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1 **Identification and quantification of tuna species in canned tunas with sunflower**
2 **medium by means of a technique based on Front Face Fluorescence**
3 **Spectroscopy (FFFS)**

4

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21 **Abstract**

22

23 The authenticity of tuna is now of great importance in the multi-step food chain, from
24 on farm production to consumer consumption. A technique based on the use of front
25 face fluorescence spectroscopy (FFFS) was employed to authenticate tuna species in
26 canned tuna produced at the pilot scale with sunflower oil medium: skipjack tuna
27 (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*Thunnus*
28 *obesus*). Tryptophan residues, aromatic amino acids and nucleic acids (AAA+NA),
29 riboflavin, nicotinamide adenine dinucleotide (NADH) and vitamin A spectra were
30 recorded on 232 canned tunas. When Factorial Discriminant Analysis (FDA) was
31 applied to the different intrinsic probes, the classification rates were not satisfactory.
32 Therefore, the first five principal components (PCs) of the PCA extracted from each
33 intrinsic probe was pooled into a single matrix and analysed again by FDA. Correct
34 classification amounting to 74.6% was observed on the calibration data sets. The
35 established models tested on 30 unknown commercial canned tunas illustrated 40%
36 rate of mislabeling. The tuna cans labelled as skipjack species were 100% correctly
37 classified, while those labelled as yellowfin and bigeye tunas seemed to be adulterated
38 since for: i) tuna cans labelled as bigeye, skipjack species were detected; and ii) tuna
39 labelled as yellowfin, bigeye, skipjack and mixtures of yellowfin and skipjack and
40 yellowfin and bigeye were found.

41

42 **Keywords:** Bigeye tuna (*Thunnus obesus*), Yellowfin tuna (*Thunnus albacares*),
43 Skipjack tuna (*Katsuwonus pelamis*), Front face fluorescence spectroscopy,
44 authentication, quantification, chemometric.

45

46 **1. Introduction:**

47 Interest in fish consumption has increased in recent years due to the wide range of
48 health benefits associated to the high polyunsaturated fatty acids content. Moreover,
49 fish presents the main source of protein in many parts of the world. Canned fish
50 represents a central item of the French diet, with an apparent yearly consumption of
51 118,000 tons in 2015, of which canned tuna represents the lion's share (55.5%)
52 (FranceAgriMer, 2016). Among the different species of tuna, the most used in
53 canning industry are yellowfin tuna (*Thunnus albacares*), bigeye tuna (*Thunnus*
54 *obesus*) and skipjack tuna (*Katsuwonus pelamis*). In addition to brine, sunflower oil is
55 usually adopted as liquid medium due to both its protective action and low price
56 compared to olive oil. In fact, sunflower oil leads to isolate the product from the air
57 which helps to ensure a more palatable product. Tuna is among the most acclaimed
58 fish by consumers. Nowadays, the identification of tuna species in seafood products is
59 of paramount importance. Generally, canned tuna are produced from frozen tuna
60 fillets imported from different countries. This make difficult to differentiate between
61 species visually, inducing some errors in the labelling of canned products. To protect
62 consumers from economic deception, the authentication of tuna species becomes
63 more and more important. Although, unintentional and intentional substitution tuna
64 species are strictly forbidden by EU Regulation 1536/92, several studies reported
65 recently that canned tunas of high price such as bluefin tuna could be adulterated with
66 cheaper ones like skipjack tuna (Sotelo et al., 2018).

67 Several methodologies have been used to authenticate canned tuna. Among them,
68 we can cited TaqMan-based qPCR (Chuang, Chen, and Shiao 2012; Bojolly et al.
69 2017), electrical properties (Etienne et al., 2000) and nucleotide sequence (Paine,
70 McDowell, & Graves, 2007). [DNA-based techniques were recently used by Sotelo et](#)

71 al., (2018) in a survey at authenticating tuna species in food products sold on EU
72 market and a mislabelling level ranging from 50% to 100% were found. Today, the
73 most promising technique used for the authentication of food products is the profiling
74 approach that does not have the ability to differentiate between the different analytes
75 present in a food product but gives rapid information based on the collected
76 information (Karoui, et al., 2006; Karoui, Downey, & Blecker, 2010; Karoui &
77 Blecker, 2011). Among these techniques, we can mention front face fluorescence
78 spectroscopy (FFFS), near and mid infrared. Nowadays, the industry is looking for
79 these rapid analytical techniques that could be used on-line and/or at-line in the
80 seafood industry. For example, FFFS has been applied for: i) monitoring the freshness
81 of different fish species (Karoui, Thomas, and Dufour 2006; Hassoun and Karoui
82 2016), ii) discriminating between fresh and frozen-thawed fish (Karoui, Hassoun, &
83 Ethuin, 2017); additionally infrared spectroscopy has been used successfully to
84 differentiate between fresh and frozen thawed fillet tunas (Reis et al., 2017).

85 Despite the numerous studies related to the use of spectroscopic technique for
86 the authentication of sea food products, at our best knowledge, no investigation has
87 assessed the potential of FFFS to authenticate species in canned tuna. Thus, the
88 objective of the present study was to investigate the potentiality of FFFS to
89 authenticate species in canned tunas produced at the pilot scale with sunflower oil
90 medium with only one tuna specie (skipjack: *Katsuwonus pelamis*, yellowfin:
91 *Thunnus albacares* or bigeye tuna: *Thunnus obesus*) or a binary mixture ratios varying
92 between 1 and 99%.

93

94 **2. Materials and methods**

95 **2.1 Canned tuna**

96 Two hundred and thirty-two (232) cans were prepared by the innovation
97 platform for aquatic products (Plateforme d’Innovation Nouvelles Vagues (PFINV),
98 Boulogne sur Mer, France) using the process technology applied in the canning
99 industry. Entire individuals of bigeye, skipjack and yellowfin tuna originated from
100 Atlantic, Pacific, and Indian Oceans were identified according to morphological
101 characters using identification keys from the FAO Species Catalogue (Collette &
102 Nauen, 1983).

103 Can samples containing [the aforementioned](#) tuna species were prepared by
104 PFINV using the technique applied in the canning industry to obtain [standardised](#)
105 cans. The frozen tuna fillets were thawed to a temperature between 0 and 2 °C. Tins
106 (diameter = 55 mm, 1/12 can) were filled with 80 g of flesh and sunflower oil. The
107 flesh has previously pre-cooked in vacuum bags (steam oven 80 °C until the flesh
108 centre reaches 65 °C). Cans were crimped and [sterilised](#) at 116 °C to the sterilising
109 value of 7 min.

110 To imitate involuntary and voluntary rate substitutions in canned tuna, tuna
111 cans of different mixtures of bigeye /yellowfin, bigeye /skipjack and yellowfin
112 /skipjack with ratios varying from 1 to 99% (**Table 1**) were prepared. The 232 tuna
113 samples were used in the calibration process to establish models. The validation of the
114 established models was determined on 30 commercial unknown tuna cans that were
115 purchased randomly from local markets.

116

117 **2.2. Fluorescence spectroscopy**

118 The content of each canned tuna was transferred into 250 ml plastic beaker;
119 then, the tuna sample was homogenised by using [homogeniser](#) (T 25 digital ULTRA-
120 TURRAX®, IKA) with a speed of 10000 rpm for 5 minutes, according to the method

121 described by Srikornkarn & Sirisomboon (2014). Approximately 3 g of the obtained
122 sample was poured in a 3 ml quartz cuvette and fluorescence spectra were recorded
123 using a Fluoromax-4 spectrofluorometer (Jobin Yvon, Horiba, NJ, USA). The
124 incidence angle of the excitation radiation was set at 60° to ensure that reflected light,
125 scattered radiation, and **depolarisation** phenomena were **minimised**. The
126 spectrofluorometer was equipped with a thermostated cell and the temperature was
127 controlled by a Haake A25 AC 200 temperature controller (Thermo-Scientific.
128 France). The emission spectra of aromatic amino acids and nucleic acids (AAA+NA),
129 tryptophan residues, nicotinamide adenine dinucleotide (NADH) and riboflavin
130 spectra were recorded with the excitation wavelengths set at 250, 290, 340, 380 nm,
131 respectively. The excitation spectra of vitamin A were acquired with the emission
132 wavelength set at 410 nm. For each sample, 3 spectra were recorded.

133

134 **2.3. Mathematical treatment of data**

135 In order to reduce scattering effects and to compare samples, fluorescence
136 spectra were **normalised** by reducing the area under each spectrum to a value of 1
137 according to others (Karoui, Dufour, & Baerdemaeker, 2006a; Karoui, Nicolaï, and de
138 Baerdemaeker 2008; Leriche et al. 2004). Mainly the shift of the peak maximum and
139 the peak width changes in the spectra were considered following this **normalisation**.

140 Then principal component analysis (PCA) was applied separately to each
141 **normalised** spectral collection. The PCA transforms the original variables into new
142 axes, or principal components (PCs). This statistical multivariate treatment was earlier
143 used to observe similarities among different samples (Karoui, Lefur, et al. 2007;
144 Karoui, Schoonheydt, et al. 2007) reducing the dimension to two or three PCs, while
145 keeping most of the original information found in the data sets.

146 In a second step, FDA was performed on the first 5 PCs resulting from the
147 PCA applied to each fluorophore, containing more than 99% of total variance. FDA
148 assessed new synthetic variables called “discriminant factors”, which were linear
149 combinations of the selected PCs, and allowed a better separation of the centres of
150 gravity of the considered groups. Each canned tuna, can be reallocated within one of
151 the defined groups. For each group, the distance from the various centres of gravity of
152 the groups is calculated. The canned tuna is assigned to the group where its distance
153 between the centres of gravity is the shortest. Comparison of the assigned group to the
154 real group is an indicator of the quality of the discrimination.

155 Then, the first 5 PCs of the PCA performed on each of the five data sets were
156 pooled into one matrix, and this new table was analysed by the FDA (Karoui et al.,
157 2004). The process consists of putting the PCs data sets of each fluorophore
158 fluorescence spectra one beside the other in the same matrix to take into account the
159 whole information collected. This concatenation approach helps to improve the
160 discrimination of the investigated cans of tuna using different fluorophores, as well as
161 to assess the ability of this technique to identify the contents of tuna cans: species and
162 the amount of each tuna species in the case of binary mixture. The robustness of the
163 established model from the concatenation technique was determined on 30 unknown
164 commercial canned tunas.

165 All analyses were performed using XLSTAT 2014 (Addinsoft SARL USA,
166 New York, NY, USA) software.

167

168 **3. Results and discussion**

169 **3.1. Fluorescence spectra**

170

171 The normalised tryptophan emission spectra recorded on canned tuna
172 containing 100% bigeye, 100% yellowfin, 100% skipjack and their binary mixtures
173 with different percentages are presented in **Figs. 1a, 1b and 1c**. These spectra
174 exhibited maxima located ~373 nm corresponding to the maximum emission of
175 tryptophan (Karoui & Hassoun, 2017). A slight red shift of the maximum emission of
176 tryptophan as a function of tuna species and their mixture levels was noted. This shift
177 could be explained by changes in the protein-protein, protein-lipid and/or protein-
178 water interactions, in agreement with the findings of Karoui, Dufour, et al., (2006a).
179 These [hypotheses](#) were reinforced by the investigation of Rossi, Colonello, &
180 Alamprese, (2001) reporting an interchange between fish lipid fraction and covering
181 oil that induce some modifications in the tryptophan environment.

182 The normalised AAA+NA emission spectra recorded on canned tuna containing 100%
183 bigeye, 100% yellowfin, 100% skipjack, and their mixtures at different percentages
184 are presented in **Figs. 1Sa, b and c**. The spectra exhibited a maximum located around
185 383 nm. It appeared that the shape of AAA+NA emission spectra was correlated with
186 the [composition of tuna cans \(one species or a binary mixture\)](#), since a shift of the
187 maximum emission of AAA+NA towards higher wavelengths was observed for tuna
188 cans with a binary mixture regardless of the level of adulteration.

189 **Figs. 2a, 2b and 2c** illustrate the emission spectra of NADH recorded on
190 canned tunas. Again, it appeared that the shape of NADH emission spectra depends
191 on the composition of tuna cans. For example, the maximum emission spectra
192 observed ~ 470 nm for canned tunas containing skipjack and Y99/B01 shifted to: i)
193 475 nm for canned tunas composed of bigeye (100%) and S01/B99; ii) and to 478 nm
194 for canned tunas with Y10/S90.

195 Although, the riboflavin emission spectra of canned tunas showed a maximum
196 ~ 470 nm, the shape of the spectra varied according to the composition of canned
197 tunas (**Figs. 3a, b and c**). The variations in the fluorescence intensity ~ 470 nm could
198 be ascribed to the structural changes in riboflavin during sterilisation. This was in
199 agreement with the findings of Karoui et al., (2017) reporting that lumichrome, a
200 photo breakdown product from riboflavin contributes to the shape of fluorescence
201 spectra in the 400–500 nm range.

202 **Figs. 4a, b and c** illustrate the normalised vitamin A excitation spectra
203 acquired on canned tunas containing bigeye, yellowfin, skipjack and their mixtures.
204 The spectra exhibited two maxima located ~ 292 and 337 nm varying with canned
205 tuna contents. A clear visual differentiation can be observed between canned tunas
206 according to their species. Indeed, different fluorescence intensities (FI) ratios ($F.I_{337}$
207 $nm/F.I_{292 nm}$) were observed as a function of tuna species since bigeye, yellowfin and
208 skipjack canned tunas presented ratios of 0.94, 1.06 and 1.17, respectively. This could
209 be explained by the difference in the molecular environment of vitamin A and/or
210 solvent viscosity (Karoui, Dufour, & De Baerdemaeker, 2006b). One explanation
211 could arise from the exchange that could be occurred between fat and water in the fish
212 and the sunflower oil (Garcia-Arias et al., 1994).

213 From the obtained results, it appeared that $F.I_{337 nm}/F.I_{292 nm}$ ratios could be
214 considered as a valuable tool for differentiating between tuna species when used in a
215 binary mixture in canned tunas.

216

217 **3.2 Global analysis of the fluorescence spectral data sets recorded on different**
218 **cans: concatenation technique**

219 **3.2.1. Development of calibration models for the authentication of tuna species in**
220 **canned tunas**

221

222 The different data sets obtained following the excitation and emission
223 wavelengths were gathered into one **matrix** and analysed by FDA. Indeed, each
224 emission **and** excitation wavelength used in the present study contains specific
225 information. For consequent, more information on canned tunas could be obtained by
226 jointly analysing the different intrinsic probes. This method is known as the
227 concatenation (Karoui et al. 2008).

228 The FDA was applied to the first 5 PCs resulting from the PCA performed on
229 each intrinsic probe containing more than 99% of total variance. The similarity map,
230 defined by the discriminant factors 1 and 2 accounting for 66.58% of the total
231 variance showed a clear differentiation of tuna cans containing only one species from
232 those made with a binary mixture of tuna species. Indeed, according to the
233 discriminant factor 1, which took into account 52.14% of the total variance, tuna cans
234 produced with 100% of bigeye, yellowfin and skipjack species were observed on the
235 negative side, whereas the other tuna cans were located mostly on the positive side
236 (**Fig. 5**). Overall correct classification rate amounting to 74.57% was obtained (**Table**
237 **1S**). Tuna cans containing binary mixtures (S90/B10) were 100% correctly classified.
238 Regarding canned tunas made with only one species, 79.17, 80.56 and 87.50% of
239 correct classification was obtained for tuna cans containing bigeye, yellowfin and
240 skipjack **species**, respectively. The low percentage of correct classification of tuna
241 cans containing bigeye species could be explained by the fact that 6 out 96 spectra
242 were classified as belonging to Y100, 3 spectra were assigned to Y75/B25 and 1
243 spectrum was ascribed to S10/B90. The obtained results were in agreement with the

244 findings obtained by Bojolly et al. (2017) and Unseld, Brandt, & Hiesel, (1995) who
245 indicated that is difficult even impossible to differentiate between yellowfin and
246 bigeye tuna species, especially at juvenile stage.

247 Regarding tuna cans containing binary mixture of bigeye and yellowfin
248 species at different percentages, correct classification varying from 33.33% to 87.50%
249 was obtained. The worst classification was observed for the Y95/B05 that could be
250 explained by the fact that 10 spectra were ascribed to tuna cans containing yellowfin
251 at a level of 99% (7 spectra to the Y99/S01 group and 3 spectra to Y99/B01 group).
252 Correct classification rates amounting to 80.00% was observed for Y90/S10 group.
253 When the amounts of skipjack tuna in the binary mixture were greater than 10%,
254 correct classifications varying from 60% to 100% were obtained (**Table 1S**).

255 For economic consideration, it is well-known that yellowfin and albacore tuna
256 cans were considered more susceptible to adulteration. Thus such finding seemed to
257 suggest that FFFS could be used as a promising tool for skipjack tuna detection in
258 yellowfin and albacore tunas. The obtained results, suggested that the methodology
259 consisting in coupling fluorescence data sets into 1 table allowed more exhaustive
260 identification of tuna species especially when used in a binary mixture.

261

262 **3.2.2. Evaluation of the ability of the established model for the authentication** 263 **and quantification of commercial tuna cans**

264 The established model **obtained** on experimental tuna cans produced by
265 PFINV with known tuna species was tested on 30 canned tunas purchased from local
266 supermarket in France and labelled as bigeye tuna ($n = 10$), yellowfin tuna ($n = 10$)
267 and skipjack tuna ($n = 10$).

268 The obtained results indicated that 40% of tuna can samples were mislabelled. The
269 tuna cans labelled as skipjack tuna were completely (100%) correctly classified
270 (**Table 1b**), in agreement with the findings of Bojolly et al. (2017) who by using
271 qPCR method succeeded to differentiate 100% skipjack tuna cans from bigeye and
272 yellowfin tuna cans. In our study, tuna cans labelled as bigeye were 70% correctly
273 classified, with 3 out 10 tuna cans were ascribed to skipjack tuna samples. The worst
274 classification was obtained for tuna cans labelled as yellowfin, **since only** 10% of
275 correct classification was observed: 15 spectra were classified as belonging to bigeye,
276 7 spectra to Y75/S25 group, 4 spectra to skipjack and 1 spectrum as Y75/B25 group.
277 From the obtained results, it could be concluded the presence of different species in
278 tuna cans labelled as yellowfin and bigeye tunas or mislabeling during the production
279 and processing, which the tuna canning industry would have to address.

280

281 **4. Conclusion**

282 Front face fluorescence spectroscopy (FFFS) along with chemometric tools
283 (PCA and FDA) has demonstrated its ability to authenticate skipjack tuna
284 (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*Thunnus*
285 *obesus*) that are the most species commonly used in canned tunas. The application of
286 FDA to the concatenated intrinsic probes appeared to be a valuable technique to
287 authenticate tuna species. A complete (100%) of correct classification was observed
288 for skipjack tuna. The canned tunas labelled as yellowfin and bigeye were found to be
289 misclassified indicating error labelling for these tuna species. The obtained results
290 suggest that **FFFS** could be considered as a rapid and non-destructive screening tool
291 to authenticate canned tunas. The technique could be used as on line screening tool
292 for the authentication of canned tuna.

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297

298

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387 **List of tables:**

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389 **Table 1:** (a) Details of canned tunas used for the calibration models and (b) the prediction of
390 commercial unknown tuna cans

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392 **Table 1S:** Cross-validation table of concatenated fluorescence spectra (Tryptophan, aromatic amino
393 acids and nucleic acids (AAA+NA), riboflavin, NADH and vitamin A) realised on canned tunas used
394 for the establishment of models

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List of figures:

Figure 1S: Normalised aromatic amino acids and nucleic acids (AAA+NA) fluorescence spectra scanned on (a): tuna cans containing 100% bigeye species, 100% yellowfin species and a binary mixture of bigeye and yellowfin species with different percentages (b) tuna cans containing 100% yellowfin species, 100% skipjack species and a binary mixture of yellowfin and skipjack species with different percentages (c) tuna cans containing 100% skipjack species, 100% bigeye species and a binary mixture of skipjack and bigeye species with different percentages

Notes:

- *The first letter is related to tuna species: B: Bigeye; S: Skipjack and Y: Yellowfin.*
- *The two numbers represent the percentage of tuna species in canned tuna*
- *The three numbers (100 %) indicated that canned tuna is produced with only 1 species*

Figure 1: Normalised tryptophan fluorescence spectra recorded on (a): tuna cans containing 100% bigeye species, 100% yellowfin species and a binary mixture of bigeye and yellowfin species with different percentages (b) tuna cans containing 100% yellowfin species, 100% skipjack species and binary mixture of yellowfin and skipjack species with different percentages (c) tuna cans containing 100% skipjack species, 100% bigeye species and a binary mixture of skipjack and bigeye species with different percentages

Notes:

- *The first letter is related to tuna species: B: Bigeye; S: Skipjack and Y: Yellowfin.*
- *The two numbers represent the percentage of tuna species in canned tuna*
- *The three numbers (100 %) indicated that canned tuna is produced with only 1 species*

Figure 2: Normalised NADH fluorescence spectra recorded on (a): tuna cans containing 100% bigeye species, 100% yellowfin species and a binary mixture of bigeye and yellowfin species with different percentages (b) tuna cans containing 100% yellowfin species, 100% skipjack species and a binary mixture of yellowfin and skipjack species with different percentages (c) tuna cans containing 100% skipjack species, 100% bigeye species and a binary mixture of skipjack and bigeye species with different percentages

Notes:

- *The first letter is related to tuna species: B: Bigeye; S: Skipjack and Y: Yellowfin.*
- *The two numbers represent the percentage of tuna species in canned tuna*
- *The three numbers (100 %) indicated that canned tuna is produced with only 1 species*

Figure 3: Normalised riboflavin fluorescence spectra recorded on (a): tuna cans containing 100% bigeye species, 100% yellowfin species and a binary mixture of bigeye and yellowfin species with different percentages (b) tuna cans containing 100% yellowfin species, 100% skipjack species and a binary mixture of yellowfin and skipjack species with different percentages (c) tuna cans containing 100% skipjack species, 100% bigeye species and a binary mixture of skipjack and bigeye species with different percentages

Notes:

- *The first letter is related to tuna species: B: Bigeye; S: Skipjack and Y: Yellowfin.*
- *The two numbers represent the percentage of tuna species in canned tuna*
- *The three numbers (100 %) indicated that canned tuna is produced with only 1 species*

446 **Figure 4:** Normalised vitamin A fluorescence spectra recorded on (a): tuna cans containing
447 100% bigeye species, 100% yellowfin species and a binary mixture of bigeye and yellowfin
448 species with different percentages (b) tuna cans containing 100% yellowfin species, 100%
449 skipjack species and a binary mixture of yellowfin and skipjack species with different
450 percentages (c) tuna cans containing 100% skipjack species, 100% bigeye species and a binary
451 mixture of skipjack and bigeye species with different percentages

452 *Notes:*

453 - *The first letter is related to tuna species: B: Bigeye; S: Skipjack and Y: Yellowfin*

454 - *The two numbers represent the percentage of tuna species in canned tuna*

455 - *The three numbers (100 %) indicated that canned tuna is produced with only 1 species*

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458 **Figure 5:** Similarity map of the factorial discriminant analysis (FDA) determined by
459 discriminant factors 1 (DF1) and 2 (DF2), applied on the 25 concatenated PCs which
460 correspond to the first 5 PCs of the PCA performed on aromatic amino acids and nucleic acids
461 (AAA+NA), tryptophan, NADH, riboflavin and vitamin A fluorescence spectra recorded on
462 tuna cans containing: 100% bigeye species (Δ), 100% yellowfin species (\diamond), 100% skipjack
463 species (o), a binary mixture of skipjack and bigeye species with different percentages (\bullet) a
464 binary mixture of yellowfin and bigeye species with different percentages (-) and a binary
465 mixture of yellowfin and skipjack species with different percentages (\times).

