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Abstract

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

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

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

I. Introduction

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

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

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

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

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

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

2. Materials and methods

2.1. Chemicals and reagents

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

2.2. Fish samples preparation

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

2.3. Physico-chemical analysis

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

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

2.4. Total volatile basic nitrogen measurements

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

2.5. Instrumental techniques

2.5.1. Color measurements

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

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

2.5.2. Texture profile analysis measurements

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

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

2.5.3. Fluorescence measurements

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

2.6. Statistical analysis

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

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

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

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

3. Results and discussion

3.1. Proximate analysis

Proximate analysis of fresh sturgeon aged 2 days showed that protein and lipid contents were 14.79% and 12.28%, respectively, in agreement, with the findings of Badiani et al. (1996) who pointed out levels of protein and lipid in the 17.60 - 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% (±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**

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

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

3.3. Evolution of thiobarbituric acid reactive substances during storage

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

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

3.4 Evolution of total volatile basic nitrogen during storage

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

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

3.5. Instrumental techniques

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

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

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

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

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

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

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

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 β-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^+, 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 CC1exhibited 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

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

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

4. Conclusion

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

Acknowledgments:

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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

Table 1a:

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

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

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

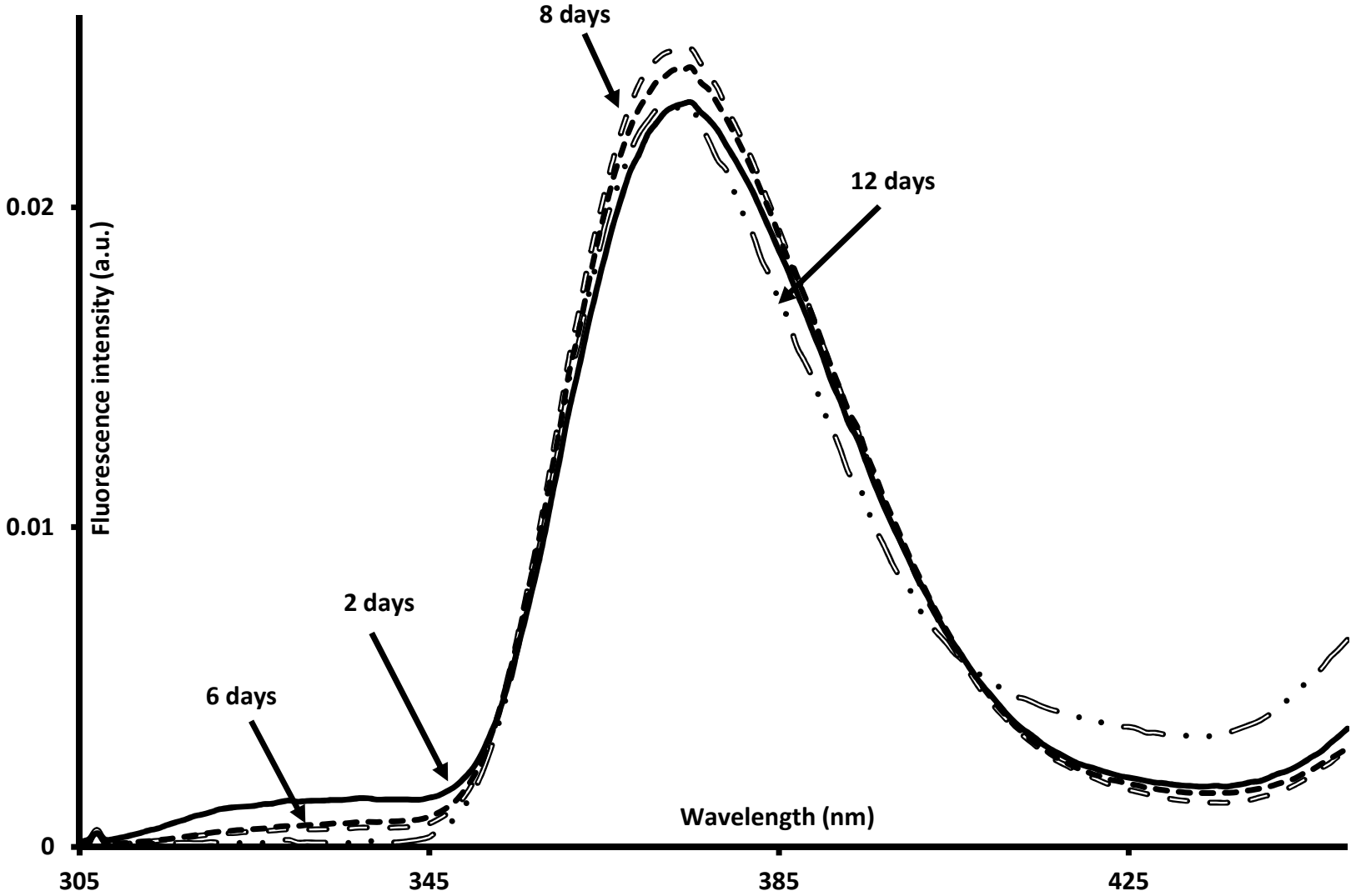
674 *Mean values and standard deviations from three replicates are presented.*
675 *Different capital letters (A, B, C, D) represent statistical differences between different storage days (P<0.05)*

List of supplementary figures:

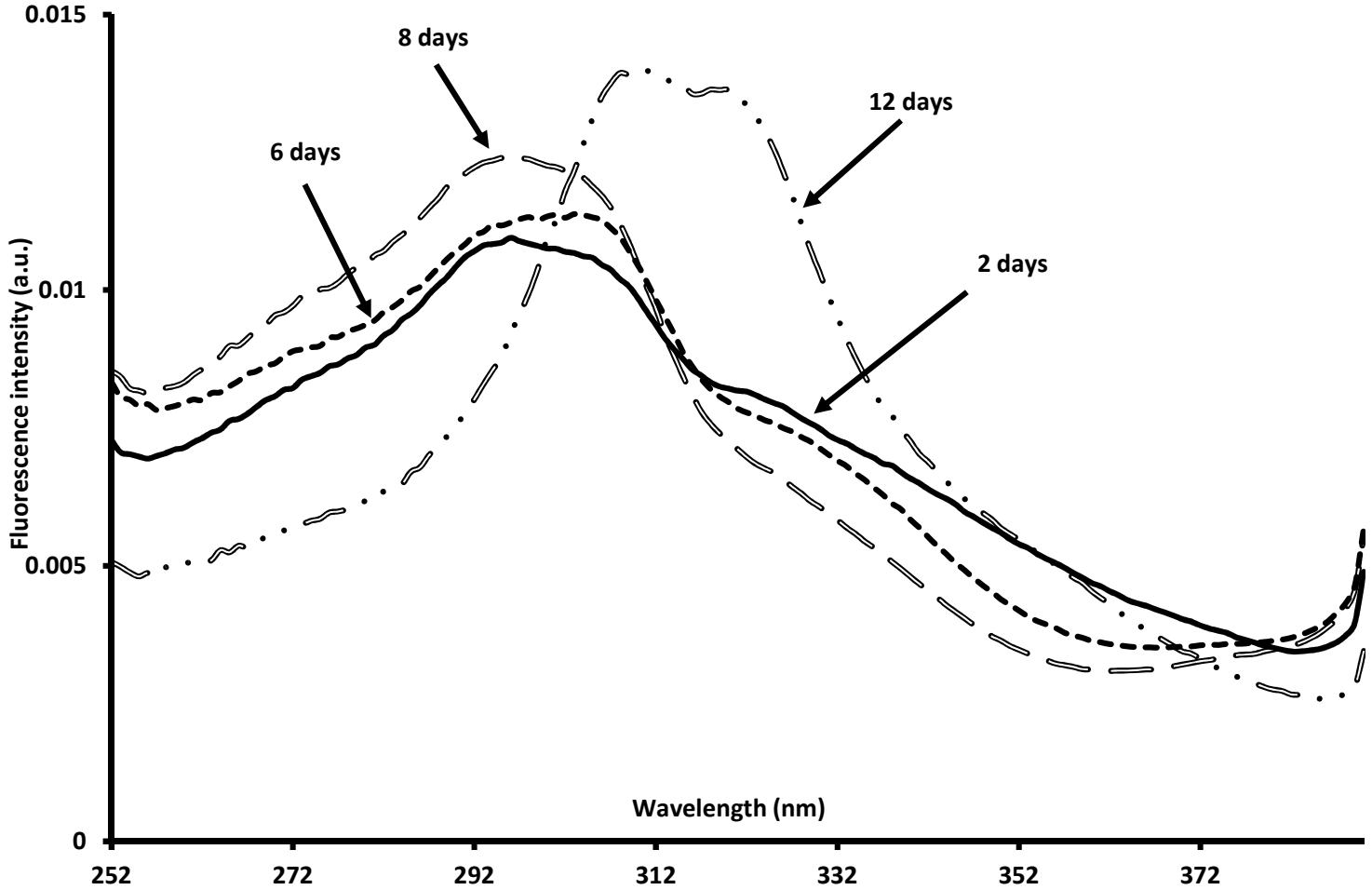
Figure 1S: Normalised fluorescence emission spectra of tryptophan (excitation: 290 nm, emission 305–450 nm) recorded on sturgeon samples stored at 4 °C up to 12 days.

