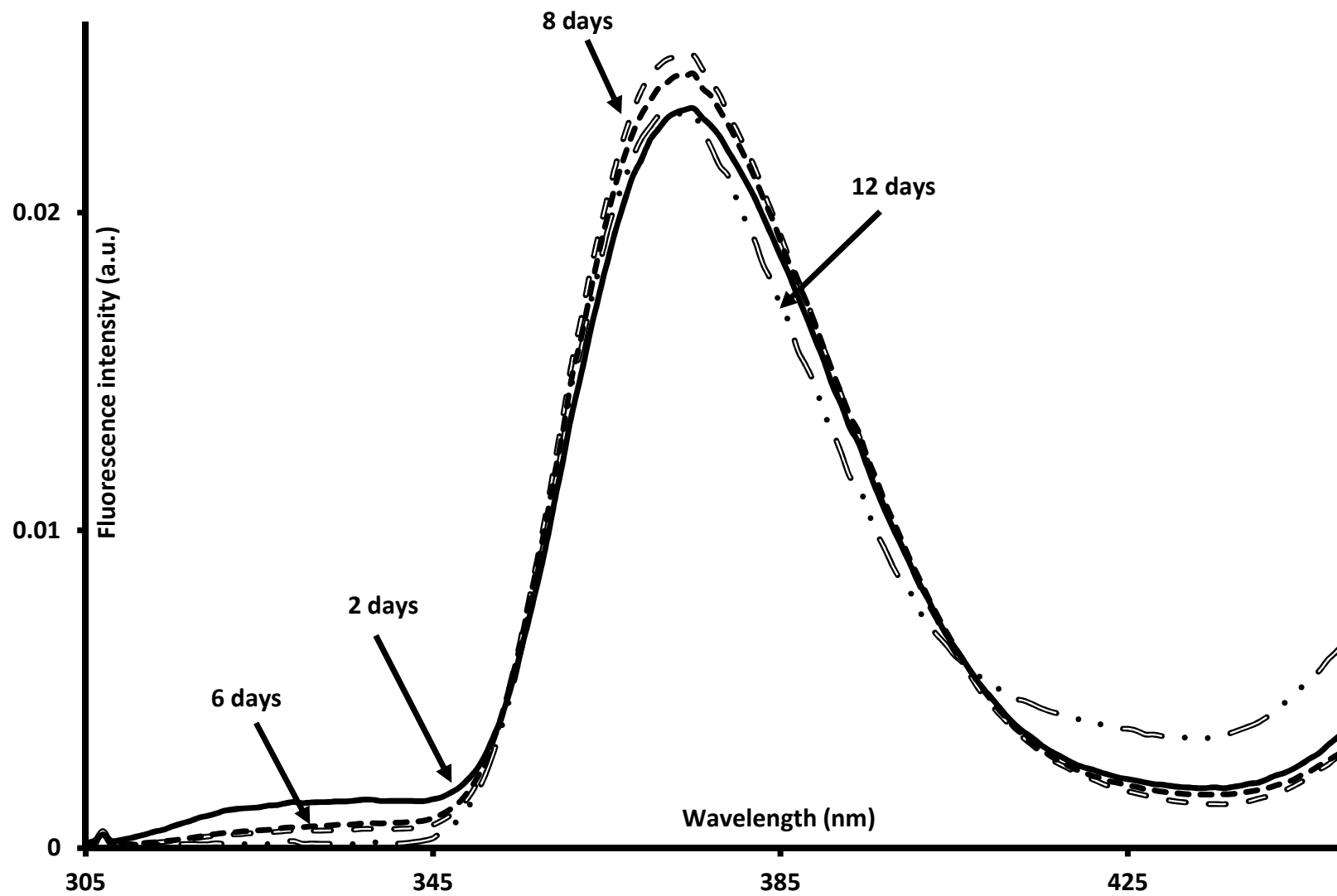
677

678 **Figure 1S:** Normalised fluorescence emission spectra of tryptophan (excitation: 290  
679 nm, emission 305–450 nm) recorded on sturgeon samples stored at 4 °C up to 12  
680 days.

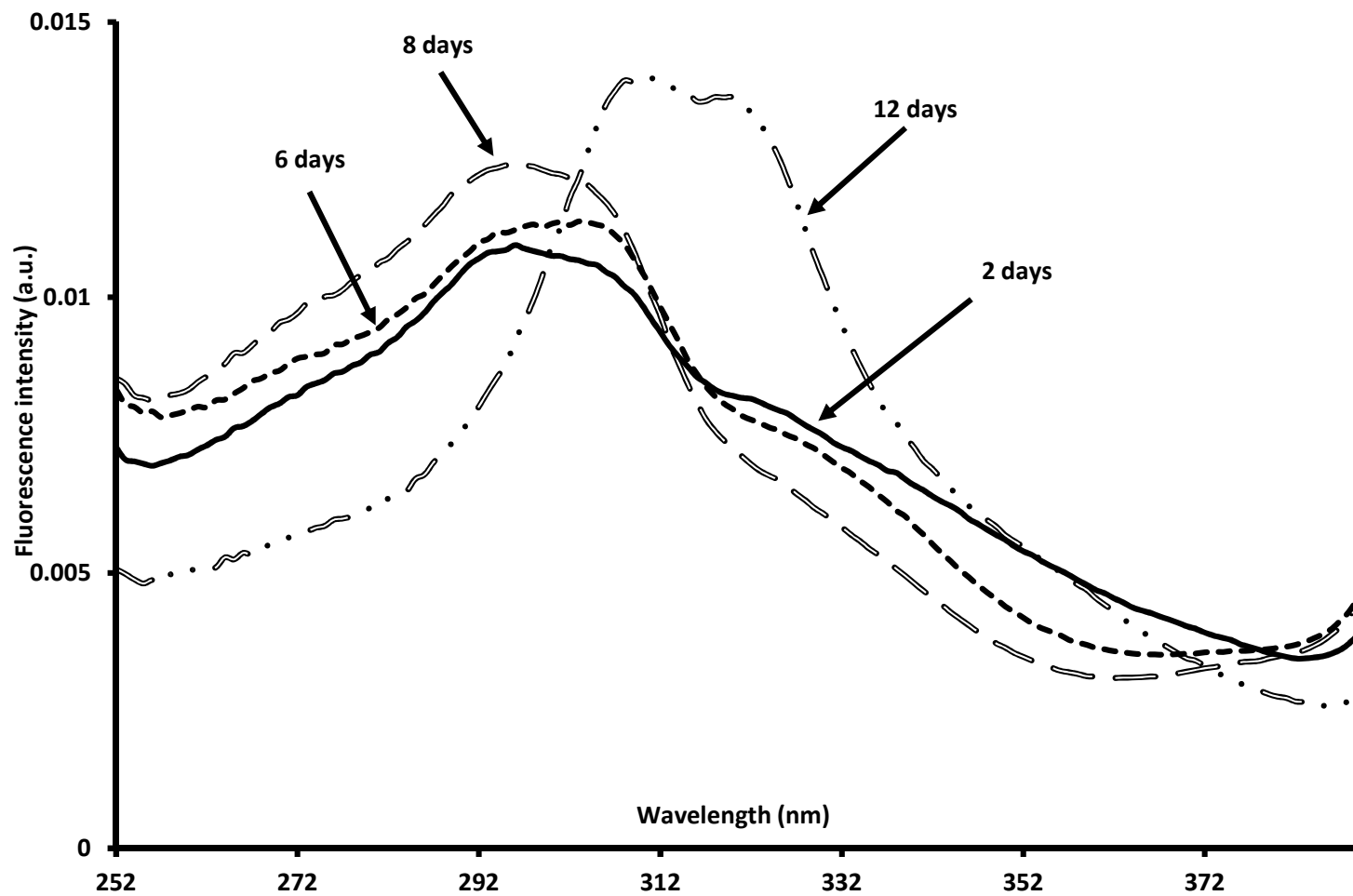
681

682 **Figure 2S:** Normalised fluorescence excitation spectra of vitamin A (emission: 420  
683 nm, emission 252–390 nm) recorded on sturgeon samples stored at 4 °C up to 12 days

684 Figure 1S:



685 Figure 2S:



686

687 **List of Figures**

688 **Figure 1:** Evolution of: (a) pH, (b) peroxide values (PV), (c) thiobarbituric acid  
689 reactive substances (TBARS) and (d) total volatile basic nitrogen (TVB-N) of  
690 sturgeon samples stored at 4 °C up to 12 days.

