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**Mid infrared spectroscopy combined with chemometric tools for the identification of  
canned tuna species in brine**

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1           **Mid infrared spectroscopy combined with chemometric tools for the**  
2                           **identification of [canned](#) tuna species in [brine](#)**

3

4

5

6 **Abstract**

7 Each tuna species appeared to have its unique price depending on landing, season, and  
8 nutritional value, hence, adulteration could occur by replacing tunas of high quality by  
9 those having low quality. This study was undertaken to explore the potential of mid  
10 infrared (MIR) spectroscopy for discriminating canned tunas containing different  
11 species: yellowfin, skipjack, bigeye, and albacore. A total of 218 canned tunas,  
12 produced at the pilot scale were scanned by MIR in the 4000-900  $\text{cm}^{-1}$ . The factorial  
13 discriminant analysis (FDA) applied to the concatenated data sets corresponding to  
14 the first 5 principal components (PCs) of the principal component analysis (PCA)  
15 applied to the 3000-2800  $\text{cm}^{-1}$ , 1700-1500  $\text{cm}^{-1}$  and 1500-900  $\text{cm}^{-1}$  spectral regions  
16 gave a model with correct classification rate amounting to 74.96%. The established  
17 model was tested on 40 commercial canned tunas; a total (100%) of correct  
18 classification was obtained for the bigeye tuna, while misclassification was observed  
19 for canned tunas labelled as skipjack, yellowfin and albacore suggesting an  
20 adulteration action or mislabelling during process technology.

21

22

23 **Keywords:** Tuna; identification; quantification; MIR; chemometry.

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27

## 28 **Introduction**

29 Tuna is rich, among other components, in protein, fat and is considered an excellent  
30 source of the essential omega-3 fatty acids, which help to lower blood pressure,  
31 cholesterol and maintain a healthy heart. The European Union, the United States, and  
32 Japan are the largest consumers of canned tunas consuming about 51%, 31%, and 6%,  
33 respectively, of the world's canned tuna products. Canned tuna is sourced  
34 predominantly from four species of tuna: skipjack (*Katsuwonus pelamis*), yellowfin  
35 (*Thunnus albacares*), albacore (*Thunnus alalunga*) and bigeye (*Thunnus obesus*). In  
36 France, the market of canned tunas reached around 112,000 tons in 2010, accounting  
37 for more than 50 % of the purchasing volume of canned aquatic products (Lindsey,  
38 2012).

39 It is well known that the tuna trade is a very sensitive sector. Each tuna species  
40 appeared to have its unique price depending on landing, season, and nutritional value.  
41 For fishermen, catching as many fish as possible may seem like a profitable practice,  
42 but overfishing has serious consequences. This trend destroys the balance of life in the  
43 oceans, as well as social and economic well-being. Several studies have detected  
44 fraud in tuna cans industry, mainly by substituting species for commercial gain or due  
45 to misidentification especially for tunas caught in mixed fisheries and with similar  
46 morphological features (Bojolly et al., 2017; Sotelo et al., 2018).

47 Indeed interest in authenticity, traceability, and safety of canned tunas are increasing  
48 to fight fraud that costs between 1 to 8 billion euros (Lindsey, 2012) and many  
49 measures have been taken from all the involved organisations to provide to the  
50 consumer authentic product that replies to his desire.

51 In particular, the Food and Agriculture Organisation (FAO) developed as a voluntary  
52 instrument, the International Plan of Action (IPOA) to eliminate illegal, unreported  
53 and unregulated fishing (Kuemlangan and Press, 2010). Later, the European Union

54 (EU) developed two complementary Regulations, the Council Regulation (EC) No.  
55 1005/2008 and the Council Regulation (EC) No. 1224/2009, both emphasising the  
56 authentication and traceability of fishery products. Indeed, Europe with these  
57 Regulations has become the zone with the most stringent legislation in this area,  
58 especially with the European Regulation ((EC) No 1536/92) that forbids the presence  
59 of different tuna species in the same tuna cans.

60 As a consequence several analytical techniques based on biomolecular DNA  
61 techniques (Unsel et al., 1995) and mitochondrial DNA (Bartlett and Davidson,  
62 1991; Paine et al., 2007) have been proposed in the literature for the authentication of  
63 tunas; however, most of these techniques are expensive, time-consuming, generate  
64 toxic wastes and are not suitable for online authentication. Furthermore, even for the  
65 professional in the seafood industry, difficulties exist for identifying at the juvenile  
66 stages between, for example, bigeye tuna and yellowfin tuna due to their similarity.

67 In addition, processed seafood may make additional difficulties, especially concerning  
68 the use of molecular markers that can be easily degraded (Etienne et al., 2000). Thus,  
69 there is currently a pressing need for a simple, fast, accurate and inexpensive  
70 technique, which can be applied in the factory online sensors to identify tuna species.  
71 In this case, spectroscopic techniques provide a great deal of information with only  
72 one test.