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

684 **Figure 1S:**



685 **Figure 2S:**



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List of Figures

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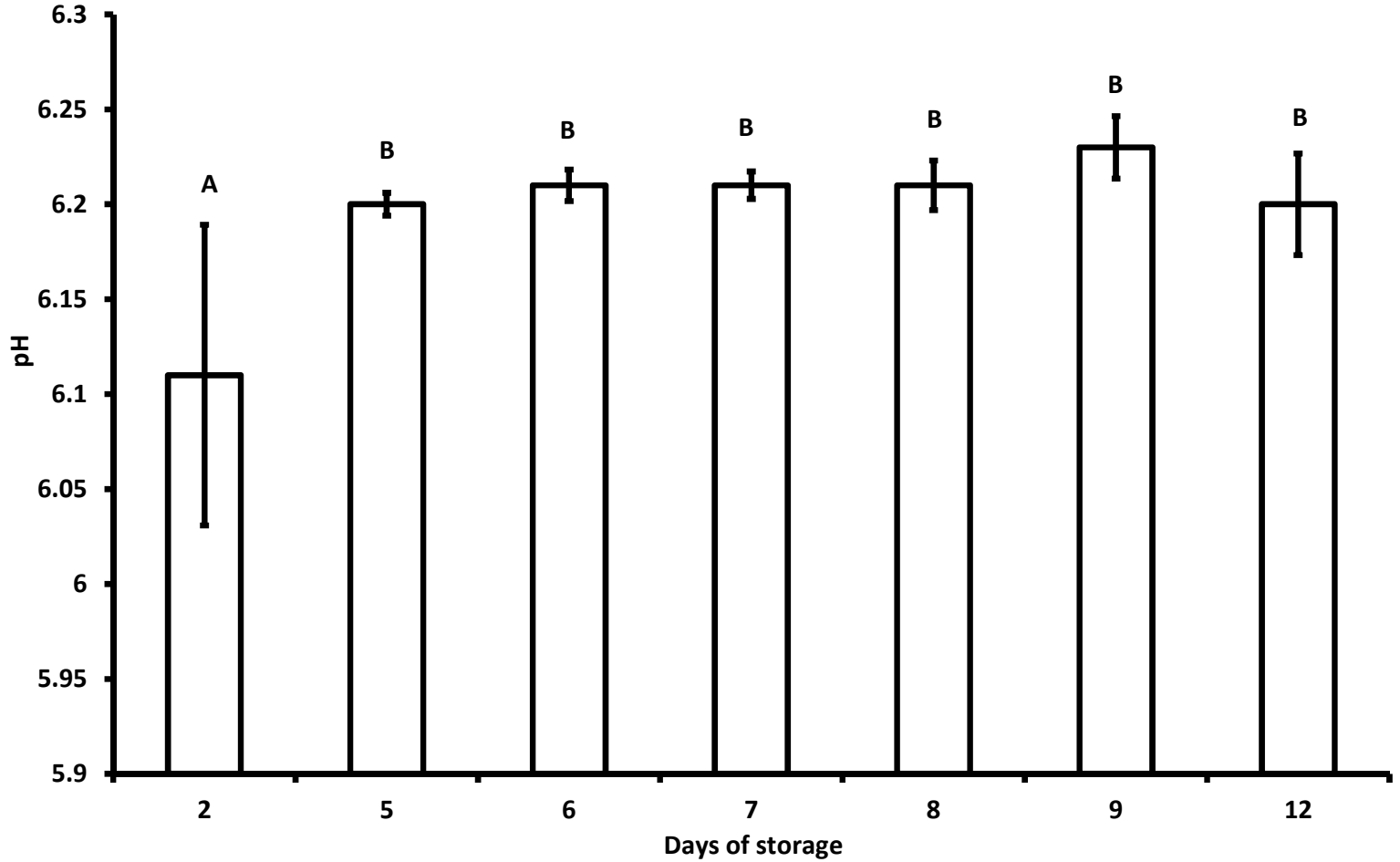

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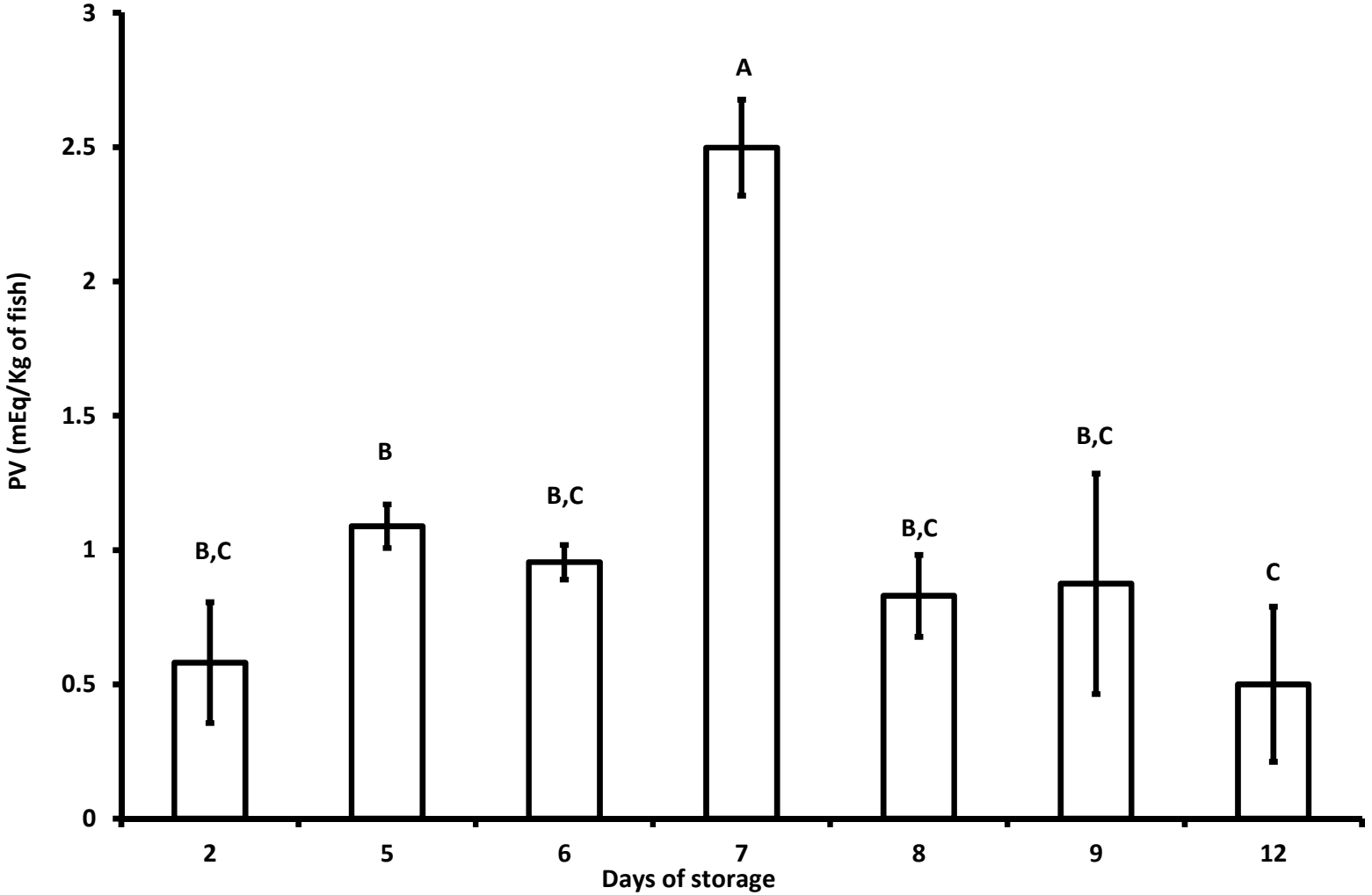