Figure 1a

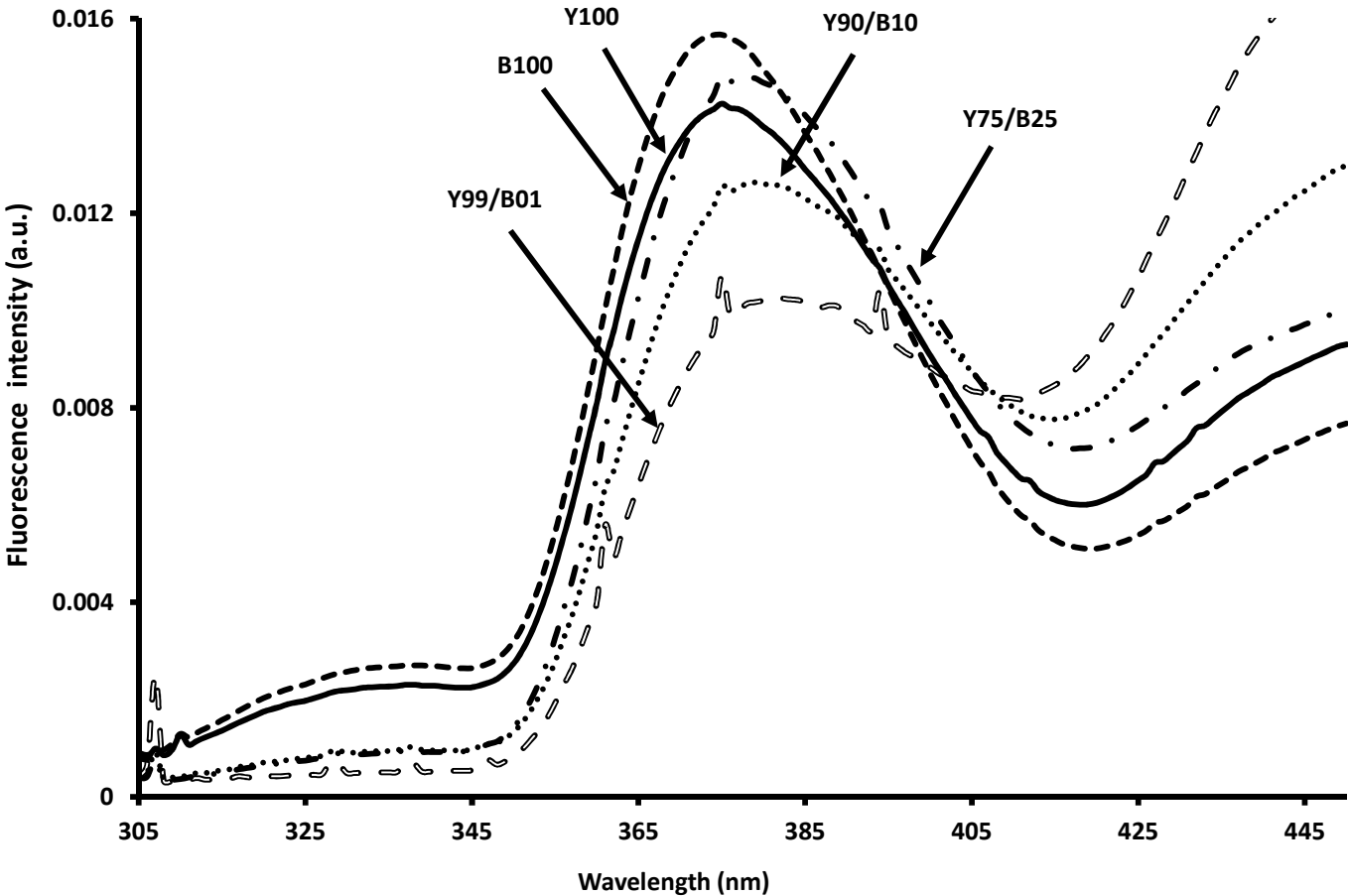


Figure 1b

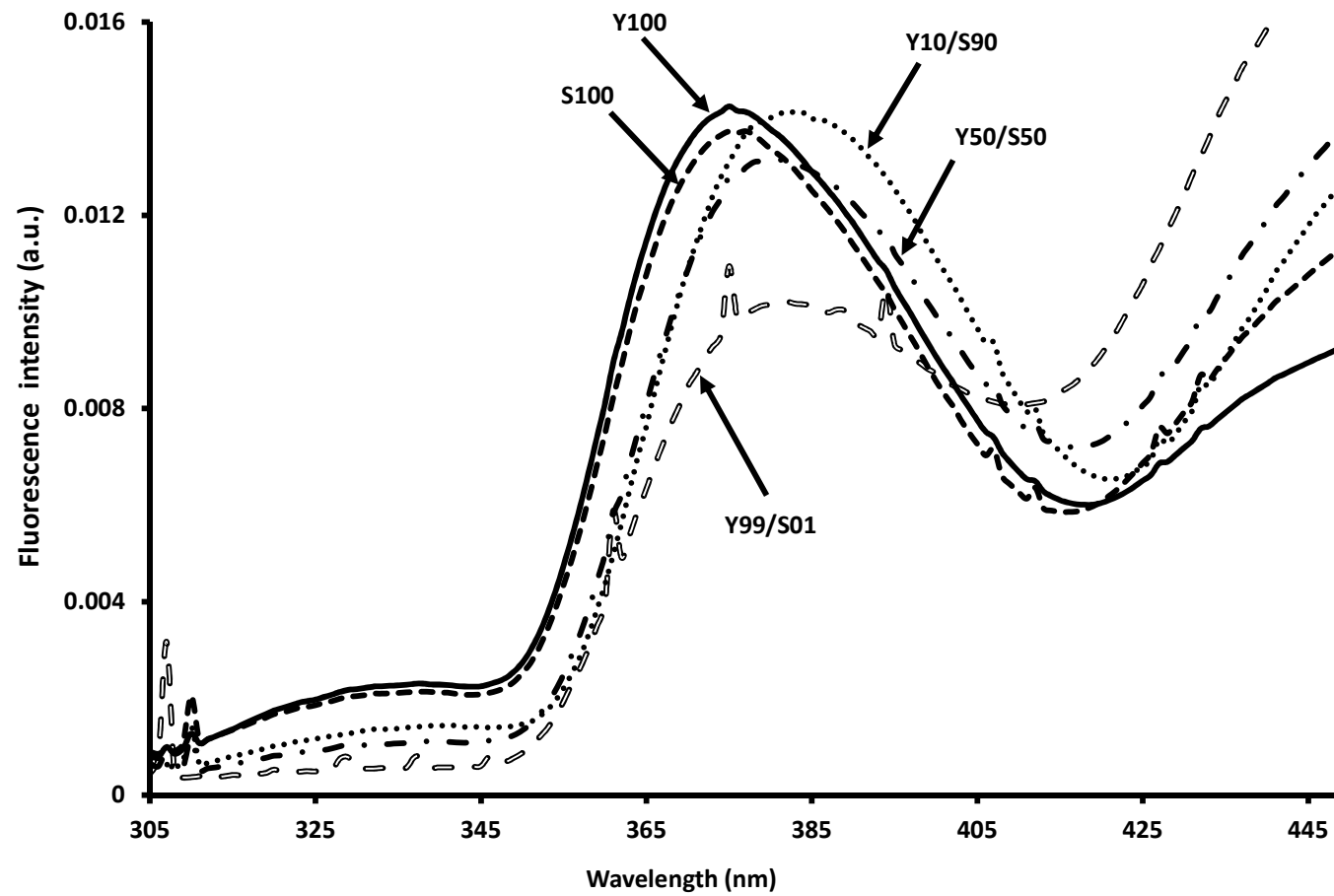


Figure 1c

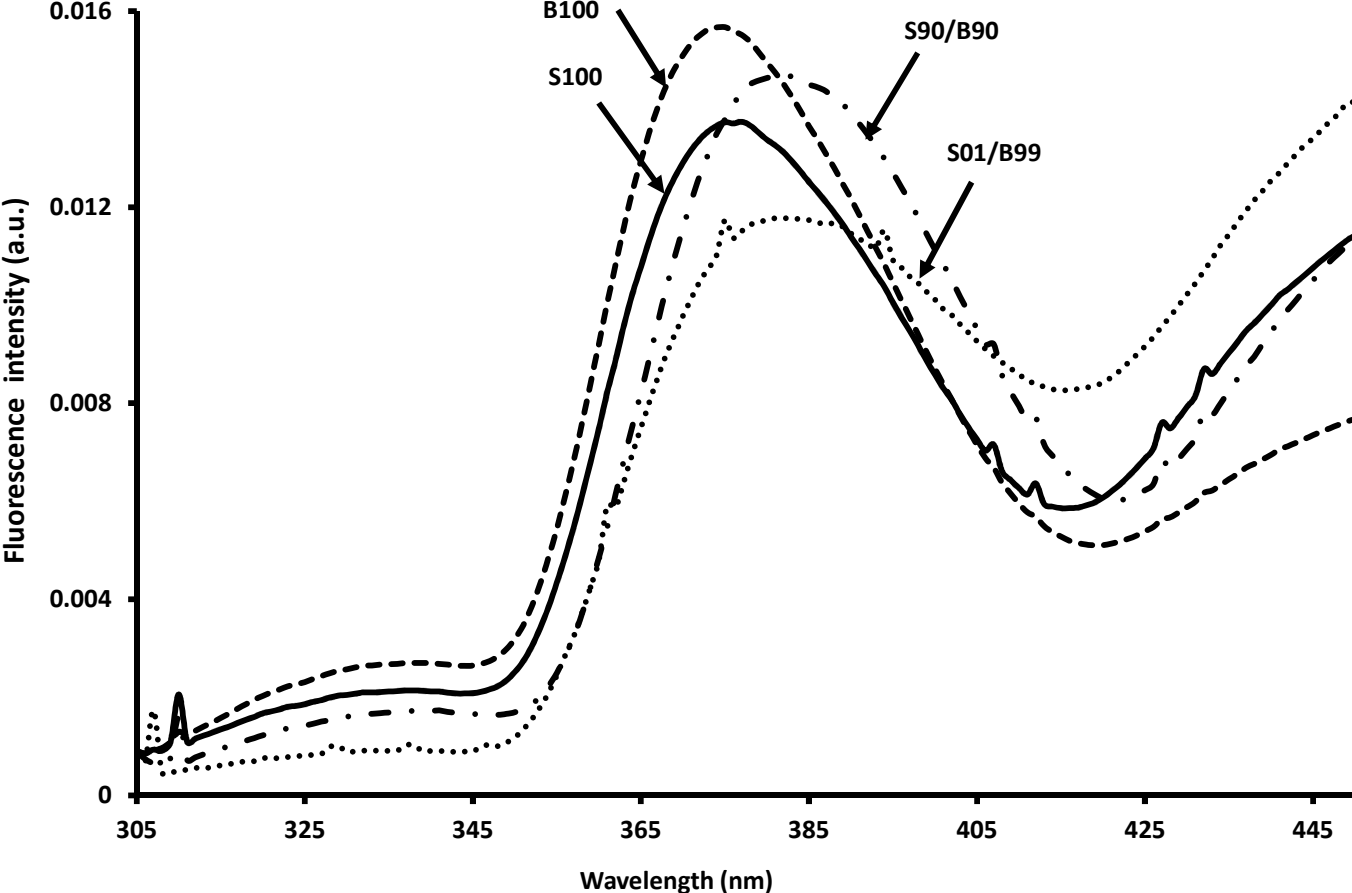


Figure 2a

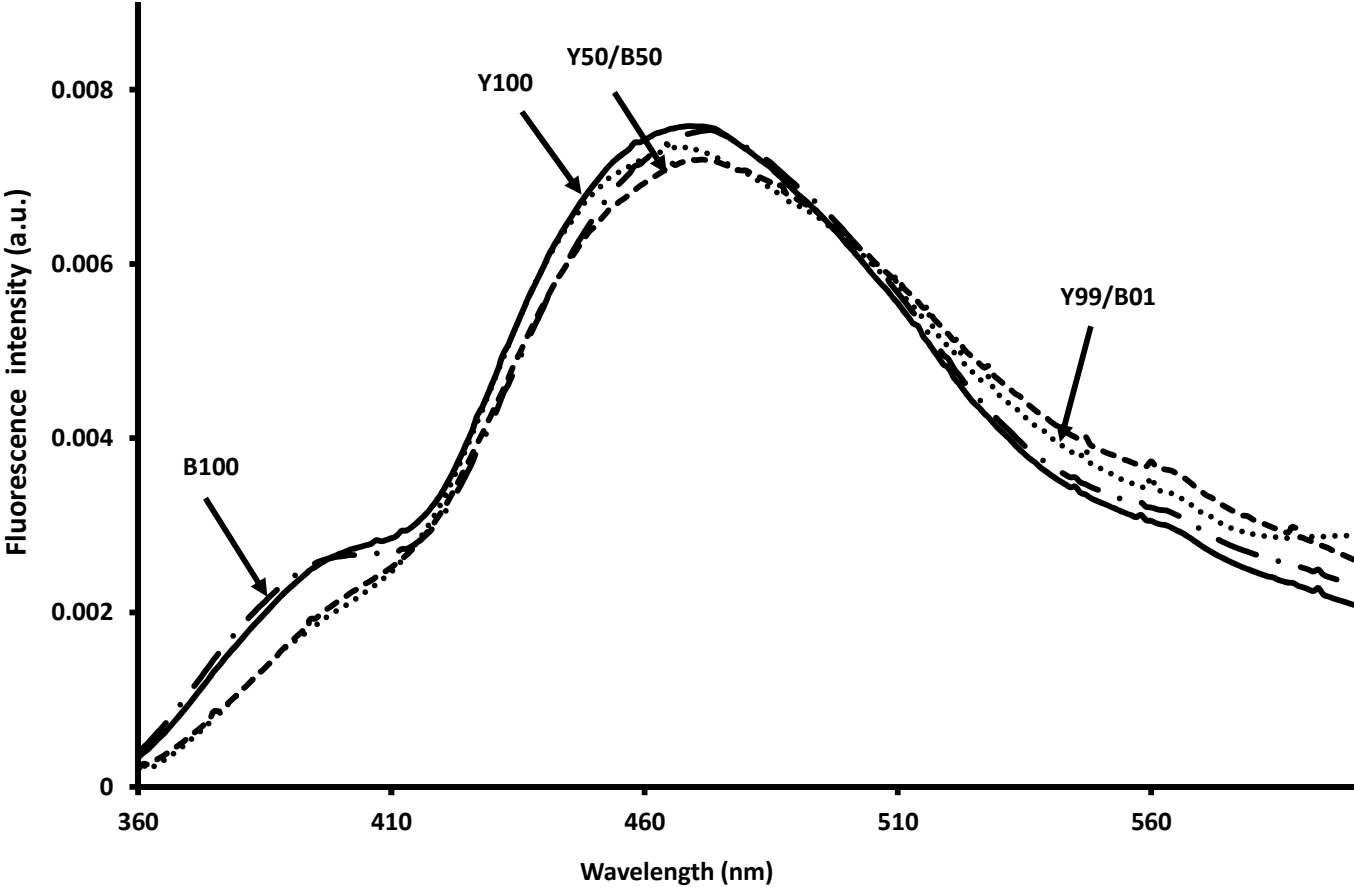


Figure 2b

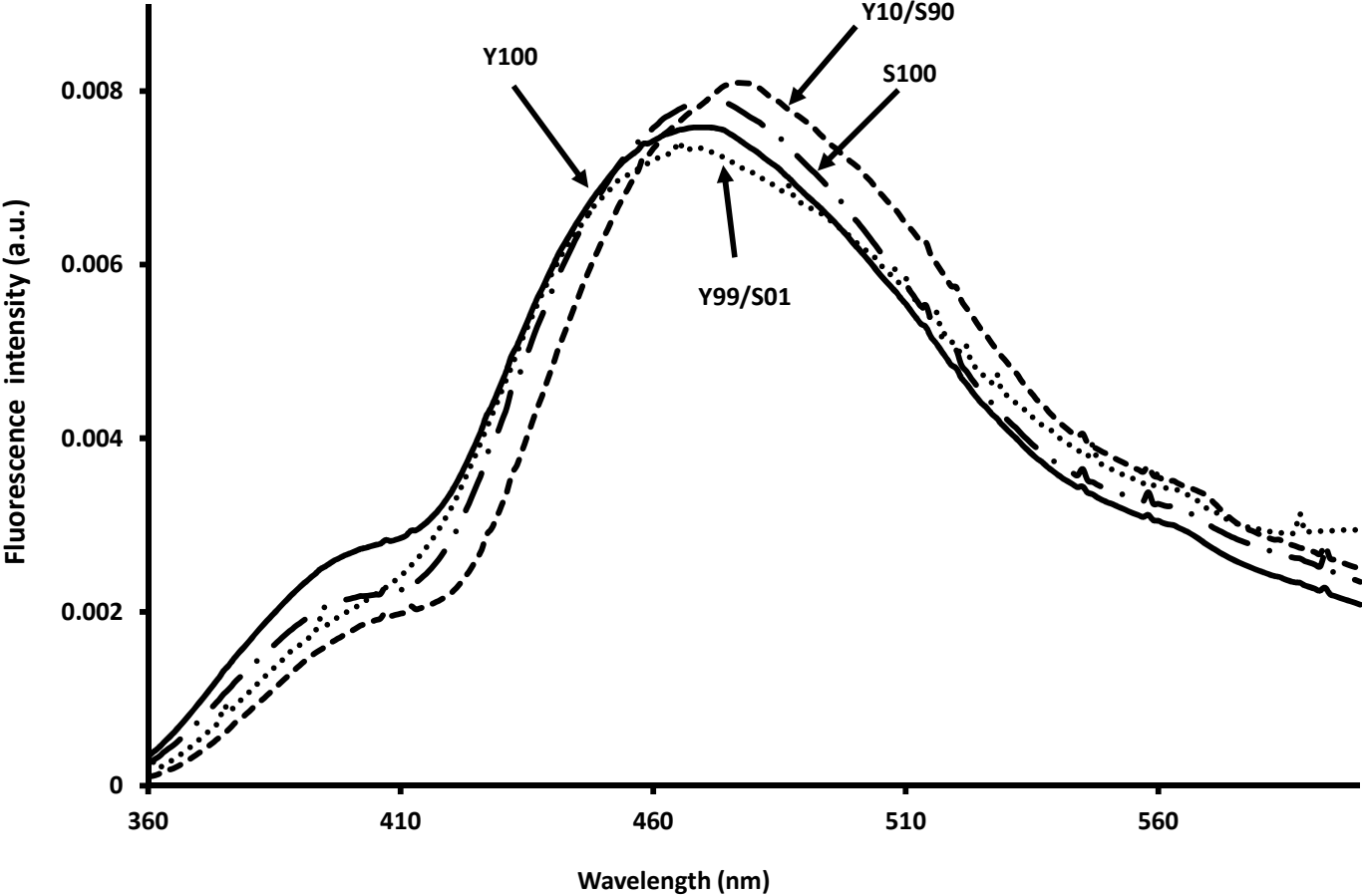


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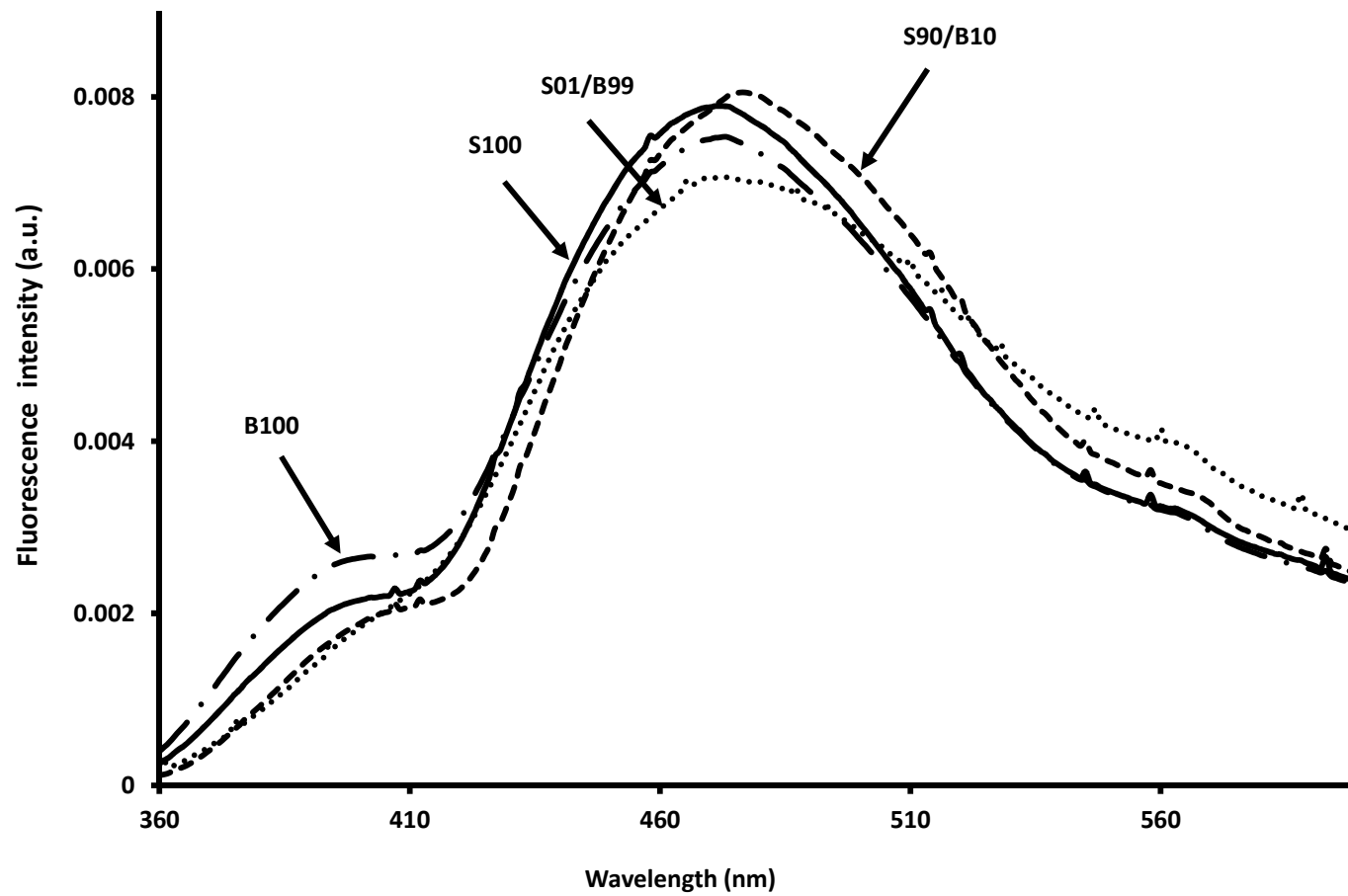