691 *The error bars represent the standard deviation obtained with three replicates.*

692 *Different capital letters (A, B, C, D, E, F, G) represent statistical differences between*  
693 *different storage days (P<0.05).*

694

695 **Figure 2:** (a) Similarity map of the principal component analysis (PCA) determined  
696 by principal components 1 (PC1) and 2 (PC2) of physico-chemical, color, and textural  
697 parameters performed on days 2 ( $\Delta$ ), 5 ( $\square$ ), 6 ( $\diamond$ ), 7 ( $\circ$ ), 8 ( $\blacksquare$ ), 9 ( $\blacktriangle$ ) and 12 ( $\bullet$ ); and  
698 (b) correlation chart of variables.

699

700 **Figure 3:** Normalised fluorescence emission spectra of: (a) AAA+NA (excitation:  
701 250 nm, emission 290–400 nm), (b) riboflavin (excitation: 380 nm, emission: 405-650  
702 nm) and (c) NADH (excitation: 340 nm, emission 360–600 nm), recorded on sturgeon  
703 samples stored at 4 °C up to 12 days.

704

705 **Figure 4:** Similarity map of the principle component analysis (PCA) determined by  
706 principal components: (a) 1 (PC1) and 2 (PC2) performed on riboflavin fluorescence  
707 spectra and (b) 2 (PC2) and 3 (PC3) performed on NADH fluorescence spectra  
708 recorded on sturgeon samples aged 2 ( $\Delta$ ), 5 ( $\square$ ), 6 ( $\diamond$ ), 7 ( $\circ$ ), 8 ( $\blacksquare$ ), 9 ( $\blacktriangle$ ), and 12 ( $\bullet$ )  
709 days.

710

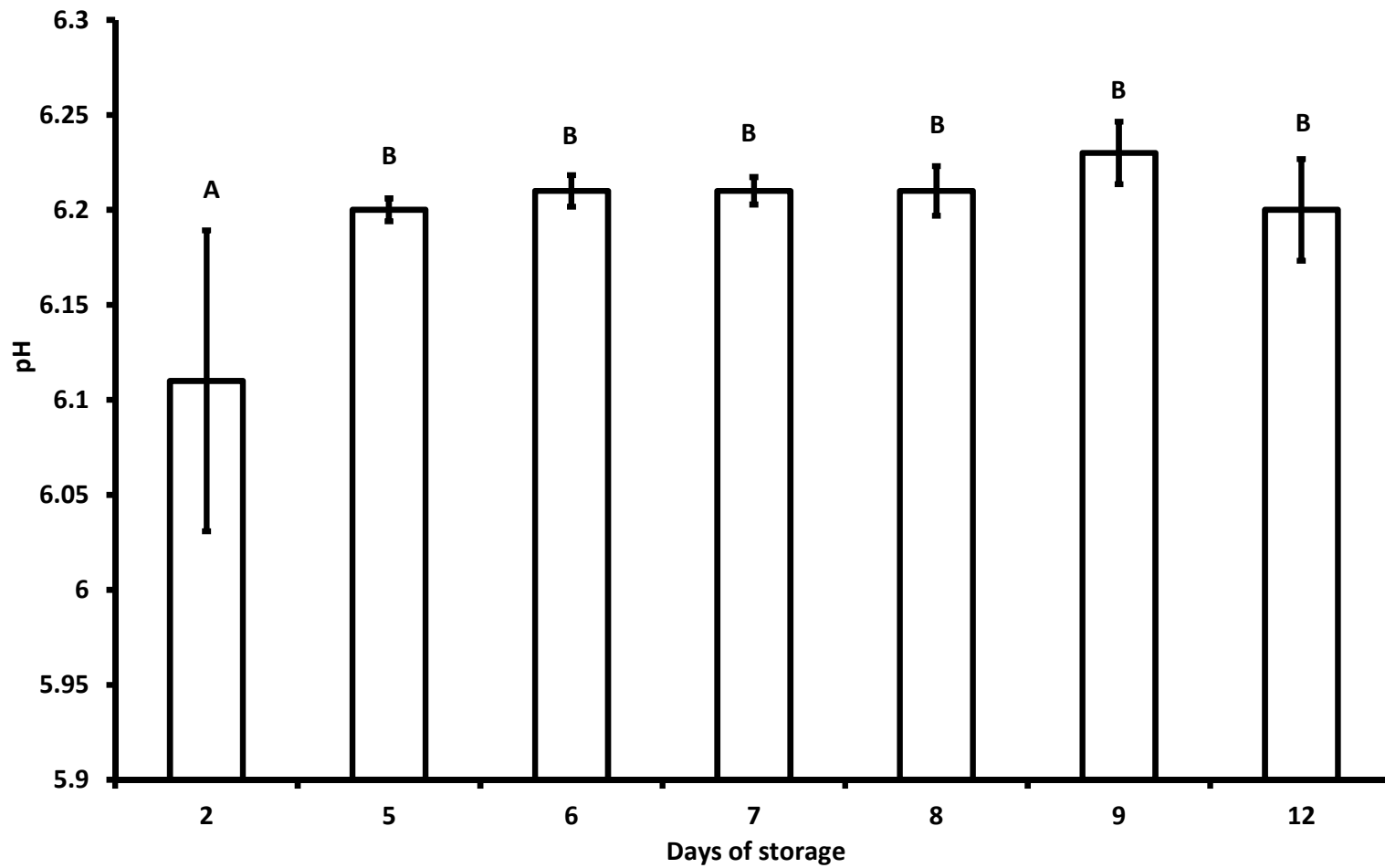
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712 **Figure 5:** Common components and specific weights analysis (CCSWA) performed  
713 on riboflavin and NADH spectra: Similarity map defined by the common components  
714 1 and 3 of sturgeon samples aged 2 ( $\Delta$ ), 5 ( $\square$ ), 6( $\diamond$ ), 7 ( $\circ$ ), 8 ( $\blacksquare$ ), 9 ( $\blacktriangle$ ), and 12 ( $\bullet$ )  
715 days (a), weights for the common components CC1 and CC3 (b), and spectral  
716 patterns of NADH (c) and riboflavin (d), associated with the common components 1  
717 (—), and 3 (⋯).

718

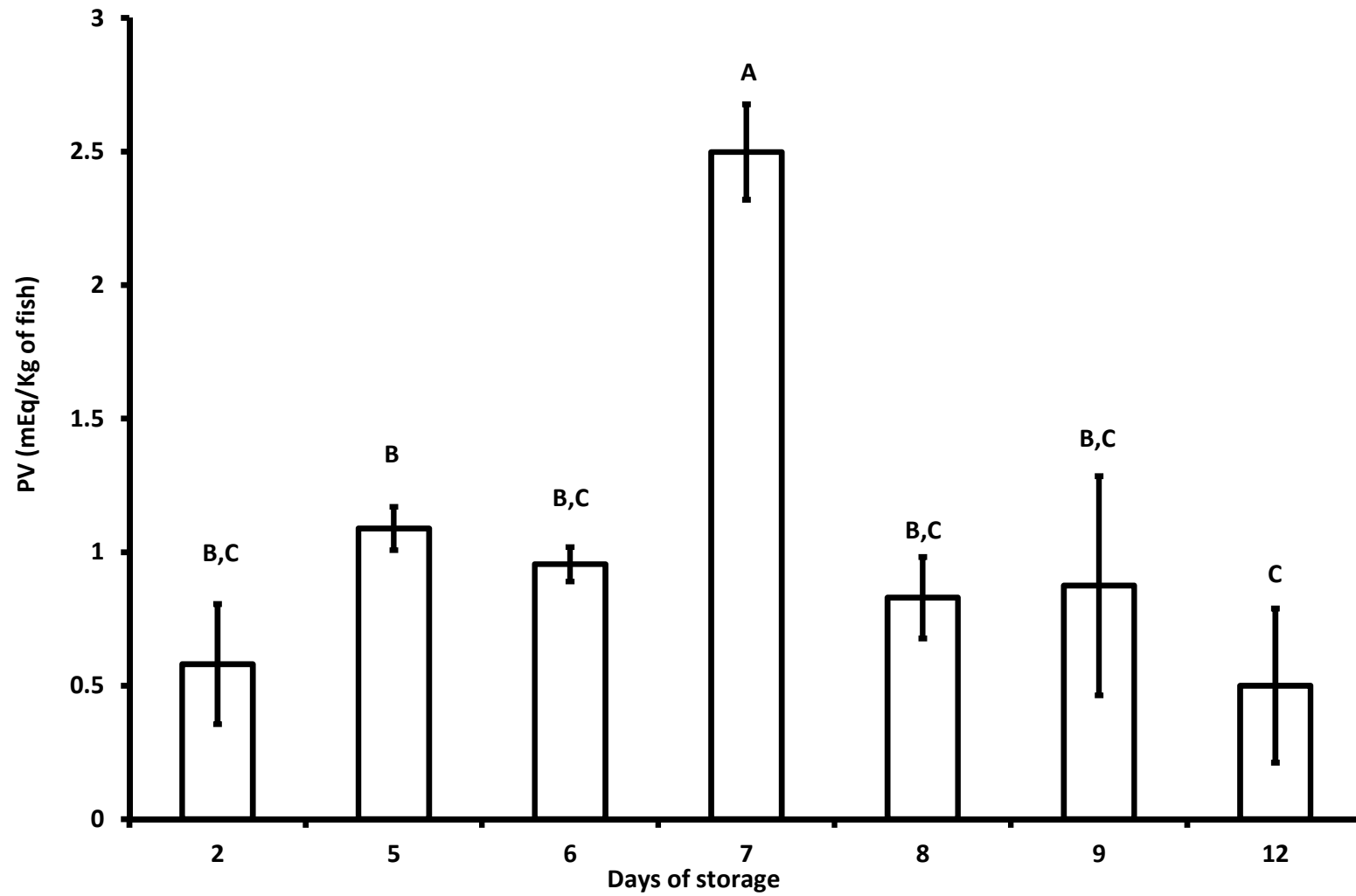
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720 **Figure 1a:**



