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Technical Note

Near infrared spectroscopic authentication of seafood

Nada O'Brien,[a] Charles A. Hulse,[a] Frank Pfeifer[b] and Heinz W. Siesler[b]

[a]JDSU, Optical Security and Performance Products, Santa Rosa, CA 95407, USA

[b]Department of Physical Chemistry, University of Duisburg-Essen, D 45117 Essen, Germany. E-mail: hw.siesler@uni-due.de

Near infrared (NIR) spectroscopic investigations of whole fish and fish fillets with a miniaturised, hand-held instrument were performed to demonstrate the feasibility of discriminating high-quality, expensive from lower-quality, less expensive, substitutes and responding to the increasing concerns regarding fraud and deception in seafood marketing. Generally, such problems can occur due to the mislabelling of products in the harvesting and processing system or species substitution at the restaurant level. To test the possibility of distinguishing superior from lower quality fish species, NIR spectra were measured in diffuse reflection from the skin and meat of the investigated fish. Subsequently, the spectra were evaluated by principal component analysis and further classified by soft independent modelling of class analogies. In the present communication, the results obtained with respect to the authentication of two different species of mullet, cod and trout, respectively, will be discussed in some detail.

Keywords: seafood authentication, mullet, cod, trout, hand-held NIR spectrometer, PCA, SIMCA

Introduction

A recently published report on one of the largest surveys conducted to date about seafood fraud revealed that one-third of seafood species purchased at restaurants and grocery stores in cities across the Unites States were mislabelled.[1] The study was conducted by Oceana, a non-profit international advocacy group, over a period of two years from 2010 to 2012, when over 1200 samples were collected from 674 retail outlets in 21 US states. DNA testing was performed on all the fish samples to correctly identify the fish species and uncover mislabelling. Similar conclusions could be drawn from a previous Congressional Research Service Report regarding combating fraud and deception in seafood marekting.[2]

Despite numerous publications on NIR spectroscopic investigations of different quality parameters of fish,[3–8] to the best of our knowledge no results regarding authentication and detection of mislabelling of very similar species with hand-held instrumentation are available in the literature. Substitution of a more expensive fish by a lower-cost type is illegal. It is motivated by monetary gains by the perpetrator, leading to negative economic, health and environmental consequences. Consumers and the honest seafood suppliers are cheated into paying higher prices for lower-cost, less-desirable substitutes. One of the most commonly substituted and more expensive fish is red snapper which is often swapped for tilapia. Second, some fish substitutes pose health hazards. For example, the Oceana study[1] found that over 90% of what was advertised as white tuna was actually escolar which is a snake mackerel species that contains toxins known to cause gastrointestinal problems. Last, some substituted fish may be a type of overfished or threatened species. One such fish is Atlantic cod which has been found to be swapped for Pacific cod in the same study.

The supply chain from "boat-to-plate" is complex and unregulated, making such illegal activities hard to track.

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Table 1. Species, sample numbers, quality and origin of the investigated fishes.

Fish species	Sample no.	Quality	Origin
Red mullet	3	Superior	France
Mullet	6	Lower	Senegal
Winter cod	3	Superior	Norway
Cod	7	Lower	Iceland
Samlet	6	Superior	Iceland
Salmon trout	5	Lower	Italy

Combating fish fraud requires traceability of the fish across the supply chain as well as increased inspection. DNA testing for inspection is time consuming and can only be done on a sampling basis. It requires taking samples of fish to a laboratory and waiting for results that could take days. Alternate methods for identifying or verifying the fish type at the point-of-sale quickly would be highly desirable and is one step towards deterring illegal activities.

Considering the excellent performance and operational characteristics of recently available hand-held near infrared spectrometers, food analysis has been launched into a new era of sample presentation and measurement flexibility in order to fulfil the increasing requirements of consumer protection by quality and process control. In this context, the investigations were performed with the aim of developing a fast, reliable and on-site measurement technique for a more efficient federal and state enforcement effort for combating fraud and misidentification in the seafood industry and marketing.