Figure 3a

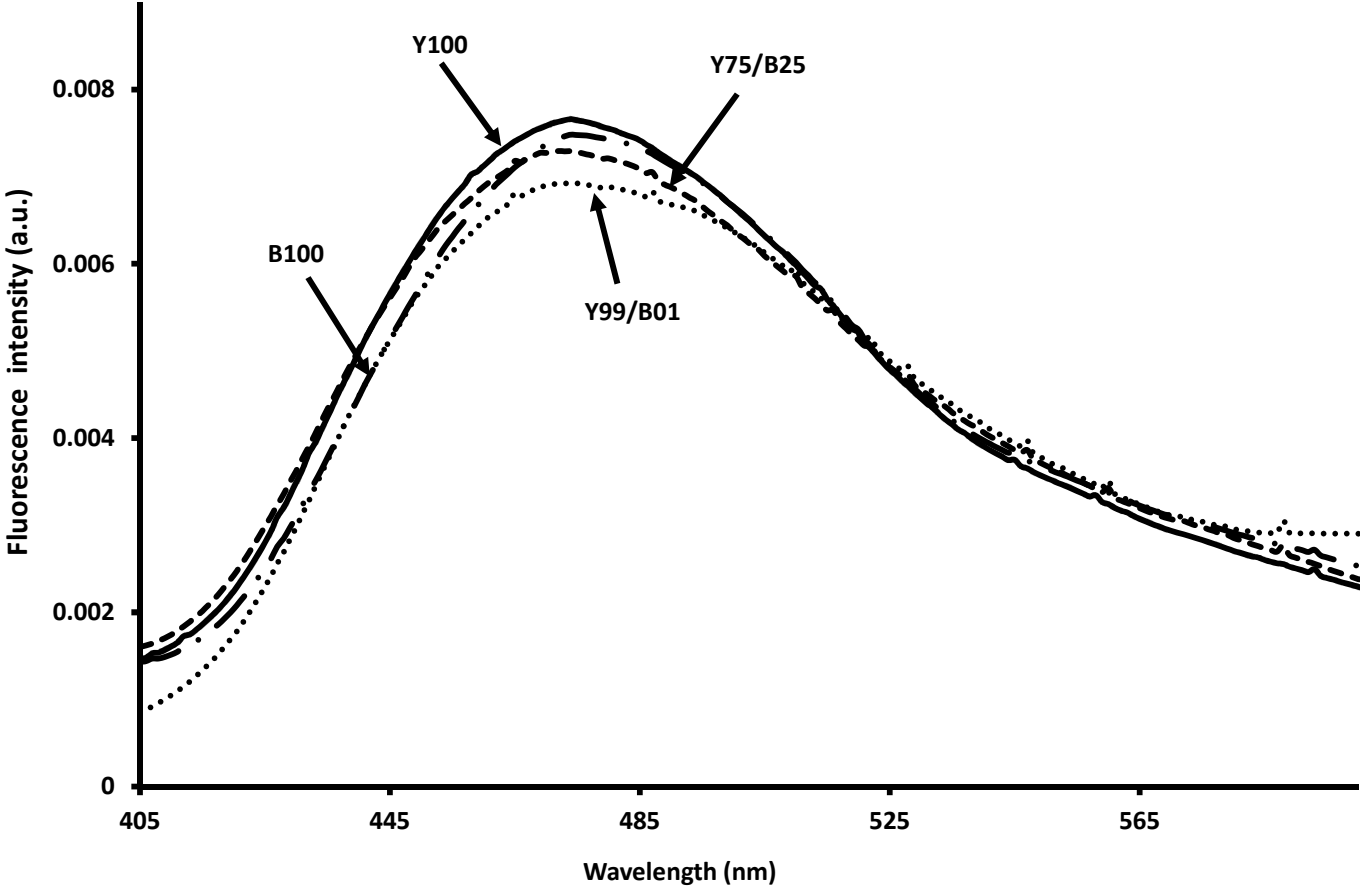


Figure 3b

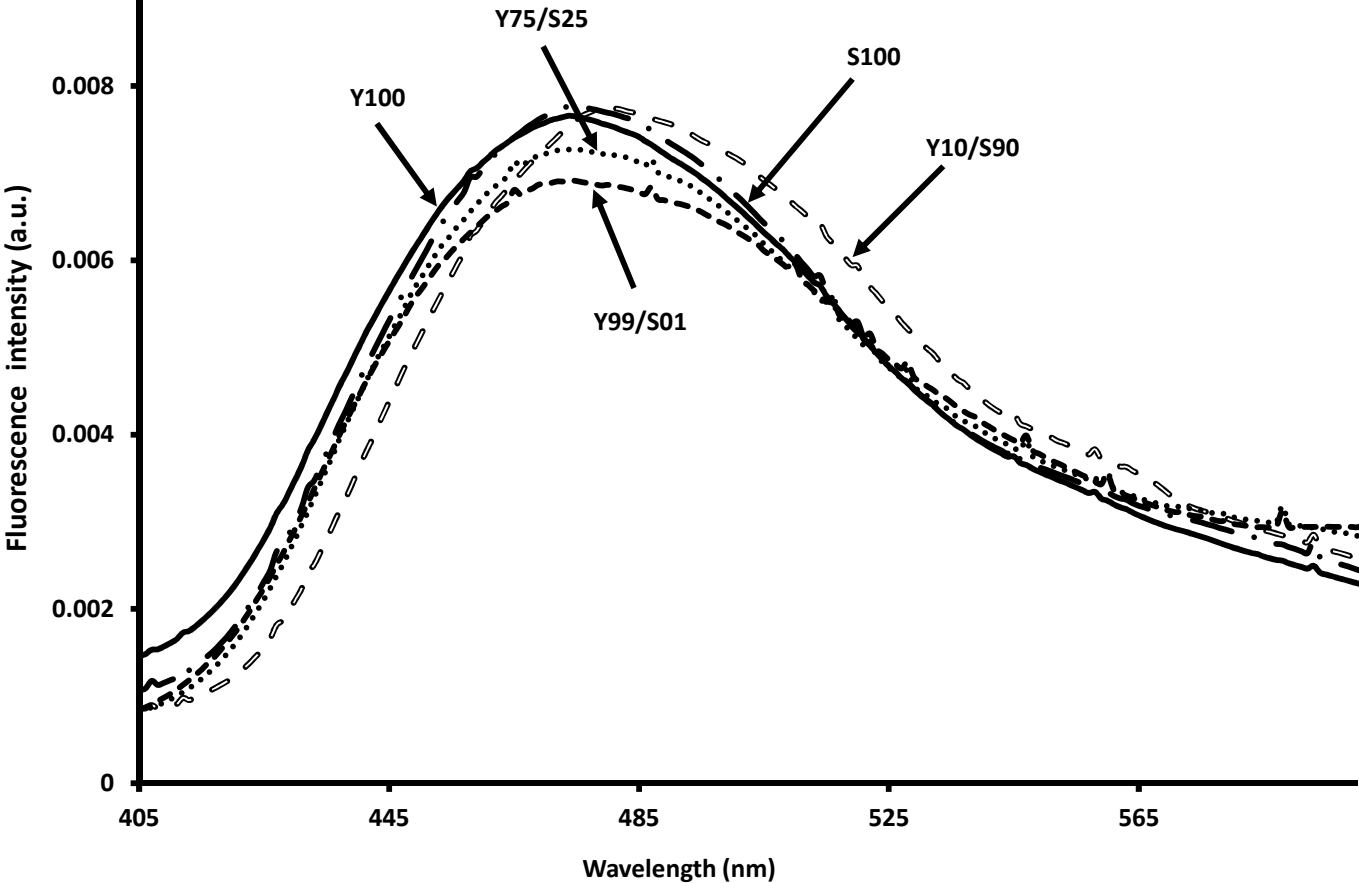


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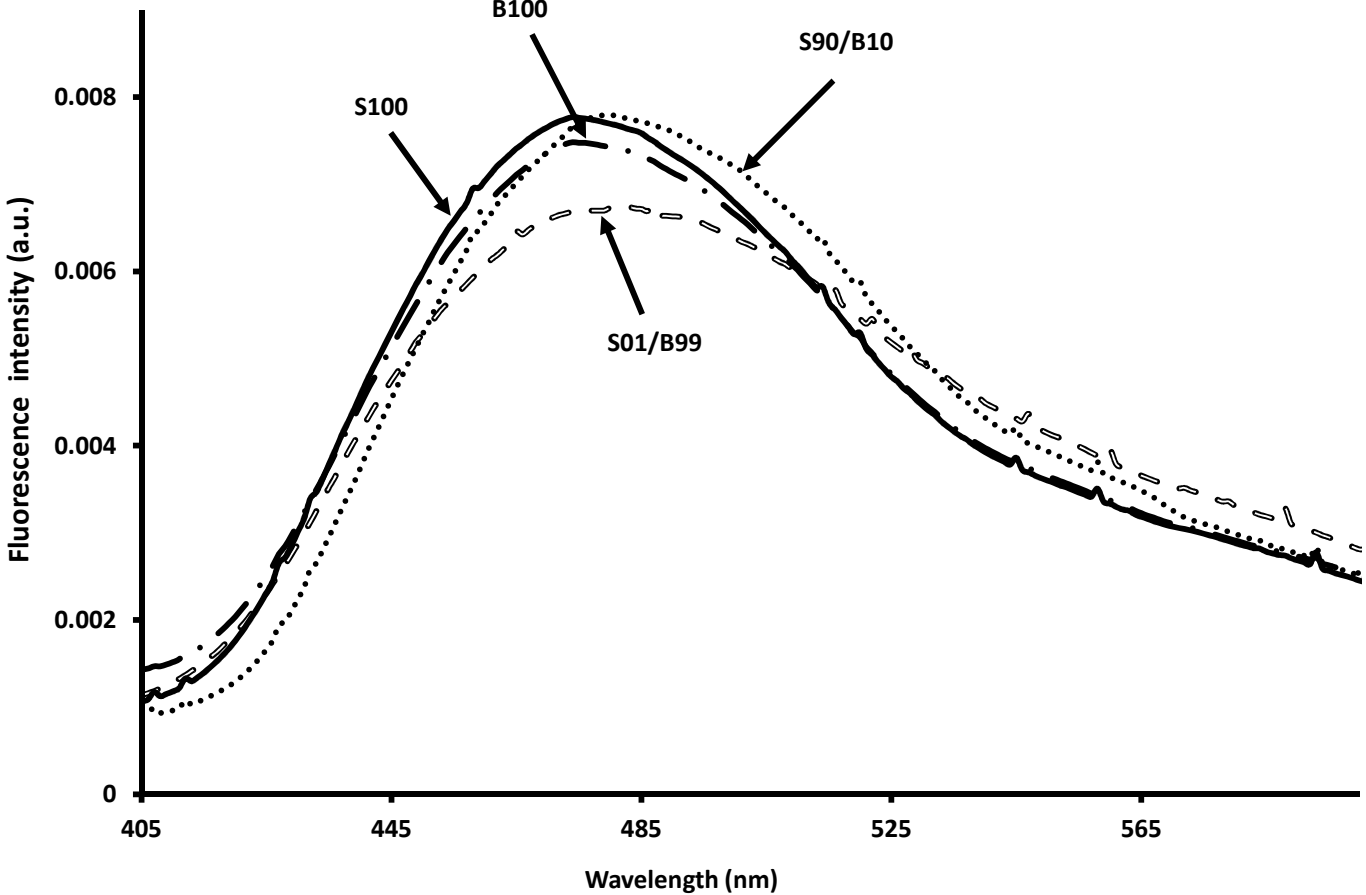