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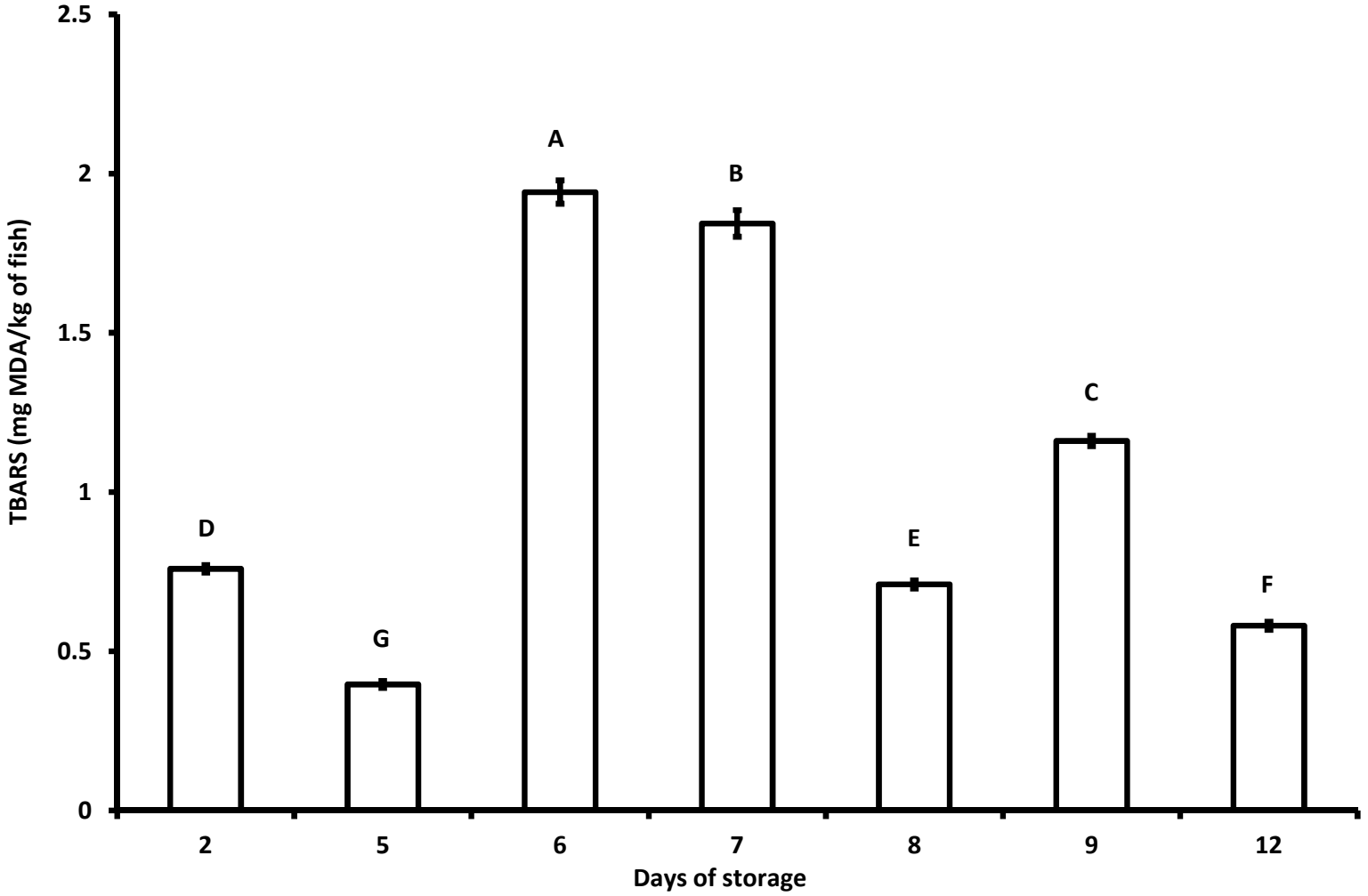

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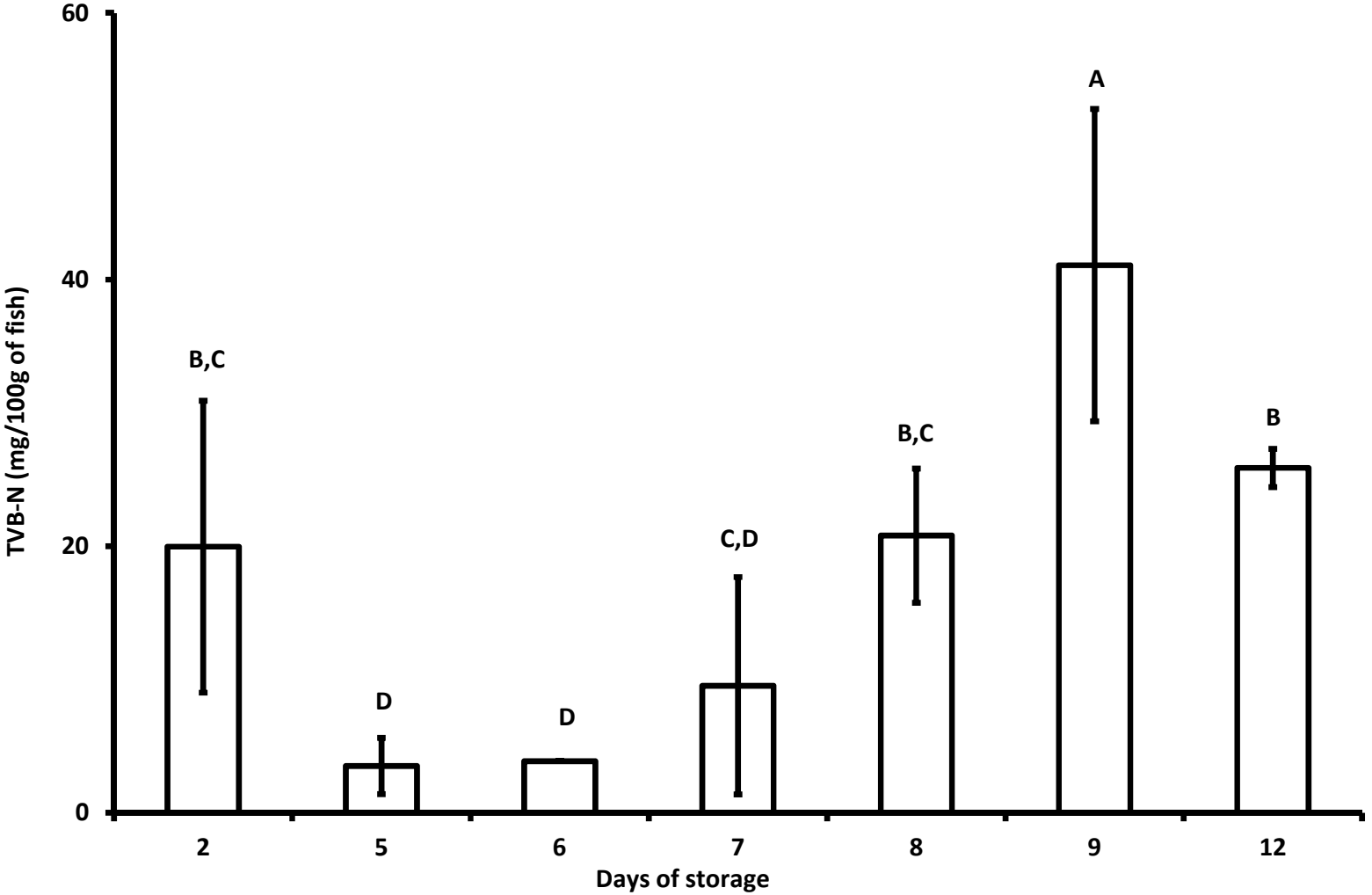