73 The application of mid-infrared (MIR) spectroscopy is widespread in the world  
74 industry over the past decade (De Fuentes Navarta et al., 2008; Woodcock et al.,  
75 2008; Wu et al., 2012). The MIR spectrum represents the absorption of all the  
76 chemical bonds between 4000 and 700  $\text{cm}^{-1}$ . The MIR spectroscopy coupled with  
77 chemometric tools has shown its ability to authenticate several food products:  
78 L'Etivaz and Gruyère cheeses produced in different geographical regions (Karoui et  
79 al., 2007a) and Emmental cheeses produced in winter and summer periods (Karoui et

80 al., 2004). Moreover, this technique has shown its potential to differentiate between  
81 beef stored under different [atmospheres](#) (Ammor et al., 2009). For fish products, MIR  
82 spectroscopy has been used to differentiate between: i) fresh and frozen fish (Karoui  
83 et al., 2007b), ii) canned fish packing oils (Dominguez-Vidal et al., 2016), iii) fish  
84 species (Alamprese and Casiraghi, 2015) and iv) fish oil with different oxidative  
85 values (Klaypradit et al., 2011).

86 Only limited research work employed the MIR to authenticate canned tunas according  
87 to the composition of liquid media (Dominguez-Vidal et al., 2016). In their research  
88 study, the authors scanned MIR spectra on 90 canned tunas that were purchased from  
89 10 different Spanish retailers (48 and 40 canned tunas produced with olive and seed  
90 oil, respectively). By applying Partial least squares discriminant analysis to the MIR  
91 spectra, the authors succeeded to differentiate between tuna samples produced with  
92 olive oil from those made with seed oils.

93 In our previous study (Boughattas et al., 2020), [our research group](#) reported the ability  
94 of MIR spectroscopy to authenticate 232 canned tuna with sunflower medium,  
95 containing bigeye, yellowfin, skipjack and their presence in a binary mixture, with  
96 90.38% of correct classification. At our best knowledge, up to date, no published  
97 work was found in the literature for the identification of canned tuna [species in brine](#)  
98 medium. Thus, the objective of the present study was to investigate, for the first time,  
99 the potential use of MIR in combination with chemometric tools to: i) identify bigeye,  
100 yellowfin, albacore, and skipjack and their binary mixture of canned tuna [species in](#)  
101 [brine](#) medium produced at the [pilot-scale](#); and ii) identify commercially tuna cans.

102

## 103 **2. Materials and methods**

### 104 **2.1. Raw materials**

105 Four tuna species (bigeye, albacore, skipjack, and yellowfin), originating from  
106 different areas (Atlantic, Pacific, and Indian oceans) were purchased from commercial  
107 fishing vessels by the innovation platform for aquatic products (Plateforme  
108 d'Innovation Nouvelles Vagues, (PFINV), Boulogne Sur Mer, France). The entire  
109 individual of tuna species was sampled and identified according to the morphological  
110 characters using identification keys from the FAO Species Catalogue (Collette and  
111 Nauen, 1983), before the filleting process.

112

## 113 **2.2 Canned tuna samples**

114 To prepare tuna cans, a thawed process was applied to previously frozen fillets at a  
115 temperature of 0 - 2 °C. Then in each tin (diameter = 55 mm, 1/12 can), 80 g of raw  
116 flesh and brine were added. Cans were crimped and sterilised at 116 °C to the  
117 sterilising value of 7 min. Tuna cans containing either yellowfin, skipjack, albacore,  
118 or bigeye tuna species were prepared using the technique applied in the canning  
119 industry to obtain standardised cans.

120 To imitate voluntary and involuntary rate substitution in canned products, two  
121 hundred eighteen (218) tuna cans of different mixtures of tuna species varying from 1  
122 to 99% (**Table 1**) were prepared. The 218 tuna samples have been used in the  
123 calibration step to establish models. The accuracy of the established models was  
124 determined on 40 unknown commercial tuna cans that were purchased randomly from  
125 local markets.

126

## 127 **2.3. Mid-infrared spectroscopy measurements**

128 The MIR spectra were recorded at room temperature (20 °C) between 4000 and 700  
129  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  on a Fourier transform spectrometer IRTracer-100  
130 (Shimadzu, Duisburg, Germany) mounted with an attenuated total reflection (ATR)



131 accessory equipped with a grip (Pike Technologies, Inc. Madison, United States). The  
132 ATR cell was made of horizontal ZnSe crystal which presented an incidence angle of  
133  $45^\circ$  and total reflection ( $n=10$ ). To prepare a representative sample for scanning, the  
134 content of each canned tuna was transferred into 250 mL plastic beaker; then, the tuna  
135 was mixed by using a homogenizer (T 25 digital ULTRA-TURRAX®, IKA) with a  
136 speed of 10000 rpm for 5 minutes, according to previous findings (Srikornkarn and  
137 Sirisomboon, 2014). Then, the ground sample was deposited on the crystal and gently  
138 pressed with a press accessory ensuring good contact between the sample and the  
139 crystal. Before each measurement, the spectrum of the ZnSe crystal was recorded and  
140 used as background. For each sample, 3 spectra were recorded.