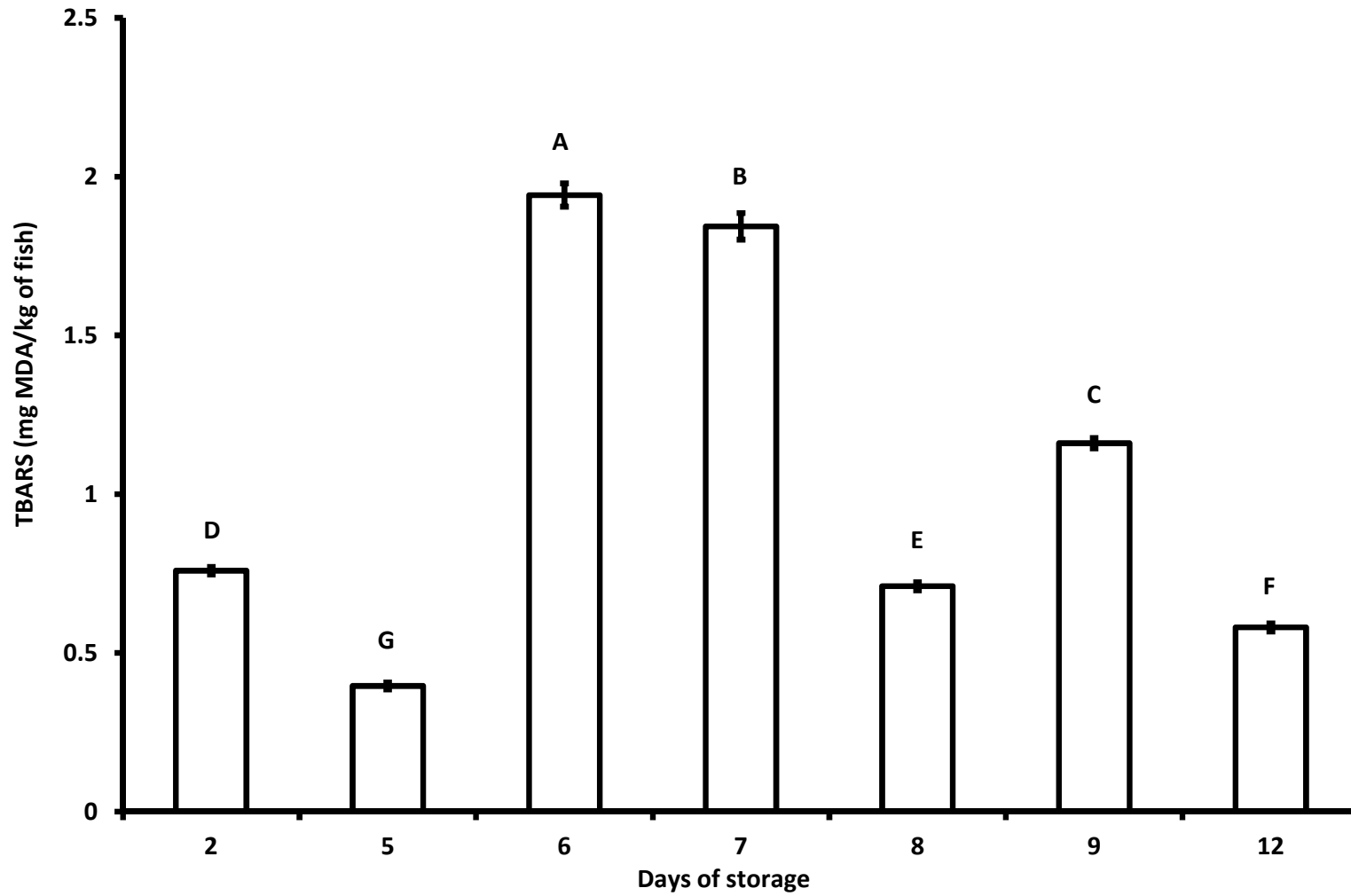
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722 Figure 1b:



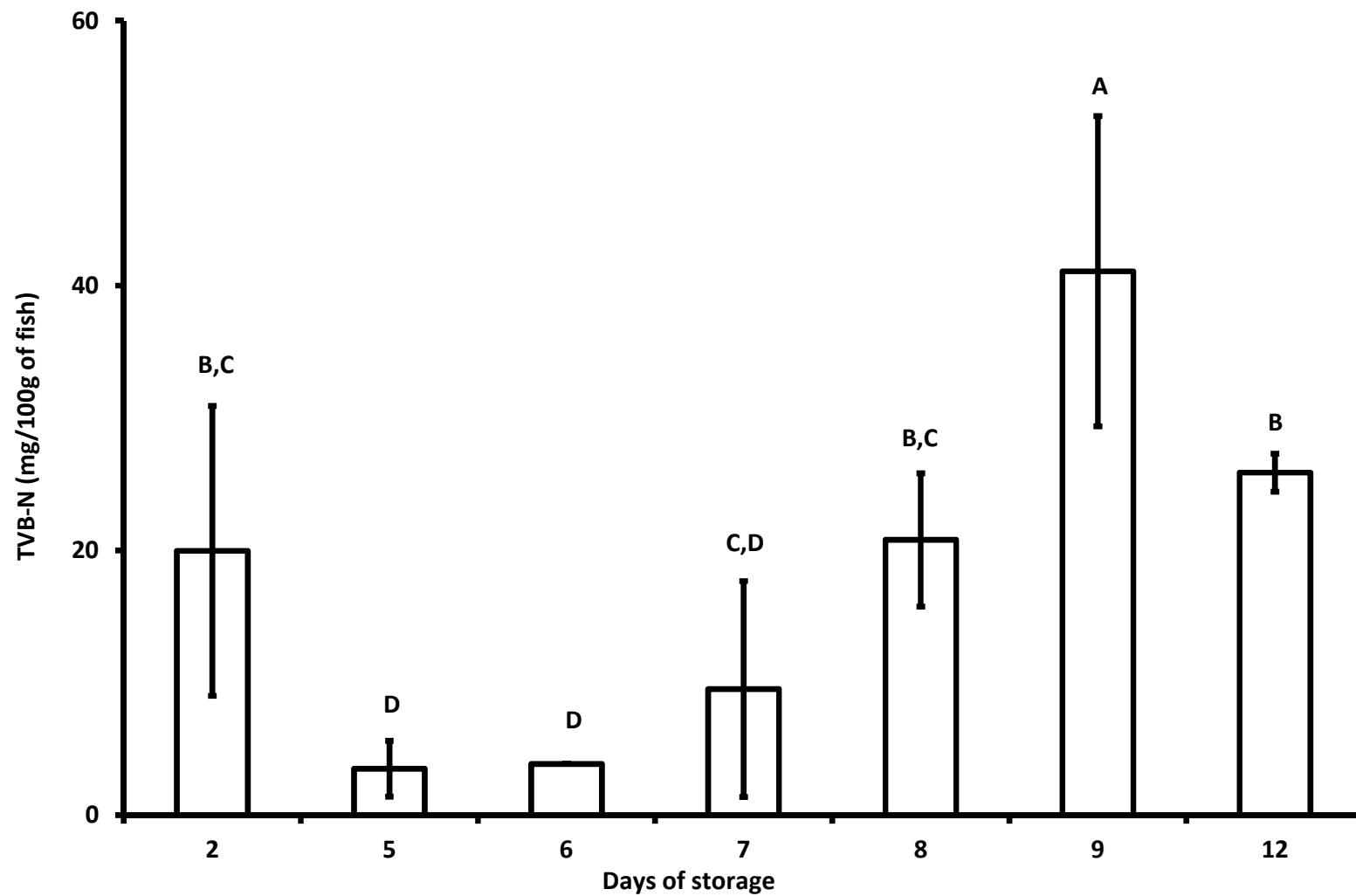
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724 **Figure 1c**