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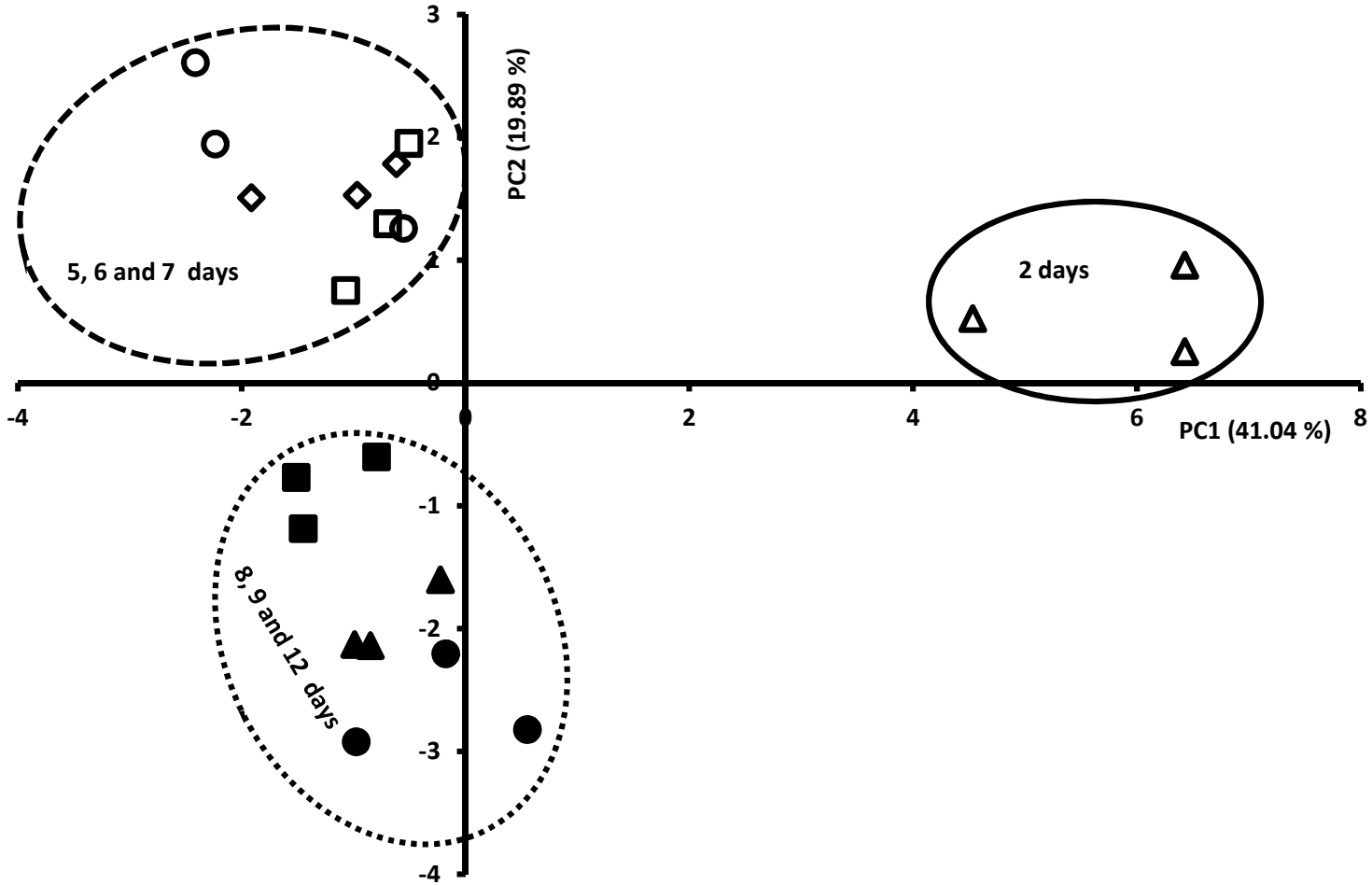

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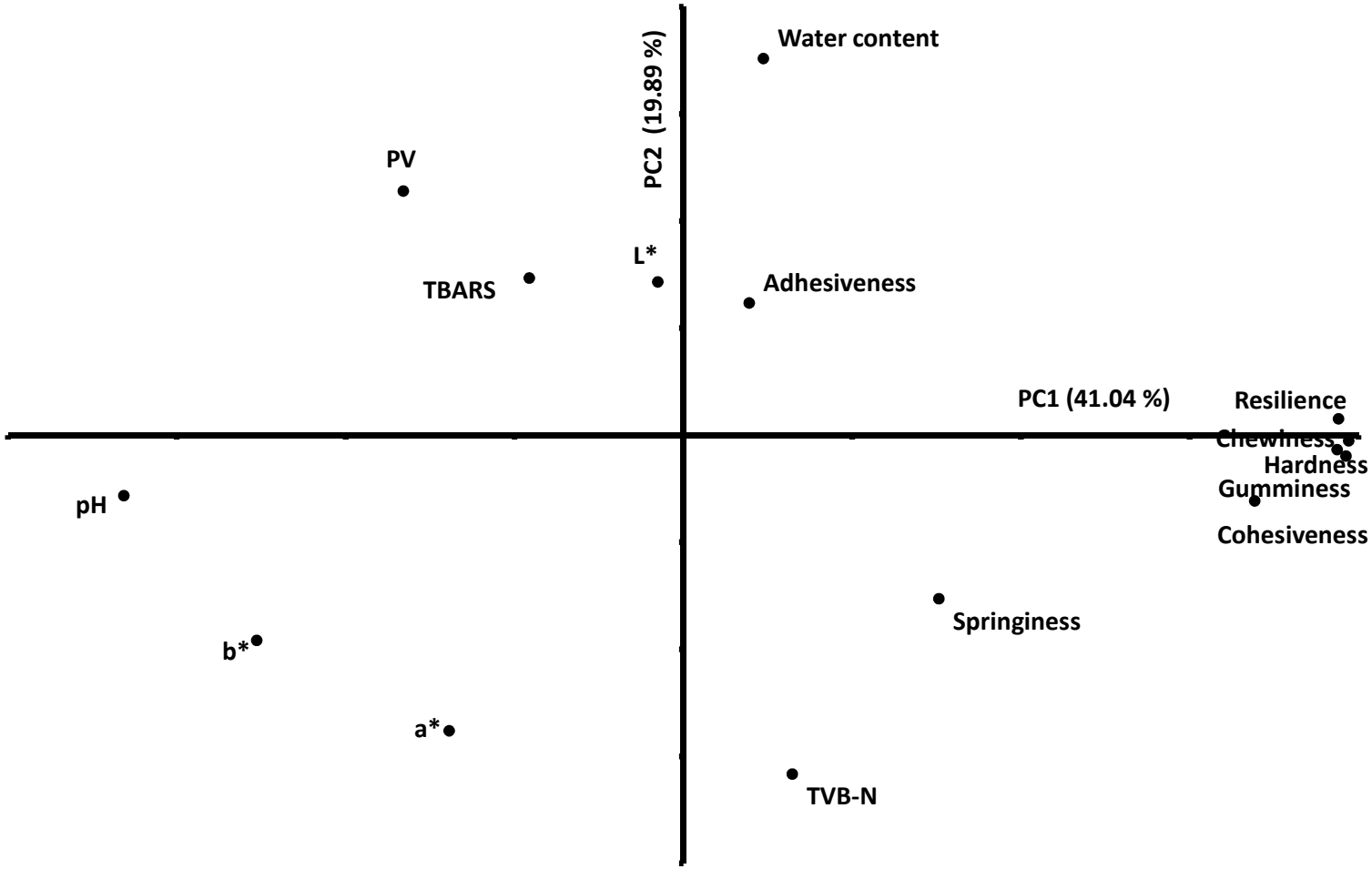