141

#### 142 **2.4. Statistical treatments of mid infrared data sets**

143 In a first step, principal component analysis (PCA) was applied to the normalised  
144 spectra to investigate differences between samples (Karoui et al., 2006b, 2006a).  
145 Then, factorial discriminant analysis (FDA) was performed on the first 5 **principal**  
146 **components (PCs)** resulting from the PCA applied, **separately**, to each of the three  
147 spectral regions ( $3000\text{-}2800\text{ cm}^{-1}$ ,  $1700\text{-}1500\text{ cm}^{-1}$  and  $1500\text{-}900\text{ cm}^{-1}$ ). **This**  
148 **techniques aims** to predict the membership of an individual to a qualitative group  
149 defined as a preliminary. The canned tuna is assigned to the group where its distance  
150 between the centres of gravity is the shortest.

151 Finally, the first 5 PCs of the PCA performed on each of the three data sets  
152 were pooled into one matrix and the new table was analysed newly by the FDA. This  
153 concatenation approach helps to improve the discrimination of the investigated  
154 canned tunas, as well as to assess the ability of this technique to determine the species  
155 and the amount of each tuna species in the can.

156 In fact, the 218 tuna samples produced at the PFNV with a known percentage  
157 of tuna species were divided into calibration and validation sets. Once the calibration  
158 model was established with well high accuracy, a validation test was realised. This  
159 procedure allows us to confirm the robustness of the established model that was used  
160 to predict the 40 samples purchased from a local supermarket.

161 A specific approach was adopted, in which the commercial samples have been  
162 used as supplementary samples when realizing the PCA. Thus, the 40 commercial  
163 samples do not participate in the creation of PCs.

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

166

### 167 **3. Results and discussion**

#### 168 **3.1 Mid-infrared spectra of canned tunas containing one unique species**

169 **Fig. 1** shows MIR spectra recorded on canned tuna containing 100% yellowfin, 100%  
170 bigeye, 100% skipjack, and 100% albacore. The spectra were dominated by two  
171 strong bands  $\sim 3270$  and  $3370\text{ cm}^{-1}$  assigned to amide A (N-H stretching)  
172 (Dominguez-Vidal et al., 2016) and water absorption (Rasmussen and Morrissey,  
173 2006), which usually represent 60% of canned tuna muscle. To authenticate tuna cans  
174 as a function of their composition, three spectral regions were studied separately:  
175  $3000\text{-}2800\text{ cm}^{-1}$ ,  $1700\text{-}1500\text{ cm}^{-1}$ , and  $1500\text{-}900\text{ cm}^{-1}$ .

176 The  $3000\text{-}2800\text{ cm}^{-1}$  spectral region (**Fig. 1a**) was characterised by the  
177 presence of bands related to the physical state of triglycerides. The spectra showed  
178 two bands located  $\sim 2925$  and  $2854\text{ cm}^{-1}$  that were assigned to the methylene anti-  
179 symmetric and symmetric stretching modes. Reliable discrimination between cans as  
180 a function of species was observed. As illustrated in **Fig. 1a**, absorption bands  
181 corresponding to cans labelled bigeye were far less intense compared to those

182 designed albacore. In addition, these bands were not observed for cans known as  
183 skipjack and yellowfin tunas. This is in line with other findings, who observed that the  
184 highest fat content in tuna was observed for albacore species (Wheeler and Morrissey,  
185 2002). Moreover, another study pointed out that bigeye, yellowfin, and skipjack  
186 contained 2.06%, 1.93%, and 0.41% of crude fat, respectively (Mahaliyana et al.,  
187 2015). From the obtained results, it could be concluded that the spectral region  
188 between 3000 and 2800  $\text{cm}^{-1}$  could be considered as a very efficient way to  
189 authenticate tuna cans.

190 **Fig. 1b** depicts the shape of the spectra in the 1700-1500  $\text{cm}^{-1}$  region that is  
191 characterised by the presence of the Amide I and II bands related to proteins. The  
192 Amide I band, which is used to investigate the secondary structure of proteins, is  
193 characterised by the symmetric stretching vibrations of the carbonyl (C=O) functional  
194 group that is observed ~ 1651, 1635 and 1646  $\text{cm}^{-1}$  (**Fig. 1B**) (Dousseau and Pézolet,  
195 1990). The absorbance ~1651  $\text{cm}^{-1}$  could be ascribed to the protein degradation  
196 during canning and is characteristic of  $\alpha$ -helical structure (Grunert et al., 2016).  
197 Regarding bands ~1635, 1646  $\text{cm}^{-1}$ , they are characteristic of  $\beta$ -plated sheet secondary  
198 structure (Grunert et al., 2016).

199 Contributions to the Amide II band, principally based on N-H bending coupled  
200 with C-N stretching, were observed ~1508, 1523, 1542, 1557, and 1573  $\text{cm}^{-1}$   
201 (Bellamy, 1975). Significant changes for both Amide I and II were observed  
202 according to the tuna species. From the aforementioned results, it could be concluded  
203 that the 1700-1500  $\text{cm}^{-1}$  region is a reliable region to discriminate albacore species  
204 from the other species.