725

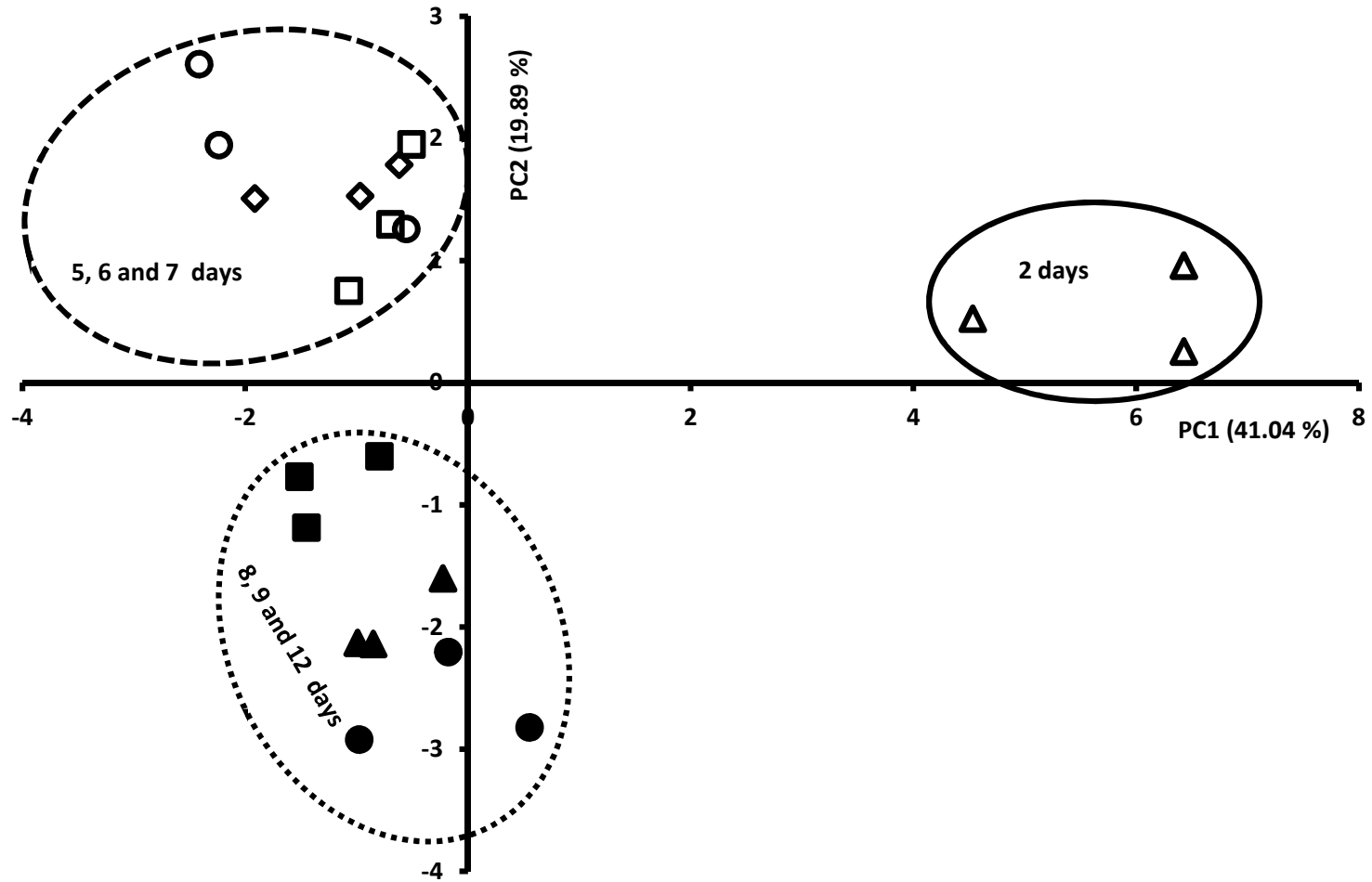
726 Figure 1d



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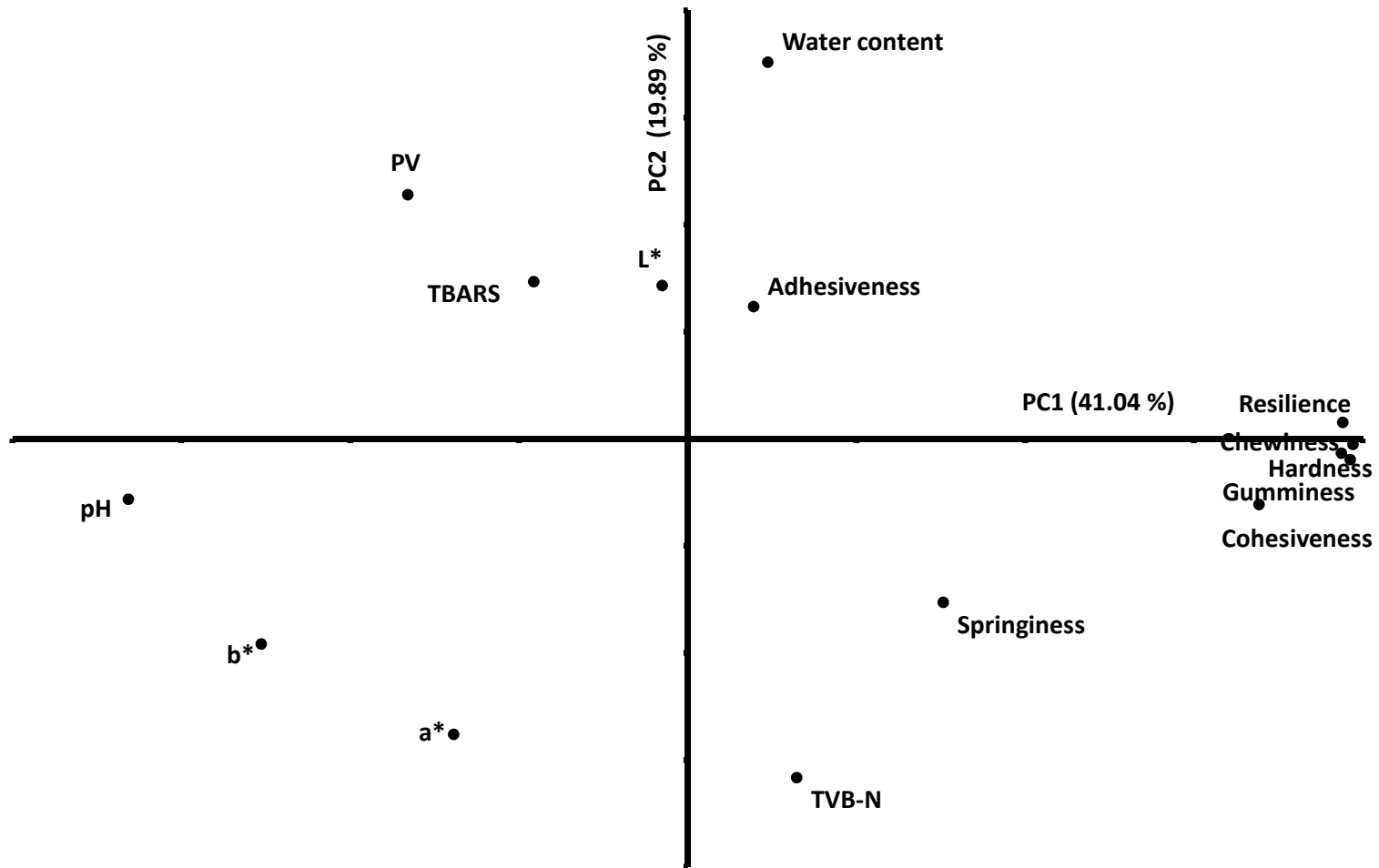