Experimental

Materials

The investigated fish, including their quality, number of samples and origin are summarised in Table 1. All samples have been collected from a minimum of three different catches and no deep-freeze or any other pre-treatments were involved. Exemplary photos of the different species of each couple are shown in Figures 1–3. As can be derived from the photos, even for a professional, visual discrimination of the whole fish and the fish fillets would be extremely difficult, let alone the public customer. To compensate the heterogeneity of the texture from each fish or fish fillet sample, 10 spectra were recorded from different positions of the skin and meat, respectively. For this purpose, the mullets were filleted after recording the spectra of the skin.

Instrumentation

For the NIR spectroscopic measurements, the JDSU MicroNIR 1700 spectrometer was used, covering the wavelength range of 887–1667 nm (11,274–6000 cm^{-1}). It is a low-cost, ultra-compact hand-held spectrometer that weighs 60 g and is less than 50 mm in diameter. The spectrometer works in diffuse reflection. The light source, dispersing element, detector and electronics are all contained in the small device which can fit in the pocket of a FDA inspector, or can be placed on the seafood counter at a grocery store or used at the receiving dock of a seafood department (Figure 4). The principle of operation of the MicroNIR spectrometer has been discussed previously.[9]

The experimental set-up of a typical measurement procedure (shown here for a samlet fillet) is represented in Figure 4. For the spectra acquisition, an integration time of 5000 µs was selected and 50 scans were accumulated resulting in an extremely short total measurement time of 0.25 s.

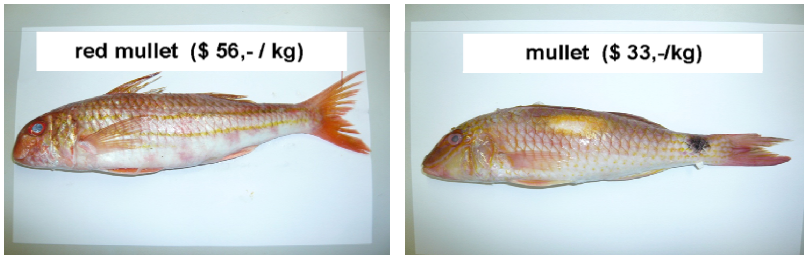

Figure 1. Pair of mullets of different gastronomical quality (after measuring in different positions of the skin the fish were filleted and measurements were performed on the meat).

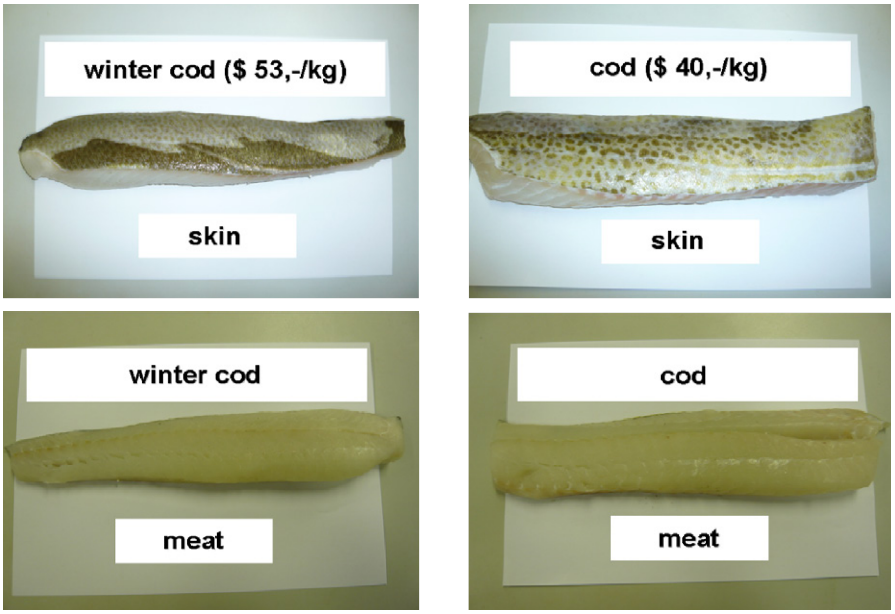

Figure 2. Winter cod and cod fillets photographed from the skin and meat sides.

Chemometric data evaluation

The data pretreatment [selection of suitable wavenumber region and extended multiplicative scatter correction (EMSC)] and the development of the principal component analysis (PCA) and soft independent modelling of class analogies (SIMCA) models were performed using the Unscrambler software (Version 9.6; CAMO AS, Oslo, Norway).

