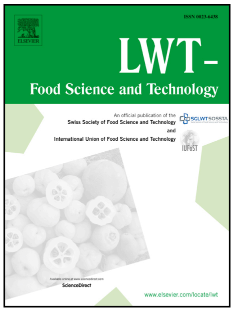
Accepted Manuscript



Application of FT-NIR and FT-IR spectroscopy to fish fillet authentication

Cristina Alamprese, Ernestina Casiraghi

PII: S0023-6438(15)00161-9
DOI: 10.1016/j.lwt.2015.03.021
Reference: YFSTL 4495

To appear in: *LWT - Food Science and Technology*

Received Date: 27 February 2014
Revised Date: 12 February 2015
Accepted Date: 9 March 2015

Please cite this article as: Alamprese, C., Casiraghi, E., Application of FT-NIR and FT-IR spectroscopy to fish fillet authentication, *LWT - Food Science and Technology* (2015), doi: 10.1016/j.lwt.2015.03.021.

Application of FT-NIR and FT-IR spectroscopy to fish fillet authentication

Cristina Alamprese*, Ernestina Casiraghi

Department of Food, Environmental and Nutritional Sciences (DeFENS)

Università degli Studi di Milano; Via Celoria 2, 20133 Milano, Italy

*Corresponding Author: Phone: +39 0250319187; Fax: +39 0250319190; E-mail: cristina.alamprese@unimi.it

Abstract

The most common frauds, carried out in different points of the fish and fish product supply chain, concern the substitution of valuable species with cheaper ones, and the selling of frozen-thawed products as fresh fish. The aim of this work was to investigate the possibility of using infrared spectroscopy as a rapid and easy tool for the identification of valuable species (i.e. red mullet and plaice) substitution with cheaper ones (i.e. Atlantic mullet and flounder). Moreover, the discrimination power of the spectroscopic techniques in identifying fresh and frozen-thawed fillets of Atlantic mullet was studied. The use of suitable chemometric strategies (Linear Discriminant Analysis, LDA; Soft Independent Modeling of Class Analogy, SIMCA) allowed to clearly distinguish Atlantic mullet fillets from those of the more valuable red mullet. In particular, LDA gave a 100% correct classification, and with SIMCA a sensitivity higher than 70% and a specificity of 100% were calculated. Good results were obtained also for plaice and flounder fillet discrimination, as well as for the recognition of Atlantic mullet fresh fillets from the frozen-thawed ones, even if with SIMCA some false positives were generated.

Keywords: Authentication, fish, IR spectroscopy, LDA, SIMCA

Abbreviations: AM, Atlantic mullet; AM-FT, frozen-thawed Atlantic mullet fillets; ATR, attenuated total reflectance; d1, first derivative; FL, flounder; FT, Fourier transform; IR, infrared; LDA, Linear Discriminant Analysis; MIR, mid infrared; MSC, multiplicative scatter correction; NIR, near infrared; PCA, Principal Component Analysis; PL, plaice; RM, red mullet; SIMCA, Soft Independent Modeling of Class Analogy; SNV, standard normal variate.

1. Introduction

In the last decades, consumers' request for fish and fish products has greatly increased, mainly due to the nutritional properties of these products. As a consequence, commercial exchanges and import/export activities have raised throughout the world, originating increased sanitary risks and commercial frauds, closely connected with the perishable nature and the economic value of fish and seafood. The most common frauds, carried out in different points of the supply chain, concern the substitution of valuable species with cheaper ones, and the selling of frozen-thawed products as fresh fish (Uddin et al., 2005). A portion of the mislabeling occurs unintentionally, because fish species identities may be easily mistaken or due to different vernacular names used for the same fish species in different regions. However, for certain species and products, fish substitution may be intentional, because of their differing values. Appearance, taste and texture of many fish species are similar, therefore it is frequently difficult to identify a species, especially if prepared in fillet form for consumption (Buck, 2010). From 2010 to 2012, in the USA the analysis of more than 1,200 samples collected from 674 retail outlets in 21 states to determine if they were honestly labeled revealed that one-third of the seafood samples were mislabeled, according to U.S. Food and Drug Administration guidelines (Oceana, 2013). Cawthorn, Steinman and Witthuhn (2012) reported that, on a total of 257 fish samples collected over a two-year period (2008-2010) in four provinces of South Africa, 9% samples from wholesalers and 31% from retailers were identified as different species to the ones indicated at the point of sale.

Freezing is a common practice used to prolong fish storage over long periods. It is effective in protecting fish against microbial deterioration, but physico-chemical and sensory properties of the product are modified. Thus, the consumer perception of thawed fish is inferior to that of the fresh material and this is reflected in the price it realizes. As a consequence, a number of frozen fish are thawed in fish shops, stored on ice, and sold as unfrozen fish without being labeled as such (Uddin et al., 2005).

Several analytical methods can help in the identification of species substitution and frozen products sold as fresh: electrophoretic, antibody, DNA, and enzymatic techniques (Arvanitoyannis, Tsitsika, & Panagiotaki, 2005). However, these techniques are time, cost, and reagent demanding and require highly skilled operators. Therefore, interest in spectroscopic techniques is continuously growing, due to high specificity, convenience, quick response, and being non-destructive, non-invasive, and cost effective. In the seafood sector, spectroscopic techniques have been used to assess composition and quality and they have shown great potential for the detection of pathogens, foreign contamination, protein structure changes, lipid oxidation, and for spoilage monitoring (Cheng et al., 2013). As regards food authenticity, to the best of our knowledge, no papers report the use of infrared (IR) spectroscopy for fresh fish species authentication. Only Dalle Zotte et al. (2013) applied near infrared spectroscopy to the genetic strain authentication of raw and cooked freeze-dried rainbow trout fillets. A preliminary work of O’Brien, Hulse, Pfeifer and Siesler (2013) aiming at distinguishing superior from lower quality fish species by using a microNIR spectrometer has been published as technical note. However, the number of tested samples is really too little (maximum 7 for fish species) to draw reliable conclusions. Also the applicability of IR spectroscopy to the discrimination between fresh and frozen-thawed fish samples is little studied (Ottavian, Fasolato, Facco, & Barolo, 2013; Uddin et al., 2005; Uddin & Okazaki, 2004). For the authentication of other food products, good potential of IR spectroscopy was already demonstrated (Alamprese, Casale, Sinelli, Lanteri, & Casiraghi, 2013; Kurz, Leitenberger, Carle, & Schieber, 2010; Lerma-García, Ramis-Ramos, Herrero-Martínez, & Simó-Alfonso, 2010; Sinelli et al., 2010; Reid, O'Donnell, & Downey, 2006).

Thus, the aim of this work was to investigate the possibility of using infrared (IR) spectroscopy as a rapid and easy tool for the identification of valuable fish species (i.e. red mullet and plaice) substitution with cheaper ones (i.e. Atlantic mullet and flounder). Moreover, the discrimination power of the spectroscopic techniques in fresh and frozen-thawed fillets of Atlantic mullet was studied.

2. Materials and methods

2.1 Materials

Industrially prepared fish fillets analyzed by IR spectroscopy for species authentication and discrimination between fresh and frozen-thawed samples are reported in Table 1. Samples were obtained by different producers. Fresh fillets were stored in ice inside a cold room (4°C) until the analyses, for a maximum of two days. The frozen fillets were stored at -18°C up to two months and before analyses they were thawed at 4°C for 48 hours.

2.2 IR spectroscopy

The near infrared (NIR) spectra were recorded (12 cm^{-1} resolution; 64 scans both for background and samples) on the flesh side of the whole fillet previously conditioned at room temperature, by using a Fourier transform (FT)-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany) fitted both with an integrating sphere (spectral range: 12500-3750 cm^{-1}) and an optical fiber (spectral range: 11000-4400 cm^{-1}).

Before mid infrared (MIR) analysis, two fish fillets at a time were minced without skin, using a heavy duty blender (Waring Laboratory, Torrington, CT) for 20 s at the highest speed. Spectra were then acquired (4000-700 cm^{-1}; 4 cm^{-1} resolution; 16 scans both for background and samples) at room temperature, by means of an FT-IR spectrometer (VERTEX 70, Bruker Optics, Ettlingen, Germany) equipped with an attenuated total reflectance (ATR) cell.

All spectra were collected in duplicate, by the software OPUS v. 6.5 (Bruker Optics, Ettlingen, Germany).

2.3 Data analysis

Replicates of spectral data were averaged, standardized by different pretreatments (MSC, multiplicative scatter correction, or SNV, standard normal variate, alone or coupled with first or second derivatives) (Barnes, Dhanoa, & Lister, 1989; Martens, Jensen, & Geladi, 1983; Savitzky & Golay, 1964), and processed with Principal Component Analysis (PCA; Cowe & McNicol, 1985). FT-NIR spectra acquired by the integrating sphere and the optical fiber were also smoothed (moving average with segment size of fifteen and twenty-one, respectively) before pretreatments. First and second derivatives were calculated by Savitzky-Golay algorithm, with second-order smoothing polynomials through thirty-one points. After selection of thirty features by the algorithm SELECT (Forina, Lanteri, Casale, & Cerrato Oliveros, 2007; Kowalski & Bender, 1976) implemented in the V-Parvus package (Forina et al., 2008), two different classification techniques were applied: Linear Discriminant Analysis (LDA; Massart et al., 1997) and Soft Independent Modeling of Class Analogy (SIMCA; Wold & Sjostrom, 1977). LDA is a probabilistic classification technique which classifies each sample in the category with the highest value of *a-posteriori* probability. The terms in the delimiter equation are the squared Mahalanobis distances from the category centroids. With SIMCA, classification is obtained on the basis of the distance of the object to be classified from the class models: each object is assigned to the class for which the Simca distance was minimum. The mathematical model of the category is based on the principal components of the category. The limit of the class model in the inner space is defined by the number of significant components obtained by double-cross validation.

Classification models were validated using three different external test sets, randomly created, each containing about 30% of the spectra used for the analysis. Objects were divided between training and prediction set, by using a random number generation routine implemented in the V-Parvus package.

Data elaboration was performed by using the software The Unscrambler X (v. 10.2, Camo Software AS, Oslo, Norway) and the V-Parvus package.

3. Results and discussion

3.1 Spectra interpretation

In order to eliminate the noisiest and the least informative regions, spectral ranges were reduced as follows: 10900-3750 cm^{-1} for FT-NIR spectra acquired by means of the integrating sphere; 3700-2640 and 2250-1000 cm^{-1} for FT-IR spectra. No reduction was necessary for NIR data collected by the optical fiber. Some examples of the averaged reduced spectra are shown in Fig. 1. FT-NIR spectra were dominated by the absorption bands of water (5200 cm^{-1}; 6900 cm^{-1}, first overtone of OH; 10200 cm^{-1}, second overtone of OH) and C-H aliphatic group (5560 cm^{-1} and 8300 cm^{-1}, first and second overtone of stretching respectively). In the FT-IR spectra, besides C-H group (absorbing in the regions 1000-1500 cm^{-1} and 2800-3000 cm^{-1}), also amines play an important role (1550 and 1640 cm^{-1}, amine I; 3300 cm^{-1}, amine II) (Workman & Weyer, 2008; Williams & Norris, 2002).

The averaged spectra were smoothed and eventually standardized by different pre-treatments (MSC or SNV alone or coupled with first or second derivative), before the chemometric analyses (see § 2.3). For the sake of brevity, only the best results obtained for each thesis will be shown.

3.2 Species identification

A preliminary FT-NIR and FT-IR data examination was performed by PCA. In the case of mullets, a good sample distinction on the basis of species was observed (Fig. 2) for all spectroscopy techniques used; the best results were obtained with the optical fiber data. The explained variance was 98% considering the first two PCs of FT-NIR data acquired by the sphere (Fig. 2a), 79% in the case of PC 1 and 3 of FT-NIR data obtained by the optical fiber (Fig. 2b), and 70% for the first and the third PC of FT-IR data (Fig. 3c). In the plaice-flounder (PL-FL) comparison, instead, score plots were a bit more confusing. The best separation of the two species was obtained with FT-IR data, pre-treated by MSC, on the plane of first two PCs (Fig. 2f).

The species authentication study was at first dealt with a classification-discriminant approach, applying the LDA to the IR data. This method is able to determine to which pre-defined class a sample belongs. Since for LDA the number of samples must be higher than the number of variables, the analysis was performed using the 30 variables with the largest classification weight, selected by means of the algorithm SELECT (Kowalski & Bender, 1976; Forina, Lanteri, Casale, & Cerreto Oliveros, 2007) implemented in the V-Parvus package. The LDA results were validated using three different external test sets, each composed of 30% of the spectra used for the analysis, randomly selected.

The discrimination between red mullet (RM) and Atlantic mullet (AM) gave a 100% correct classification percentage in prediction, irrespective the spectroscopic technique considered. Optimal results were obtained also for PL and FL, with percentages of correct classification in prediction higher than 92%, 88%, and 100% with integrating sphere, optical fiber and FT-IR data, respectively. Selected features were mainly associated to the O-H bond of water, to the C-H methyl of fatty acid aliphatic chains and to N-H from amines and amides (Workman & Weyer, 2008), thus reflecting the different composition of the two species.

The species authentication problem was then faced by means of a class-modeling strategy. This approach is more appropriate than classification-discriminant techniques in addressing most questions of authenticity (Di Egidio, Oliveri, Woodcock, & Downey, 2011). Spectral data were thus used for SIMCA, a class-modeling method aiming at establishing if a sample X, which claims to belong to a certain species, does actually belong to that species. For comparison's sake, also in this case, only the thirty wavenumbers with the highest classification weights were used. Models were obtained with 7 PCs and they were validated as for the LDA models, using the same external test sets.

As shown in Table 2, the best model for mullet species identification was obtained using the selected features of the smoothed FT-NIR spectra collected with the fiber-optic: in prediction, a sensitivity higher than 70% and a specificity of 100% were calculated ($p<0.05$). Sensitivity refers to the percentage of objects in the external prediction set known to belong to the modeled class which are correctly accepted by the model developed using the objects in the calibration set.

Specificity is the percentage of objects in the external prediction set which do not belong to the modeled class and which are correctly rejected by the model developed using objects in the calibration set (Di Egidio et al., 2011). As shown by the Cooman's plot reported in Fig. 3, the model of the AM class (the vertical rectangle on the left) did not accept any object of the RM class, as well as the model of the RM class (the horizontal rectangle on the bottom) did not accept any sample of the AM class, in accordance with the average calculated 100% specificity value of the two classes. Only few samples (those in the big upper square) were not accepted by any of the two class models, resulting in a satisfactory average sensitivity value.

Less good results were obtained applying SIMCA to plaice and flounder discrimination (Table 3). However, due to the high severity of this class-modeling methods, results are acceptable. FT-IR spectroscopy showed the best discrimination power, with a prediction ability higher than 83% and a specificity of 100%. The low sensitivity calculated for the external test set no. 1 and 3 means that about half of the validation samples of each class (i.e. 8-10 samples) was rejected by the corresponding model. Thus, the model generated some false negatives. In our opinion, this is a less dangerous error in an authentication issue than false positive creation. False negatives could in fact be further analyzed by means of more sensitive techniques, while false positive would be considered as authentic samples, without any other examination.

3.3 Fresh and frozen-thawed fillet discrimination

Fresh and frozen-thawed fillet discrimination was studied considering only Atlantic mullets. As for species authentication, also in this case the data sets were firstly examined by performing PCA (data not shown). Sample distribution on the first two PCs plane appeared quite confused, with no clear separation between the two kinds of samples, notwithstanding an explained variance range of 75-99%. The best separation of fresh fillets from frozen-thawed samples was observed using FT-NIR smoothed data collected by the optical-fiber.

The classification-discriminant approach (LDA) gave optimal results, with a prediction ability of 100% for frozen-thawed (AM-FT) fillets and higher than 97.2% for the fresh ones (AM). In this case, the thirty selected variables mainly referred to the O-H bond of water in FT-NIR data, and also to amines and carboxylic acids in FT-IR data (Workman & Weyer, 2008).

In the class modeling (SIMCA) of fresh and frozen fillets, carried out with the same conditions used for species authentication, the best results were achieved with the selected variables of the MSC pre-treated FT-IR spectra: specificity and sensitivity values in prediction were higher than 95% and 60%, respectively (Table 4). As already observed for PL-FL comparison, also in this case some false negatives were generated. In fact, in the Cooman's plot reported in Fig. 4, referred to the External Test Set 2, some samples (those in the big upper square) were not accepted by any of the models, according to the sensitivity values obtained. As it can be seen, samples are distributed along the axes of the two class models, instead of being localized far from the origin of the axes as happened for red mullet *vs.* Atlantic mullet fillets. A few fresh samples (those in the small square on the bottom) were accepted not only by the model of AM, but also by the model of AM-FT.

Conclusion

The potential for IR spectroscopy to rapidly and easily identify commercial frauds in fish marketing was demonstrated. In particular, the use of suitable chemometric strategies allowed to clearly distinguish Atlantic mullet fillets from those of the more valuable red mullet. Good results were obtained also for plaice and flounder fillets discrimination, as well as for the recognition of Atlantic mullet fresh fillets from the frozen-thawed ones.

FT-IR spectroscopy showed a better classification ability both for species and fresh/thawed fillet identification, but it needs a sample preparation although simple. On the other hand, NIR spectroscopy, implemented in portable instruments, could be a valid pre-screening technique, in order to verify the authenticity of fish fillets.

Consumer protection against adulterations and fraudulent claims would be thus improved by the possibility of examining a high number of samples in a short time. Moreover, commercial customers could use IR instruments in order to test their suppliers. In case of a suspected fraud, more sophisticated analyses could be carried out in order to legally assess the fraudulent claims. The actual models could be improved, considering the different sources of sample variability and the interests of the food chain actors involved in fish authentication.

Acknowledgements

Authors are grateful to Chiara Corbetta for her assistance in the experimental work and to Orobica Pesca S.p.A. (Bergamo, Italy) and Eurofishmarket S.r.l. (Bologna, Italy) for sample supply.

References

Alamprese, C., Casale, M., Sinelli, N., Lanteri, S., & Casiraghi, E. (2013). Detection of minced beef adulteration with turkey meat by UV-VIS, NIR and MIR spectroscopy. *LWT-Food Science & Technology, 53,* 225-232.

Arvanitoyannis, I.S., Tsitsika, E.V., & Panagiotaki, P. (2005). Implementation of quality control methods (physico-chemical, microbiological and sensory) in conjunction with multivariate analysis towards fish authenticity. International *Journal of Food Science and Technology, 40,* 237-263.

Barnes, R.J., Dhanoa, M.S., & Lister, S.J. (1989). Standard normal variate transformation and detrending of near-infrared diffuse reflectance spectra, *Applied Spectroscopy, 43*, 772-777.

Buck, E.H. (2010). Seafood Marketing: Combating Fraud and Deception. *CRS Report for Congress. Congressional Research Service.* http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL34124.pdf

Cawthorn, D.M., Steinman, H.A., & Witthuhn, R.C. (2012). DNA barcoding reveals a high incidence of fish species misrepresentation and substitution on the South African market. *Food Research International, 46,* 30-40.

Cheng, J.H., Dai, Q., Sun, D.W., Zeng, X.A., Liu, D., & Pu, H.B. (2013). Applications of non-destructive spectroscopic techniques for fish quality and safety evaluation and inspection. *Trends in Food Science & Technology, 34,* 18-31.

Cowe, I., & McNicol, J. (1985). The use of principal components in the analysis of near infrared spectra. *Applied Spectroscopy, 39*, 257-266.

Dalle Zotte, A., Ottavian, M., Concollato, A., Serva, L., Martelli, R., & Parisi, G. (2013). Authentication of raw and cooked freeze-dried rainbow trout (*Oncorhynchus mykiss*) by means of near infrared spectroscopy and data fusion. *Food Research International*, in press. http://dx.doi.org/10.1016/j.foodres.2013.10.033.

Di Egidio, V., Oliveri, P., Woodcock, T., & Downey G. (2011). Confirmation of brand identity in foods by near infrared transflectance spectroscopy using classification and class-modelling chemometric techniques – The example of a Belgian beer. *Food Research International, 44,* 544-549.

Forina, M., Lanteri, S., Armanino, C., Casolino, C., Casale, M., & Oliveri, P. (2008). V-PARVUS 2008, University of Genova, http://www.parvus.unige.it

Forina, M., Lanteri, S., Casale, M., & Cerrato Oliveros, M.C. (2007). Stepwise orthogonalization of predictors in classification and regression techniques: an "old" technique revisited. *Chemometrics and Intelligent Laboratory Systems, 87,* 252-261.

Kowalski, B.R., & Bender, C.F. (1976). An orthogonal feature selection method. *Pattern Recognition, 8*, 1-4.

Kurz, C., Leitenberger, M., Carle, R., Schieber, A. (2010). Evaluation of fruit authenticity and determination of the fruit content of fruit products using FT-NIR spectroscopy of cell wall components. *Food Chemistry, 119,* 806-812.

Lerma-García, M.J., Ramis-Ramos, G., Herrero-Martínez, J.M., & Simó-Alfonso, E.F. (2010). Authentication of extra virgin olive oils by Fourier-transform infrared spectroscopy. *Food Chemistry, 118,* 78-83.

Martens, H., Jensen, S.A., & Geladi, P. (1983). Multivariate linearity transformation for near infrared reflectance spectra of meat. *Application Spectroscopy, Proceedings of the Nordic Symposium, Applied Statistics* (pp. 235-267). Stavanger: Stockholm Forlag Publication.

Massart, D.L., Vandeginste, B.G.M., Buydens, L.M.C., De Jong, S., Lewi, P.L., & Smeyers-Verbeke, J. (1997). Supervised pattern recognition. In B.G.M. Vandeginste & S.C. Rutan (Eds.). *Handbook of Chemometrics and Qualimetrics: Part A* (Vol. 20A, pp. 280-282). Amsterdam: Elsevier.

O'Brien, N., Hulse, C.A., Pfeiferb, F., & Siesler, H.W. (2013). Near infrared spectroscopic authentication of seafood. Technical Note. *Journal of Near Infrared Spectroscopy, 21,* 299-305.

Oceana, 2013. Oceana Study Reveals Seafood Fraud Nationwide. http://oceana.org/sites/default/files/National_Seafood_Fraud_Testing_Results_Highlights_FINAL.pdf.

Ottavian, M., Fasolato, L., Facco, P., & Barolo, M. (2013). Foodstuff authentication from spectral data: Toward a species-independent discrimination between fresh and frozen–thawed fish samples. *Journal of Food Engineering, 119*, 765-775.

Reid, L.M., O'Donnell, C.P., & Downey, G. (2006). Recent technological advances for the determination of food authenticity. *Trends in Food Science & Technology, 17*, 344-353.

Savitzky, A., & Golay, M.J.E. (1964). Smoothing and differentiation of data by simplified least-squares procedures. *Analytical Chemistry, 36,* 1627-1639.

Sinelli, N., Casale, M., Di Egidio, V., Oliveri, P., Bassi, D., Tura, D., Casiraghi, E., 2010. Varietal discrimination of extra virgin olive oils by near and mid infrared spectroscopy. *Food Research International*, *43*, 2126-2131.

Uddin, M., & Okazaki, E. (2004). Classification of fresh and frozen-thawed fish by near-infrared spectroscopy. *Journal of Food Science*, *69(8),* C665-C668.

Uddin, M., Okazaki, E., Turza, S., Yumiko, Y., Tanaka, M., & Fukuda, Y. (2005). Nondestructive visible/NIR spectroscopy for differentiation of fresh and frozen-thawed fish. *Journal of Food Science, 70,* 506-510.

Williams, P., & Norris, K. (2002). *Near-Infrared Technology in the Agricultural and Food Industries.* St. Paul: America Association of Cereal Chemist.

Wold, S., & Sjostrom, M. (1977). SIMCA: a method for analyzing chemical data in terms of similarity and analogy. *ACS Symposium Series 52.*

Workman, J.Jr., & Weyer, L. (2008). *Practical Guide to Interpretative Near-Infrared Spectroscopy.* Boca Raton: CRC Press, Taylor & Francis Group.

Figure captions

Fig. 1. Examples of reduced IR spectra obtained from the different fish samples: RM, red mullet; AM, Atlantic mullet; PL, plaice; FL, flounder; AM-FT, frozen-thawed Atlantic mullet. a) FT-NIR integrating sphere; b) FT-NIR optical fiber; c) FT-IR.

Fig. 2. Score plots of IR data. Red mullet (RM) *vs.* Atlantic mullet (AM): a) smoothed FT-NIR data acquired by integrating sphere; b) SNV pre-treated FT-NIR data acquired by optical-fiber; c) FT-IR data, pre-treated by SNV and first derivative. Plaice (PL) *vs.* flounder (FL): d) FT-NIR data acquired by integrating sphere, pre-treated by SNV; e) FT-NIR data acquired by optical-fiber, pre-treated by SNV and first derivative; f) MSC pre-treated FT-IR data.

Fig. 3. Red mullet (RM) *vs.* Atlantic mullet (AM): Cooman's plot obtained from the 30 selected features of the smoothed FT-NIR spectra collected with the fiber-optic (external test set no. 3). ●, RM samples of the calibration set; ○, RM samples of the external prediction set; ▲, AM samples of the calibration set; Δ, AM samples of the external prediction set.

Fig. 4. Atlantic mullet fresh fillets (AM) *vs.* Atlantic mullet frozen-thawed fillets (AM-FT): Cooman's plot obtained from the 30 selected features of the MSC pre-treated FT-IR spectra (external test set no. 2). ■, AM-FT samples of the

ACCEPTED MANUSCRIPT

calibration set; □, AM-FT samples of the external prediction set; ▲, AM samples of the calibration set; Δ, AM samples of the external prediction set.

Table 1.

Samples of fresh and frozen-thawed fillets analyzed by IR spectroscopy.

Code	Species	Trivial name	Status	No. of fillets
RM	*Mullus surmuletus*	Red mullet	Fresh	132
AM	*Pseudupeneus prayensis*	Atlantic mullet	Fresh	165
PL	*Pleuronectes platessa*	Plaice	Fresh	124
FL	*Platichthys flesus flesus*	Flounder	Fresh	134
AM-FT	*Pseudupeneus prayensis*	Atlantic mullet	Frozen-thawed	180

Table 2.

Results in prediction of SIMCA applied to IR spectral data for mullet species identification (red mullet *vs.* Atlantic mullet).

Data	External Test Set	Classification ability (%)	Prediction ability (%)	Sensitivity (%)	Specificity (%)
FT-NIR integrating sphere - smoothed	1	99.50	96.88	70.83	100
	2	99.50	97.87	69.15	100
	3	99.49	97.00	72.00	100
FT-NIR optical fiber - smoothed	1	100	100	70.11	100
	2	100	98.86	72.73	100
	3	100	98.84	80.23	100
FT-IR – SNV+d1	1	100	91.67	56.25	100
	2	100	91.58	51.58	100
	3	100	94.95	55.56	100

SNV, standard normal variate; d1, first derivative.

Table 3.

Results in prediction of SIMCA applied to IR spectral data for plaice and flounder discrimination.

Data	External Test Set	Classification ability (%)	Prediction ability (%)	Sensitivity (%)	Specificity (%)
FT-NIR integrating sphere - SNV	1	77.97	73.08	70.51	73.08
	2	84.95	81.16	66.67	84.05
	3	80.81	78.31	84.34	73.49
FT-NIR optical fiber – SNV+d1	1	79.46	78.07	75.34	75.34
	2	84.97	75.29	72.94	64.71
	3	79.33	67.09	74.68	68.35
FT-IR – MSC	1	98.96	93.94	51.52	100
	2	100	89.47	73.68	100
	3	98.91	83.78	45.95	100

SNV, standard normal variate; d1, first derivative; MSC, multiplicative scatter correction

Table 4.

Results in prediction of SIMCA applied to IR spectral data for fresh and frozen-thawed Atlantic mullet fillet discrimination.

Data	External Test Set	Classification ability (%)	Prediction ability (%)	Sensitivity (%)	Specificity (%)
FT-NIR integrating sphere - MSC	1	93.33	88.12	73.27	77.23
	2	93.88	89.58	70.83	75.00
	3	94.96	88.35	68.93	77.67
FT-NIR optical fiber – smoothed	1	88.19	86.11	85.19	57.41
	2	92.59	82.35	88.24	60.78
	3	87.34	87.04	88.89	60.19
FT-IR – MSC	1	98.28	97.78	64.44	95.56
	2	98.20	98.04	70.59	100
	3	98.20	88.24	60.78	100

MSC, multiplicative scatter correction

Figure 1

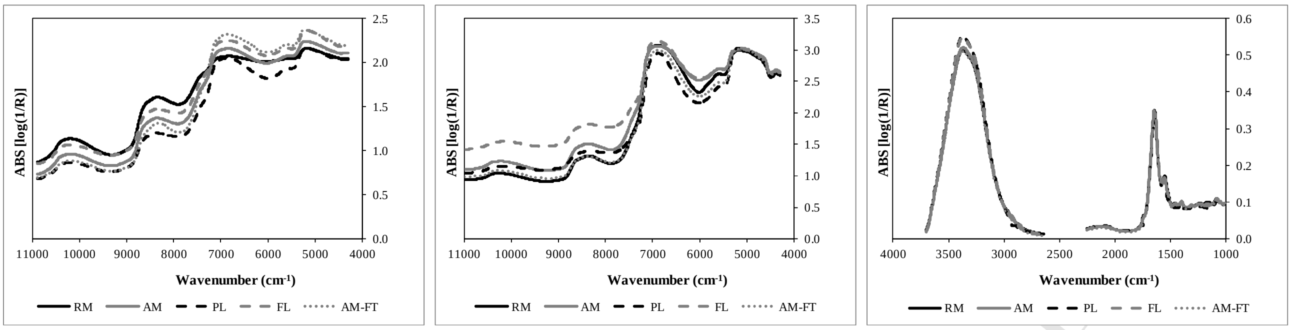


Figure 2.