728 Figure 2a



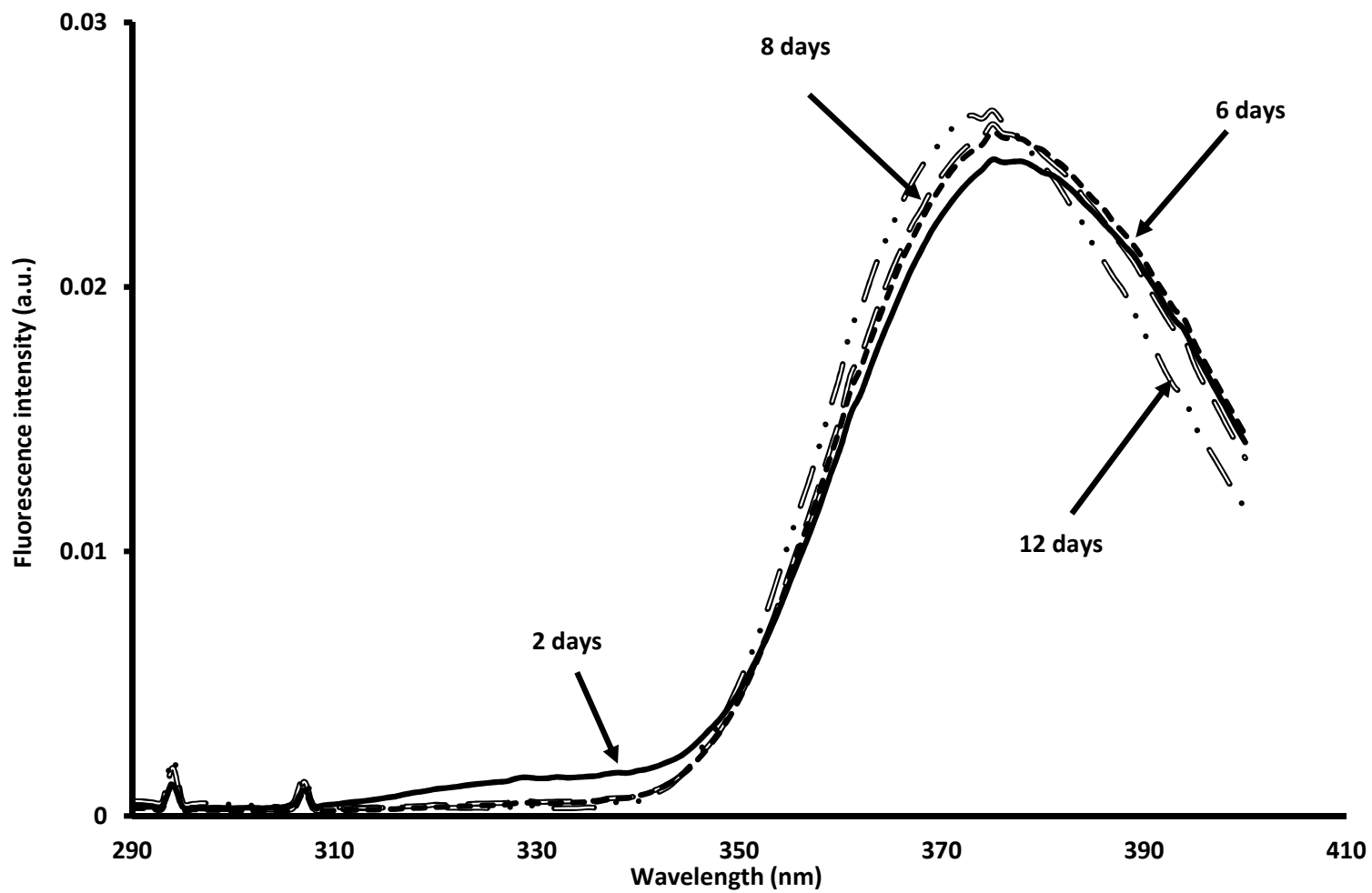
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730 **Figure 2b**



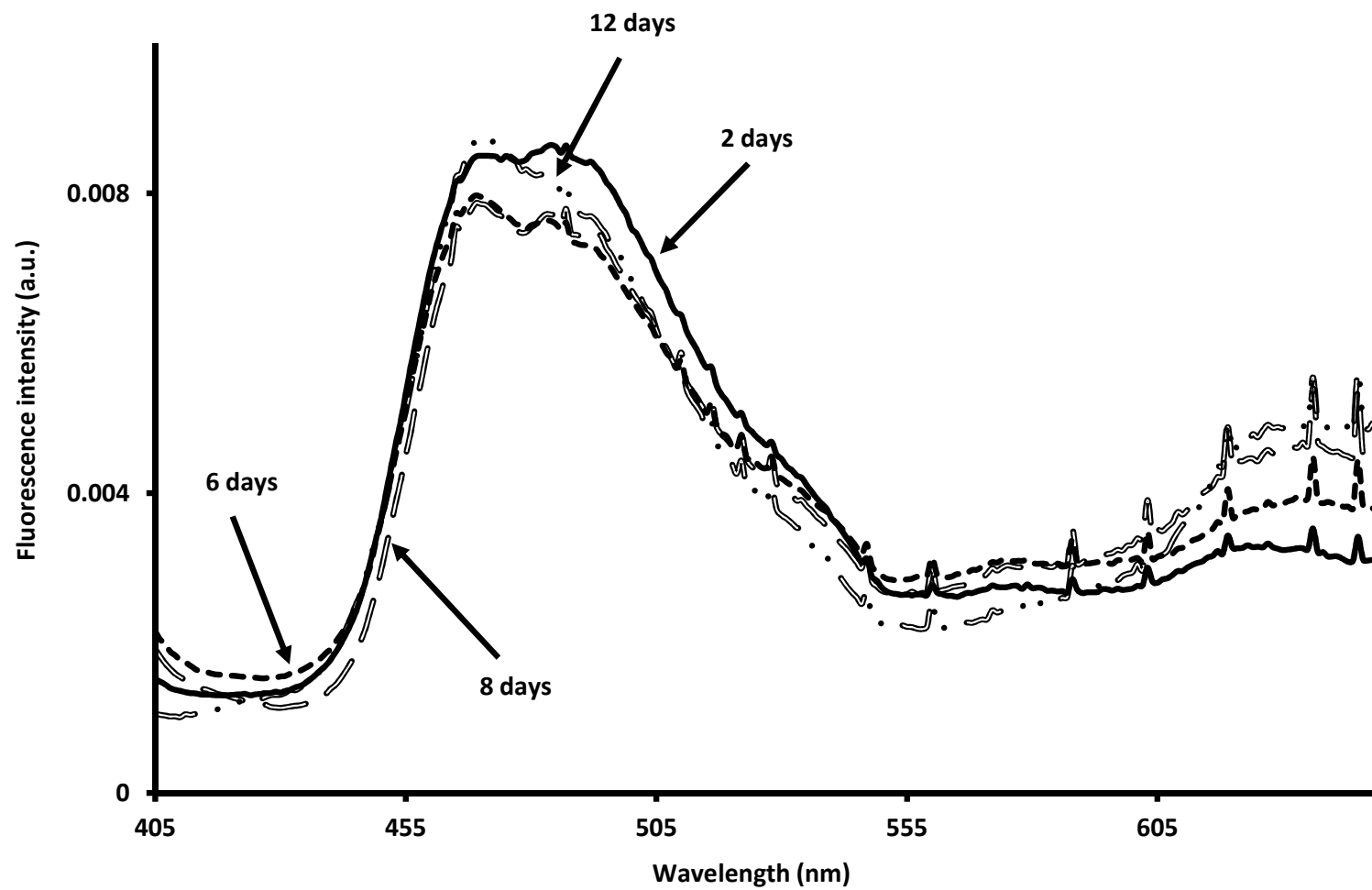
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732 Figure 3a



