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Classification of sea bream (*Sparus aurata*) fillets subjected to freeze-thaw cycles by using front-face fluorescence spectroscopy

Abbreviated running title: Classification of sea bream fillets using fluorescence spectroscopy

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Abstract

The most common frauds carried out in different points of fish and fish product supply chain concern the selling of freeze-thaw fish as fresh fish. Moreover, fluctuation of temperature during the storage process, transportation, and sale modified the fish quality and increased the frozen-thawed cycles. In this study, front face fluorescence spectroscopy (FFFS) was used to determine the effect of freeze-thaw cycles on the quality of sea bream fillets. The application of factorial discriminant analysis (FDA) to the concatenated fluorescence spectra allowed to distinguish clearly between the numbers of freeze-thaw cycles applied to sea bream fillets since correct classification rate amounting to 91.67% was observed, regardless of the initial raw quality. This could be explained by changes in the tertiary structure of tryptophan and the oxidation state of NADH. The obtained result was confirmed following the application of partial least square regression (PLSR) since an excellent prediction of the number of freeze-thaw cycles was obtained (R^2 = 0.99; RPD = 7.13). Therefore, FFFS could be used as a rapid screening technique to detect with high accuracy between fresh, once and twice freeze-thaw sea bream fillets.

Keywords: Sea bream (*Sparus aurata*); Freeze-thaw cycles; Quality; Fluorescence; Chemometry.

I. Introduction:

Fish is known to present a good source of digestible proteins, vitamins and lipids with a high level of polyunsaturated fatty acids, particularly the eicosapentaenoic acids and docosahexaenoic acids (Karami et al., 2013). As freshness and quality decline rapidly after the death of fish, different preservation methods are used. Freezing process is one of the most frequently used techniques over the world due to its ability to stop microbial spoilage (Alvarez and Canet, 2001; Cheng et al., 2015). In the study conducted by Boran et al. (2006), it was pointed out that fish oil samples stored at -18°C had almost twice longer shelf life than those kept at +4 °C. Freezing process allow to: i) adjust the fish supply to the processing rate, and/or ii) transport fish to distribution networks and importing countries (Cheng et al., 2015). However, it is well known that fresh fish presented a big price difference compared to the frozen-thawed ones. Due to this difference, the history of fishery products should be mentioned on the label since the sale of frozen-thawed fish as a fresh product is forbidden and considered as a fraudulent practice. Moreover, during storage, transportation, distribution, and sale of fish, the temperature may undergo large fluctuations intentionally or unintentionally, leading to an increase of the frozen-thawed cycles that triggered a series of physical and chemical changes in fish products. During freezing, the fish muscle undergoes several texture changes since ice crystal formation increases the solute concentration (Estévez et al., 2011). Then, during the thawing process, muscle cells disrupted, resulting in the release of enzymes from mitochondria into sarcoplasm (Duflos et al., 2002). Indeed, it has been observed that frozen-thawed fish presented a higher cutting force than the fresh one, which is not appreciated by customers (Xia et al., 2009). The increase of cutting force in the

frozen-thawed fish was ascribed to the myosin degradation as well as to the aggregation of myofibrillar proteins, leading to the formation of disulphide bonds. The freeze-thaw cycles promote also a decrease in the water holding capacity (Estévez et al., 2011; Wang et al., 2018) and lipid and protein oxidation (Velioglu et al., 2015). Thus, all these undesirable changes reduce consumer acceptability of frozen-thawed fish.

A large variety of classical methods has been proposed in the literature including physical (e.g., torrymeter magnetic, physiological (eye lenses examination, rate of penetration of salt in muscle)), microbiological methods, and sensorial techniques to detect frozen-thawed products sold as fresh ones (Cai et al., 2014; Duflos et al., 2002). However, these methods are destructive, laboratory-based, time-consuming and expensive (Karoui et al., 2006). For example, sensorial methods need a high number of untrained panels or a limited trained panel for the evaluation of fish freshness and microbiological methods require a sample preparation step and several days for analyses. Moreover, as the change in fish state or fish substitution could be made in different points of the supply chain, there is a need for simple and rapid techniques to differentiate between fresh and frozen-thawed fish and seafood products.

Spectroscopic techniques are emerging methods that overcame these drawbacks mentioned above since they demonstrated their potential to determine the quality of meat and fish products. Spectroscopic techniques provide a great deal of information with only one scan. For example, the near infrared spectroscopy has demonstrated its ability to detect the number of the freeze-thaw cycles of frozen tilapia (*Oreochromis*) fillets (Wang et al., 2018) and to differentiate between fresh and frozen-thawed fish fillets (Zhu et al., 2013). In a similar approach, Frelka et al. (2019) pointed out the

usefulness of the magnetic resonance imaging techniques to assess frozen-thawed damage in the chicken breast meat; Cheng et al. (2015) succeeded, by using hyperspectral imaging technique in a rapid and non-invasive manner in the 400-1000 nm, to predict the colour distribution of grass carp fillets during cold storage. Moreover, front-face fluorescence spectroscopy (FFFS) has demonstrated its potential to: i) differentiate between fish samples according to their storage time (ElMasry et al., 2016; Hassoun and Karoui, 2016); ii) discriminate between fresh and one frozen-thawed sea bass fillets having different initial raw quality (Karoui et al., 2017). However, the ability of FFFS to differentiate between fresh fish and those submitted to different freeze-thaw cycles fish is up to date not investigated. Therefore, the objective of the present study was to assess, for the first time, the ability of FFFS coupled with multivariate statistical methods to differentiate between fresh, once freeze-thaw and twice freeze-thaw sea bream fillets.

2. Materials and methods

2.1. Fish samples preparation

Farmed sea bream (*Sparus aurata)* samples were collected from the Graveline Aquanord sea farm (Boulogne Sur Mer, France). The temperature and pH of breeding conditions are fixed to 18 ± 6 °C and 8.2, respectively. The fish were killed in Aquanord sea farm by asphyxiation/hypothermia and kept on ice (0-2 °C) in expanded polystyrene boxes for 1 h of transport. The fish samples were bought from the company after their death. The fish samples were then eviscerated and filleted. The 48 fillet samples were packaged in a plastic bag (polyamide / polyethylene) (PA10/PE90) and divided randomly into three groups: i) twenty-four samples were

119 stored on ice inside a cold room kept at 4 °C up to 9 days and analysed day 0, 1, 2, 3,
120 4, 7, 8 and 9 (F group); ii) sixteen fresh samples previously refrigerated at 4° C up to
121 9 days (0, 1, 2, 3, 4, 7, 8 and 9 days) were frozen at – 18 ° C for 2 months, thawed at
122 4° C for 24 h and analysed immediately (R F-T1 group); and iii) eight fresh samples
123 were kept at 4°C up to 9 days (0, 1, 2, 3, 4, 7, 8 and 9 days), then frozen at -18°C for 2
124 weeks and thawed at 4°C for 24 h; the frozen-thawed samples were frozen again at -
125 18°C for 6 weeks and then thawed at 4°C for 24 h and analysed immediately (R F-T2
126 group). The difference in the number of samples in each group is due to the limited
127 number of fillets originating from the same batch.
128 The labels of sea bream fillets used in this study are detailed in **Table 1**.
129
130 **2.2. Fluorescence measurements**
131 The spectra were scanned with a Fluoromax-4 spectrofluorometer (Jobin Yvon,
132 Horiba, NJ, USA) equipped with a front-angle cell holder fixed at 60° to reduce
133 reflected light. Slices of approximately 2 cm length, 1 cm width, and 0.5 cm thickness
134 were cut off manually in the middle of sea bream fillets. Fish samples mounted
135 between two quartz slides and fluorescence spectra were recorded at 20 °C. The
136 emission spectra of aromatic amino acids and nucleic acids (AAA + NA) (emission:
137 290-400 nm), tryptophan residues (305-450 nm), nicotinamide adenine dinucleotide
138 (NADH) (360-600 nm), riboflavin (405-650 nm) and other unknown fluorescent
139 compounds (emission: 462-650 nm) and (emission: 403-800 nm) were recorded with
140 the excitation wavelengths sets at 250, 290, 340, 380, 430 and 390 nm, respectively.
141 The excitation spectra of vitamin A (excitation: 252-390 nm) were acquired with the
142 emission wavelength set at 410 nm. All the measurements were determined in
143 duplicate.

144 **2.3. Mathematical analysis of data**

145 The normalised fluorescence spectra were analysed separately using principal
146 component analysis (PCA), an unsupervised tool oriented toward reducing a large
147 number of variables to a much smaller number of uncorrelated principal components
148 (PCs) that capture the majority of variance in the original data sets (Karoui et
149 al.,2008). The first 5 PCs of the PCA applied to each fluorophore accounting for more
150 than 99% of the total variance were pooled into one matrix and the new table was
151 analysed by the:

152 i) Factorial discriminant analysis (FDA) with leave-one-out cross-validation (Karoui
153 et al. 2011).

154 ii) Partial least squares regression (PLSR) to predict the number of freeze-thaw cycles
155 (0, 1, 2).

156 As the successful establishment of the PLSR model consisted of modelling and
157 validation procedures, the leave-one-out cross-validation method was used according
158 to the PLSR modelling procedure. The first group designated as a calibration set was
159 composed of 86 spectra, representing all the classes of sea bream fillets (fresh, and
160 those submitted to 1 and 2 frozen-thawed cycles). Moreover, the leave-one-out cross-
161 validation of the prediction model was performed using the calibration data set. The
162 calibration equation was selected based on several parameters.

163 The purpose was to seek the best PLS model with the high squared correlation
164 coefficient (R^2). In fact, R^2 indicates the percentage of the variance in the Y variable
165 that is accounted for by the X variable. A value of R^2 between 0.50 and 0.65 indicates
166 that more than 50% of the variance in Y is accounted for variance X, so that
167 discrimination between high and low concentrations can be made. A value for R^2
168 between 0.66 and 0.81 indicates approximate quantitative predictions, whereas, a

169 value of R^2 between 0.82 and 0.90 reveals good prediction. Calibration models having
170 a value of R^2 above 0.91 are considered to be excellent.
171 The robustness of the model was evaluated also by determining the latent variables
172 (LV) and the squared correlation ratio of the standard deviation (SD) to the root mean
173 square error of prediction (RMSEP), called the ratio of prediction to deviation (RPD).
174 This latter determines the factor, by which the prediction accuracy has been increased
175 compared to using the mean composition for all samples. This ratio is desired to be
176 larger than 2 for a good calibration. An RPD ratio of less than 1.5 indicates poor
177 predictions and the model cannot be used for further prediction (Karoui et al., 2006a;
178 2006b). To validate the calibration model, an independent set of 10 samples,
179 representing all fish freshness classes, were randomly selected from all sets
180 representing different class quality of sea bream fillets and the number of frozen-
181 thawed cycle was predicted.
182
183 The PCA and FDA were performed using XLSTAT 2014 (Addinsoft SARL USA,
184 New York, NY, USA) software, while the PLSR was determined using the
185 Unscramble X (V.10.4, Camo Software AS, Oslo, Norway).
186
187 **3. Results and discussion**
188 **3.1. Evolution of fluorescence spectra**
189 Fish is a complex mixture of several constituents making it susceptible to different
190 mechanisms of fish muscle denaturation and the formation of Maillard-reaction
191 compounds during refrigeration and freeze-thaw process. As fluorescent molecules
192 are highly sensitive to their local environment, different intrinsic probes were studied.

193 An example of the normalised emission spectra recorded on sea bream fillets between
194 305 and 450 nm after excitation set ~ 290 nm is showed in **Fig. 1a**. The spectra
195 exhibited a peak ~ 373 nm corresponding to the maximum emission of tryptophan
196 (Hassoun and Karoui, 2015). According to **Fig. 1a**, it appeared that the shape of
197 tryptophan emission spectra was correlated with the storage time and the number of
198 freeze-thaw cycle(s) of sea bream fillets. Indeed, an increase in the fluorescence
199 intensity (FI) with the increase of the freeze-thaw cycles was observed, since fresh
200 samples (F0) had the lowest FI ~373 nm (i.e., 0.014 a.u.), while fresh fish submitted
201 to twice freeze-thaw cycles (R0 F-T2) exhibited the highest one (i.e., 0.021 a.u.). The
202 variation of FI according to the storage time was in line with the findings of Hassoun
203 and Karoui (2016) who pointed out a high correlation between the FI ~ 370 nm after
204 excitation set ~ 290 nm and the thiobarbituric acid reactive substances (TBARS) level
205 ($0.8 \leq r \leq 0.85$), which is an effective indicator of fish lipid oxidation. The change of
206 tryptophan FI can be also due to other changes in the protein molecule than oxidation
207 such as other conformational changes due to interaction with lipids (lipid-lipid and/or
208 lipid-protein interactions) as has been depicted by Viljanen et al. (2005). Thus, it
209 could be concluded that repeated freeze-thaw cycles promote lipid oxidation, causing
210 the degradation of fish quality, in agreement with the findings of Rahman et al. (2014)
211 who pointed out that lipid oxidation of beef meat, determined by TBARS, increased
212 with the repeated freeze-thaw cycles.
213 A general trend of FI decrease was observed among the repeated freeze-thaw group
214 (R F-T2) with the degradation of the sea bream fillets quality since, FI of 0.021 and
215 0.018 a.u. were observed for R0 F-T2 and R9 F-T2 groups, respectively. This could
216 be explained by the fact that during storage of R F-T2 group, protein could undergo
217 several and intense oxidative reactions such as, the loss of functional groups by the

218 degradation of amino acids residues, the modification of side chains and the
219 aggregation and/or the polymerisation of the protein, which could decrease the
220 tryptophan fluorescence quantum yield (Estévez et al., 2011; Karoui et al., 2006c).
221 Additionally, after 9 days of storage, the maximum emission shifted from ~ 373 nm
222 for F group to 374 nm and 375 nm for fillets submitted to 1 and 2 freeze-thaw
223 cycle(s). From the obtained result, it could be concluded that tryptophan was in a
224 more polar environment for samples subjected to the freeze-thaw cycles, in
225 agreement with the findings of Wang et al. (2018) who pointed out a decrease of
226 water holding capacity and a concurrent increase in thawing loss for samples
227 subjected to the freeze-thaw cycles. It is well known that in a more polar environment,
228 tryptophan in an excited state will relax to a lower energy state. Indeed, for frozen-
229 thawed samples, the maximum emission of tryptophan spectra shifted towards higher
230 wavelengths as indicated in **Fig. 1a**.
231 An example of the normalised emission spectra recorded on sea bream fillets
232 belonging to three different groups, between 360 and 600 nm after excitation set ~
233 340 nm is showed in **Fig. 1b**. It appeared that the shape of NADH emission spectra
234 was correlated with both the number of freeze-thaw cycles and the storage time, since
235 fresh fish (F0) exhibited a maximum ~ 468 nm, which could be ascribed to the
236 maximum emission of NADH, and a shoulder ~ 408 nm, whereas all the other
237 samples (F9, R F-T1, R F-T2) showed two maxima ~ 390 and 528 nm. **Fig.1S**
238 illustrates the evolution of NADH emission spectra recorded on sea bream samples of
239 fresh group according to storage time. As could be observed, since the second day of
240 storage, a shift of the maximum emission from 468 nm to 528 nm has occurred which
241 could be due to changes in the molecular environment of NADH.
242

243 Regarding samples aged of 0 day and belonging to the three groups (F0, R0 F-T1 and
244 R0 F-T2), a significant decrease ($P < 0.05$) in the FI ~ 468 nm was observed, which is
245 in accordance with the findings of Rahman et al. (2019) who pointed out that the
246 NADH spectrum was correlated with the freshness state of horse mackerel (*Trachurus*
247 *japonicus*), allowing an accurate prediction of fish quality. Indeed, several studies
248 pointed out that during refrigeration and/or freeze-thaw cycles, the oxidation of
249 NADH and/or the reduction of NAD^+ induced the formation of high metmyoglobin
250 that induced changes in both the colour and post-mortem degradation in fish muscle
251 (Karoui et al., 2017; Rahman et al., 2019). Moreover, the formed products are not
252 fluorescent at 468 nm which may induce a decrease of the FI around 468 nm and the
253 appearance of a new peak around 528 nm.

254 Regarding the peak observed ~ 390 nm that could be attributed to the Maillard
255 reaction products originating from the reaction between free amino groups and
256 carbonyl compounds, an increase in the FI was observed with the increase of the
257 storage time and freeze-thaw cycles, since FI of 0.002, 0.0031, 0.0035 and 0.0044 a.u.
258 was observed for F0, R0 F-T2, F9 and R9 F-T2, respectively.
259 Similar results were obtained with the other fluorophores since the shape of spectra
260 displayed a variation according to the storage time and the number of freeze-thaw
261 cycles (0, 1, and 2).
262

263 **3.2. Evaluation of the discriminant ability of fluorescence spectra recorded on**
264 **sea bream fillets according to the number of freeze-thaw cycles and the initial**
265 **raw quality**

266 Before applying the FDA with leave-one-cross validation: i) 24 groups corresponding
267 to sea bream fillets submitted to different freeze-thaw cycle (s) (0, 1, 2) with different

268 initial raw quality (**Table 1**) and ii) 3 groups corresponding to the number of freeze-
269 thaw cycle(s) (0, 1, 2) regardless of the initial raw quality of sea bream fillets, were
270 created (**Table 1**). The similarity map of the FDA realised on the 24 groups is showed
271 in **Fig 2a.** According to the discriminant factor 1, taking into account 52.21% of the
272 total variance, a clear separation was observed among samples belonging to the fresh
273 group. It is interesting to note that fillet samples not subjected to the freeze-thaw cycle
274 showed clear separation according to their storage time. Indeed, samples of 8 days
275 (F8) or less presented positive scores according to discriminant factor 1, while those
276 aged 9 days (F9) exhibited negative scores. Besides, a clear separation between F0,
277 F1, F2 and F3, and F4, F7 and F8, was observed on the map. The obtained results
278 were in agreement with those of Hassoun and Karoui (2015) who observed a clear
279 differentiation between whiting fish (*Merlangius merlangus*) according to their
280 storage time. Also, a separation between sea bream fillets according to the number of
281 the freeze-thaw cycles was noted since samples belonging to the fresh group, having
282 mostly positive coordinates according to the discriminant factor 1, were differentiated
283 from those subjected to 1 and 2 freeze-thaw cycles, located on the negative side.
284 Indeed, fillet samples kept for 9 days at 4 °C and then subjected to 1 freeze-thaw
285 cycle (R9 F-T1) showed different trend than the other samples. Moreover, as could be
286 observed in **Fig. 2a**, samples of 9 days belonging to F group (F9) were positioned
287 close to those of R2 F-T2 and R0 F-T1 groups. This trajectory could be ascribed to
288 the similarity of the molecular environment of these samples.
289 An overall correct classification rate of 90.63% was obtained (**Table 2a**). As could be
290 observed, 18 out of 24 groups were totally (100%) correctly classified. For instance,
291 sea bream fillets subjected to freezing treatment immediately after death were 100%
292 correctly classified (R0 F-T1, R0 F-T2).

293 Regarding the FDA applied to the 3 groups, the similarity map provided clear
294 discrimination between sea bream fillets according to the number of freeze-thaw
295 cycle(s) (0, 1, and 2) regardless of their initial raw quality (**Fig. 2b**). All the samples
296 belonging to the F group presented positive score values according to the discriminant
297 factor 1, in opposition to those subjected to the freeze-thaw cycle(s). The discriminant
298 factor 2 allowed clear separation between samples subjected to 1 freeze-thaw cycle
299 from those subjected to 2 freeze-thaw cycles since the former presented mostly
300 negative score values, whereas the latter had, mostly, positives ones. Overall, 91.67%
301 of the samples belonging to the 3 groups were correctly classified (**Table 2b**). In
302 addition, no sample subjected to the freeze-thaw cycle(s) was ascribed to the fresh
303 group. Out 32 and 16 spectra subjected to 1 and 2 freeze-thaw cycles, 4 and 4 spectra
304 were classified as belonging to the group submitted to 2 and 1 freeze-thaw cycles,
305 respectively. The high level of correct classification of samples subjected to the
306 freeze-thaw cycle(s) could be ascribed to the impact of ice crystal formation during
307 freezing on cell membrane stability causing significant damage in muscle tissue that
308 increases with the freeze-thaw cycles. The obtained results confirmed that FFFS could
309 be used to differentiate sea bream fillets according to the number of freeze-thaw
310 cycle(s) regardless of their initial raw quality.
311

312 **3.3. Performance of PLSR models to predict the number of freeze-thaw cycle(s)**
313 **applied to sea bream fillets**

314 The PLSR was applied to the 35 concatenated PCs and the number of freeze-thaw
315 cycle(s) applied to sea bream fillets (0, 1 and 2). **Table 2c** provided an excellent
316 prediction of the number of freeze-thaw cycle(s) applied to sea bass fillets, since R^2
317 and RPD values were of 0.92 and 3.35, respectively. The prediction of the validation

set yielded a model with R^2 = 0.99 (**Fig. 3**) and RPD = 7.13 (**Table 2c)** indicating the capability of FFFS to differentiate between sea bream fillets subjected to 0, 1, and 2 freeze-thaw cycle (s) with excellent accuracy.

Indeed, despite the relatively small number of samples, the performance of the developed model applied, for the first time, to sea bream fillets was satisfactory and suggested the potential use of the FFFS as a powerful tool to screen rapidly the differentiation between sea bream fillets according to the number of freeze-thaw cycle(s) (0, 1, and 2) regardless of their initial raw quality. This hypothesis should be confirmed on a large number of samples belonging to different fish species.

4. Conclusion

The potential use of FFFS to identify commercial frauds in fish marketing and to solve some authentication issues was demonstrated. In particular, the use of suitable chemometric strategies allowed to differentiate between fresh, and freeze-thaw cycle(s) (1 and 2) applied to sea bream fillets. Indeed, the correct classification rate of 91.67% was achieved following the application of the FDA to the concatenated excitation and emission spectra scanned on sea bream fillets regardless of their raw quality. This result was confirmed following the application of PLSR since excellent results were obtained for the prediction model with leave-one-out-cross validation (R^2 = 0.99; RMSEP =0.13, RPD = 7.13). Although further investigations would be required on other fish species and a high number of repeated freeze-thaw cycles, the FFFS has a promising to be used a

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Reference:

Alvarez, M.D., Canet, W. (2001). Influence of cooking and freeze-thawing cycles on viscoelastic properties of vegetable purees. *LWT - Food Science and Technology, 34,* 549-555.

Boran, G., Karaçam, H., Boran, M., 2006. Changes in the quality of fish oils due to storage temperature and time. Food Chem. 98, 693–698.

Cai, L., Wu, X., Li, X., Zhong, K., Li, Y., Li, J., 2014. Effects of different freezing treatments on physicochemical responses and microbial characteristics of Japanese sea bass (Lateolabrax japonicas) fillets during refrigerated storage. LWT - Food Sci. Technol. 59, 122–129.

Cheng, J.-H., Sun, D.-W., Pu, H.-B., Chen, X., Liu, Y., Zhang, H., Li, J.-L., 2015. Integration of classifiers analysis and hyperspectral imaging for rapid discrimination of fresh from cold-stored and frozen-thawed fish fillets. J. Food Eng. 161, 33–39.

Duflos, G., Fur, B. Le, Mulak, V., Becel, P., Malle, P., 2002. Comparison of methods of differentiating between fresh and frozen-thawed fish or fillets. J. Sci. Food Agric. 82, 1341–1345.

ElMasry, G., Nakazawa, N., Okazaki, E., Nakauchi, S., 2016. Non-invasive sensing of freshness indices of frozen fish and fillets using pretreated excitation-emission matrices. Sensors Actuators, B Chem. 228, 237–250.

Estévez, M., Ventanas, S., Heinonen, M., Puolanne, E., 2011. Protein Carbonylation and Water-Holding Capacity of Pork Subjected to Frozen Storage : Effect of Muscle Type , Premincing , and Packaging. Agric. Food Chem. 5435–5443.

Frelka, J.C., Phinney, D.M., Yang, X., Knopp, M. V, Heldman, D.R., Wick, M.P., Vodovotz, Y., 2019. Assessment of chicken breast meat quality after freeze/thaw

abuse using magnetic resonance imaging techniques. J. Sci. Food Agric. 99, 844–853.

Hassoun, A., Karoui, R., 2016. Monitoring changes in whiting (Merlangius merlangus) fillets stored under modified atmosphere packaging by front face fluorescence spectroscopy and instrumental techniques. Food Chem. 200, 343–353.

Hassoun, A., Karoui, R., 2015. Front-face fluorescence spectroscopy coupled with chemometric tools for monitoring fish freshness stored under different refrigerated conditions. Food Control 54, 240–249.

Karami, B., Moradi, Y., Motallebi, A.A., Hosseini, E., Soltani, M., 2013. Effects of frozen storage on fatty acids profile , chemical quality indices and sensory properties of red tilapia (Oreochromis niloticus × Tilapia mosambicus) fillets. Iran. J. Fish. Sci. 12, 378–388.

Karoui, R., Hammami, M., Rouissi, H., Blecker, C., 2011. Mid infrared and fluorescence spectroscopies coupled with factorial discriminant analysis technique to identify sheep milk from different feeding systems. Food Chem. 127, 743-748.

Karoui, R., Hassoun, A., Ethuin, P., 2017. Front face fluorescence spectroscopy enables rapid differentiation of fresh and frozen- thawed sea bass (Dicentrarchus labrax) fillets. J. Food Eng. 202, 89–98. https://doi.org/10.1016/j.jfoodeng.2017.01.018

Karoui, R., Kemps, B., Bamelis, F., De Ketelaere, B., Merten, K., Schoonheydt, R., Decuypere, E., and De Baerdemaeker, J. 2006. Development of a rapid method based on front-face fluorescence spectroscopy for the monitoring of egg freshness: 2 - Evolution of egg yolk. Eur. Food Res. Technol., 223, 180–188.

Springer.

Karoui, R., Mouazen, A.M., Dufour, É., Pillonel, L., Picque, D., Bosset, J.-O., De Baerdemaeker, J., 2006a. Mid-infrared spectrometry: A tool for the determination of chemical parameters in Emmental cheeses produced during winter. Lait 86, 83–97.

Karoui, R., Mouazen, A.M., Dufour, E., Pillonel, L., Picque, D., De Baerdemaeker, J., Bosset, J.-O. 2006b. Application of the MIR for the determination of some chemical parameters in European Emmental cheeses produced during summer. Eur. Food Res.Technol. 222, 165-170.

Karoui, R., Thomas, E., Dufour, E., 2006c. Utilisation of a rapid technique based on front-face fluorescence spectroscopy for differentiating between fresh and frozen-thawed fish fillets. Food Res. Int. 39, 349–355.

Karoui, R., Nicolaï, B., de Baerdemaeker, J.Monitoring the egg freshness during storage under modified atmosphere by fluorescence spectroscopy, (2008) Food Bioproc. Technol. 1, 346-356.

Mouazen, A.M., Karoui, R., De Baerdemaeker, J., Ramon, H. Classification of soil texture classes by using soil visual near infrared spectroscopy and factorial discriminant analysis techniques, (2005) J. Near Infrared Spectrosc. 13, 231-240.

Rahman, M.H., Hossain, M.M., Rahman, S.M.E., Hashem, M.A., Oh, D.-H., 2014. Effect of repeated freeze-thaw cycles on beef quality and safety. korean J. food Sci. Anim. ressources 34, 482–495.

Rahman, M.M., Shibata, M., Elmasry, G., Nakazawa, N., Nakauchi, S., Hagiwara, T., Osako, K., Okazaki, E., 2019. Expeditious prediction of post- fingerprints Expeditious prediction of post-mortem changes in frozen fish meat using three-dimensional fluorescence fingerprints. Biosci. Biotechnol. Biochem. 1–13.

423 Velioglu, H.M., Temiz, H.T., Boyaci, I.H., 2015. Differentiation of fresh and frozen-
424 thawed fish samples using Raman spectroscopy coupled with chemometric
425 analysis. Food Chem. 172, 283–290.
426 Viljanen, K., Halmos, A.L., Sinclair, A., Heinonen, M., 2005. Effect of blackberry
427 and raspberry juice on whey protein emulsion stability. Eur. Food Res. Technol.
428 221, 602–609.
429 Wang, W., Chen, W., Tian, H., Liu, Y., 2018. Detection of frozen-thawed cycles for
430 frozen tilapia (oreochromis) fillets using near infrared spectroscopy. J. Aquat.
431 Food Prod. Technol. 27, 1–10.
432 Xia, X., Kong, B., Liu, Q., Liu, J., 2009. Physicochemical change and protein
433 oxidation in porcine longissimus dorsi as influenced by different freeze-thaw
434 cycles. Meat Sci. 83, 239–245.
435 Zhu, F., Zhang, D., He, Y., Liu, F., Sun, D.-W., 2013. Application of visible and near
436 infrared hyperspectral imaging to differentiate between fresh and frozen-thawed
437 fish fillets. Food Bioprocess Technol. 6, 2931–2937.
438

List of table:

Table1: Details of sea bream fillets used in the study

Table 2: (a) Classification table for sea bream fillets under different storage time and frozen-thawed cycles; and **(b)** Classification table for sea bream fillets subjected to different freeze-thaw cycle(s) (0, 1, 2) regardless of their initial raw quality; and (c) Cross-validation results of the freeze-thaw cycle numbers using partial least square regression (PLSR) of the calibration and validation models.

447 **Table1:**

Studied sea bream fillets	Label of 24 groups	Label of 3 groups
0 day of storage at 4°C	**F0**	**F**
1 day of storage at 4°C	**F1**	**F**
2 day of storage at 4°C	**F2**	**F**
3 day of storage at 4°C	**F3**	**F**
4 day of storage at 4°C	**F4**	**F**
7 day of storage at 4°C	**F7**	**F**
8 day of storage at 4°C	**F8**	**F**
9 day of storage at 4°C	**F9**	**F**
0 day of storage at 4°C + 1 freeze-thaw cycle	**R0 F-T1**	**R F-T1**
1 day of storage at 4°+ 1 freeze-thaw cycle	**R1 F-T1**	**R F-T1**
2 day of storage at 4°C + 1 freeze-thaw cycle	**R2 F-T1**	**R F-T1**
3 day of storage at 4°C + 1 freeze-thaw cycle	**R3 F-T1**	**R F-T1**
4 day of storage at 4°C + 1 freeze-thaw cycle	**R4 F-T1**	**R F-T1**
7 day of storage at 4°C + 1 freeze-thaw cycle	**R7 F-T1**	**R F-T1**
8 day of storage at 4°C + 1 freeze-thaw cycle	**R8 F-T1**	**R F-T1**
9 day of storage at 4°C + 1 freeze-thaw cycle	**R9 F-T1**	**R F-T1**
0 day of storage at 4°C + 2 freeze-thaw cycles	**R0 F-T2**	**R F-T2**
1 day of storage at 4°+ 2 freeze-thaw cycles	**R1 F-T2**	**R F-T2**
2 day of storage at 4°C + 2 freeze-thaw cycles	**R2 F-T2**	**R F-T2**
3 day of storage at 4°C + 2 freeze-thaw cycles	**R3 F-T2**	**R F-T2**
4 day of storage at 4°C + 2 freeze-thaw cycles	**R4 F-T2**	**R F-T2**
7 day of storage at 4°C + 2 freeze-thaw cycles	**R7 F-T2**	**R F-T2**
8 day of storage at 4°C + 2 freeze-thaw cycles	**R8 F-T2**	**R F-T2**
9 day of storage at 4°C + 2 freeze-thaw cycles	**R9 F-T2**	**R F-T2**

448 **Table 2a**:

Predicted/ Observed*	R0 F-T2	R1 F-T2	R2 F-T2	R3 F-T2	R4 F-T2	R7 F-T2	R8 F-T2	R9 F-T2	R0 F-T1	R1 F-T1	R2 F-T1	R3 F-T1	R4 F-T1	R7 F-T1	R8 F-T1	R9 F-T1	F0	F1	F2	F3	F4	F7	F8	F9	% Correct classification
R0 F-T2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R1 F-T2	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00%
R2 F-T2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R3 F-T2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R4 F-T2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R7 F-T2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R8 F-T2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R9 F-T2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R0 F-T1	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R1 F-T1	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	75.00%
R2 F-T1	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R3 F-T1	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	100.00%
R4 F-T1	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	100.00%
R7 F-T1	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	100.00%
R8 F-T1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0	0	0	0	0	0	75.00%
R9 F-T1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	100.00%
F0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	100.00%
F1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5	0	0	0	0	0	0	83.33%
F2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	100.00%
F3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	0	0	0	66.67%
F4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	1	0	0	66.67%
F7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	100.00%
F8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	100.00%
F9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	100.00%
Total	2	0	2	2	2	2	2	2	4	4	5	4	6	4	3	4	7	5	6	5	6	7	6	6	**90.63%**

449 **: See Table 1 for labelling*

450 **Table 2b:**

Predicted/ Observed*	F	R F-T1	R F-T2	% Correct classification
F	**48**	0	0	100.00%
R F-T1	0	**28**	4	87.50%
R F-T2	0	4	**12**	75.00%
Total	48	32	16	**91.67%**

451

452 **: See Table 1 for labelling*

453

Table 2c:

	Calibration				validation			
Component	**R^2**	**RMSEC (number of freeze-thaw cycle(s))**	**LV**	**RPD**	**R^2**	**RMSEP (number of freeze-thaw cycle(s))**	**LV**	**RPD**
Number of freeze-thaw cycles	0.92	0.21	12	3.35	0.99	0.13	12	7.13

RMSEC: Root mean square error of calibration*; RMSEP:* Root mean square error of prediction; *RPD:* Ratio of prediction deviation; LV: Number of latent variables

459 **List of figures:**

460 **Figure 1:** Normalised fluorescence spectra acquired on sea bream fillets under different
461 storage time and subjected to different freeze-thaw cycle(s) (0, 1, 2) after: **(a)** excitation: 290
462 nm, emission: 305-450 nm; and **(b)** excitation: 340 nm, emission: 360-600 nm.

463 **Figure 2:** Similarity map of the factorial discriminant analysis (FDA) determined by
464 discriminant factors 1 (F1) and 2 (F2) applied on the 35 concatenated principal components
465 (PCs) of: **(a)** 24 groups; and **(b)** 3 groups.

466 **Figure 3:** Scatter plot of measured *versus* predicted freeze-thaw cycles obtained for sea
467 bream fillets with full- cross validation after partial least square regression (PLSR).

468

Figure 1a:

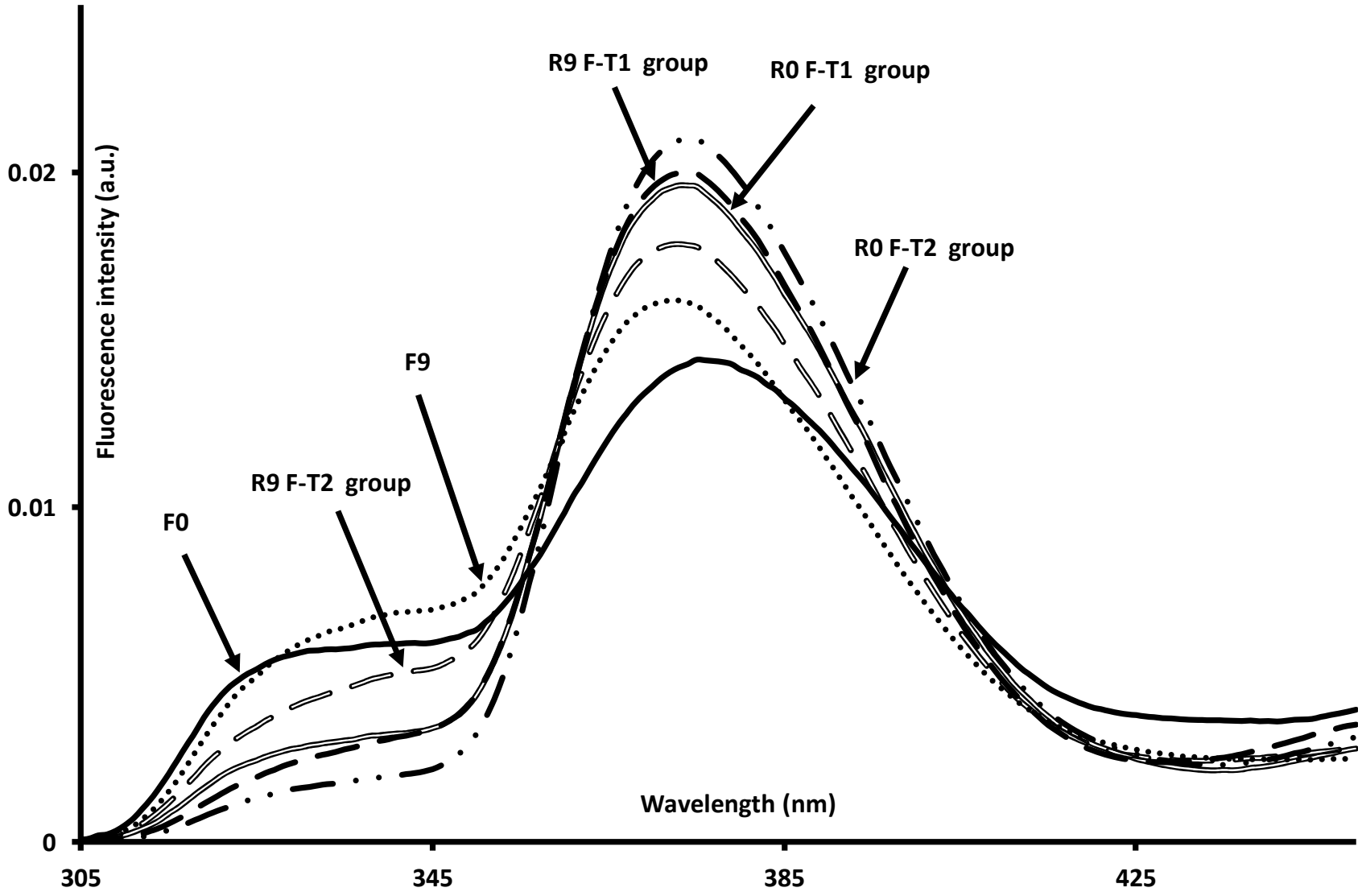

R9 F-T1 group
R0 F-T1 group
R0 F-T2 group
F9
R9 F-T2 group
F0
Fluorescence intensity (a.u.)
0.02
0.01
0
Wavelength (nm)
305
345
385
425

Figure 1b:

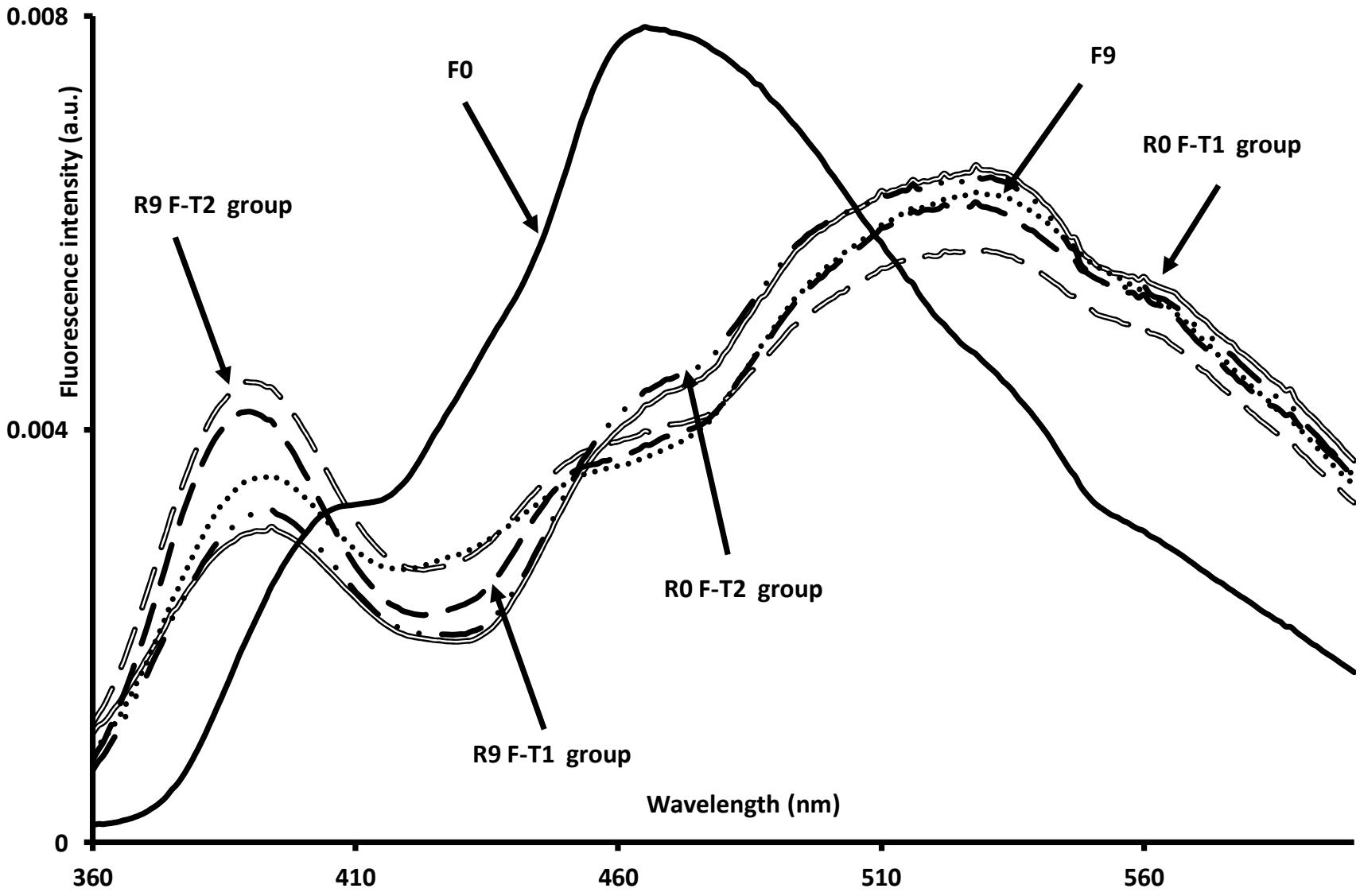

0.008
0.004
0
Fluorescence intensity (a.u.)
F0
F9
R0 F-T1 group
R9 F-T2 group
R0 F-T2 group
R9 F-T1 group
Wavelength (nm)
360
410
460
510
560

Figure 1S:

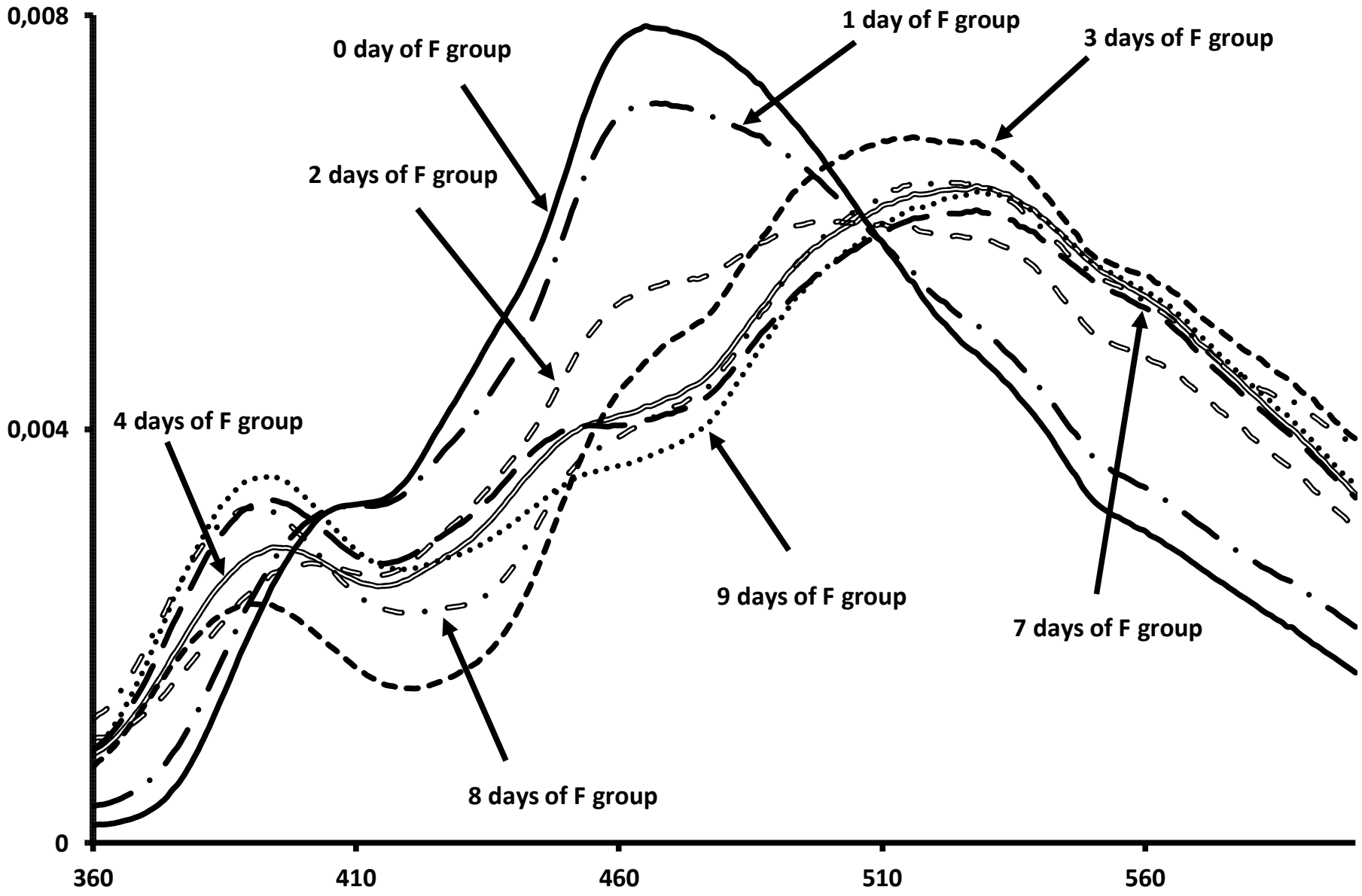

0,008
0,004
0
360
410
460
510
560
0 day of F group
1 day of F group
3 days of F group
2 days of F group
4 days of F group
9 days of F group
7 days of F group
8 days of F group

Figure 2a:

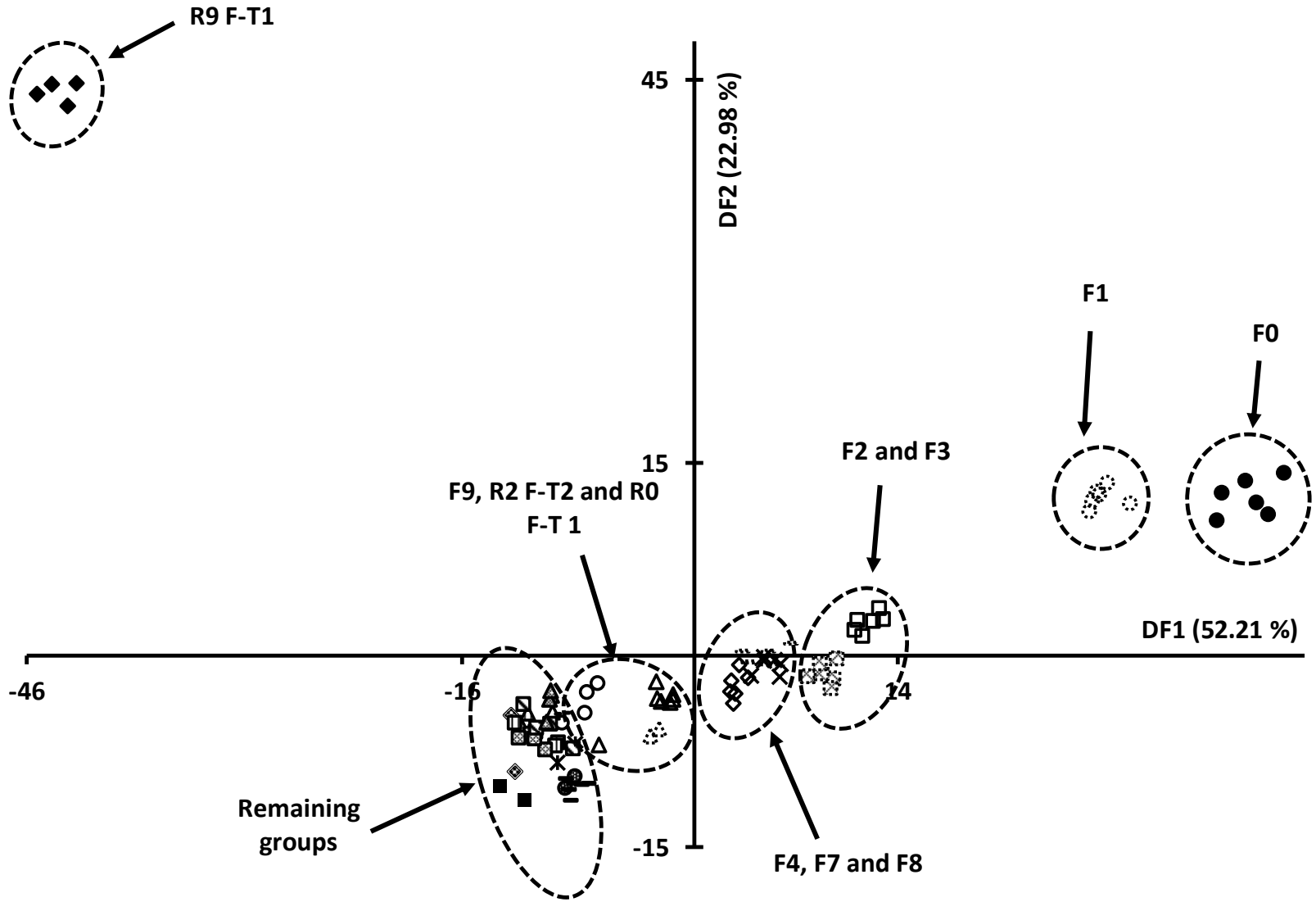

R9 F-T1
45
DF2 (22.98 %)
F1
F0
15
F2 and F3
F9, R2 F-T2 and R0
F-T 1
DF1 (52.21 %)
-46
-16
14
Remaining
groups
-15
F4, F7 and F8

Figure 2b:

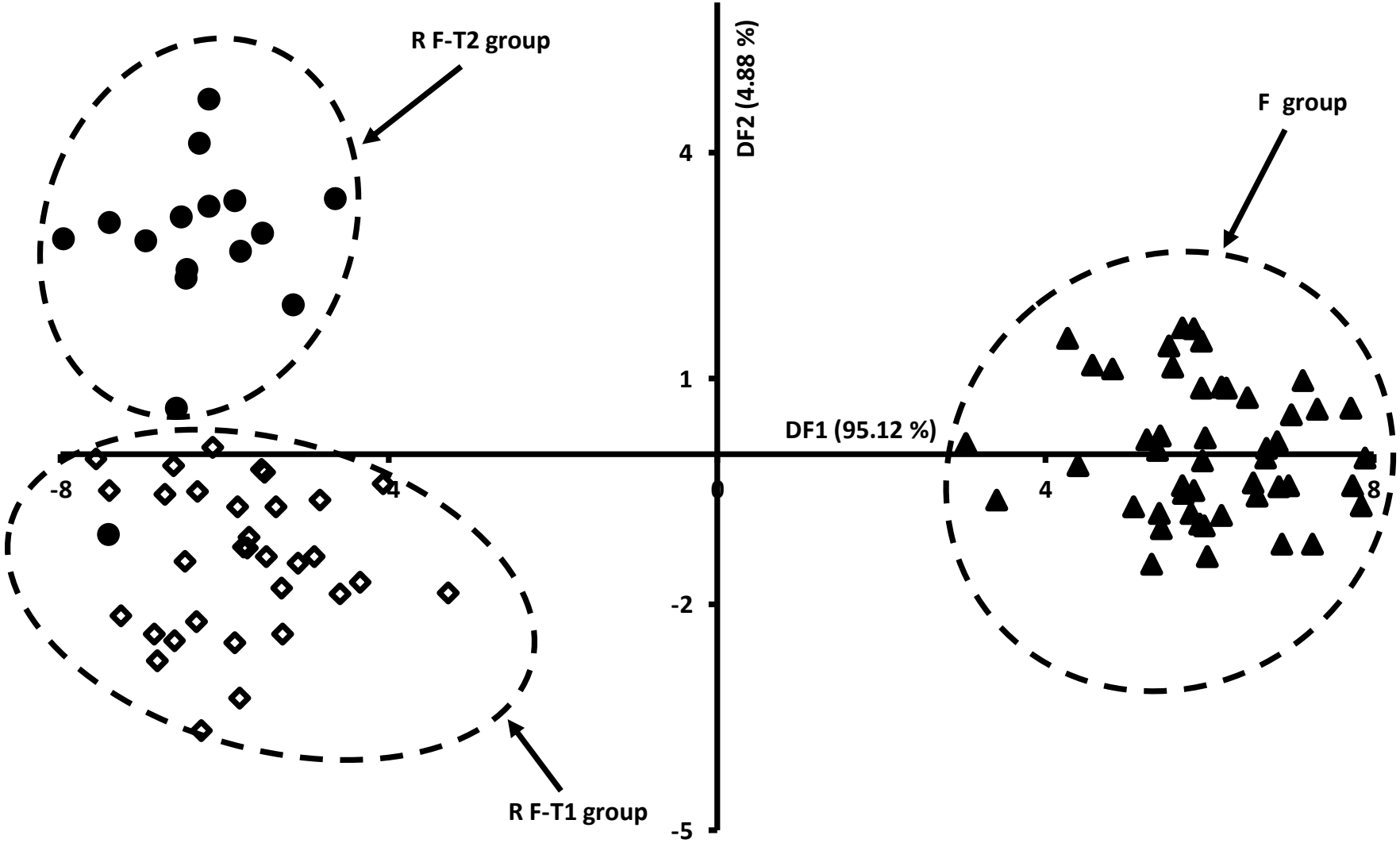
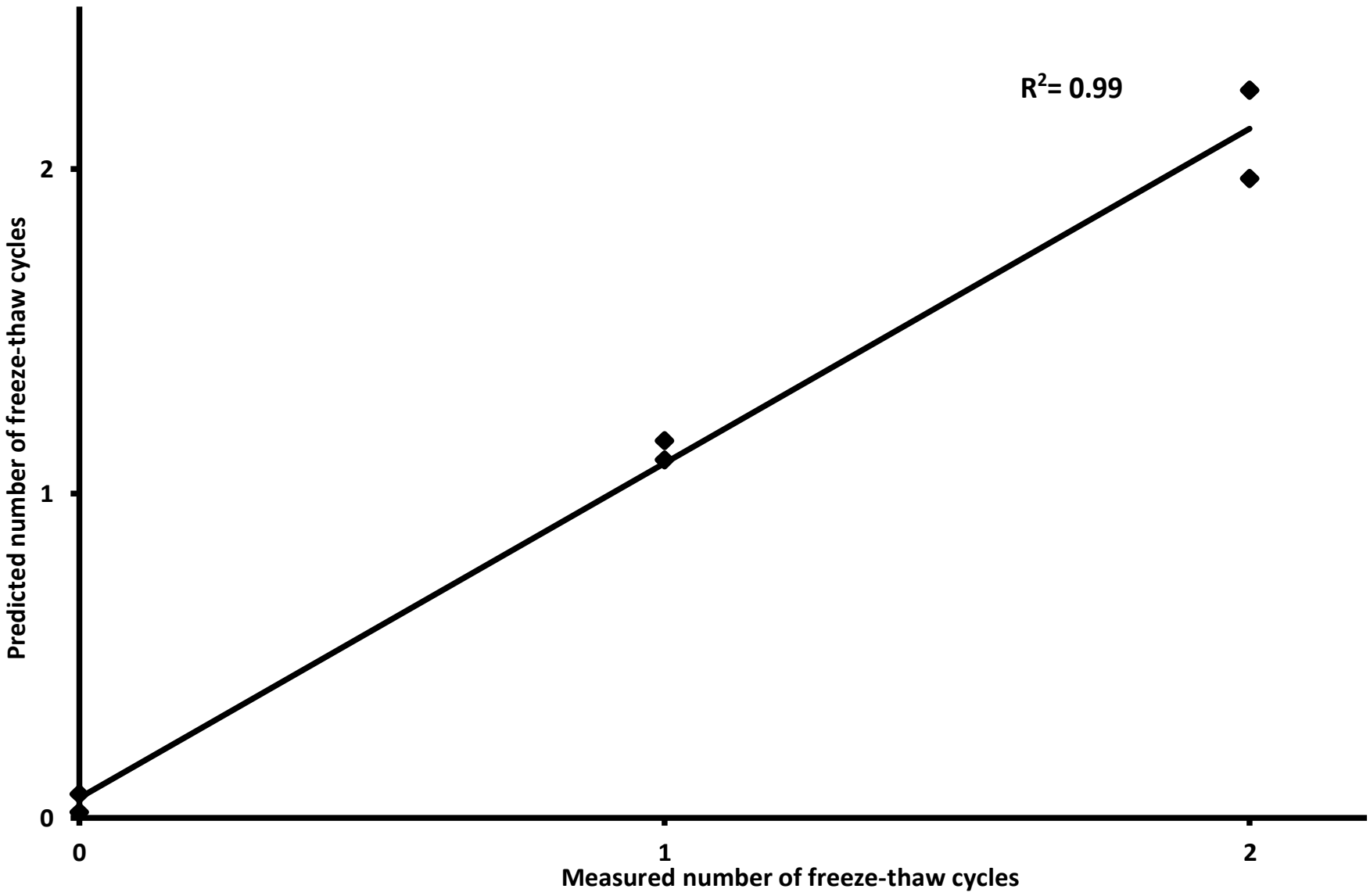


Figure 3:


R2= 0.99
Predicted number of freeze-thaw cycles
0
1
2
0
1
2
Measured number of freeze-thaw cycles