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## ► To cite this version:

Ferdaous Boughattas, Romdhane Karoui. Mid infrared spectroscopy combined with chemometric tools for the identification of canned tuna species in brine. Journal of Food Composition and Analysis, 2021, 96, pp.103717 - 10.1016/j.jfca.2020.103717 . hal-03494080

# HAL Id: hal-03494080 https://hal.science/hal-03494080

Submitted on 2 Jan 2023

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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
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#### 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 cm<sup>-1</sup>. 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 cm<sup>-1</sup>, 1700-1500 cm<sup>-1</sup> and 1500-900 cm<sup>-1</sup> 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 classification was obtained for the bigeye tuna, while misclassification was observed 18 19 for canned tunas labelled as skipjack, yellowfin and albacore suggesting an 20 adulteration action or mislabelling during process technology.

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

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#### 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

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

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

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

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

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

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

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

112

#### 113 **2.2 Canned tuna samples**

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

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

126

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

128 The MIR spectra were recorded at room temperature (20  $^{\circ}$ C) between 4000 and 700 129 cm<sup>-1</sup> at a resolution of 4 cm<sup>-1</sup> 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 45 ° and total reflection (n=10). To prepare a representative sample for scanning, the 133 134 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 components (PCs) resulting from the PCA applied, separately, to each of the three 146 spectral regions (3000-2800 cm<sup>-1</sup>, 1700-1500 cm<sup>-1</sup> and 1500-900 cm<sup>-1</sup>). This 147 148 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.

Finally, the first 5 PCs of the PCA performed on each of the three data sets were pooled into one matrix and the new table was analysed newly by the FDA. This concatenation approach helps to improve the discrimination of the investigated canned tunas, as well as to assess the ability of this technique to determine the species and the amount of each tuna species in the can. In fact, the 218 tuna samples produced at the PFNV with a known percentage of tuna species were divided into calibration and validation sets. Once the calibration model was established with well high accuracy, a validation test was realised. This procedure allows us to confirm the robustness of the established model that was used 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**

Fig. 1 shows MIR spectra recorded on canned tuna containing 100% yellowfin, 100% bigeye, 100% skipjack, and 100% albacore. The spectra were dominated by two strong bands ~ 3270 and 3370 cm<sup>-1</sup> assigned to amide A (N-H stretching) (Dominguez-Vidal et al., 2016) and water absorption (Rasmussen and Morrissey, 2006), which usually represent 60% of canned tuna muscle. To authenticate tuna cans as a function of their composition, three spectral regions were studied separately: 3000-2800 cm<sup>-1</sup>, 1700-1500 cm<sup>-1</sup>, and 1500-900 cm<sup>-1</sup>.

The 3000-2800 cm<sup>-1</sup> spectral region (**Fig. 1a**) was characterised by the presence of bands related to the physical state of triglycerides. The spectra showed two bands located ~ 2925 and 2854 cm<sup>-1</sup> that were assigned to the methylene antisymmetric and symmetric stretching modes. Reliable discrimination between cans as a function of species was observed. As illustrated in **Fig. 1a**, absorption bands 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 highest fat content in tuna was observed for albacore species (Wheeler and Morrissev. 184 185 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 between 3000 and 2800 cm<sup>-1</sup> could be considered as a very efficient way to 188 authenticate tuna cans. 189

Fig. 1b depicts the shape of the spectra in the 1700-1500 cm<sup>-1</sup> region that is 190 characterised by the presence of the Amide I and II bands related to proteins. The 191 192 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 group that is observed ~ 1651, 1635 and 1646 cm<sup>-1</sup> (Fig. 1B) (Dousseau and Pézolet, 194 1990). The absorbance  $\sim 1651 \text{ cm}^{-1}$  could be ascribed to the protein degradation 195 196 during canning and is characteristic of  $\alpha$ -helical structure (Grunert et al., 2016). 197 Regarding bands ~1635, 1646 cm<sup>-1</sup>, 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 cm<sup>-1</sup> 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 cm<sup>-1</sup> region is a reliable region to discriminate albacore species 204 from the other species.

The 1500-900 cm<sup>-1</sup> region (**Fig. 1c**) is known as the fingerprint region and refers to C–O and C–C stretching modes (1176-900 cm<sup>-1</sup>) and the bending modes of O–C–H, C–C–H, C–O–H in the 1481-1199 cm<sup>-1</sup> (Sivakesava and Irudayaraj, 2001). The bands

located ~ 1048 cm<sup>-1</sup> could be ascribed to the primary alcohol (C–O stretching mode), 208 while bands ~1127 and 1159 cm<sup>-1</sup> were assigned to the tertiary alcohol (C-O 209 210 stretching mode). The band observed ~ 1239 cm<sup>-1</sup> was attributed to the  $PO_2^{-1}$ 211 asymmetric stretching of phospholipids and Amide III band (C-H and N-H). The peak ~ 1080 cm<sup>-1</sup> is related to the PO<sub>2</sub><sup>-</sup> symmetric stretching, and C–C and C–O to 212 lipids and proteins (Hernández-Martínez et al., 2014). Those located ~1460 cm<sup>-1</sup> are 213 related to the  $-CH_2$  and  $-CH_3$  scissoring, and that ~ 1396 cm<sup>-1</sup> to symmetric 214 215 stretching of COO<sup>-</sup> group. The = C-H (cis) bending (rocking) is observed ~ 1419 cm<sup>-</sup> <sup>1</sup> in agreement with the previous findings (Vidal et al., 2013). 216