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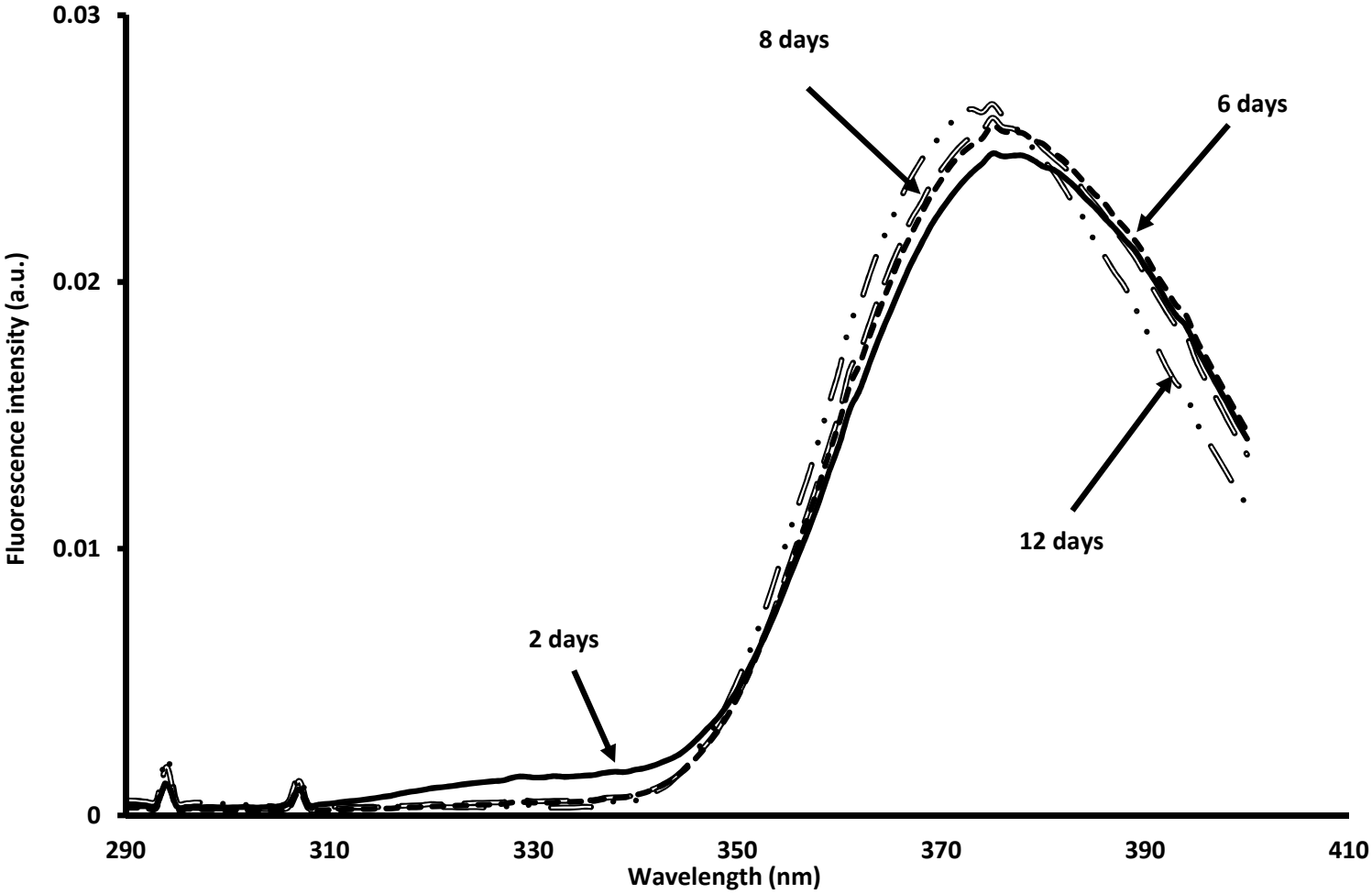

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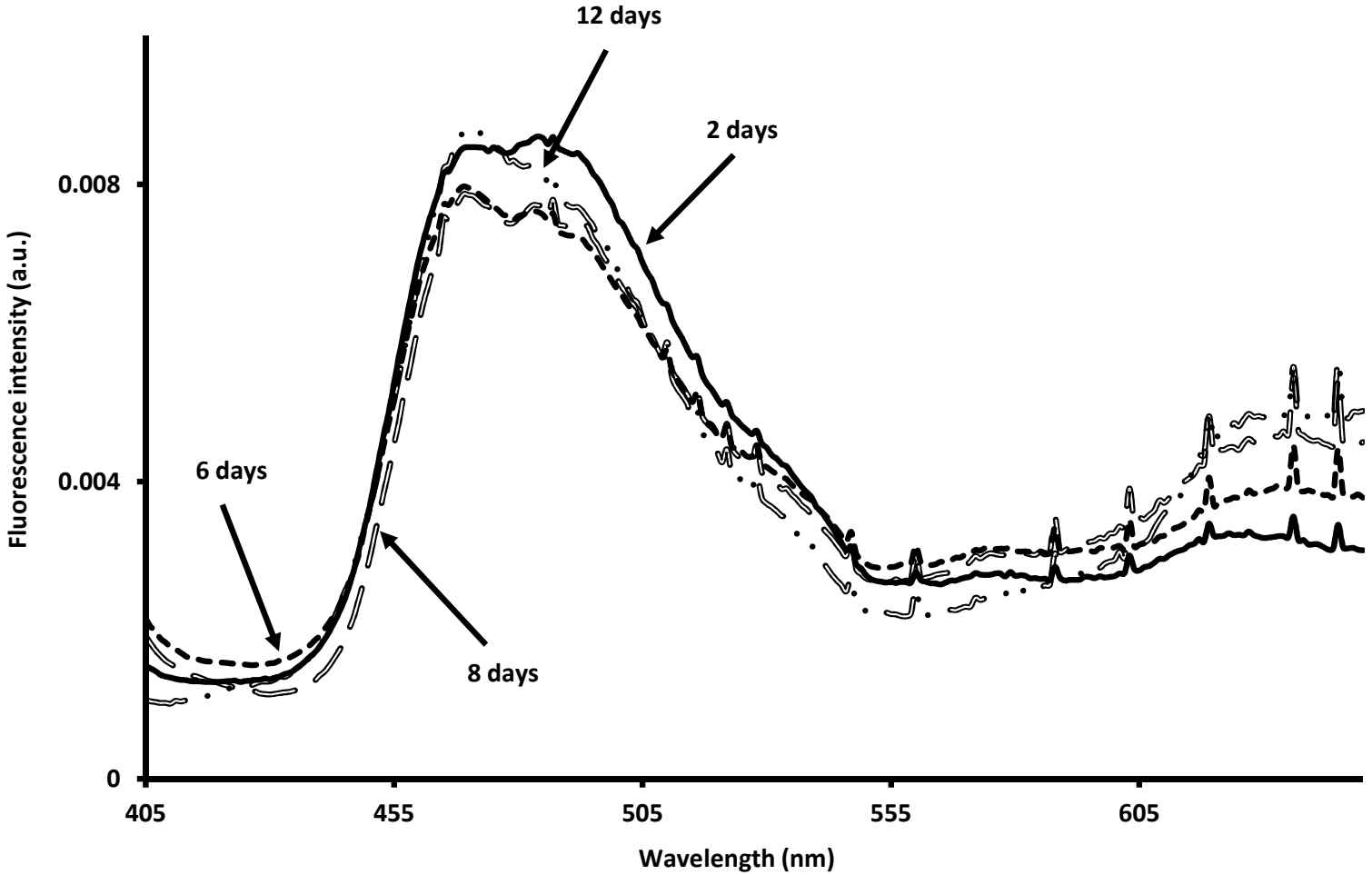
734 **Figure 3b**