205 The 1500-900  $\text{cm}^{-1}$  region (**Fig. 1c**) is known as the fingerprint region and refers to  
206 C–O and C–C stretching modes (1176-900  $\text{cm}^{-1}$ ) and the bending modes of O–C–H,  
207 C–C–H, C–O–H in the 1481-1199  $\text{cm}^{-1}$  (Sivakesava and Irudayaraj, 2001). The bands

208 located  $\sim 1048\text{ cm}^{-1}$  could be ascribed to the primary alcohol (C–O stretching mode),  
209 while bands  $\sim 1127$  and  $1159\text{ cm}^{-1}$  were assigned to the tertiary alcohol (C–O  
210 stretching mode). The band observed  $\sim 1239\text{ cm}^{-1}$  was attributed to the  $\text{PO}_2^-$   
211 asymmetric stretching of phospholipids and Amide III band (C–H and N–H). The  
212 peak  $\sim 1080\text{ cm}^{-1}$  is related to the  $\text{PO}_2^-$  symmetric stretching, and C–C and C–O to  
213 lipids and proteins (Hernández-Martínez et al., 2014). Those located  $\sim 1460\text{ cm}^{-1}$  are  
214 related to the  $-\text{CH}_2$  and  $-\text{CH}_3$  scissoring, and that  $\sim 1396\text{ cm}^{-1}$  to symmetric  
215 stretching of  $\text{COO}^-$  group. The = C–H (cis) bending (rocking) is observed  $\sim 1419\text{ cm}^{-1}$   
216 <sup>1</sup> in agreement with the previous findings (Vidal et al., 2013).

217

### 218 **3.2 Mid-infrared spectra of canned tunas containing binary mixture species**

219 The MIR spectra of tuna cans contain information on compounds, which are present  
220 at level  $> 0.1\%$  (w/w) (Karoui et al., 2007a). Thus the ability of MIR to differentiate  
221 between tuna cans containing binary mixtures with different percentages of bigeye,  
222 skipjack, and yellowfin tunas were assessed (Figs. 2a, b, and c).

223 **Fig. 2a** depicts the shape of the spectra recorded on canned tunas containing  
224 yellowfin, bigeye, and their binary mixtures with different percentages in the  $3000 -$   
225  $2800\text{ cm}^{-1}$  region. The spectra exhibited two bands ( $\sim 2925$  and  $2854\text{ cm}^{-1}$ ) where  
226 their absorbance showed significant differences ( $p < 0.05$ ) between tuna cans as a  
227 function of their containing. As mentioned above, these bands are due to methylene  
228 anti-symmetric and symmetric stretching vibrations, respectively. As showed in **Fig.**  
229 **2a**, the absorbance of these bands ( $\sim 2925$  and  $2854\text{ cm}^{-1}$ ) are slightly higher in tuna  
230 cans containing 100% bigeye species than those containing 100% yellowfin,  
231 Y95/B05, and Y90/B10. Previous studies on fish oil proved that the absorbance  
232 intensity of this band is closely related to the percentage of saturated acyl groups  
233 constituting the fish oil (Vidal et al., 2013). Additionally, a previous study pointed out

234 that yellowfin and bigeye species have different amounts of saturated acyl groups  
235 (Sardenne et al., 2017).

236 To differentiate between canned tunas containing bigeye species, skipjack  
237 species, and their mixtures, the fingerprint region ( $1500 - 900 \text{ cm}^{-1}$ ) was studied. As  
238 illustrated in **Fig. 2b**, a large difference between samples was observed. The band ~  
239  $1395 \text{ cm}^{-1}$ , indicating salt bridges, showed a higher absorbance for canned tunas  
240 containing 100% skipjack, 100% bigeye, S75/B25, and S25/B75. The band ~  $965 \text{ cm}^{-1}$   
241 corresponding to the bending out of the plane of  $-\text{HC}=\text{CH}-$  was distinctly observed  
242 in the spectra of 100% skipjack, and S75/B25, while it is absent for spectra scanned  
243 on tuna containing 100% bigeye, and S25/B75. This finding could be explained by the  
244 fact that each tuna species presents a specific fatty acid profile since composition,  
245 length, and unsaturated degree of fatty acids, as well as the position of the C=C bond  
246 in the chain, differ from tuna species to another. These results were in agreement with  
247 previous studies in which those areas or heights of bands were used to investigate the  
248 degree of unsaturation of fatty acids (Pu et al., 2016).

249 **Fig. 2c** illustrates the  $1500-1700 \text{ cm}^{-1}$  region acquired on canned tuna  
250 containing 100% skipjack, 100% yellowfin, and their mixtures. Clear discrimination  
251 was observed according to the composition of canned tunas. A special focus is made  
252 on the spectral changes ~  $1523 \text{ cm}^{-1}$ . The absorbance of 0.62, 0.58, 0.51, and 0.47 was  
253 observed for Y10/S90, 100% skipjack, 100% yellowfin, and Y75/S25, respectively.  
254 The difference could be due to changes in the protein structure of tunas, especially  $\beta$ -  
255 sheet structure (Cheng et al., 2013).

256

257 **3.3 Global analysis of the infrared spectral data sets recorded on tuna cans:**  
258 **concatenation technique**

259

### 260 3.3.1. Development of calibration models for the authentication of tuna species in

#### 261 canned tunas

262 Due to the complexity of the spectra, the univariate analysis does not allow us to  
263 differentiate between canned tunas. Consequently, better discrimination of these  
264 samples produced with only one species or a binary mixture of tuna species could be  
265 obtained by using a multivariate statistical approach. Therefore, the concatenation  
266 method was applied to the first 5 PCs of the PCA applied to the 3000-2800  $\text{cm}^{-1}$ ,  
267 1700-1500  $\text{cm}^{-1}$ , and 1500-900  $\text{cm}^{-1}$  spectral regions. The total (15 PCs)  
268 corresponding to the three spectral data were gathered into one matrix that was  
269 analysed by the FDA.

270 To establish a robust model that could be used later for the classification of unknown  
271 canned tunas, misclassified samples were deleted from the databases, and the FDA  
272 was applied again. As a result, the classification rate was improved. We obtained  
273 74.96% of correct classification (**Table 2a**). Canned tunas produced with 100%  
274 albacore, Y99/S01, Y75/S25, S01/B99, and S10/B90 were 100% correctly classified.  
275 The worst classification was observed for S90/B10 and S95/B05 since correct  
276 classification rates of 30.33% and 40% were observed, respectively.

277 Despite the high rate of misclassification, correct classification amounting to 75 and  
278 99% of skipjack species was observed for both mixtures (S90/B10 and S95/B05).

279 Regarding the other canned tunas, correct classification in the range of 50 – 93.33%  
280 was obtained (**Table 2a**). It is interesting to notice that the MIR spectra succeeded to  
281 classify samples containing 100% bigeye, 100% yellowfin, and 100% skipjack with  
282 correct classification rates of 77.78, 79.59, and 93.33%, respectively. This was  
283 confirmed by further synthetic parameters such as the sensitivity, the specificity, the  
284 accuracy, and the error rate that were calculated from the confusion matrix. As shown  
285 in **Table 2b**, an accuracy rate varying between 95.06 and 100% was observed.

286 Interestingly, a perfect specificity rate (100%) was observed for Y75/B25, Y95/S05,  
287 S05/B95, Y90/S10, S10/B90, S25/B75, and S90/B10.

288 From the obtained results, it appeared that the approach based on using the  
289 concatenation of the three spectral regions allowing to identify bigeye, yellowfin,  
290 albacore, and skipjack tuna species and their amount in canned tunas.

291

### 292 **3.3.2. Evaluation of the ability of the established model for the authentication of** 293 **commercially tuna cans**

294 The established [models](#) realised on experimental canned tunas with known samples  
295 and their binary mixtures [were](#) tested on 40 unknown commercially tuna cans labelled  
296 as bigeye ( $n = 10$ ), yellowfin ( $n = 10$ ), albacore ( $n = 10$ ), and skipjack ( $n = 10$ ) (**Table**  
297 **2c**).

298 The results indicate that the 10 commercial tuna cans labelled bigeye are 100%  
299 correctly classified and contained effectively bigeye species. The 10 commercial  
300 canned tuna labelled skipjack were 100% misclassified and were ascribed to albacore  
301 and yellowfin tunas. Regarding canned tunas labelled as yellowfin, [a correct](#)  
302 classification rate of 70% was obtained since 3 out of 10 canned tunas seemed to  
303 contain albacore and mixture of yellowfin and bigeye tunas. A better classification  
304 was obtained for canned tuna labelled albacore since [a correct](#) classification level of  
305 93.33% was obtained.

306 These results that should be strengthened on a large number of commercially canned  
307 tunas suggested mislabelling during the production, which the tuna canning industry  
308 will have to address. The obtained results are in agreement with: (i) our previous  
309 investigations reporting a rate of mislabelling amounting to 40% in commercially  
310 canned tunas with sunflower medium (Boughattas et al., 2020), and (ii) [with the](#)  
311 [findings of Bojolly et al. \(2017\) who pointed out a mislabelling rate of canned tuna](#)

312 species in species in brine using a qPCR method. It would be interesting that these  
313 findings would be confirmed by reference methods used in the canned industry.

#### 314 **4. Conclusion**

315 This study provides proof of principle of the application of the MIR spectroscopy  
316 coupled with chemometric tools as a rapid technique to discriminate between skipjack  
317 tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), albacore tuna  
318 (*Thunnus alalunga*) and bigeye tuna (*Thunnus obesus*) that are the species most  
319 commonly used for producing canned tunas.

320 Due to the complexity of data sets contained in MIR spectra, several multivariate  
321 statistical techniques were applied. The application of a multi-block method based on  
322 the concatenation technique was found to be a potential suitable technique to  
323 authenticate tuna cans according to their containing. Thus, the MIR spectroscopy may  
324 represent a promising tool to be used as a rapid screening analytical tool to ascertain  
325 rapidly labelling of canned tuna species in brine. Before its possible application, it  
326 would be interesting to determine the potential of the MIR technique on a large  
327 number of canned tuna species with other liquid media (olive oil, soybean oil, etc.).  
328 This investigation would be performed on tuna samples collected from different  
329 fishing areas in combination with reference methods used in canned tunas  
330 authentication. This identification would help laboratories to determine canned tunas  
331 traceability.