PCA modelling

For the development of PCA calibration models for each of the three fish pairs (red mullet/mullet, winter cod/cod, samlet/salmon trout), the spectra measured on the skin and meat of all available samples were used. In order to take into account the variations of the surface textures, every five spectra were averaged before the development of the PCA models. According to this replication schedule for each fish sample,

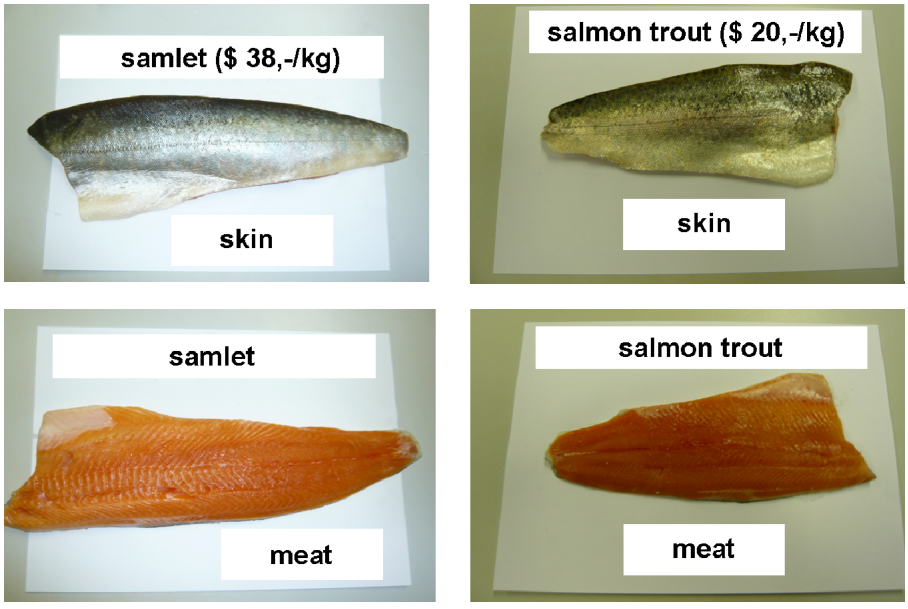

Figure 3. Samlet and salmon trout fillets photographed from the skin and meat sides.

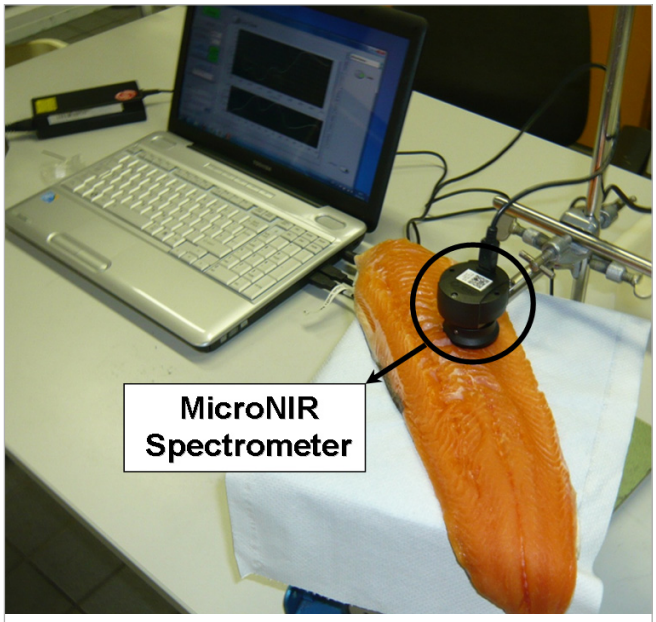

Figure 4. Practical measurement set-up for a samlet fillet (meat side).

four spectra (two spectra of the skin/two spectra of the meat) were finally available.

SIMCA classification

SIMCA is a classification method analysing similarities.[10–12] It is based on separate PCA models (disjointed modelling) of the investigated classes (in the present case the high- and low-quality classes of a fish pair). Depending on the availability of the number of fish samples for the development of these PCA models, which serve as precursors for subsequent SIMCA classification, the spectra of one or two fish/fillet samples of the high/low quality species have been selected as test data. The SIMCA classification step then uses the PCA models of the residual high- and low-quality fish/fillet spectra to assess which class the spectra of a test fish belong to, independently of whether they were measured on the skin or on the meat. The classification result is represented in a so-called Coomans plot. Authenticity is achieved if the spectra of the test fish/fillet are assigned to the relevant quadrant defined by the SIMCA model.