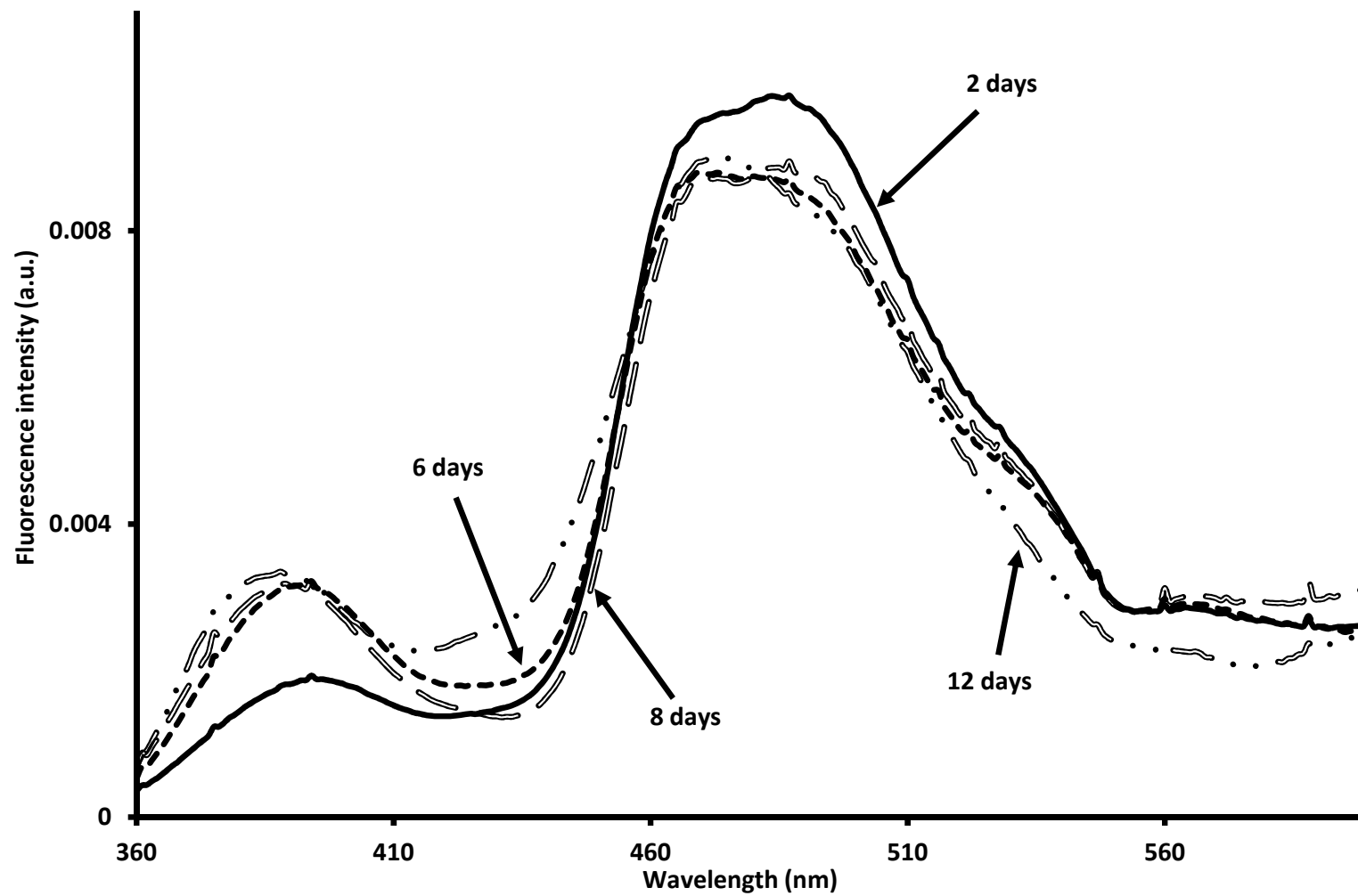
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734 Figure 3b



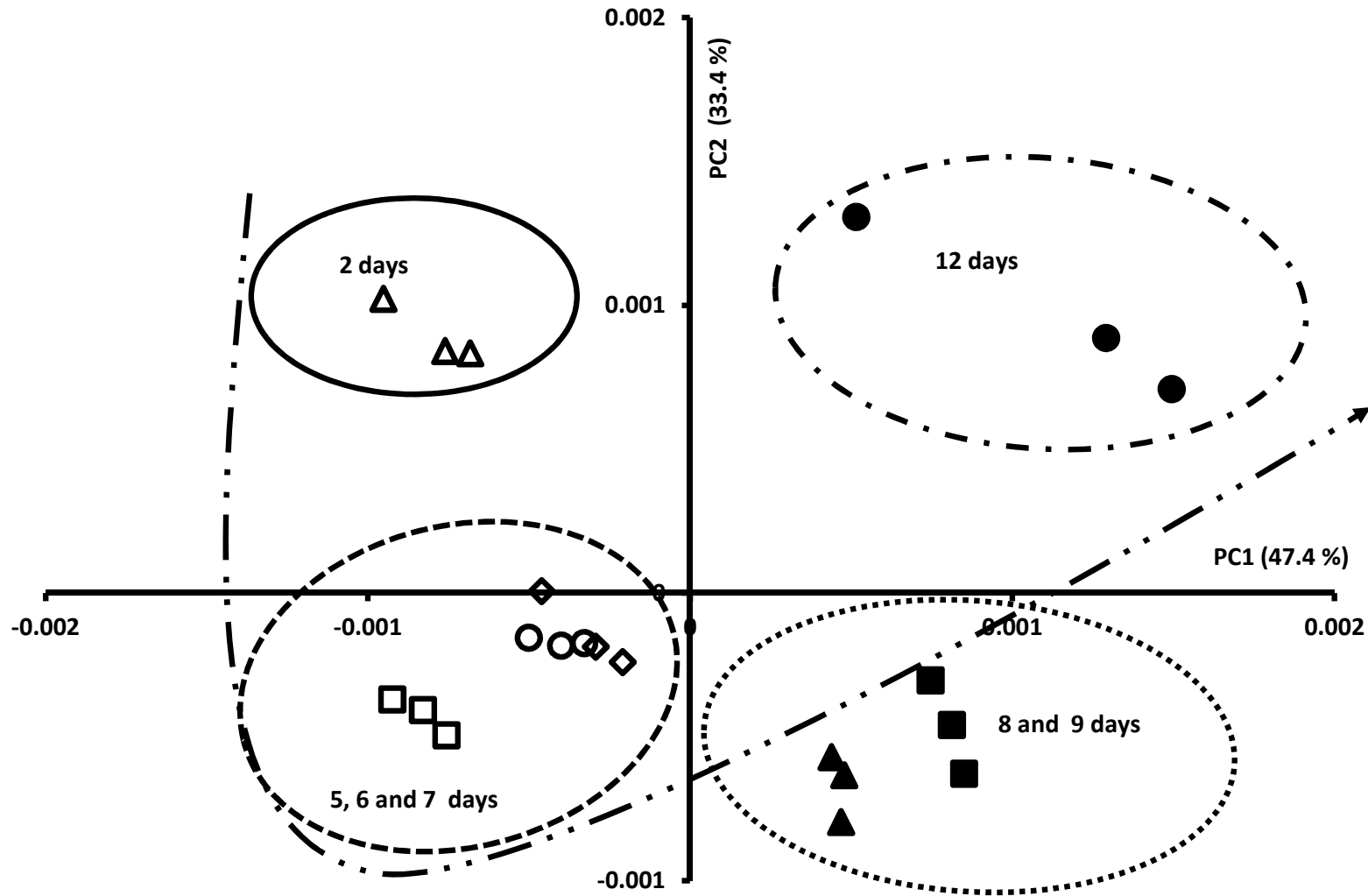
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736 Figure 3c



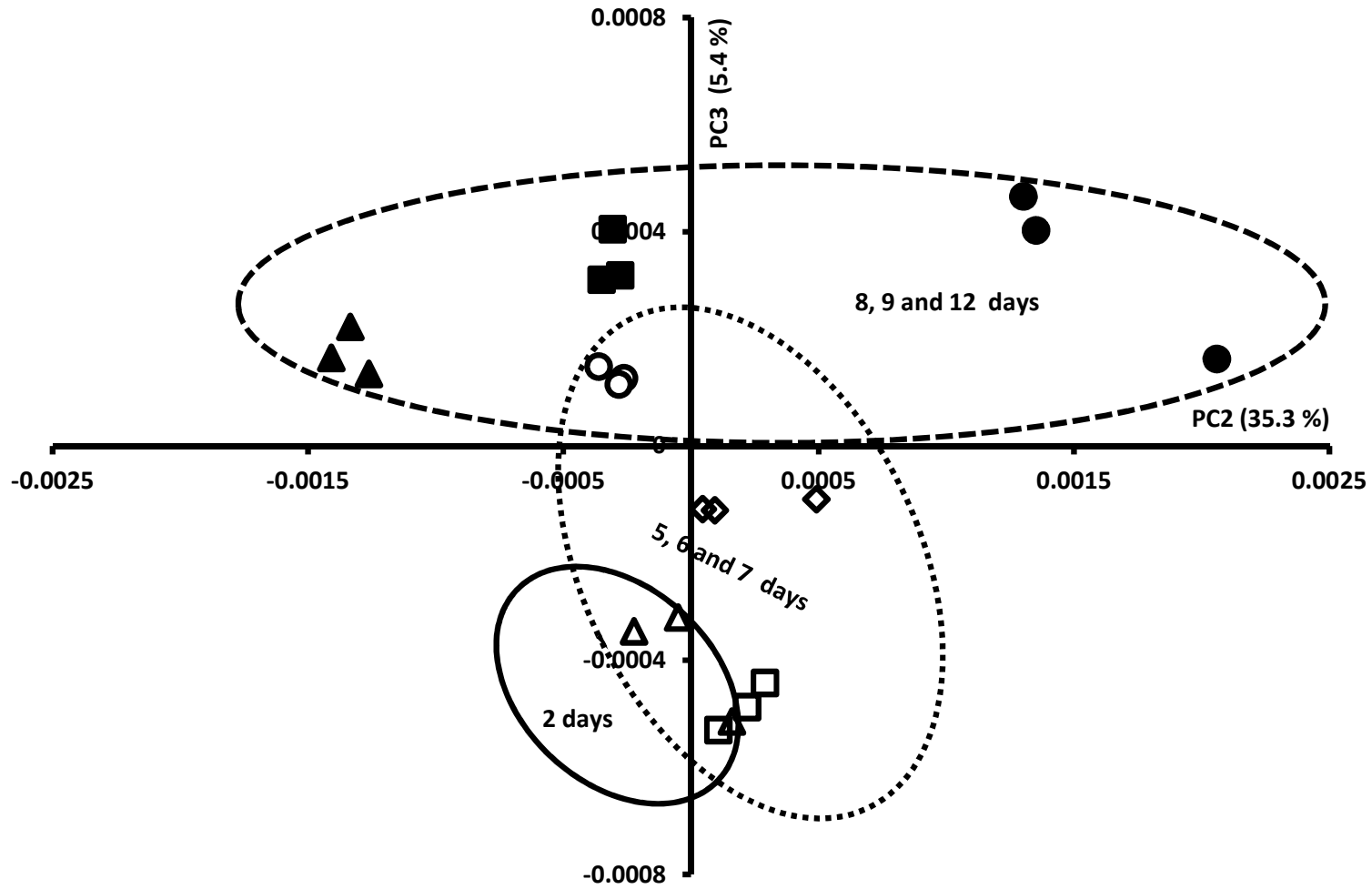
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738 Figure 4a