Figure 4a

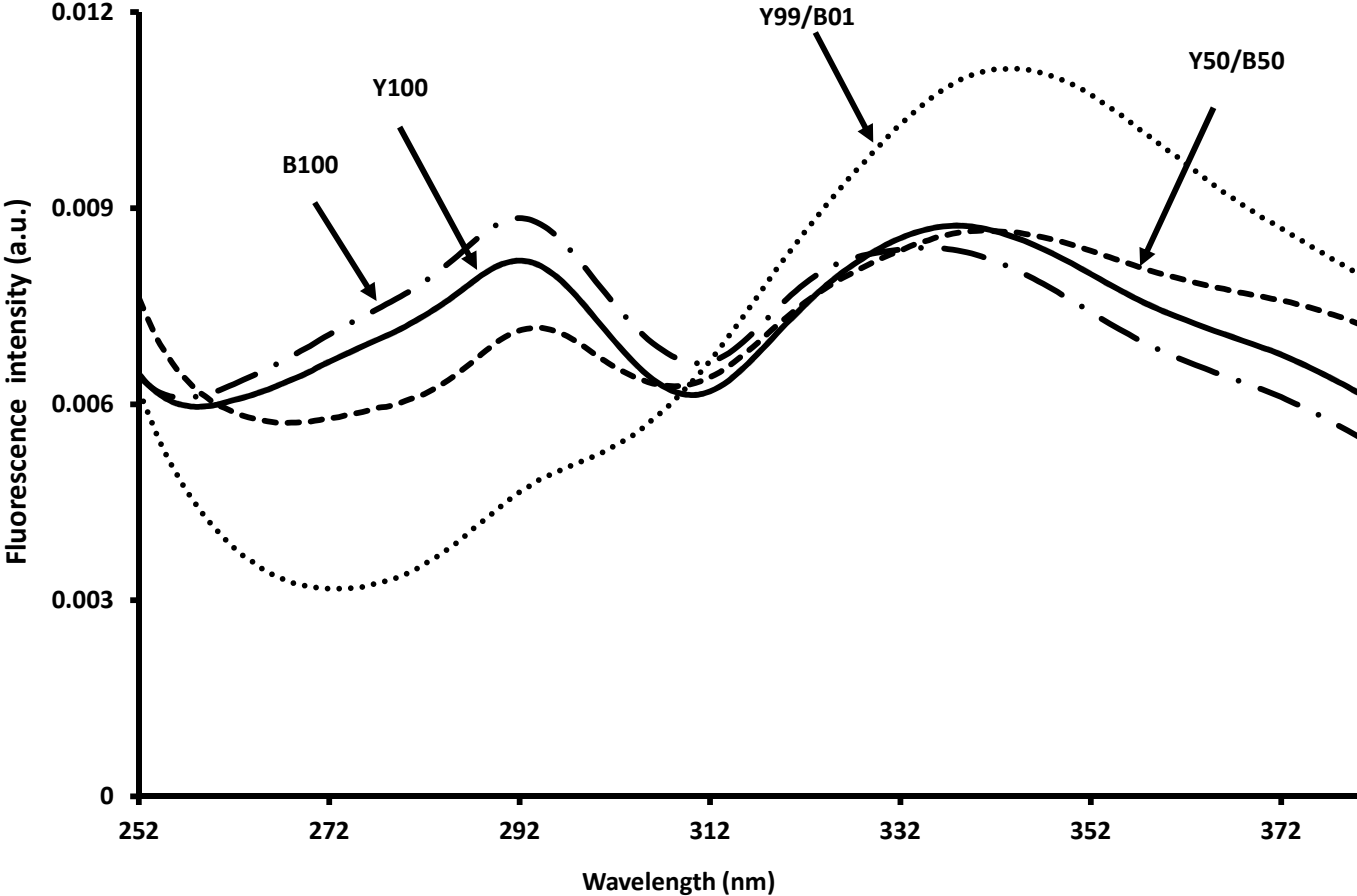


Figure 4b

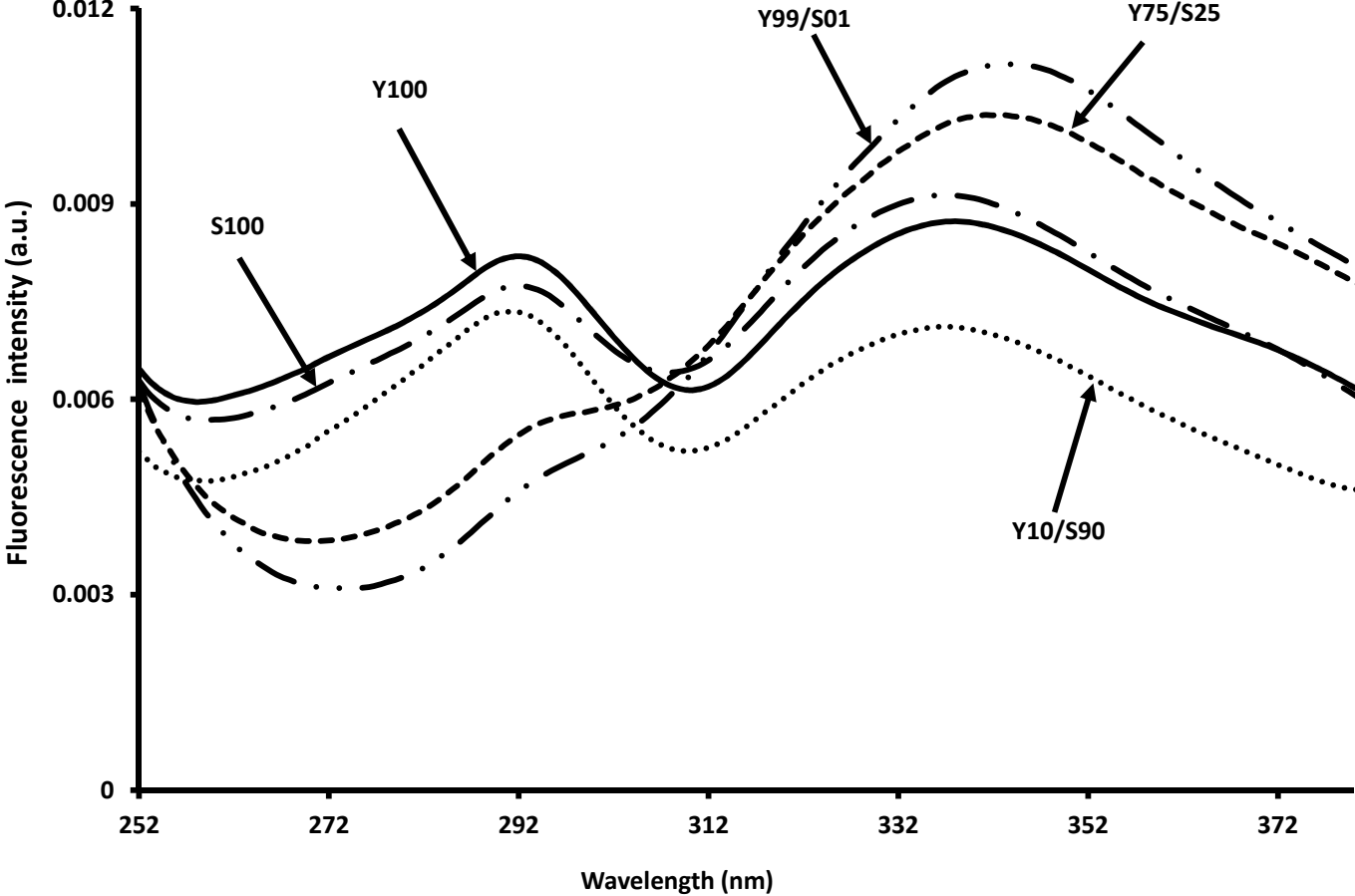


Figure 4c

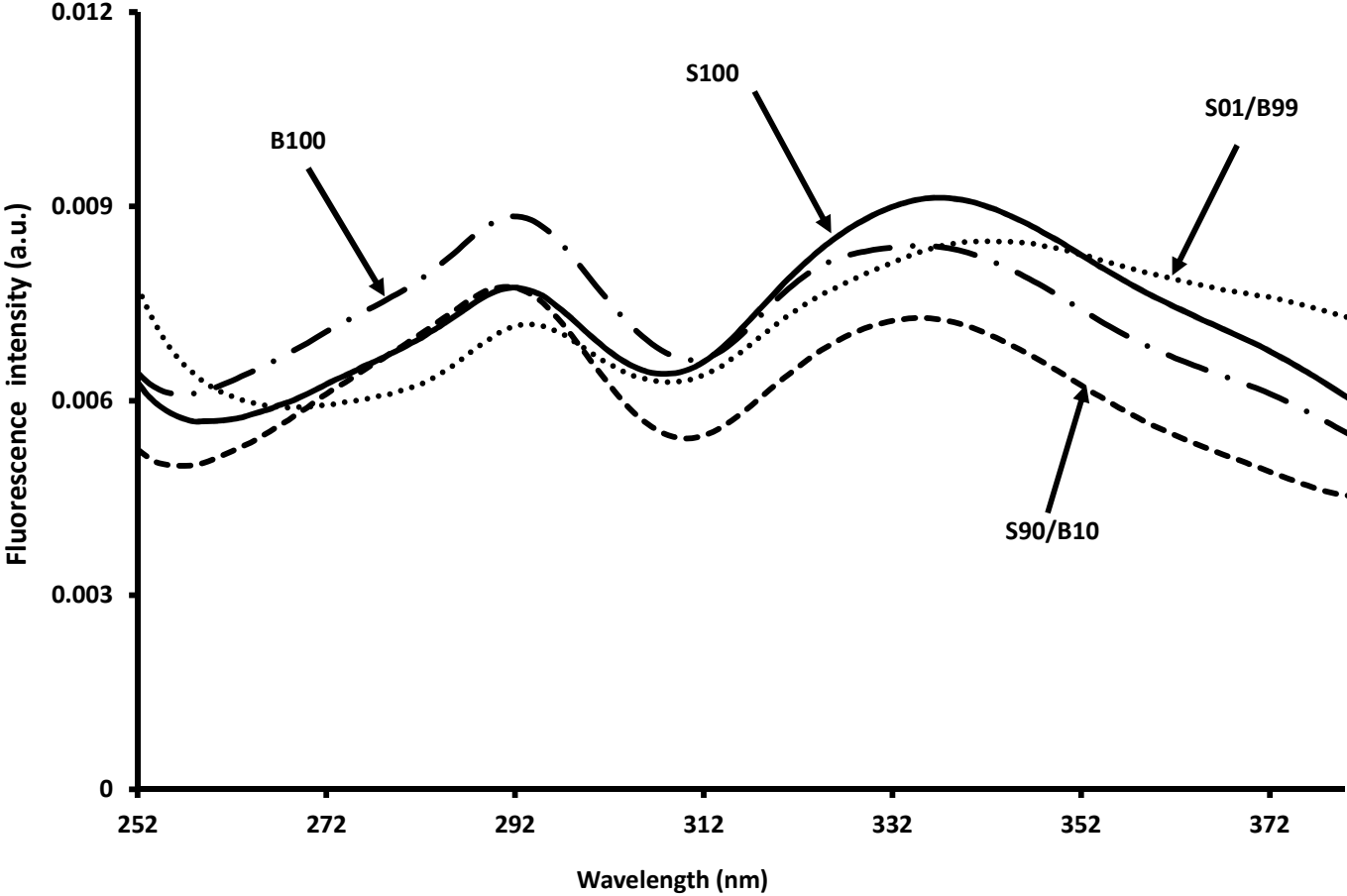
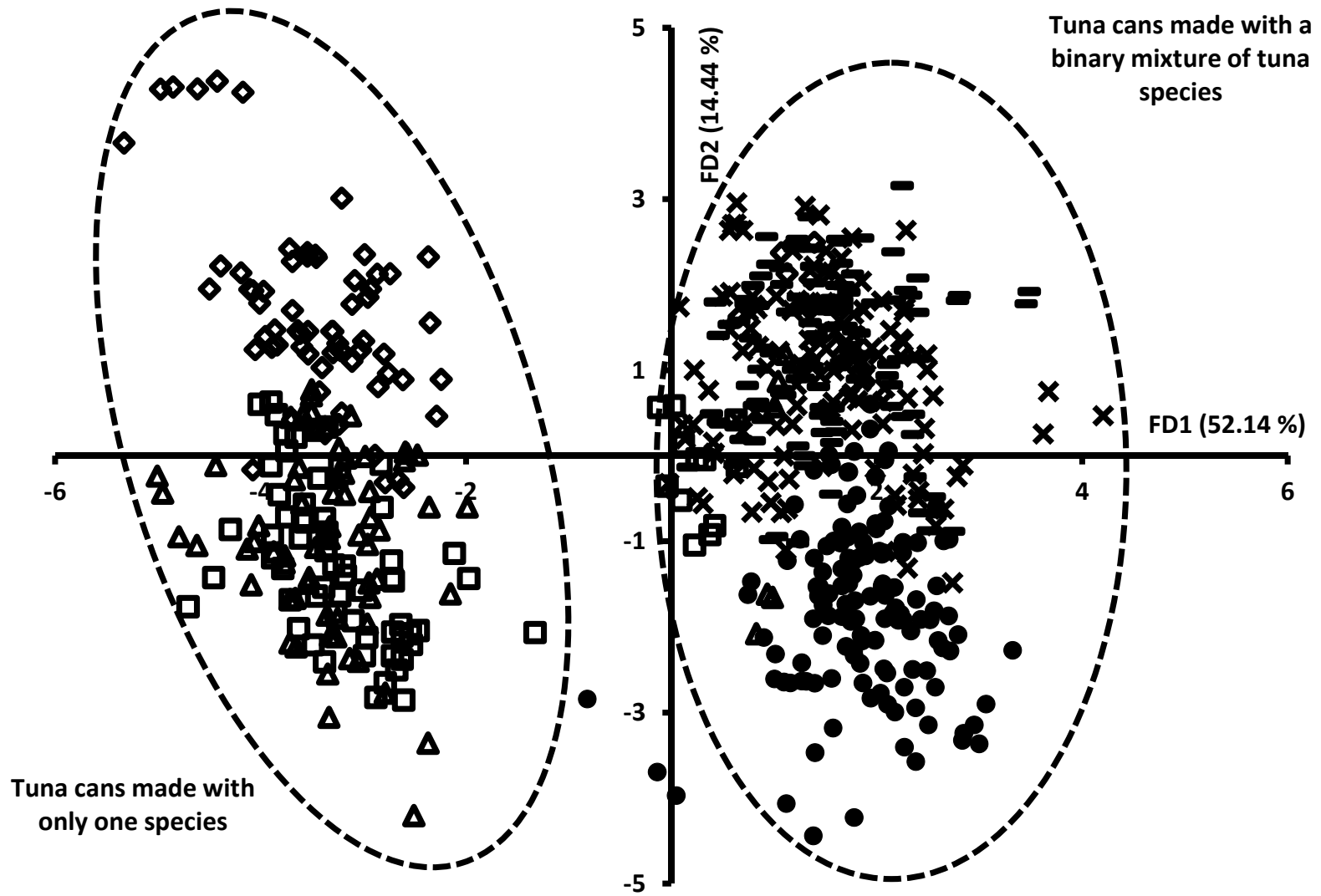


Figure 5



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Table 1a:

Composition of canned tuna	code	number of cans
100% of yellowfin tuna	Y100	24
100% of Skipjack tuna	S100	48
100% of Bigeye tuna	B100	32
99% of yellowfin tuna and 01% of bigeye tuna	Y99/B01	8
95% of yellowfin tuna and 05% of bigeye tuna	Y95/B05	8
90% of yellowfin tuna and 10% of bigeye tuna	Y90/B10	8
75% of yellowfin tuna and 25% of bigeye tuna	Y75/B25	8
50% of yellowfin tuna and 50% of bigeye tuna	Y50/B50	8
99% of yellowfin tuna and 01% of skipjack tuna	Y99/S01	8
95% of yellowfin tuna and 05% of skipjack tuna	Y95/S05	8
90% of yellowfin tuna and 10% of skipjack tuna	Y90/S10	5
75% of yellowfin tuna and 25% of skipjack tuna	Y75/S25	8
50% of yellowfin tuna and 50% of skipjack tuna	Y50/S50	10
10% of yellowfin tuna and 90% of skipjack tuna	Y10/S90	5
90% of skipjack tuna and 10% of bigeye tuna	S90/B10	5
50% of skipjack tuna and 50% of bigeye tuna	S50/B50	10
25% of skipjack tuna and 75% of bigeye tuna	S25/B75	8
10% of skipjack tuna and 90% of bigeye tuna	S10/B90	5
05% of skipjack tuna and 95% of bigeye tuna	S05/B95	8
01% of skipjack tuna and 99% of bigeye tuna	S01/B99	8
Total		232

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6 **Table 1b**
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Commercial tuna cans	Prediction	% of Correct classification
Yellowfin (<i>n=10</i>)	15 spectra belonging to bigeye 7 spectra belonging to Y75/S25 4 spectra belonging to skipjack 3 spectra belonging to yellowfin 1 spectra belonging to Y75/B25	10
Bigeye (<i>n =10</i>)	21 spectra belonging to bigeye 9 spectra belonging to skipjack	70
Skipjack (<i>n =10</i>)	30 spectra belonging to skipjack	100
Total	90	60

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