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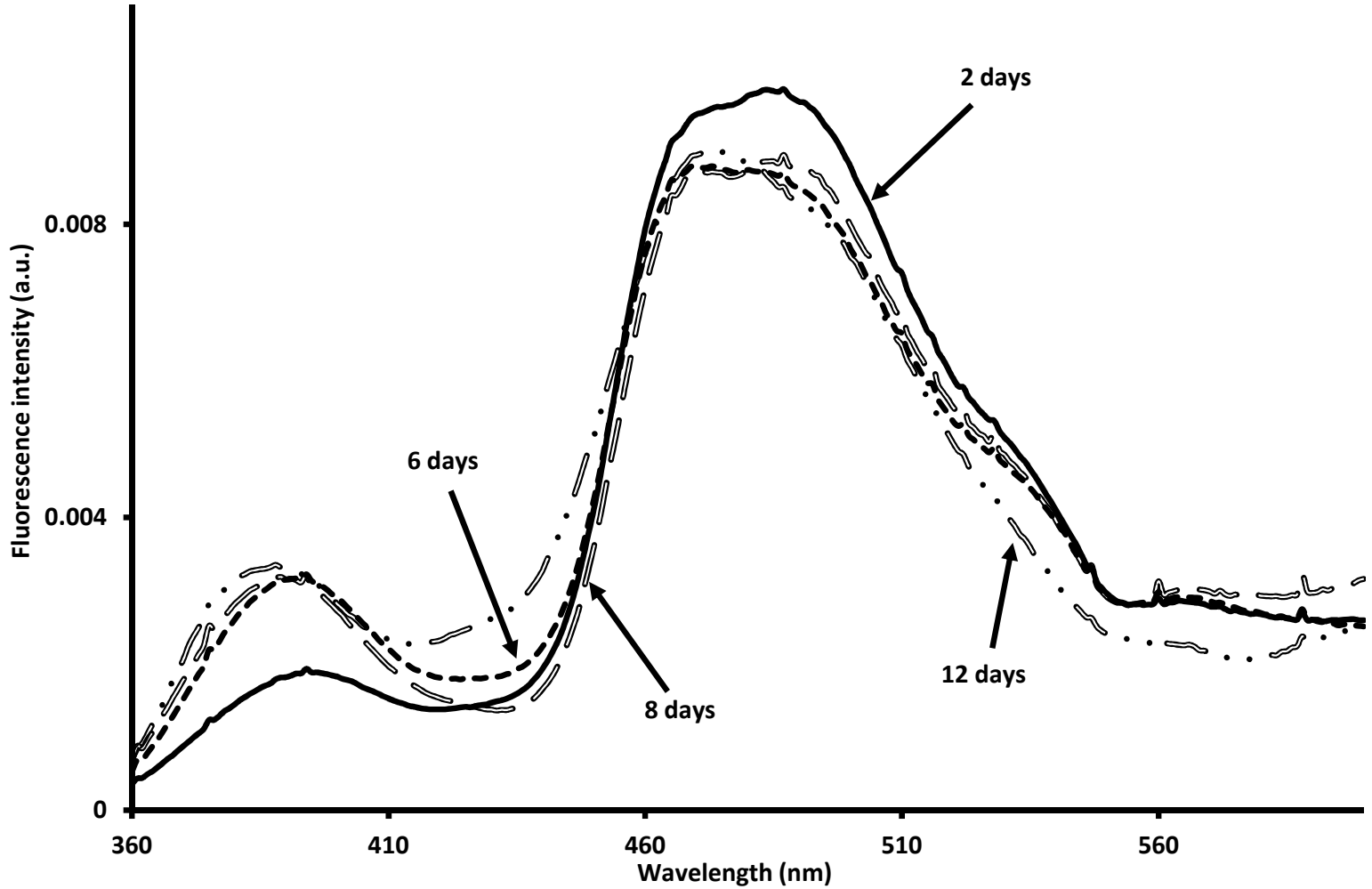

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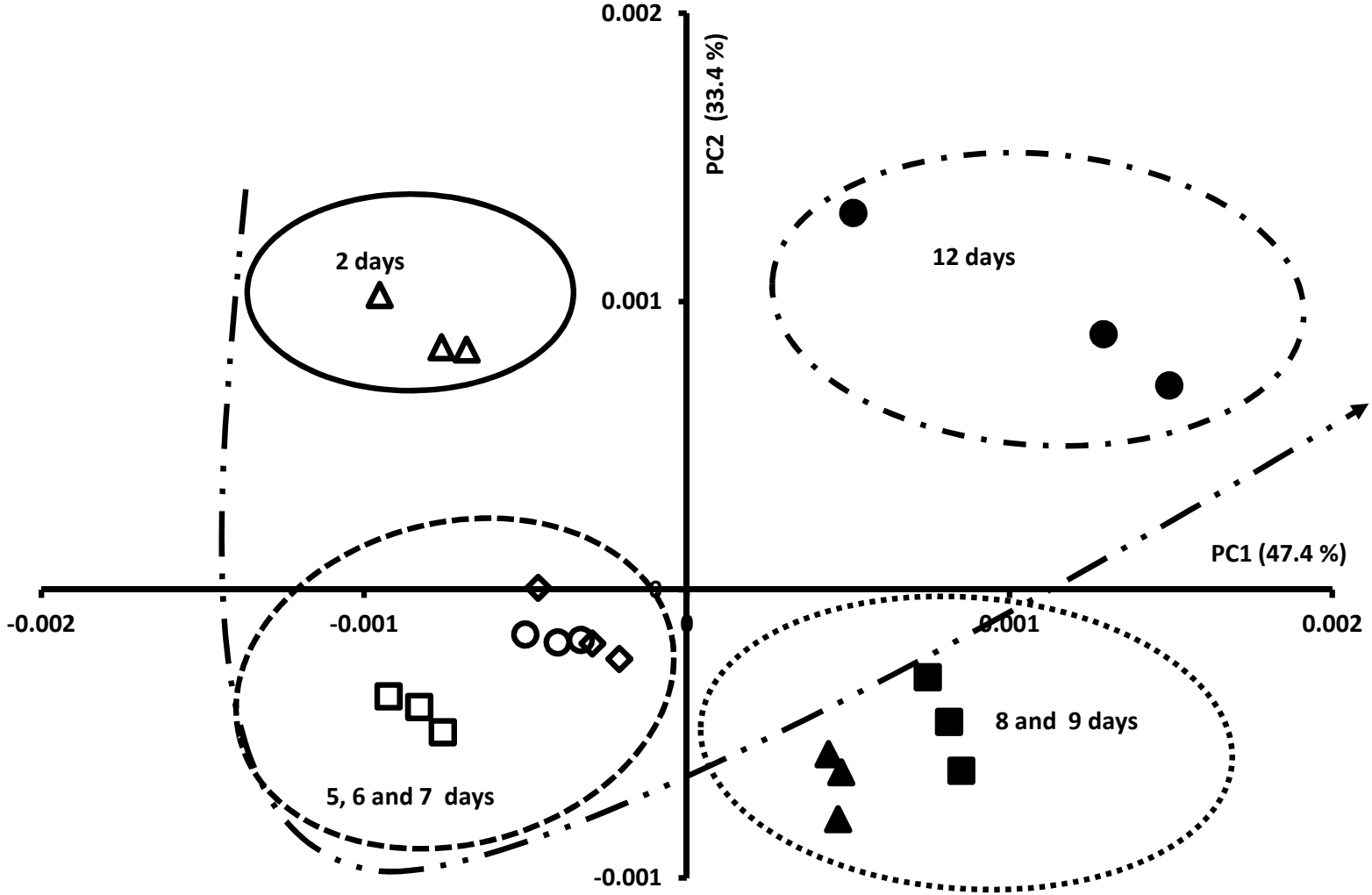

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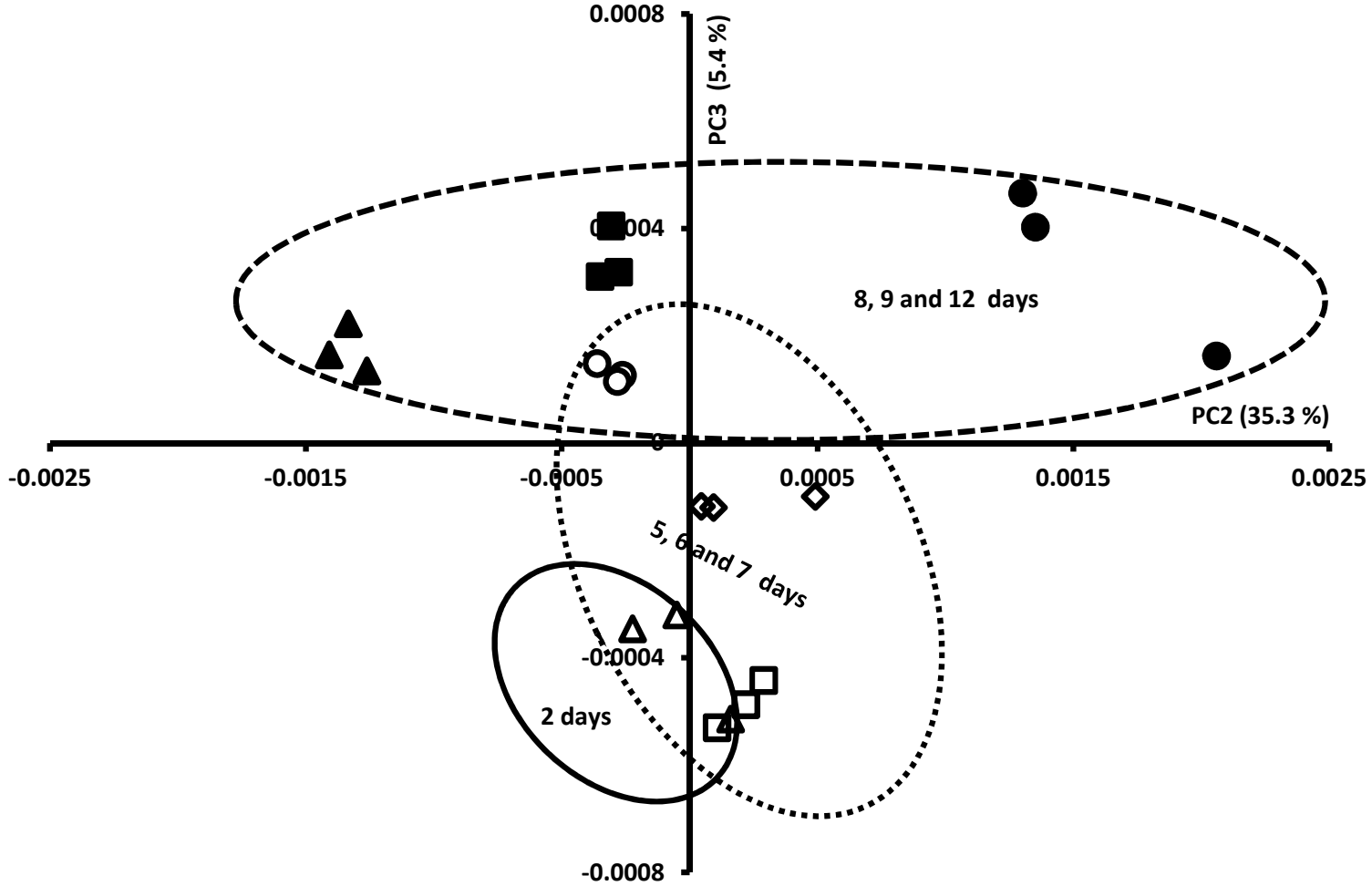