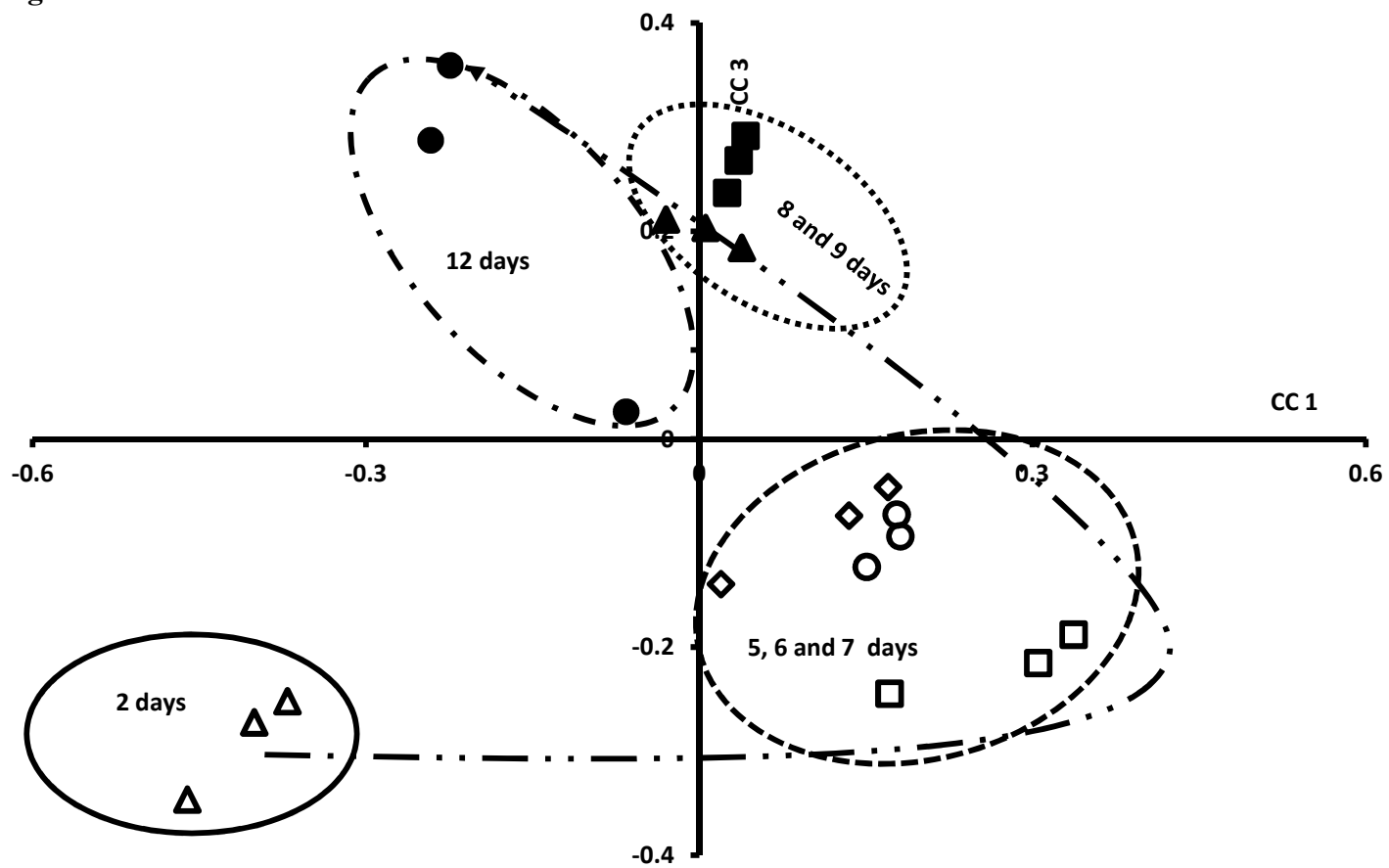
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740 Figure 4b