Results and discussion

Red mullet/mullet

In Figure 5, the spectra measured on the skin and meat of the three red mullets and the six mullets are shown. Prior to calibration modelling, they have been reduced to the 11,038–6068 cm^{-1} (906–1648 nm) region and subjected to an EMSC. Visually, the spectra of the skin and the meat of both species can be discriminated. The 3D score plot based on the PCA analysis (Figure 6) demonstrates that the red mullets and the

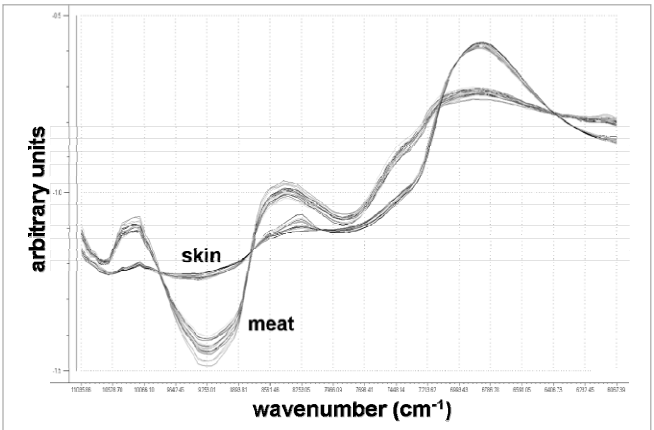

Figure 5. Diffuse reflection spectra (11,038–6068 cm^{-1}) of the investigated mullets after EMSC (extended multiplicative scatter correction) pretreatment.

mullets can be readily discriminated by their skin measurements but no clear separation was achieved by the measurements of the meat. By performing a SIMCA analysis with two mullets and one red mullet as test fishes, respectively, however, a clear separation of superior from lower quality fish is achieved, irrespective of whether a mullet of unknown quality is measured on the skin or on the meat (Figure 7). It is interesting to note that completely analogous results regarding the authentication of mullet fillet species have recently been reported at the International Conference on Near Infrared Spectroscopy (NIR 2013),[13] based on a much larger calibration sample set of 180 fishes, using a benchtop spectrometer.

Winter cod/cod

The spectra measured on the skin and meat of seven cod and three winter cod fillets are shown in Figure 8 and the 3D score plot derived from the PCA based on these spectra is represented in Figure 9. Here too, the skin and meat spectra are separated but, contrary to the mullets, both overlap for the two

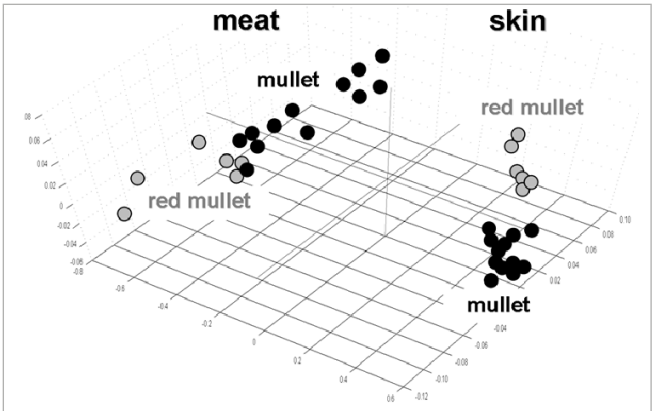

Figure 6. 3D score plot of the PCA (principal component analysis) model for the discrimination of the different mullets.

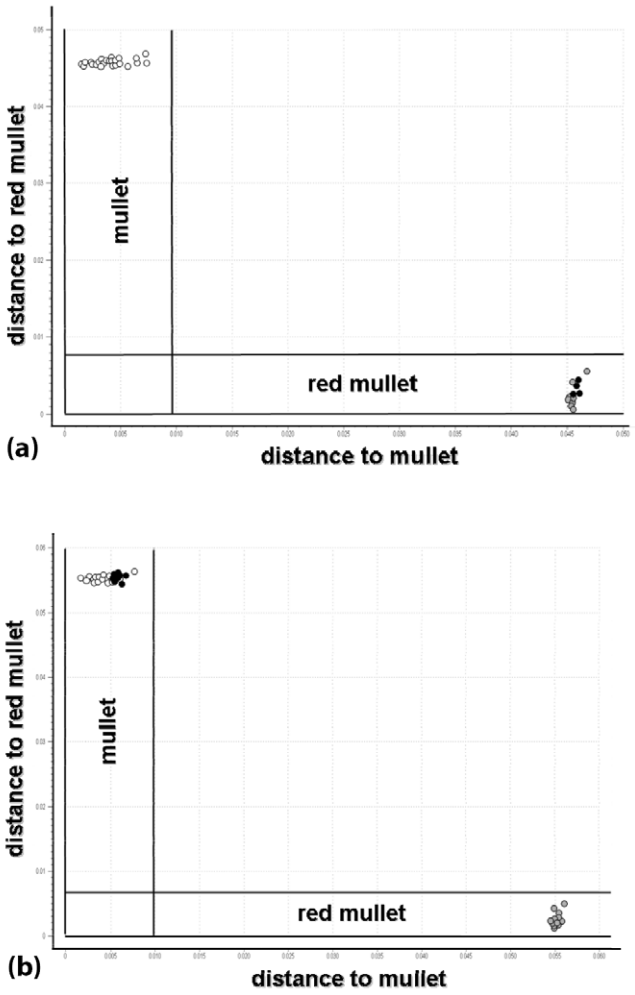

Figure 7. Coomans plots of SIMCA (soft independent modelling of class analogies) analyses (5% significance) demonstrating the correct identifications of (a) a red mullet test fish and (b) two mullet test fishes [the spectra of the test fishes are represented by the symbol (•)].

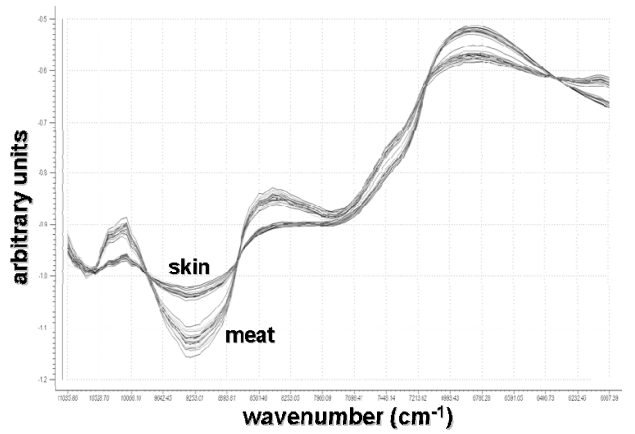

Figure 8. Diffuse reflection spectra of the skin and meat of the investigated cod species (11,038–6068 cm^{-1}) after EMSC pretreatment.

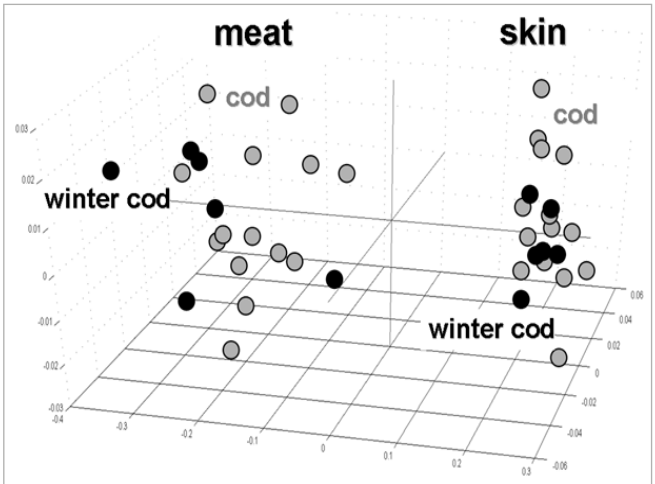

Figure 9. 3D score plot of the PCA model for the discrimination of the different cod fillets.

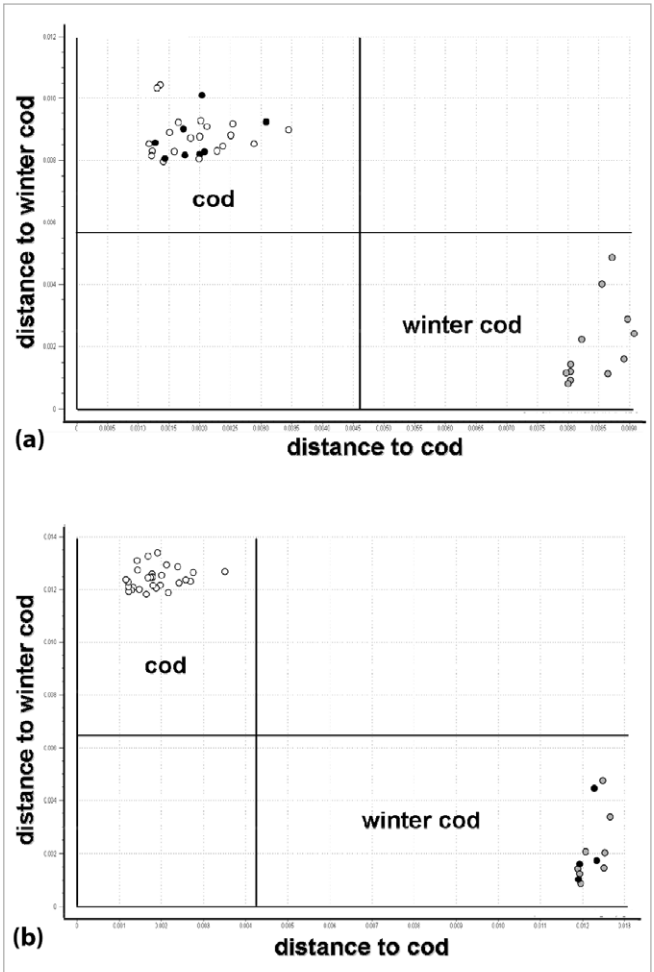

Figure 10. Coomans plots of SIMCA analyses (5% significance) demonstrating the correct identification of the test spectra (skin and meat) of (a) two cod fillets and (b) one winter cod fillet [the spectra of the test fishes are represented by the symbol (•)].

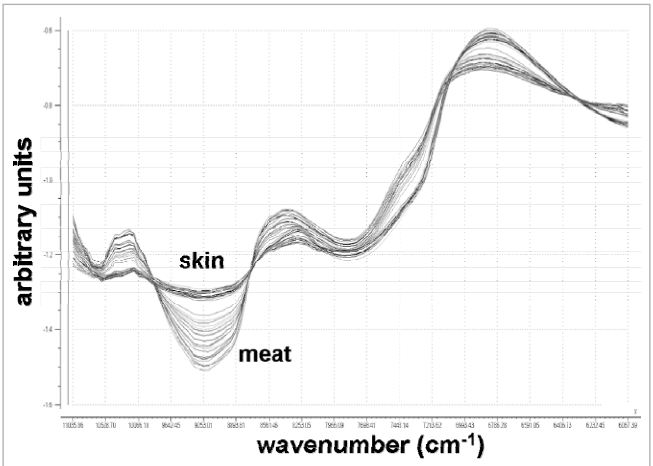

Figure 11. Diffuse reflection spectra of the skin and meat of the investigated samlet and salmon trout fillets in the 11,038–6068 cm^{-1} wavenumber region after EMSC pretreatment.

cod species. Notwithstanding this failure, the SIMCA predictions (Figure 10) performed with two cods and one winter cod again provided a correct assignment of the test fishes.

Samlet/salmon trout

As a final example, the results obtained with six samlet and five salmon trout fillets will be discussed. The skin and meat spectra of the two species are given in Figure 11 and differ in the same wavenumber ranges compared to the previous cases. Similarly, a separation in skin- and meat-specific clusters is achieved; however, no differentiation regarding the superior/lower quality fish fillets could be derived from the 3D score plot of the spectra (Figure 12). However, despite a few minor misclassifications of test spectra [Figure 13(a) and 13(b)] the SIMCA analysis again provided a suitable tool to authenticate the two samlet and two salmon trout test fish fillets.

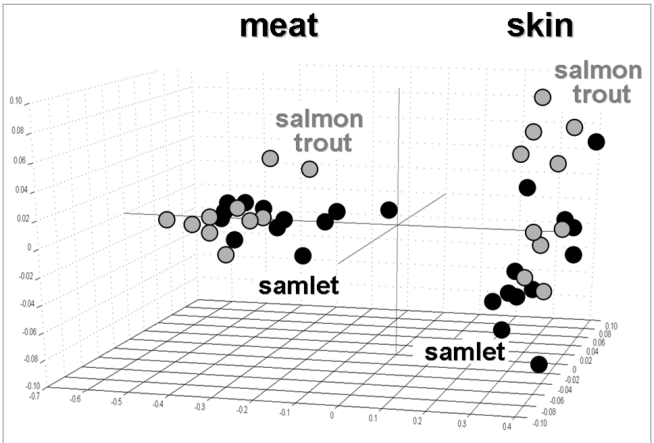

Figure 12. 3D score plot of the PCA model for the discrimination of the samlet and salmon trout fillets.

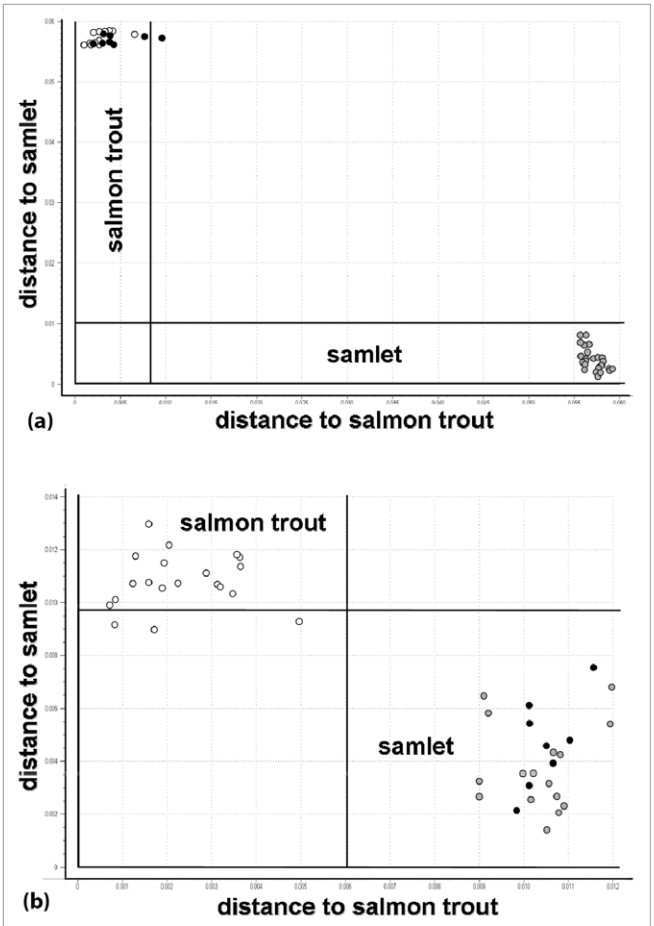

Figure 13. Coomans plots of SIMCA analyses (5% significance) demonstrating the correct identification of the test spectra (skin and meat) of (a) two salmon trout fillets and (b) two samlet fillets [the spectra of the test fishes are represented by the symbol (•)].

Conclusions

The investigations clearly demonstrate that, on the basis of spectra measured with the JDSU MicroNIR spectrometer 1700 on the skin or meat of whole fish or fish fillets, a subsequent SIMCA analysis provides a suitable analytical tool for the correct assignment of the spectra and authentication of the corresponding test fish. It has to be clearly stated, however, that the presented investigations refer to the feasibility of authenticating pairs of fresh fish/fish fillets representing a superior species and a cheaper substitute with defined origins and without any pre-treatments. The fact that the data obtained for one of the presented fish couples (red mullet/mullet) have been recently supported by the results of an independent research group give hope that, in the near future, the situation regarding commercial fraud in seafood marketing by mislabelling can be alleviated significantly. In view of the flexibility of the experimental measurement set-up, corporate enforcement bodies would have a very fast on-site measurement tool at hand to distinguish lower quality from superior quality seafood in mislabelling attempts.

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