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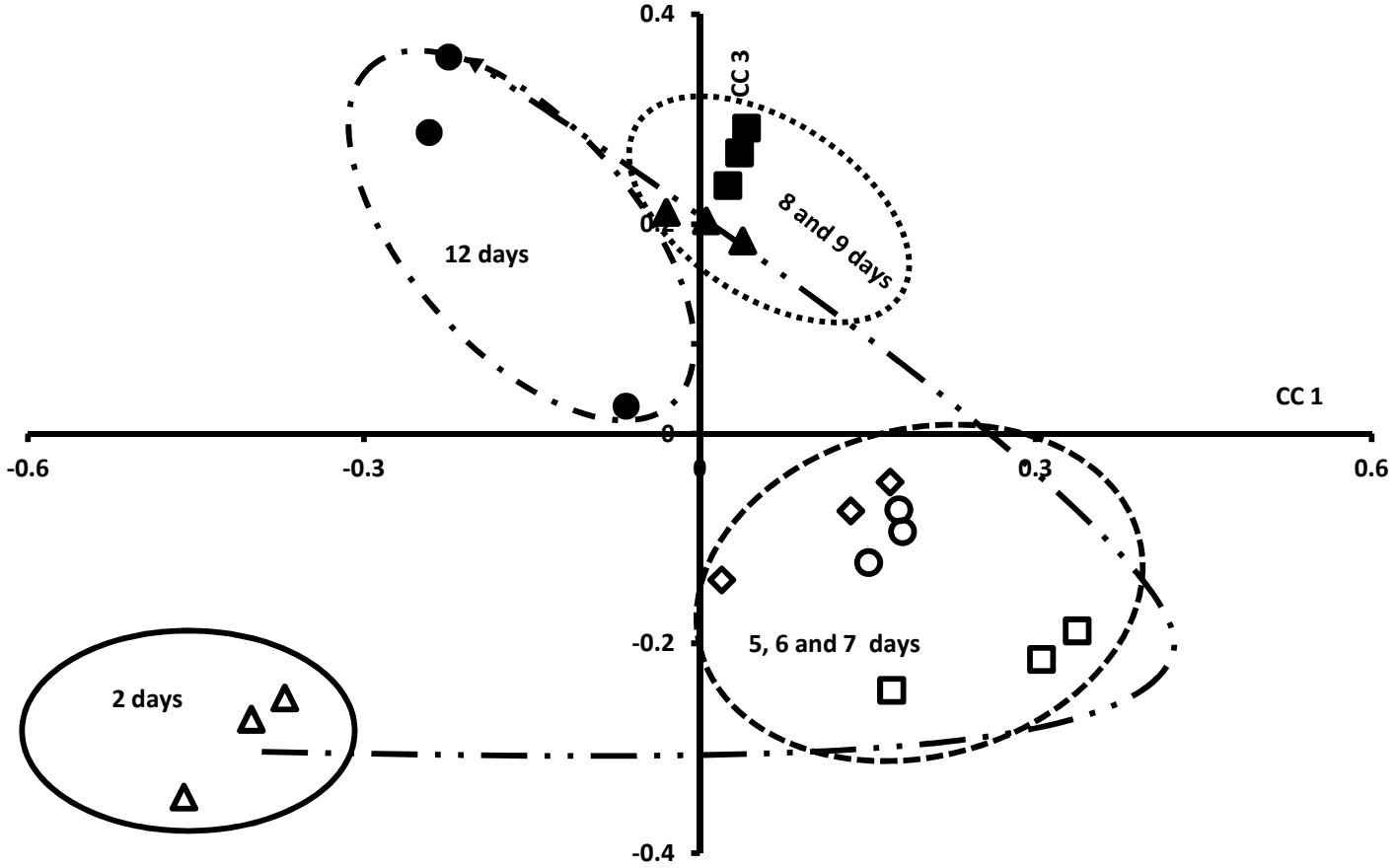

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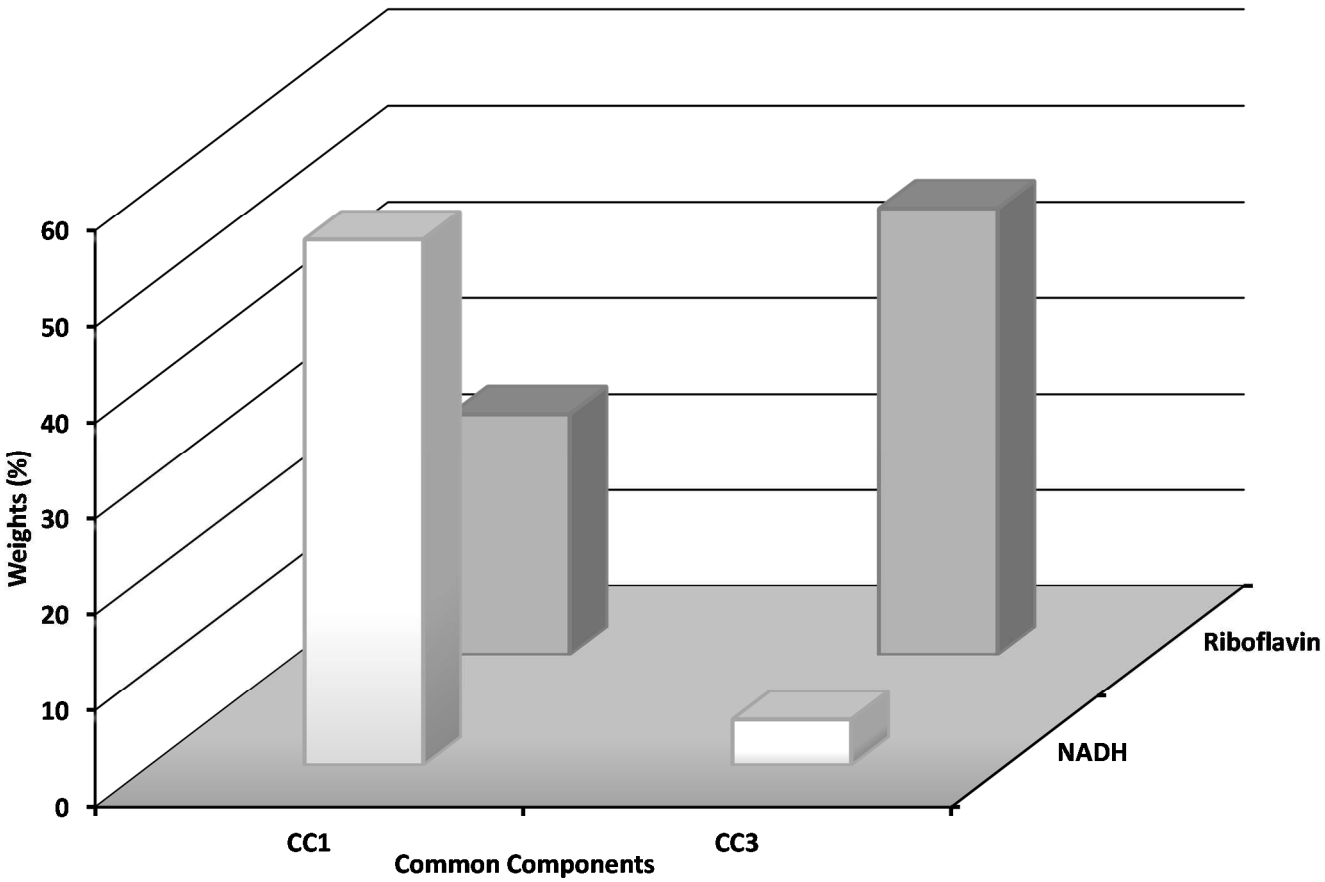
745 **Figure 5b**