741

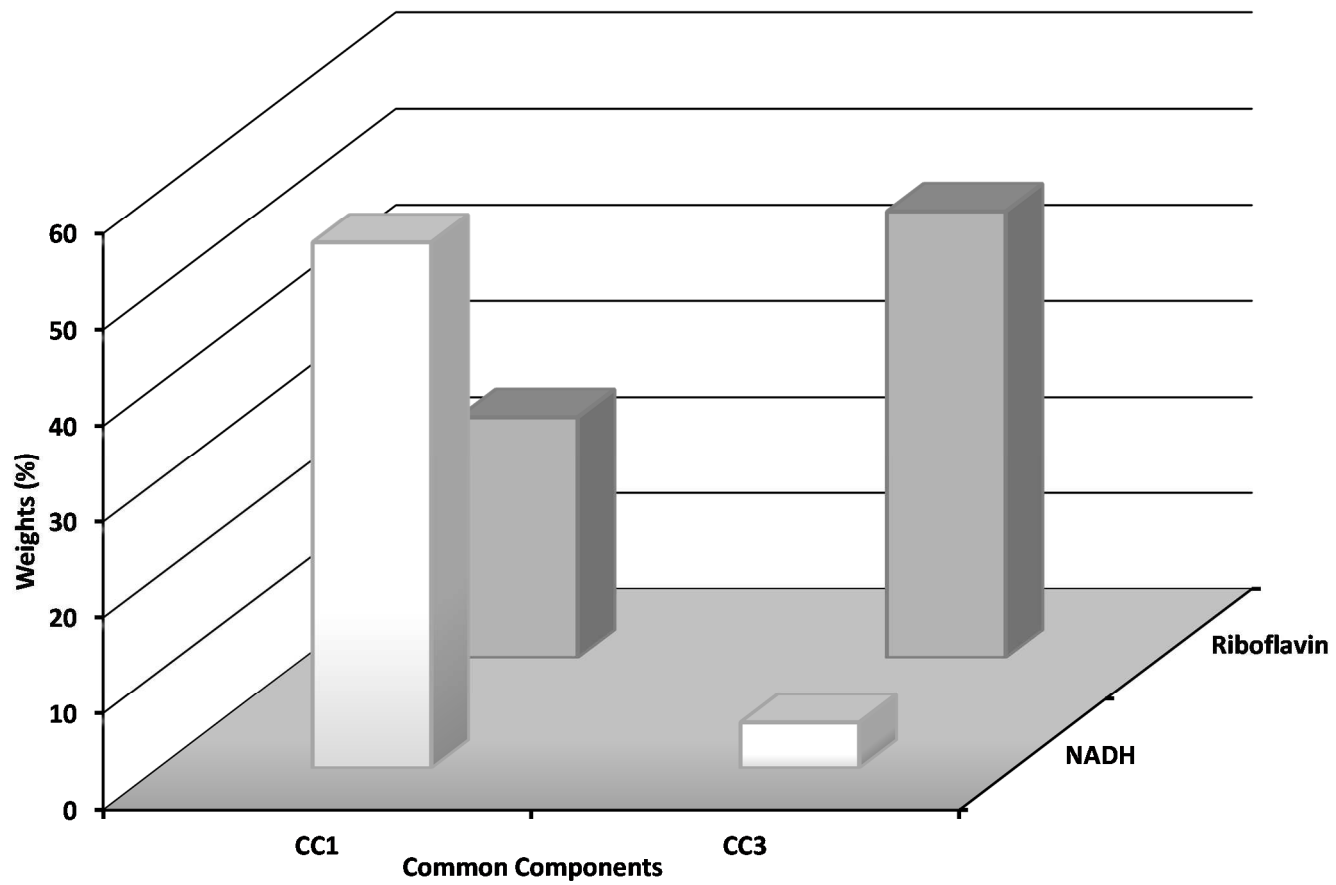
742 Figure 5a



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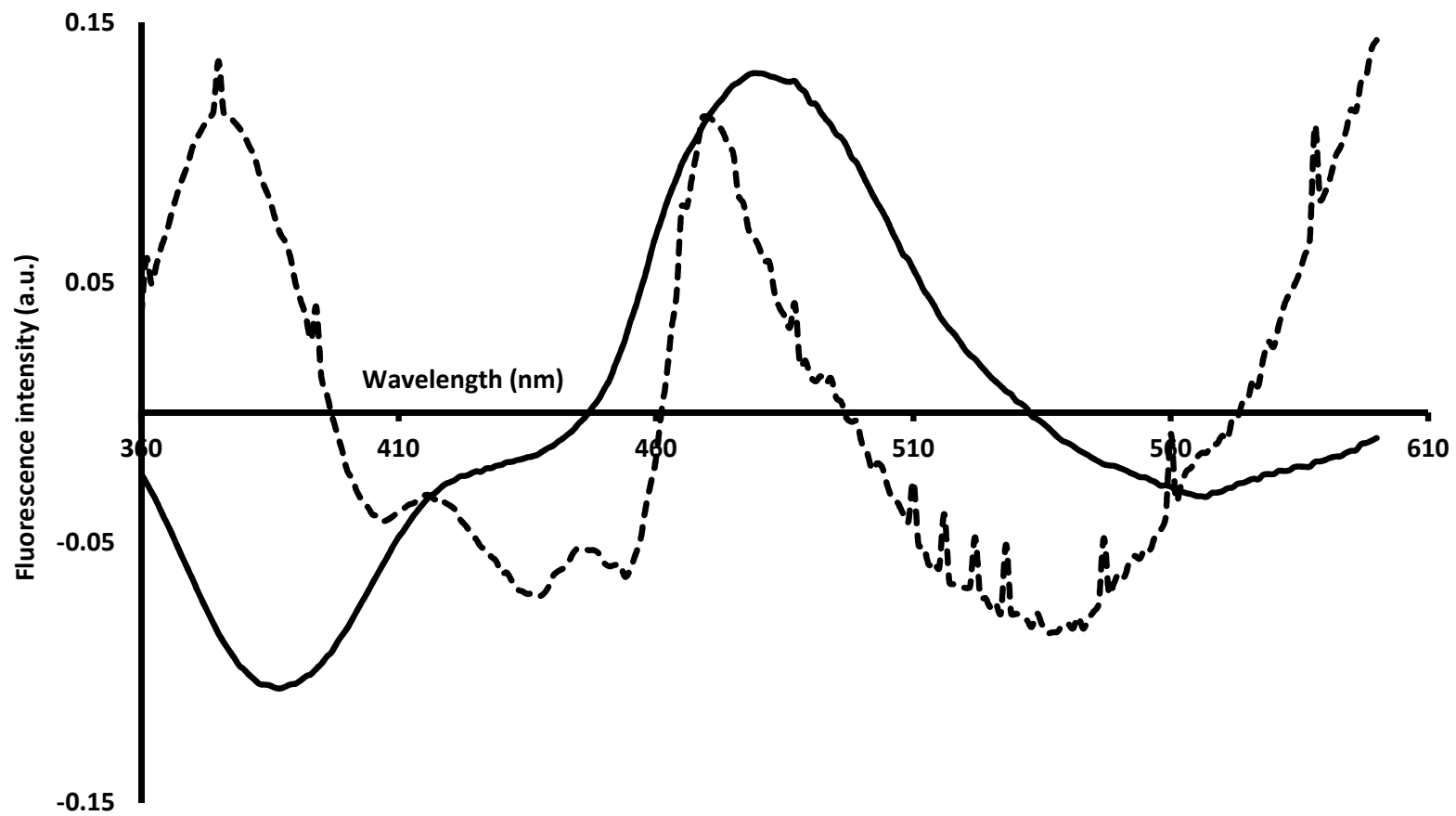


745 **Figure 5b**



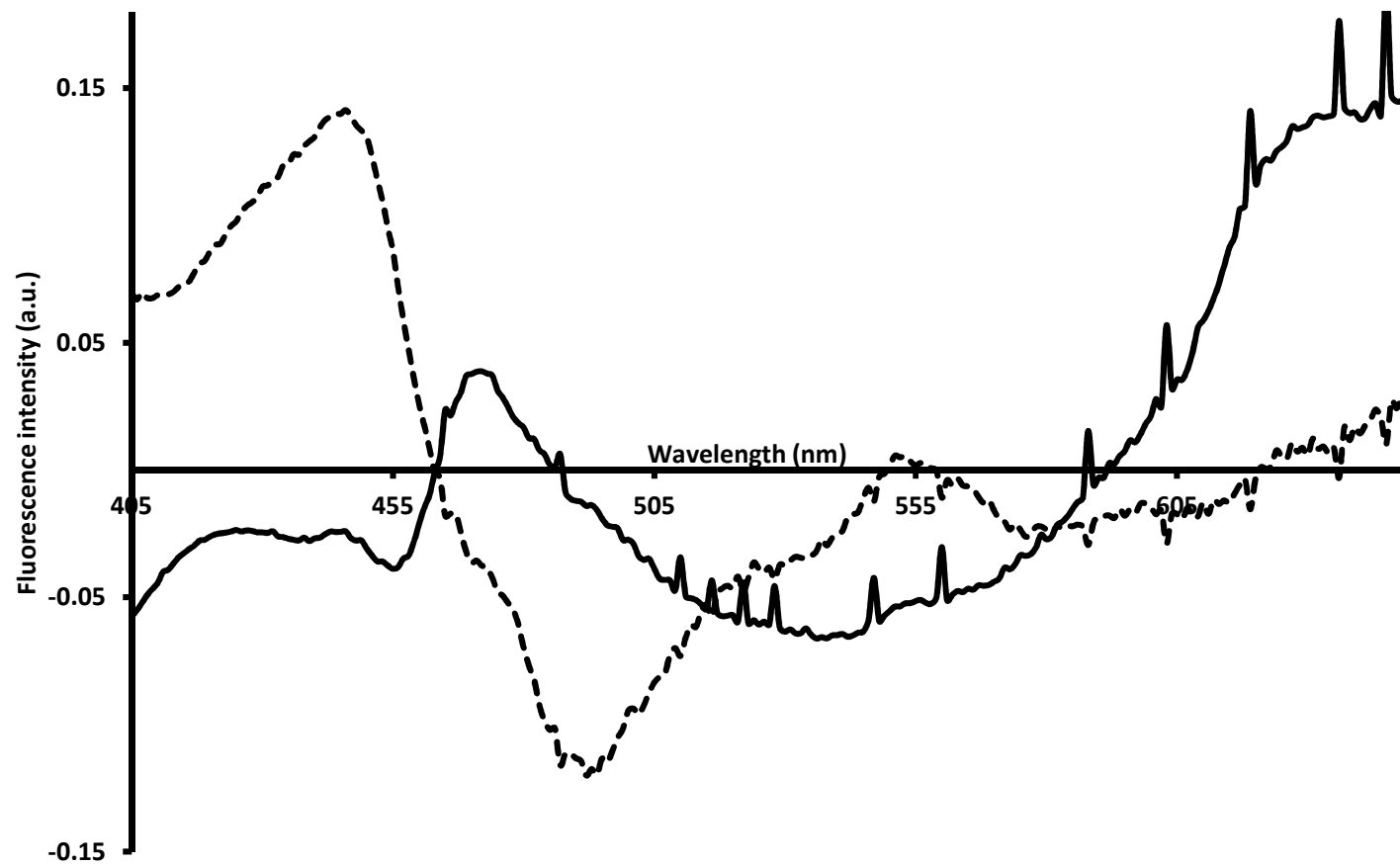
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747 Figure 5c



748

749 Figure5d



750