217

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

The MIR spectra of tuna cans contain information on compounds, which are present at level > 0.1% (w/w) (Karoui et al., 2007a). Thus the ability of MIR to differentiate between tuna cans containing binary mixtures with different percentages of bigeye, 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 cm<sup>-1</sup> region. The spectra exhibited two bands (~ 2925 and 2854 cm<sup>-1</sup>) where their absorbance showed significant differences (p < 0.05) between tuna cans as a 226 227 function of their containing. As mentioned above, these bands are due to methylene anti-symmetric and symmetric stretching vibrations, respectively. As showed in Fig. 228 229 **2a**, the absorbance of these bands (~ 2925 and 2854  $\text{cm}^{-1}$ ) are slightly higher in tuna cans containing 100% bigeye species than those containing 100% yellowfin, 230 231 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 that yellowfin and bigeye species have different amounts of saturated acyl groups(Sardenne et al., 2017).

To differentiate between canned tunas containing bigeye species, skipjack 236 237 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  $\sim$ 1395 cm<sup>-1</sup>, indicating salt bridges, showed a higher absorbance for canned tunas 239 240 containing 100% skipjack, 100% bigeye, S75/B25, and S25/B75. The band ~ 965 cm<sup>-</sup> <sup>1</sup> corresponding to the bending out of the plane of –HC=CH– was distinctly observed 241 in the spectra of 100% skipjack, and S75/B25, while it is absent for spectra scanned 242 on tuna containing 100% bigeye, and S25/B75. This finding could be explained by the 243 244 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 in the chain, differ from tuna species to another. These results were in agreement with 246 247 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).

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

256

3.3 Global analysis of the infrared spectral data sets recorded on tuna cans:
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 cm<sup>-1</sup>, 1700-1500 cm<sup>-1</sup>, and 1500-900 cm<sup>-1</sup> spectral regions. The total (15 PCs) 267 corresponding to the three spectral data were gathered into one matrix that was 268 269 analysed by the FDA.

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

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

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.

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

291

# **3.3.2.** Evaluation of the ability of the established model for the authentication of

### 293 commercially tuna cans

The established models realised on experimental canned tunas with known samples and their binary mixtures were tested on 40 unknown commercially tuna cans labelled as bigeye (n = 10), yellowfin (n = 10), albacore (n = 10), and skipjack (n = 10) (**Table 2**97 **2c**).

298 The results indicate that the 10 commercials 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.

These results that should be strengthened on a large number of commercially canned tunas suggested mislabelling during the production, which the tuna canning industry will have to address. The obtained results are in agreement with: (i) our previous investigations reporting a rate of mislabelling amounting to 40% in commercially canned tunas with sunflower medium (Boughattas et al., 2020), and (ii) with the 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

This study provides proof of principle of the application of the MIR spectroscopy coupled with chemometric tools as a rapid technique to discriminate between skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), albacore tuna (*Thunnus alalunga*) and bigeye tuna (*Thunnus obesus*) that are the species most 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

authentication. This identification would help laboratories to determine canned tunas

331 traceability.

332

#### 333 Acknowledgements

This study is a part of the IDThon project supported by the French Region of Hautsde-France and Bpi France. We are grateful to the Hauts-de-France Council for its financial support and to Bruno Lefur (PFNV) for providing us canned tuna samples. This work has been carried out in the framework of the ALIBIOTECH project, which

- 338 is financed by the European Union, the French State and the French Region of Hauts-
- de-France.

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

**Table 2:** Cross-validation table of concatenated MIR spectra regions (1500-900 cm<sup>-1</sup>,

474 1700-1500 cm<sup>-1</sup> and 3000-2800 cm<sup>-1</sup>) 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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480	
481	Figure 1: Raw mid infrared spectra recorded on canned tunas containing:
482	yellowfin $(-\cdot \cdot)$ , bigeye $(\cdot \cdot \cdot)$ , skipjack $()$ and albacore tuna $(-)$ .
483	
484	Figure 2: Raw mid infrared spectra recorded on canned tunas containing (a):
485	bigeye, yellowfin and binary mixtures of bigeye and yellowfin with different
486	percentages; (b) skipjack, bigeye and binary mixtures of skipjack and bigeye
487	with different percentages; and (c) skipjack, bigeye and binary mixtures of
488	skipjack and bigeye with different percentages
489	

Composition of canned tuna	code	number of cans
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
Total		218

### 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	<b>0</b> 8/80	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	56	72	77.78%
Total	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	5% 1.76 <b>9%8</b> .24	% 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:

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



Wavenumbers (cm<sup>-1</sup>)

Figure 2:





