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Handheld NIR device: A non-targeted approach to assess authenticity of fish fillets and patties

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ABSTRACT

This study evaluates the reliability of a handheld NIR device in distinguishing fillets and patties of Atlantic cod (n = 80) from those of haddock (n = 90), in comparison with a FT-NIR benchtop spectrometer. The authentication issue was faced by Linear Discriminant Analysis (LDA) and Soft Independent Modelling of Class Analogy (SIMCA), pre-treating spectral data with different algorithms, and validating models both internally and externally. The best LDA models gave 100% correct classification in prediction. Sensitivity >65% and specificity >74% in prediction were calculated for the best SIMCA models. No significant differences ($P > .05$) were found between the two instruments by McNemar test. Thus, the work demonstrated that a handheld NIR device can be a simple, cost-effective, and reliable alternative to benchtop spectrometers in fish fillet and patty authentication. These important findings can help in improving commercial fraud fight, extending the possibility to authenticate fish species also in processed products.

1. Introduction

Demand for proper analytical methods for authenticity assessment of food products is currently increasing dramatically, representing one of the major concerns for different stakeholders. Food industries and consumers demand to control bodies a careful monitoring of counterfeited products together with the enforcement of the existing regulations. However, it is clear that the well-established and accepted methods for food authentication (e.g. species-specific DNA-based tags) are time and money consuming (Huck, Pezzei, & Huck-Pezzei, 2016), causing their implementation as routine monitoring systems to be fruitless.

The most known and documented cases of food frauds involve fish and seafood products (Jacquet & Pauly, 2008). In particular, economic frauds along the whole fish supply chain (e.g. the substitution of valuable species with cheaper ones) are the most frequent. The mentioned frauds particularly impair fish fillets and ready-to-eat products, such as fish patties, which cannot be recognised through the traditional morphological analysis.

In this context, Stadler, Tran, Cavin, Zbinden, and Konings (2016) published a brief overview of the main analytical approaches and practices to determine food authenticity, stressing the need of portable technologies for rapid and non-destructive food testing not only at the agricultural or livestock level, but also for their implementation from the factory gate, where the material is delivered, up to the retail channels, where consumers possibly will have the ability to scan products directly in stores before purchasing. Near infrared (NIR) handheld devices can respond to this need of fast, reliable, non-destructive, and *in situ* analyses for food authentication. Indeed, even though commercial portable instruments have reduced accuracy in measurement and performance reliability if compared with large, stationary benchtop instruments, the loss of information is compensated for time and cost reduction, low power consumption, user-friendliness and tailored design. Moreover, the slightly reduced resolution in chemical information could influence regression models in which the intent is to predict a specific compound, while it should not affect the results when handheld devices are implemented as non-targeted tools for food authentication (Pustjens, Weesepoel, & van Ruth, 2015).

In recent years, the interest in NIR spectroscopy applied to food frauds has gained importance; the number of publications about vibrational spectroscopy for authentication purposes is permanently increasing and a review on food frauds (Huck et al., 2016) reports a total of 17082 papers published between 2010 and 2015. Despite this literature growth, just few authors investigated fish authentication by NIR

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spectroscopy as detailed in some recent reviews (Qu et al., 2015; Liu, Zeng, & Sun, 2013) and most of them focused on the discrimination between fresh and frozen-thawed fish. To the best of our knowledge, only one technical note deals with handheld instrumentation applied to fish species authentication (O'Brien, Hulse, Pfeifer, & Siesler, 2013). In a previous work (Alamprese & Casiraghi, 2015), the possibility of using Fourier-transform (FT)-NIR 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) was demonstrated. Here, the aim of the work was to test the capability of a handheld NIR device in distinguishing fillets and patties of Atlantic cod (*Gadus morhua*) from those of haddock (*Melanogrammus aeglefinus*) in comparison with a FT-NIR benchtop spectrometer.

2. Materials and methods

2.1. Samples

Fresh fillets of *Gadus morhua* (Gm, n = 80) and *Melanogrammus aeglefinus* (Ma, n = 90) were provided by a trusted supplier (Copromar S.r.l., Milan, Italy) in thirteen different batches from March to June 2016. Qualified personnel extracted the left fillets from the whole fishes. After spectroscopic analyses (see § 2.2.), each fillet was minced by means of a grinder (Braun-AG 4261, Frankfurt, Germany) for 30 s at the highest speed. The minced fish was then shaped into two patties of 150 g each (10 cm diameter, 1.5 cm thickness) using a manual burger press.

2.2. Spectra acquisition

A MicroNIR OnSite (VIAVI, Santa Rosa, CA) and a benchtop FT-NIR (MPA, Bruker Optics, Ettlingen, Germany) spectrometer equipped with an optical fibre were used to analyse each fillet on the flesh side in six different points equally spaced-out: 3 on the right and 3 on the left of the lateral line. Similarly, the two patties obtained from each fillet were analysed with both the instruments in three equally spaced-out points each, for a total of six acquisition points.

All spectra were acquired in diffuse reflectance. When using MicroNIR, a spectral range of 950–1650 nm, a 12.5 μs integration time, and 200 scans at 80 Hz were applied. For the benchtop instrument, the analytical conditions were as follows: diffuse reflectance solid probe IN 263 (1.5 m, 100/100 × 0.1 mm, Bruker Optics, Ettlingen, Germany), 12500–4500 cm^{-1} spectral range, 12 cm^{-1} resolution, and 64 scans both for sample and background. As the two instruments (FT-NIR and MicroNIR) have different spectral range and resolution, a resampling strategy, through combination of adjacent variables, was applied in order to be able to compare the information, overcoming the different linearity of the spectra by expressing all data as a function of wavelengths.

2.3. Data analysis

The six spectra collected from each sample were averaged before data elaboration, with the exception of FT-NIR spectra recorded on the back end of the fish fillets, which were discarded due to a different absorption behaviour linked to the fillet thinness in the terminal area. Each dataset, after outlier removal (5 Gm samples), was divided in a calibration set comprised of about 70% of the whole collected spectra (i.e. 54 Gm samples and 64 Ma samples) and an external test set represented by 4 out of the 13 sampling batches (30% of the collected spectra; i.e. 21 Gm samples and 26 Ma samples). Each set was independently pre-treated with standard normal variate (SNV), or multiplicative scatter correction (MSC), or smoothing (Savitzky-Golay, 5 wavelengths gap size and 2nd order polynomial) coupled with first or second derivative (Savitzky-Golay, 5 wavelengths gap size and 2nd order polynomial).

Linear Discriminant Analysis (LDA) and Soft Independent Modelling of Class Analogy (SIMCA) were applied for the calculation of classification models (V-Parvus package; Forina et al., 2008), which were validated both in cross validation (5CV) and in prediction using the created batch-wise external test set.

LDA is a supervised pattern recognition technique based on discriminant canonicals on which the matrix covariance centre is calculated and the covariance of each class included, enabling variance maximisation in-between- and lessen within- classes (Forina, Lanteri, Casale, & Cerrato Oliveros, 2007). Referring to a class *a priori* assigned to each sample of fish fillets or patties, LDA permits the construction of the optimal *a posteriori* classification rule. The model obtained searched for directions (canonical variables) with the maximum separation among categories, improving the class separability.

SIMCA is a supervised classification method (Wold & Sjostrom, 1977) that consists of a first step of independent principal component analysis (PCA) applied to the spectral variables of the calibration sets for the two considered classes (i.e. Gm and Ma). Then, for each class the number of principal components needed in order to reach a minimum threshold of 90% explained variance is chosen and the standard deviation of residuals is calculated. Thereafter, in the PCA-reduced space, SIMCA builds a multidimensional space where the classification of the external test set samples is performed based on the distance between each sample and the models. Since SIMCA constructs as many models as the number of classes, the assigned membership of a sample could be: (1) exclusively assigned to one class; (2) it does not belong to any class; (3) it fits two or more classes. When all samples fall in the first case, the highest sensitivity (100%) of the model is reached as, by definition, sensitivity defines the percentage of samples (objects) in the external test set belonging to the modelled class that are correctly accepted by the SIMCA rule developed with the calibration set, i.e. true positives (TP). In case samples are rejected from a class which they actually belong, type II errors (false negatives, FN) are present. Whereas, the third case represents a lack in specificity, because some objects in the external test set that do not belong to the modelled class are not rejected from the model constructed with the calibration set (false positives, FP). All these errors should be taken into account while evaluating a SIMCA model, through the calculation of the following figures of merit: sensitivity = TP/(TP + FN); specificity = TN/(TN + FP).

Since for LDA the number of samples must be higher than the number of variables, classification models were calculated using only the 15 most discriminative variables of each dataset. For the sake of comparison, the same 15 variables were used also for SIMCA models. Feature selection was performed applying the algorithm SELECT (Forina et al., 2007; Kowalski & Bender, 1976) implemented in the V-Parvus package (Forina et al., 2008). SELECT searches for the variable with the largest Fisher weight, selects it decorrelating then the other predictors, and iterates until a fixed number of variables is chosen (15 in this study).

Instrument performances were compared in terms of model predictive abilities by applying the 'testcholdout' function implemented in Matlab (v. 2016a, Mathworks, Inc., Natick, MA), which performs one-tailed, mid *P*-value McNemar test (Fagerlan, Lydersen, & Laake, 2013), a particular case of Fisher's sign test that verifies if two models A and B have the same error rate (null hypothesis) (Roggo, Duponchel, & Huvenne, 2003). It permitted to assess whether the accuracies of the classification models obtained by using the spectra collected with the two different instruments were different, thus revealing if one instrument performed better than the other. In particular, LDA models were compared on the basis of *P*-value: *P*-values higher than 0.05 confirm the acceptance of the null hypothesis. The performances of SIMCA

models were compared by the calculation of McNemar's value (χ^2) on the prediction results, according to Eq. (1).

$$\chi^2 = \frac{(n_{12} - n_{21} - 1)^2}{n_{12} + n_{21}} \quad (1)$$

where n_{12} represents the number of samples misclassified (both false positives and false negatives) by the model of the class 1 (Gm) and n_{21} stands for the number of samples misclassified (both false positives and false negatives) by the class 2 (Ma) model. The coefficient -1 imposes a continuity correction. Being 3.84 the χ^2 critical value when considering $P < .05$ and type I error, lower McNemar's values indicate that the null hypothesis is accepted and the two models are significantly comparable. The calculation of χ^2 was necessary to evaluate SIMCA results as the approach considers each class separately and it calculates one model for each defined class, thus not allowing a direct *P*-values comparison as it was performed for LDA models.

3. Results and discussion

3.1. Fish fillet and patty spectra

The averaged spectra of the fresh fish fillets of *G. morhua* (Gm) and *M. aeglefinus* (Ma) collected with both MicroNIR and FT-NIR are shown in Fig. 1. Generally, MicroNIR spectra showed slightly reduced resolution in chemical information from 1400 nm to higher energy region, however this seems not to represent a stumbling block when infrared spectroscopy is applied for authentication purpose. Spectra collected with both instruments and pre-treated by SNV or MSC (Fig. 1a and b) were characterized by absorption bands at 970 nm and 1450 nm ascribable to the first and second overtone of OH, respectively; it is also present an absorbtion band at 1200 nm linked to the second overtone of C—H aliphatic group stretching. Spectra collected by FT-NIR presented absorptions at 1800 nm linked to the first overtone of C—H aliphatic group stretching (Williams & Norris, 2002; Workman & Weyer, 2008). Derivative transformations of the spectra (Fig. 1c and d) highlighted further peaks at 1035 nm, 1600 nm, and 2220 nm originated from proteic fraction absorption, i.e. N—H first and second overtone and the combination of N—H and C═O signal (Workman & Weyer, 2008).

Spectra collected from patties obtained from the same fish fillet sample were averaged and pre-treated according to the same approach adopted for the fillet analysis. Patterns very similar to those of spectra collected from whole fish fillets were observed (data not shown). Indeed, absorption peaks ascribable to water and protein were remarked, together with a less sensitive absorption of spectra collected through the handheld device.

In all cases, negligible differences could be observed between average spectra of the two considered fish species, calling for a chemometric approach intended for authentication purpose.

3.2. Fish authentication by Linear Discriminant Analysis

A classification-discriminant approach by LDA was first applied to the MicroNIR spectral data. A 100% correct classification rate in calibration was obtained with MSC spectra for both fillets and patties (Table 1). The further validation step performed by both internal cross-validation and prediction (external test set) confirmed the goodness of the constructed classification rules, reaching correct classi-

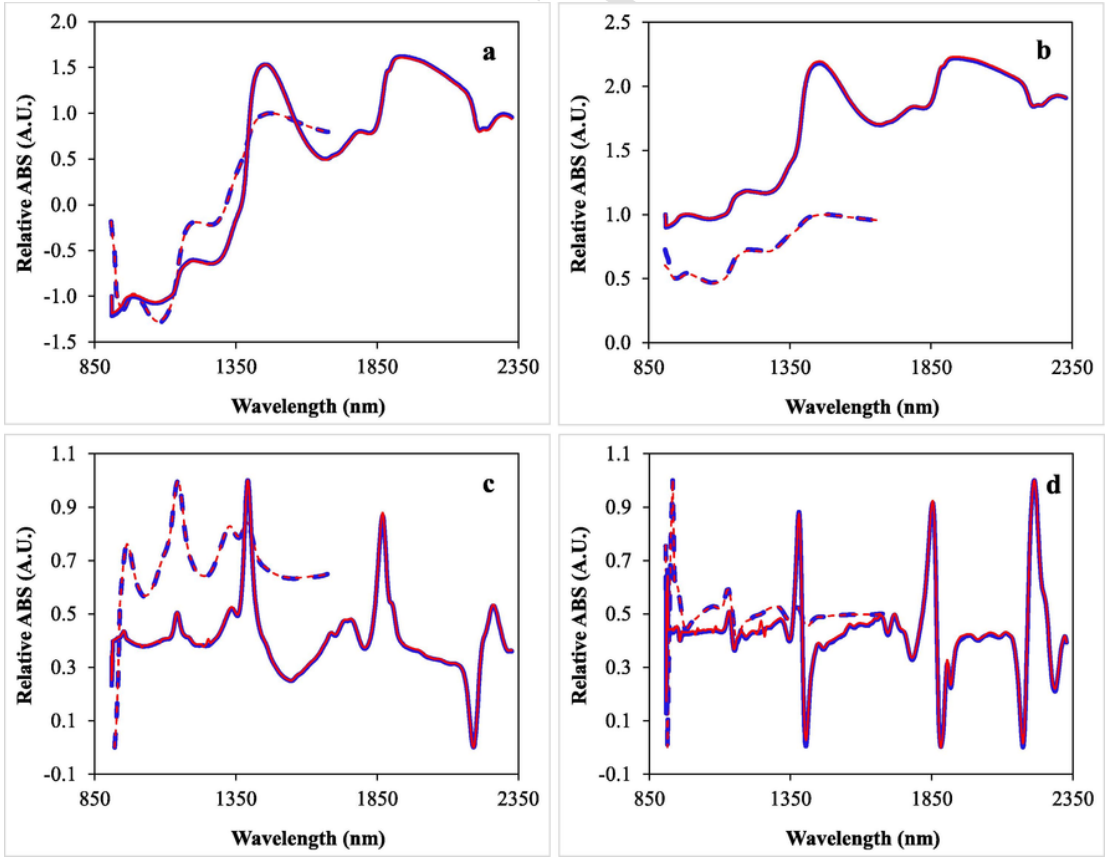

Fig. 1. Averaged spectra (relative absorbance) of fillets of *Gadus morhua* (black) and *Melanogrammus aeglefinus* (gray) collected with FT-NIR (straight line) and MicroNIR (dashed line) spectrometers, transformed with different pre-treatments: a) SNV, b) MSC, c) smoothing + first derivative, d) smoothing + second derivative.

Table 1
Results of Linear Discriminant Analysis for authentication of fillets and patties of *Gadus morhua* and *Melanogrammus aeglefinus*: average correct classification percentages of models based on MicroNIR and FT-NIR data after different mathematical pre-treatments.

		MicroNIR		FT-NIR	
		Fillets	Patties	Fillets	Patties
SNV	Calibration	99.16	100.00	100.00	100.00
	CV	99.16	100.00	100.00	100.00
	Prediction	76.60	100.00	100.00	100.00
MSC	Calibration	100.00	100.00	100.00	100.00
	CV	99.16	100.00	100.00	100.00
	Prediction	100.00	100.00	100.00	100.00
Smooth + d1	Calibration	96.13	96.13	100.00	100.00
	CV	92.44	89.92	100.00	100.00
	Prediction	89.36	91.49	100.00	100.00
Smooth + d2	Calibration	97.31	94.62	100.00	100.00
	CV	94.96	89.92	100.00	100.00
	Prediction	82.98	76.69	100.00	100.00

SNV, standard normal variate; MSC, multiplicative scatter correction; d1, smoothing + first derivative; d2, smoothing + second derivative; CV, cross-validation.

fication rates up to 100%, irrespective of the considered kind of sample (Table 1).

Subjecting FT-NIR spectra to LDA, reliable models were obtained with all mathematical pre-treatments and for both fish fillets and patties, being correct classification percentages always equal to 100% in calibration, cross-validation, and prediction (Table 1). In a previous work, Alamprese and Casiraghi (2015) demonstrated the same reliability of the FT-NIR benchtop spectrometer in discriminating Atlantic mullet fillets from those of the more valuable red mullet (100% of correct classification percentage).

The McNemar test confirmed that most of the LDA models obtained by data collected with the two instruments after different pre-treatments were comparable in terms of classification error rate in prediction ($P > .05$ in Table 2). In particular, classification models calculated with SNV and MSC pre-treated data were not significantly ($P > .05$) different based on the instrument used. On the contrary, after spectral derivative transformations, models obtained by the two instruments for both fillets and patties proved to be significantly different ($P < .05$) in terms of error rate, being the FT-NIR models more accurate. These results are in agreement with the higher resolution of the FT-NIR spectra with respect to the MicroNIR ones.

The models based on MSC or SNV pre-treated spectra collected by MicroNIR both on fillets and patties can be considered as the best LDA classification results since they combine a soft mathematical pre-treatment with the advantages of a handheld device, giving results analogous to those calculated with any pre-treated data of a benchtop FT-NIR spectrometer.

The use of a classification-discriminant approach such as LDA can be really useful in the detection of fish frauds in which a valuable species is substituted with one or more specific cheaper species. Indeed, the chemometric approach forces one sample to belong to one of the known species class. This could lead to misleading results when one or more of the species used as substitute have not been considered in the classification model construction. A similar problem can arise also when a regression approach is used instead of classification. For instance, Gayo and Hale (2007) demonstrated that with Vis-NIR spectroscopy coupled with Partial Least-Squares regression it is possible to detect Atlantic blue crabmeat adulterated with different percentages of blue swimmer crabmeat with less than ±6% error. However, the developed regression models are of course valid only for these two crabmeat species.

3.3. Fish authentication by Soft Independent Modeling of Class Analogy

SIMCA class-modeling strategy was applied to overcome possible misleading results obtained by LDA. Indeed, SIMCA is a supervised classification method of great interest in facing food authenticity issues, because it aims at establishing if a sample actually belong to the claimed species, without forcing the belonging to a specific class (Alamprese & Casiraghi, 2015).

Reliable SIMCA models for species identification in fillets or patties were obtained using both MicroNIR and FT-NIR spectra (Table 3). In particular, the SNV transformation gave optimal models for the discrimination of fish fillets, with correct classification rates in prediction

Table 2
Results of McNemar test: *P*-value resulting from pair comparison of the LDA models calculated with MicroNIR and FT-NIR spectra for fillet and patty authentication of *Gadus morhua* and *Melanogrammus aeglefinus*.

			Micro NIR							
			Fillets				Patties			
			SNV	MSC	d1	d2	SNV	MSC	d1	d2
FT-NIR	Fillets	SNV	0.070	–	–	–	0.549	–	–	–
		MSC	–	0.344	–	–	–	0.018	–	—
		d1	–	–	0.002	–	–	–	0.000	–
		d2	–	–	–	0.001	–	–	–	0.000
	Patties	SNV	0.625	–	–	–	0.453	–	–	–
		MSC	–	1.000	–	–	–	0.688	–	–
		d1	–	–	0.019	–	–	–	0.003	–
		d2	–	–	–	0.064	–	–	–	0.001

SNV, standard normal variate; MSC, multiplicative scatter correction; d1, smoothing + first derivative; d2, smoothing + second derivative.

Table 3
Results of Soft Independent Modeling of Class Analogy for authentication of fillets and patties of *Gadus morhua* and *Melanogrammus aeglefinus*: correct classification percentage, sensitivity and specificity values of models based on MicroNIR and FT-NIR data after different mathematical pre-treatments.

		MicroNIR						FT-NIR					
		Fillets			Patties			Fillets			Patties		
		C.A.	Sens	Spec	C.A.	Sens	Spec	C.A.	Sens	Spec	C.A.	Sens	Spec
SNV	Calibration	99.16	94.95	99.06	100.00	92.44	100.00	100.00	84.03	100.00	100.00	83.19	100.00
	CV	99.16	59.66	99.27	99.16	76.47	100.00	99.16	78.15	100.00	100.00	73.11	100.00
	Prediction	76.60	59.57	82.98	100.00	55.32	100.00	100.00	76.59	100.00	91.49	72.10	100.00
MSC	Calibration	100.00	94.12	100.00	100.00	90.76	100.00	100.00	85.59	100.00	100.00	83.19	100.00
	CV	100.00	60.50	100.00	100.00	57.14	100.00	98.30	77.12	100.00	100.00	73.11	100.00
	Prediction	100.00	0.00	100.00	100.00	0.00	100.00	97.92	41.67	100.00	91.49	72.10	100.00
Smooth + d1	Calibration	96.64	68.32	77.64	95.80	96.63	75.95	100.00	92.44	100.00	100.00	89.07	100.00
	CV	86.56	47.90	94.49	86.56	62.19	90.35	100.00	67.23	100.00	100.00	72.27	100.00
	Prediction	70.21	65.96	76.60	87.23	65.95	74.47	100.00	68.08	100.00	100.00	72.34	100.00
Smooth + d2	Calibration	94.12	97.48	84.87	94.96	96.64	75.82	100.00	87.39	100.00	100.00	94.12	100.00
	CV	89.08	55.46	94.29	81.38	54.62	91.32	100.00	61.34	100.00	100.00	55.46	100.00
	Prediction	59.57	61.70	57.45	70.21	57.45	65.96	100.00	82.98	100.00	100.00	78.72	100.00

SNV, standard normal variate; MSC, multiplicative scatter correction; d1, smoothing + first derivative; d2, smoothing + second derivative; CV, cross-validation; C.A., classification ability; Sens, sensitivity, Spec, specificity.

of 77% and 100% for MicroNIR and FT-NIR spectrometer, respectively. Sensitivity values in prediction were 60% and 76% for MicroNIR and FT-NIR, respectively, while specificity values in prediction were higher than 83%. Concerning species authentication in patties, good results were also obtained with SNV pre-treatment, but better models were calculated with first derivative data: in prediction, 87% classification