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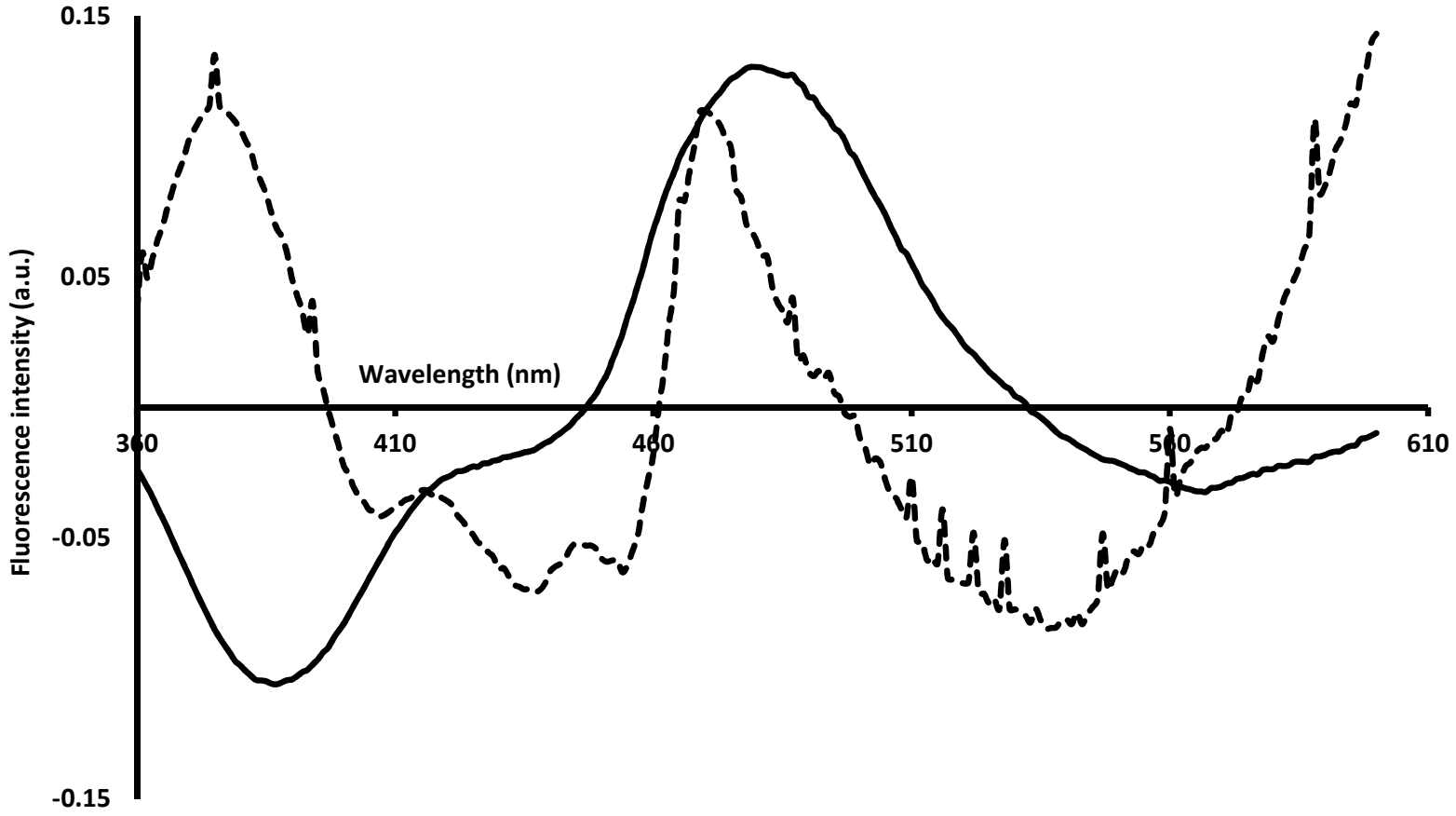

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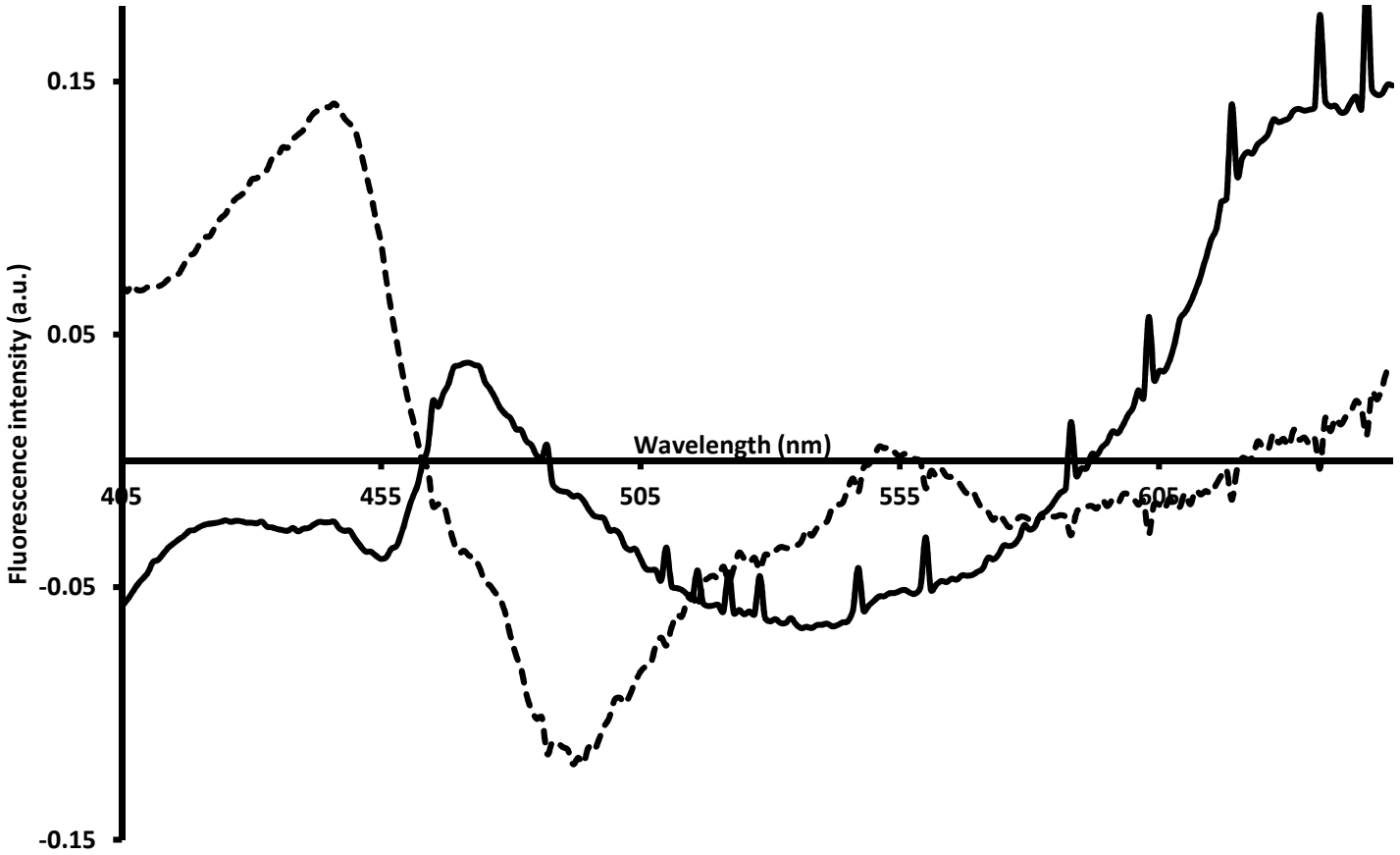

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