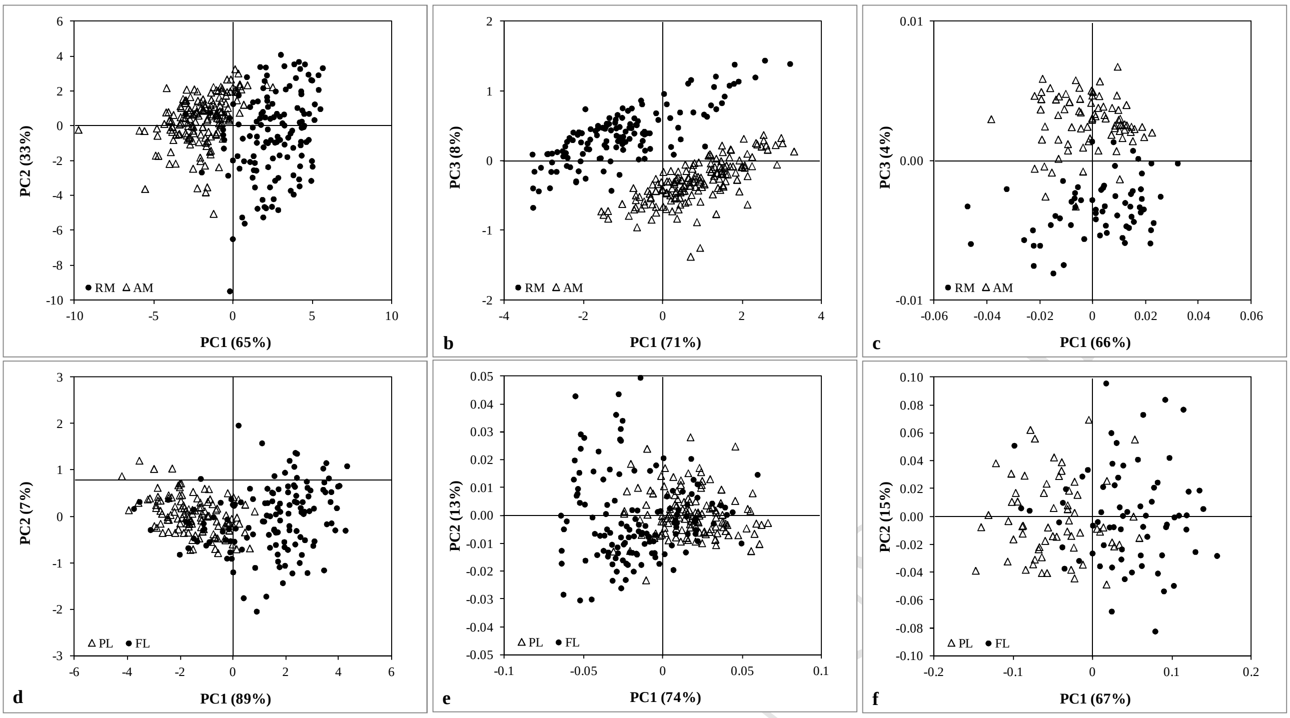

PC2 (33%)
PC1 (65%)
● RM △ AM
PC3 (8%)
PC1 (71%)
b
PC3 (4%)
PC1 (66%)
c
PC2 (7%)
PC1 (89%)
△ PL ● FL
d
PC2 (13%)
PC1 (74%)
e
PC2 (15%)
PC1 (67%)
f

Figure 3.

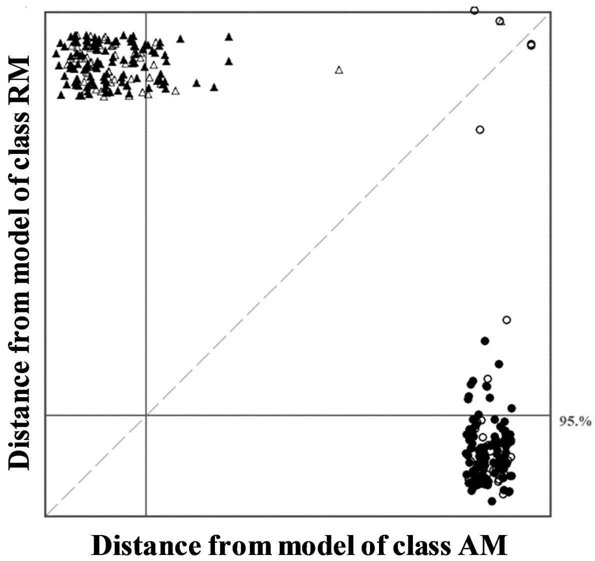
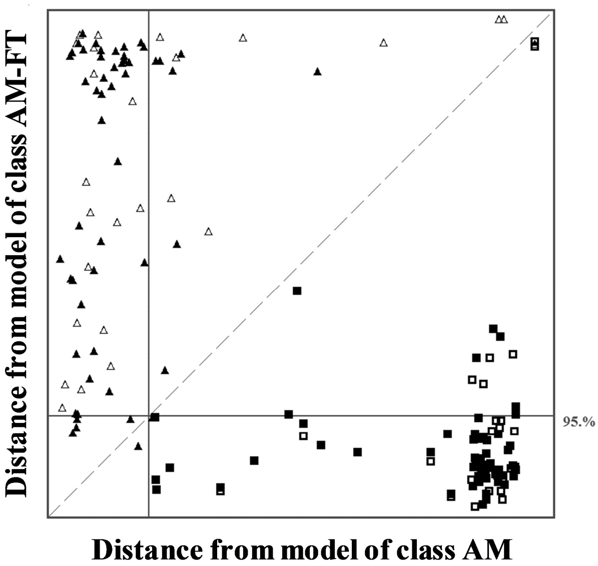

Distance from model of class RM
95.%
Distance from model of class AM

Figure 4.


Distance from model of class AM-FT
95.%
Distance from model of class AM

HIGHLIGHTS

- Application of NIR and MIR spectroscopy to fish fillet authentication
- Use of two different classification approaches: LDA and SIMCA
- Discrimination of valuable fish species from the cheaper ones gave good results
- Recognition of fresh fillets from the frozen-thawed ones was possible
- NIR and MIR spectroscopy is a valid pre-screening tool in fish authentication