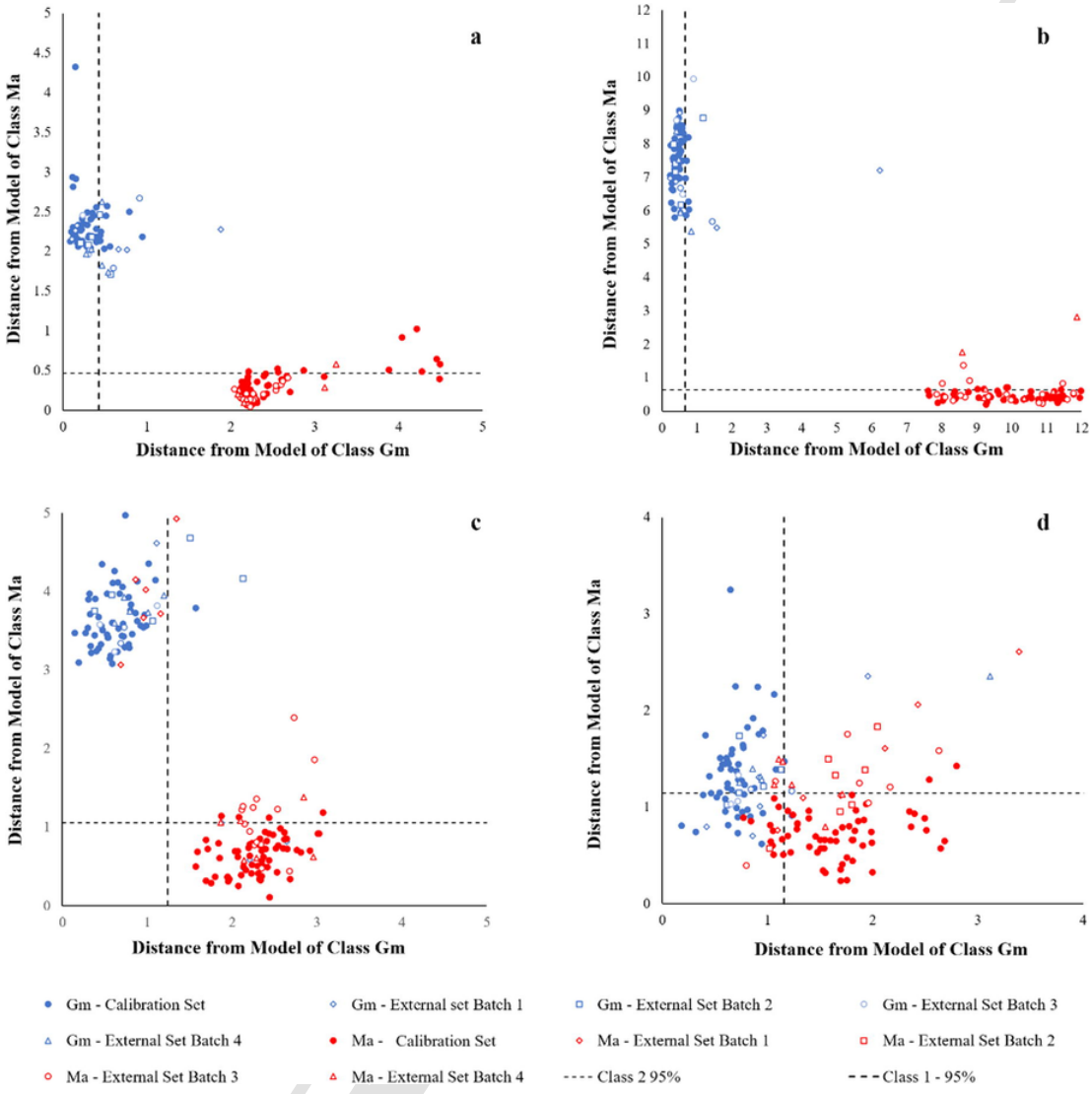

Fig. 2. Cooman's plot obtained for the best SIMCA models: a) Fish fillets SNV-spectra collected with the FT-NIR optic probe, b) Patties smoothed and first derivative pre-treated data collected with the FT-NIR optic probe, c) Fish fillets SNV-spectra collected with the MicroNIR, d) Patties smoothed and first derivative pre-treated data collected with the MicroNIR. Gm, *Gadus morhua*; Ma, *Melanogrammus aeglefinus*.

Table 4
McNemar's values (χ^2) resulting from pair comparison of the SIMCA prediction results for the authentication of fillets and patties of *Gadus morhua* and *Melanogrammus aeglefinus* by using a handheld MicroNIR device or a benchtop FT-NIR spectrometer.

			Micro NIR							
			Fillets				Patties			
			SNV	MSC	d1	d2	SNV	MSC	d1	d2
FT-NIR	Fillets	SNV	1.633	–	–	–	2.531	–	–	–
		MSC	–	0.000	–	–	–	0.000	–	–
		d1	–	–	0.138	–	–	–	0.138	–
		d2	–	–	–	3.115	–	–	–	4.321
	Patties	SNV	3.704	–	–	–	1.441	–	–	–
		MSC	–	18.150	–	–	–	18.150	–	–
		d1	–	–	–0.138	–	–	–	0.138	–
		d2	–	–	–	1.750	–	–	–	2.700

SNV, standard normal variate; MSC, multiplicative scatter correction; d1, smoothing + first derivative; d2, smoothing + second derivative.

ability, 66% sensitivity and 74% specificity were obtained with MicroNIR, whereas FT-NIR gave 100%, 72% and 100% for classification ability, sensitivity and specificity, respectively. MSC models showed low sensitivity values in prediction, in particular for MicroNIR data; these results could be ascribable to the column-wise MSC pre-treatment, that can considerably affect results obtained when two different sets of spectral data are separately pre-treated (calibration and test sets).

Comparable results in prediction were found by Alamprese and Casiraghi (2015), who discriminated red mullet fillets from those of Atlantic mullet as well as plaice from flounder fillets using FT-NIR spectra coupled with SIMCA. The best results were obtained for mullets, with 100% classification ability and specificity, and 74% sensitivity. In any case, also results about plaice and flounder were quite good, being the average figures of merit 81%, 74% and 70% for classification ability, sensitivity and specificity, respectively.

For a better discussion of SIMCA results, the Cooman's plots of the best models are presented in Fig. 2. The plots related to FT-NIR data for fish fillets (Fig. 2a) and patties (Fig. 2b) show that the model of the Gm class did not accept any object of the Ma class, reflecting the 100% specificity value reported in Table 3. Only few samples of fish fillets of the calibration (9 Gm and 10 Ma) and test sets (10 Gm and 1 Ma) were not accepted by any of the two class models, laying in the big upper square, i.e. out of the 95% acceptability boundaries (Fig. 2a). Concerning fish patties, 48 out of 55 samples of Gm class were accepted by the Gm model and 58 out of 64 samples of Ma class were correctly classified by Ma model in calibration (Fig. 2b). The mean sensitivity in prediction (72.34%) results from 71.43% (15 out of 21 samples) of specificity for Gm class and 73.08% (19 out of 26) for Ma class. This means that in prediction there were only 11 and 13 false negatives for fillets and patties, respectively, accounting for the high sensitivity values calculated (Table 3).

The Cooman's plots obtained for fish fillets considering MicroNIR data after SNV transformation (Fig. 2c) showed that 5 Ma samples of the external test set (belonging to batch 1) were erroneously classified as Gm, as well as 5 Gm samples of the external test set (belonging to batch 1) were misclassified; this is why a sensitivity value of 59.6% was calculated (Table 3). However, the model demonstrates high specificity, since only 8 out of 47 samples considered in the external set were not accepted by any of the two class model. Due to the high severity of this class-modeling method, results are considered acceptable and relevant for screening purposes.

The best model obtained with MicroNIR data collected for patties was the one calculated after smoothing and transformation with first derivative (Table 3). However, from the respective Cooman's plot (Fig. 2d) it is possible to observe that a portion of samples (15 Gm and 16 Ma, considering both calibration and test sets) lay in the small bottom rectangle designed by the 95% confidence lines. These samples are confused, i.e. they are classified as belonging to both considered classes, decreasing the classification ability of the model. In addition, 16 external test set samples were rejected from the class which they actually belong, leading to false negatives or type II error. In agreement with Alamprese and Casiraghi (2015), the presence of false negatives is not so dangerous in authentication screening, because they can be subjected to more sensitive techniques (e.g. DNA analysis) to verify the species. In any case, the number of suspicious samples to be re-analysed is very small compared to the total number of correctly classified samples, thus the presented results, even if they need further improvements, can be considered of remarkable relevance for fish authentication along the whole supply chain.

The suitability of MicroNIR data elaboration with SIMCA for fish species authentication has been indicated also in the technical note by O'Brien et al. (2013). However, due to the very limited number of samples used in that research, it is not possible to make any result comparison.

To compare the performances of the SIMCA models obtained with MicroNIR and FT-NIR data, the McNemar's values (χ^2) were calculated on the prediction results (Table 4). As reported in § 2.3, models were considered significantly comparable ($P > .05$) for values lower than 3.84. All the models obtained with MicroNIR data gave as accurate results in prediction as the FT-NIR corresponding models. The only exception is represented by the model calculated with MSC pre-treated spectra of patties, which resulted significantly different ($P < .05$) for the two instruments, due to the failure of the prediction models developed with MicroNIR data, already commented. Thus, the McNemar's results demonstrated that even if SIMCA models calculated with MicroNIR data seemed less reliable, they can be considered as good as the FT-NIR models for prediction purposes.

The SIMCA class-modeling method is in general more appropriate than classification-discriminant techniques, such as LDA, in addressing questions of authenticity when the most valuable species could be substituted with more than one different species. Indeed, it permits to identify if a new analysed sample belongs to the claimed species or if it should be considered as a fraudulent substitution.

4. Conclusions

The work demonstrated the good performances of a handheld NIR device in the authentication of fillets and patties of *Gadus morhua* and *Melanogrammus aeglefinus*. In particular, LDA and SIMCA models developed with MicroNIR spectra proved to be as reliable as those calculated using spectra acquired by a benchtop FT-NIR spectrometer. This is an important finding, because the use of a simple, portable and cost-effective tool such as the handheld NIR device can help in fighting commercial frauds in fish market, increasing the number of controlled samples and giving the possibility to make control throughout the entire commercial chain, directly *in-situ*.

The results of this study are of outmost importance because they were obtained both on fish fillets and patties, meaning that there is the possibility to identify fish species even when the morphology is no more evident, thus allowing fish authentication also in processed products.

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Conflict of interest

The Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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