332

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469 **List of tables:**

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471 **Table 1:** Details of canned tunas used for the calibration models

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473 **Table 2:** Cross-validation table of concatenated MIR spectra regions ( $1500\text{-}900\text{ cm}^{-1}$ ,  
474  $1700\text{-}1500\text{ cm}^{-1}$  and  $3000\text{-}2800\text{ cm}^{-1}$ ) realised on canned tunas for the: (a) calibration  
475 model and (b) performance parameters calculated from the confusion matrix and (c)  
476 prediction model

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**List of figures:**

**Figure 1:** Raw mid infrared spectra recorded on canned tunas containing: yellowfin (— · ·), bigeye (···), skipjack (===) and albacore tuna (====) .

**Figure 2:** Raw mid infrared spectra recorded on canned tunas containing **(a)**: bigeye, yellowfin and binary mixtures of bigeye and yellowfin with different percentages; **(b)** skipjack, bigeye and binary mixtures of skipjack and bigeye with different percentages; and **(c)** skipjack, bigeye and binary mixtures of skipjack and bigeye with different percentages

**Table 1:**

<b>Composition of canned tuna</b>	<b>code</b>	<b>number of cans</b>
100% of yellowfin tuna	Yellowfin	38
100% of skipjack tuna	Skipjack	18
100% of albacore tuna	Albacore	9
100% of Bigeye tuna	Bigeye	28
99% of yellowfin tuna and 01% of bigeye tuna	Y99/B01	7
95% of yellowfin tuna and 05% of bigeye tuna	Y95/B05	7
90% of yellowfin tuna and 10% of bigeye tuna	Y90/B10	7
75% of yellowfin tuna and 25% of bigeye tuna	Y75/B25	7
50% of yellowfin tuna and 50% of bigeye tuna	Y50/B50	7
99% of yellowfin tuna and 01% of skipjack tuna	Y99/S01	4
95% of yellowfin tuna and 05% of skipjack tuna	Y95/S05	4
90% of yellowfin tuna and 10% of skipjack tuna	Y90/S10	4
75% of yellowfin tuna and 25% of skipjack tuna	Y75/S25	4
50% of yellowfin tuna and 50% of skipjack tuna	Y50/S50	9
25% of yellowfin tuna and 75% of skipjack tuna	Y25/S75	5
10% of yellowfin tuna and 90% of skipjack tuna	Y10/S90	5
05% of yellowfin tuna and 95% of skipjack tuna	Y05/S95	5
01% of yellowfin tuna and 99% of skipjack tuna	Y01/S99	5
99% of skipjack tuna and 01% of bigeye tuna	S99/B01	5
95% of skipjack tuna and 05% of bigeye tuna	S95/B05	5
90% of skipjack tuna and 10% of bigeye tuna	S90/B10	5
75% of skipjack tuna and 25% of bigeye tuna	S75/B25	5
50% of skipjack tuna and 50% of bigeye tuna	S50/B50	9
25% of skipjack tuna and 75% of bigeye tuna	S25/B75	4
10% of skipjack tuna and 90% of bigeye tuna	S10/B90	4
05% of skipjack tuna and 95% of bigeye tuna	S05/B95	4
01% of skipjack tuna and 99% of bigeye tuna	S01/B99	4
<b>Total</b>		<b>218</b>

Table 2a:

Predicted/ Observed	Y50/B50	Y75/B25	Y90/B10	Y95/B05	Y99/B01	Yellowfin	Albacore	Y99/S01	S01/B99	Y95/S05	S05/B95	Y90/S10	S10/B90	Y75/S25	S25/B75	Y50/S50	S50/B50	Y25/S75	S75/B25	Y10/S90	S90/B10	Y05/S95	S95/B05	Y01/S99	S99/B0	Skipjack	Bigeye	Total	% Correct classification	
Y50/B50	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	15	93.33%
Y75/B25	0	9	0	5	1	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	50.00%
Y90/B10	0	0	12	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	15	80.00%
Y95/B05	0	0	0	13	7	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	61.90%
Y99/B01	0	0	0	6	12	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	57.14%
Yellowfin	0	0	6	0	0	78	4	0	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	4	98	79.59%
Albacore	0	0	0	0	0	0	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	100.00%
Y99/S01	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	100.00%
S01/B99	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	100.00%
Y95/S05	0	0	0	0	0	0	0	0	0	9	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	75.00%
S05/B95	2	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	60.00%
Y90/S10	0	0	0	0	0	0	0	1	0	0	0	6	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	50.00%
S10/B90	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	100.00%
Y75/S25	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	100.00%
S25/B75	0	0	1	0	0	1	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	6	66.67%
Y50/S50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	1	0	0	0	0	2	0	0	0	0	15	80.00%
S50/B50	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	15	0	0	0	0	0	0	0	3	0	0	0	21	71.43%
Y25/S75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	9	0	3	0	0	0	0	0	0	0	0	15	60.00%
S75/B25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	3	0	0	0	12	75.00%
Y10/S90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	10	0	0	0	0	1	1	0	0	15	66.67%
S90/B10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	5	0	3	5	0	0	0	0	15	33.33%
Y05/S95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	6	0	0	0	0	15	60.00%
S95/B05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	6	5	1	0	0	0	15	40.00%
Y01/S99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	9	2	0	0	0	15	60.00%
S99/B01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	0	0	0	0	1	9	0	0	0	15	60.00%
Skipjack	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	42	0	45	93.33%
Bigeye	0	0	0	0	0	7	0	0	6	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	56	72	77.78%
<b>Total</b>	16	9	19	24	20	86	30	15	21	9	3	6	12	33	4	13	21	16	20	13	5	13	9	28	19	43	60	567	74.96%	



Table 2b:

	Sensitivity	Specificity	Error rate	Accuracy
Y50/B50	93.33 %	99.64 %	0.53 %	99.47 %
Y75/B25	50 %	100%	1.59 %	98.41 %
Y90/B10	80 %	98.73 %	1.76 %	98.24 %
Y95/B05	61.9 %	97.99 %	3.35 %	96.65 %
Y99/B01	57.14 %	98.53 %	3 %	97 %
Yellowfin	79.59 %	98.29 %	4.94 %	95.06 %
Albacore	100 %	99.26 %	0.71 %	99.29 %
Y99/S01	100 %	99.46 %	0.53 %	99.47 %
S01/B99	100 %	98.38 %	1.59 %	98.41 %
Y95/S05	75 %	100 %	0.53 %	99.47 %
S05/B95	60 %	100 %	0.35 %	99.65 %
Y90/S10	50 %	100 %	1.06 %	98.94 %
S10/B90	100 %	100 %	0 %	100 %
Y75/S25	100 %	96.22 %	3.7 %	96.3 %
S25/B75	66.67 %	100 %	0.35 %	99.65 %
Y50/S50	80 %	99.82 %	0.71 %	99.29 %
S50/B50	71.43 %	98.9 %	2.12 %	97.88 %
Y25/S75	60 %	98.73 %	2.29 %	97.71 %
S75/B25	75 %	98.02 %	2.47 %	97.53 %
Y10/S90	66.67 %	99.46 %	1.41 %	98.59 %
S90/B10	33.33 %	<b>100%</b> 1.76 %	1.76 %	98.24 %
Y05/S95	60 %	99.28 %	1.76 %	98.24 %
S95/B05	40 %	99.46 %	2.12 %	97.88 %
Y01/S99	60 %	96.56 %	4.41 %	95.59 %
S99/B01	60 %	98.19 %	2.82 %	97.18 %
Skipjack	93.33 %	99.81 %	0.71 %	99.29 %
Bigeye	77.78 %	99.19 %	3.53 %	96.47 %

**Table 2c:**

<b>Commercial tuna cans</b>	<b>Prediction</b>	<b>% of correct classification</b>
Yellowfin ( <i>n=10</i> )	21 spectra belonging to yellowfin 3 spectra belonging to Y90/B10 6 spectra belonging to albacore	70
Bigeye ( <i>n=10</i> )	30 spectra belonging to bigeye	100
Skipjack ( <i>n=10</i> )	20 spectra belonging to yellowfin 10 spectra belonging to albacore	0
Albacore ( <i>n=10</i> )	28 spectra belonging to albacore 1 spectra belonging to Y50/S50 1 spectra belonging to yellowfin	93.33
Total		65.83

Figure 1:

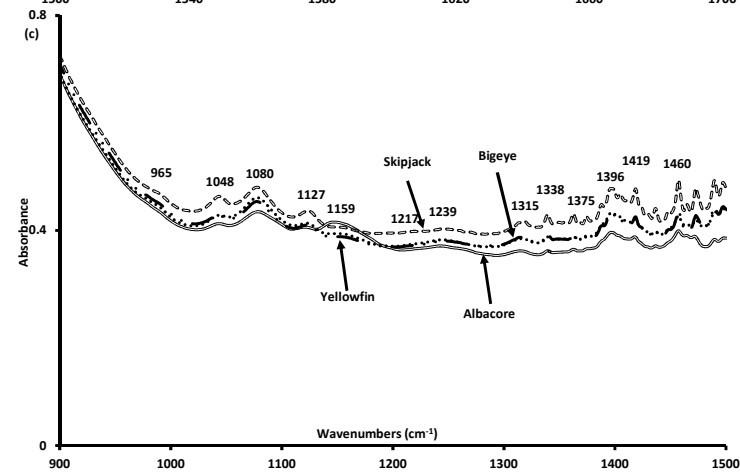
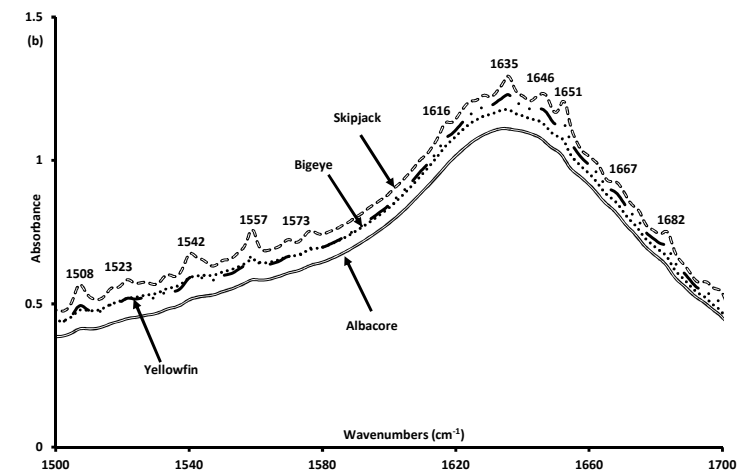
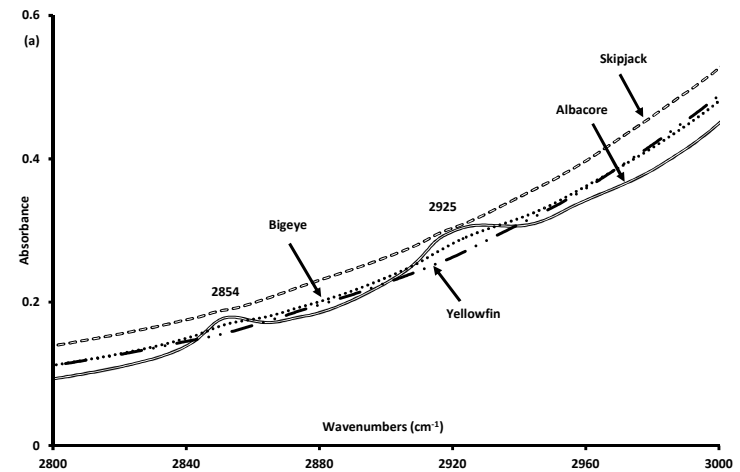
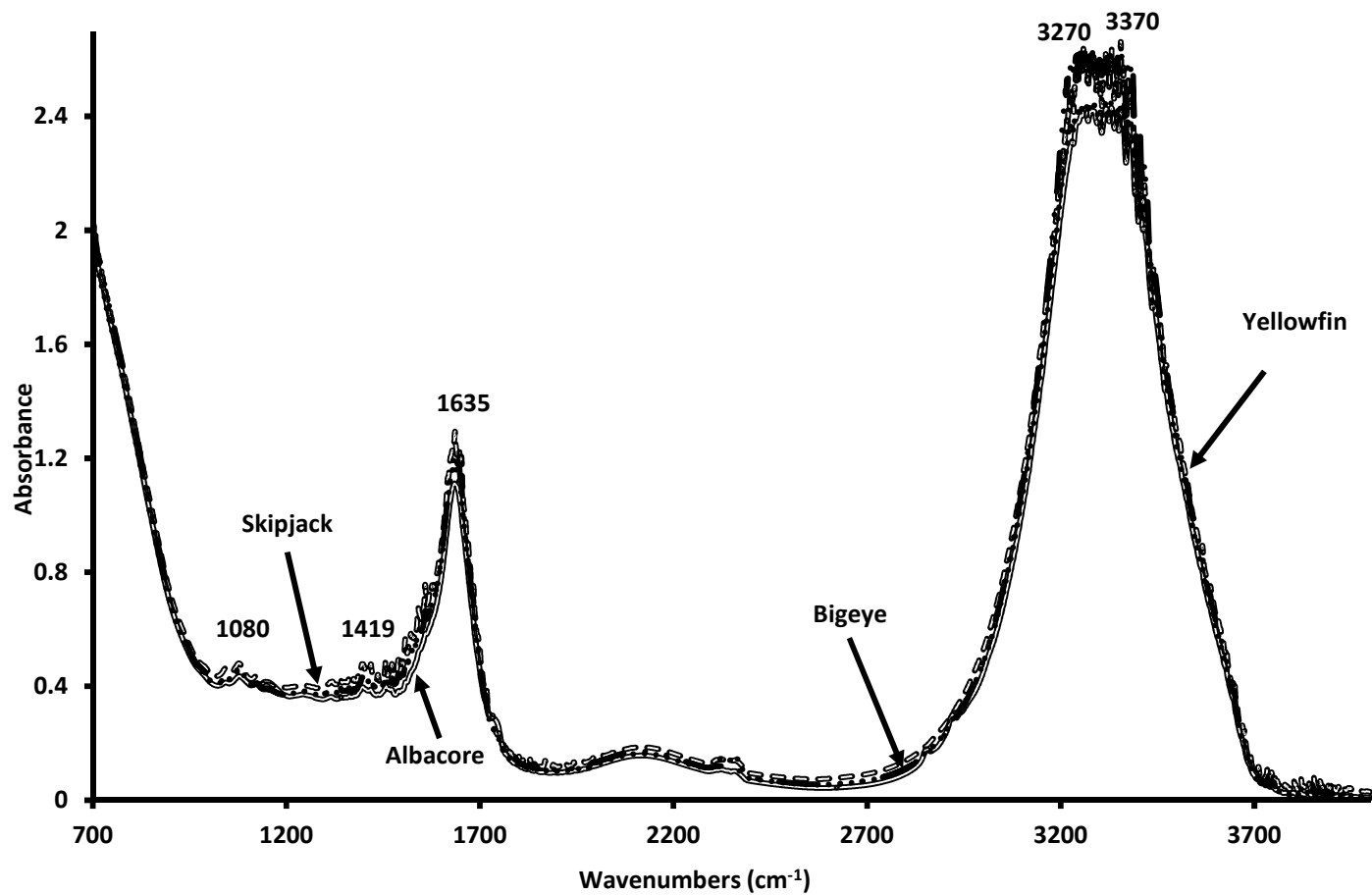


Figure 2:

