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# Application of FT-NIR spectroscopy as a rapid tool for analysis of the fish fillet chemical composition

*Lucia Benešová, Silvia Jakabová*, Ladislav Ondruš, Jozef Golian*

*Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*

**Corresponding author: silvia.jakabova@uniag.sk*

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**Abstract:** The basic chemical composition of the meat of fifteen freshwater and saltwater fish species was studied. Fourier transform-near-infrared (FT-NIR) spectroscopy was applied for determination of the content of fat, protein and dry matter in the fish meat samples. The fish species analysed differed significantly in dry matter, fat, and protein content ($P < 0.05$). The highest fat percentage was determined in the samples of *Cyprinus carpio* (17.14 ± 0.53%) and the lowest value was found in the samples of *Lophius piscatorius* (0.06 ± 0.003%). *Thunnus albacares* samples had the highest content of proteins (24.26 ± 1.89%), whereas the lowest protein content was observed in *Oreochromis niloticus* (14.73 ± 0.87%). The results of the measured dry matter varied from the highest content in *C. carpio* samples (35.73 ± 0.47%) to the lowest content of *L. piscatorius* samples (15.64 ± 0.43%). Principal component analysis (PCA) extracted three major groups, which differentiate the analysed samples based on their protein, lipid and dry matter content. Partial least squares discriminant analysis (PLSDA) confirmed the use of three variables (protein, fat, dry matter) measured by FT-NIR to separate the observed fish species.

**Keywords:** fish meat; fat content; protein content; dry matter; Fourier transform-near-infrared spectroscopy

Fish and shellfish are important food sources for the human diet, valuable for proteins containing the essential amino acids, lipids rich in polyunsaturated fatty acids, enzymes, and other bioactive compounds such as minerals and vitamins (Cahu et al. 2004; Petricorena 2015). Since fish are an available food source to many people globally, it contributes significantly to human nutrition (Sonawane 2013). The World Health Organization (2007) recommends unsaturated lipids from fish that are preferred over saturated lipids from other types of fatty meat. Eating habits related to fish meat are influenced by social, cultural, and geographic characteristics (Pieniak et al. 2011).

Various factors affect the chemical composition of fish meat, chiefly the species of fish, its sex, age, habitat, season, composition of feed, and environmental conditions. Although fish meat has a stable protein content, the ash and fat content fluctuate (Bhaskar et al. 2008).

Fish muscle accounts for 50–60% of the fish weight. The main components of the fish muscle are proteins (16–21%), lipids (0.5–2.3%), ash (1.2–1.5%), and water (52–82%). The content of carbohydrates is low (only 0.3%) and it is found mainly in the form of glycogen not only in the muscles but also in the liver (Popelka 2021).

Fish have two types of muscles: light and dark. Fatty fish usually have a high content of dark muscle, but

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in general light muscle is more abundant in fish (Petricorena 2015). Fish muscle contains three classes of proteins differing in solubility: myofibrillar proteins are the most abundant, sarcoplasmic ones are found especially in the sarcoplasm of myofibres, and stromal proteins are dominated by collagen (Strasburg and Xiong 2017). The proteins in fish meat are biologically complete since they contain all the essential amino acids and are therefore comparable with those of other terrestrial meat species. The protein content is reported to be in the range of 17.5–19.7% in freshwater fish and 17.7–18.2% in selected marine fish species (Kopřiva et al. 2010).

The total content of lipids in fish is not constant; it depends on the aforementioned variables (Bhaskar et al. 2008) as well as on geographic regions, individual maturity, and whether the fish come from aquacultures or from the wild. Generally, farmed fish contain higher fat levels compared to wild ones, sometimes as much as twice more (Yeşilayer and Genç 2013). In general, however, fish have less fat than red meats. Fish lipids are stored primarily in the liver, muscle, and perivisceral and subcutaneous tissues.

Near-infrared (NIR) spectroscopy is often used in the identification of structures of molecules since the method is able to provide information on functional groups of molecules through the assignment of certain absorption bands (Cen and He 2007; Chen et al. 2011). The use of Fourier transform-near-infrared (FT-NIR) analysis of fish meat and fish products was applied for various purposes (Azizian et al. 2010; Alamprese et al. 2016; Agyekum et al. 2020).

Our study investigated the chemical composition (proteins, lipids, and dry matter content) of freshwater and saltwater fish species intended for retail and its possible variability, in order to provide important information about their chemical composition to consumers. Information on the chemical composition of different fish species was an important part of the initial study for development of new fish-containing products for food-processing companies focused on fish products. The novelty of the present study is the comparison of fish species based on the chemical characteristics of fish tissues using FT-NIR spectroscopy and the application of chemometric statistical methods as a contribution to the possibilities of fish species differentiation. The work has potential use as a contribution to the possibilities of authentication of fish species intended for the Slovak market.

## MATERIAL AND METHODS

All fish samples were obtained from a retail store (Metro Ltd., Žilina, Slovak Republic). Fifteen species of fish were included in this study. Fresh samples (skinless fillets) were homogenised using a Bosch MSM 67170 mixer (Germany) and compressed into 90 mm Petri dishes. Samples of freshwater and saltwater fish (Table 1) were analysed by an FT-NIR Tango spectrometer (Bruker Optics, Germany) in the attenu-

Table 1. Description of fish species analysed

| English name | Latin name | Type | Food habits | Country of origin |
|---|---|---|---|---|
| Nile tilapia | *Oreochromis niloticus* | FW | O | Poland |
| African sharptooth catfish | *Clarias gariepinus* | FW | C | the Netherlands |
| Coalfish | *Pollachius virens* | SW | C | NE Atlantic Ocean |
| Rainbow trout | *Oncorhynchus mykiss* | FW | C | Italy |
| Nile perch | *Lates niloticus* | FW | O | Denmark |
| European bass | *Dicentrarchus labrax* | SW | C | Greece/Croatia |
| Gilt-head bream | *Sparus aurata* | SW | C | Greece/Croatia |
| Swordfish | *Xiphias gladius* | SW | C | Indian Ocean |
| Atlantic salmon | *Salmo salar* | SW/FW | C | Norway |
| Yellowfin tuna | *Thunnus albacares* | SW | C | Western and Central Pacific Ocean |
| Angler | *Lophius piscatorius* | SW | C | NE Atlantic Ocean |
| Atlantic mackerel | *Scomber scombrus* | SW | C | NE Atlantic Ocean |
| Greenland halibut | *Reinhardtius hippoglossoides* | SW | C | NW Atlantic Ocean |
| Haddock | *Melanogrammus aeglefinus* | SW | C | NE Atlantic Ocean |
| Common carp | *Cyprinus carpio* | FW | O | Czech Republic |

FW – freshwater; SW – saltwater; O – omnivorous; C – carnivorous; NE – North East; NW – North West

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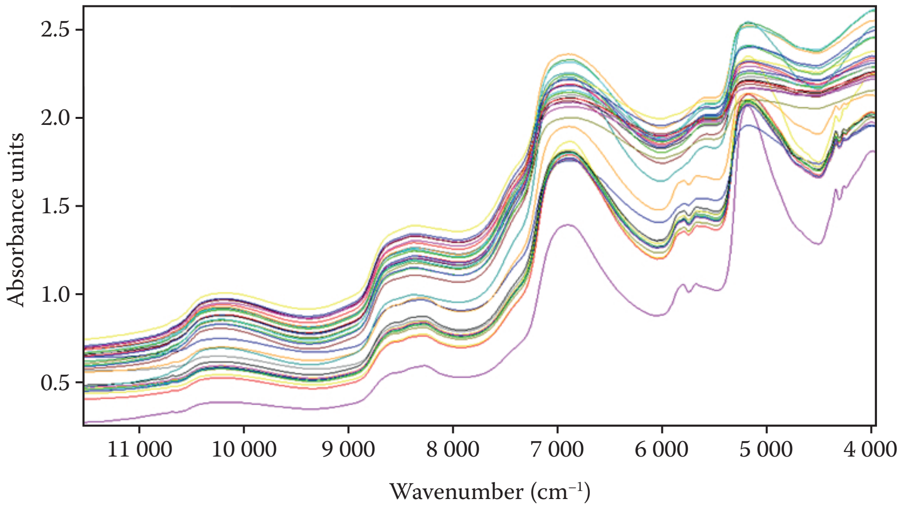


Figure 1. Fourier transform-near-infrared (FT-NIR) spectrum for fish samples

ated total reflectance (ATR) mode for solid samples. The signal was recorded within the range of wavenumbers from 11 500 $cm^{-1}$ to 4 020 $cm^{-1}$ (Figure 1). The corresponding wavenumbers for individual parameters were as follows: the ranges for the proteins were from 9 960 $cm^{-1}$ to 9 288 $cm^{-1}$ and from 6 088 $cm^{-1}$ to 4 120 $cm^{-1}$, the range for the lipids was from 8 568 $cm^{-1}$ to 7 152 $cm^{-1}$, and the range for the dry matter content was from 9 984 $cm^{-1}$ to 8 568 $cm^{-1}$. Calibration was performed by the FT-NIR owner for the purpose of chemical composition screening. Evaluation of FT-NIR results was carried out together with results for each parameter for the same samples from standard analytical methods (accredited laboratory of State Veterinary and Food Institute in Dolný Kubín, Slovak Republic). Accredited analyses were based on the standards STN 57 0146-18 (dry matter at 105 °C), STN 57 0146-20 (fat content, direct analysis), and STN 46 1011-17 (total proteins, Kjeldahl method). Results for fish parameters from accredited analyses and FT-NIR analyses were tested to determine the bias that stands for a systematic error – a difference between the expected value of the parameter and the true value of the parameter being measured. The bias values for each parameter were used to adjust the calibration settings of the FT-NIR spectrometer. Homogenisation of fish fillets was performed on the same samples several times from approximately 2–3 fillets per one homogenate at least from 15 different fishes of the same fish species together. Homogenised material was stored in closed plastic boxes and applied on the dishes prior to the analysis. Ten Petri dishes (Duroplan Petri dishes, DWK Life Sciences, Germany) were filled with the homogenised material in a 1 cm thick layer and the measurements were performed in the dishes without cover in triplicate at laboratory temperature (20 ± 1 °C). The duration of one sample measurement was 1 min.

Statistical analysis of the data was performed in XLSTAT 2020.4.1 (Addinsoft, France). Principal component analysis (PCA) and partial least squares discriminant analysis (PLSDA) were applied to test if the three variables enable the species to be defined, and to visualise the observations on a two-dimensional map which reflects the separation of individual groups of samples.

## RESULTS AND DISCUSSION

Currently, applying FT-NIR spectroscopy in the analysis of samples of animal origin is receiving growing interest and attention. To extract valuable information on the sample's chemical properties, it is necessary to process spectral data by chemometric tools mathematically (Berzaghi and Riovanto 2009). A NIR analyser (Bruker Optics, Germany) was also used to provide fast information on the content of fat, protein, and dry matter. Figure 1 is an output (NIR spectrum) containing absorption bands of different molecular groups at typical wavelengths. A comparison of results for dry matter, fat and protein content was prepared from data for fish samples from an accredited laboratory and from data measured on the FT-NIR spectrometer. Calibration of the method was carried out on the fish meat samples based on the results of individual determinations of proteins, fat content and dry matter content by standard analytical approaches and then by the FT-NIR method. The calibration curves were constructed and the coefficients of determination were calculated for proteins, fat and dry matter content. The bias, a difference between expected values and true values of the parameters, was estimated based on the difference be-

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tween the measured data from standard methods (true values) and the corresponding values (expected values), measured by FT-NIR. The results of calibration parameters are shown in Table 2. Bias values, linked with overestimation or underestimation of results, were determined for each parameter in order to adjust the calibration settings of the FT-NIR analyser. The chemical composition of fresh fish consumed in Slovak Republic is listed in Table 3.

FT-NIR method evaluation is referred to have a high potential to replace more expensive and time-consuming methods for the determination of the chemical composition of meat samples. Calibration models are usually created for these purposes using a partial least squares algorithm and cross-validation to verify the FT-NIR method application in practice. In many works, FT-NIR is usually evaluated for the samples of a limited number of animal species (Mlček et al. 2006; Alamprese and Casiraghi 2015; Schmutzler et al. 2015; Alamprese et al. 2016) or of one type of the product (Procházková et al. 2010; Schmutzler et al. 2015). Mabood et al. (2020) analysed the NIR spectrum to differentiate pork meat from other 7 meat species and they obtained the coefficient of determination for calibration from 0.740 to 0.977, depending on different ranges of NIR spectra. From this point of view, the range of spectra and the number of species analysed could contribute to the value of the coefficient of determination in the calibration.

**Protein content in fish species.** The protein content and composition are characteristic of each fish species regardless of the content in feed (Morris 2001; Shearer 2001; Khalili Tilami and Sampels 2018). Protein content varied at levels that were in agreement with the stated values for fish flesh. As observed in the fish, protein levels were relatively high in most species; the highest was in yellowfin tuna (the mean content was 24.26% in fresh material). The lowest was observed in Nile tilapia (14.73% in fresh material) (Table 3).

Hossain et al. (1999) reported that the fish species' muscle protein content varied widely (16.16–22.28%, analysed by the Kjeldahl method). Tilami et al. (2018)

Table 2. Calculated calibration parameters

| Parameter | $R^2$ | RMSECV | Bias |
|---|---|---|---|
| Dry matter | 82.01 | 2.250 | –0.0232 |
| Fat | 97.32 | 0.956 | 0.0121 |
| Proteins | 64.39 | 1.060 | –0.1480 |

RMSECV – root mean standard error of cross validation; Bias – systematic error

reported the protein content in freshwater fish species in the Czech Republic to be relatively stable (17.1 ± 1.55 to 19.2 ± 2.20% in the fillet, analysed by the Kjeldahl method). Linhartová et al. (2018) investigated the proximate composition of the most common freshwater fish species in the Czech Republic. The fish were sampled from intensive aquacultures, semi-intensive culture systems and extensive culture systems. Protein content was determined by the Kjeldahl method. When compared to our results, the content of proteins in common carp and African sharptooth catfish is in agreement, although a slightly higher content of proteins was found in rainbow trout compared to the above-mentioned survey; however, samples of Nile tilapia differed by more than 3.2%. Erkan and Özden (2007) mentioned that the protein composition of the fish could vary according to the species, size, season and gender, but usually the content is not higher than 20%. In most of the species investigated, the protein content ranged between 15% and 20%, which supports the findings of Gjedrem et al. (2012), Zotos and Vouzanidou (2012), and Linhartová et al. (2018).

Two species (Nile tilapia and gilt-head bream) had a slightly lower mean protein content than 15%. The chemical composition of 14 commercialised fish species captured in the northeastern Atlantic was reported by Nogueira et al. (2013). The content of proteins was analysed by the Kjeldahl method in the gilt-head sea bream and Atlantic mackerel. The protein content was higher than in our case (20.64 ± 1.37% and 28.71 ± 2.11%, respectively).

**Dry matter content in fish species.** Our study found the dry matter content to vary between 18.17 ± 0.32% in haddock and 35.73 ± 0.47% in common carp (Table 3). Memon et al. (2010) published the moisture content determined by a drying technique in an oven at a temperature of 105 °C in freshwater fish from the Indus River varying between 59.95% and 79.45%, which corresponds to dry matter content between 40.05% and 20.55%. Bogard et al. (2015) determined the moisture content by a drying technique in the raw edible parts of common carp and Nile tilapia to be 80.0% and 77.6%, which indicates that 20.0% and 22.4% are dry matter. Our results differ from other authors in the case of common carp. Linhartová et al. (2018) determined that the African sharptooth catfish had more than 2% higher dry matter content than in our study. The analysis was performed with the use of the drying technique. Rainbow trout and Nile tilapia in our study contained less dry matter than published by Linhartová et al. (2018). In the case of rainbow trout from extensive aquacultures, the

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Table 3. The chemical composition of fish species analysed ($n$ = 30) (%)

| Fish species | Protein | | Fat | | Dry matter | |
|---|---|---|---|---|---|---|
| | mean | SD | mean | SD | mean | SD |
| Nile tilapia | 14.73 | 0.87 | 3.38 | 0.62 | 20.06 | 0.63 |
| African sharptooth catfish | 15.70 | 0.37 | 11.51 | 0.78 | 28.61 | 0.67 |
| Coalfish | 17.16 | 0.33 | 1.03 | 0.34 | 20.68 | 0.40 |
| Rainbow trout | 18.39 | 0.35 | 4.80 | 0.58 | 24.98 | 0.31 |
| Nile perch | 16.87 | 1.30 | 1.30 | 0.37 | 18.69 | 0.73 |
| European bass | 15.68 | 0.61 | 8.58 | 0.51 | 26.11 | 0.34 |
| Gilt-head bream | 14.99 | 0.43 | 11.33 | 0.79 | 29.59 | 0.34 |
| Swordfish | 17.09 | 0.56 | 9.87 | 0.62 | 27.92 | 0.42 |
| Atlantic salmon | 17.79 | 0.31 | 11.23 | 0.54 | 31.98 | 0.39 |
| Yellowfin tuna | 24.26 | 1.89 | 1.09 | 0.15 | 17.19 | 0.87 |
| Angler | 16.29 | 0.44 | 0.06 | 0.003 | 15.64 | 0.43 |
| Atlantic mackerel | 15.22 | 0.84 | 15.44 | 0.89 | 31.61 | 0.90 |
| Greenland halibut | 19.23 | 0.46 | 4.28 | 0.42 | 26.27 | 0.51 |
| Haddock | 16.23 | 0.64 | 0.59 | 0.48 | 18.17 | 0.32 |
| Common carp | 16.11 | 0.21 | 17.14 | 0.53 | 35.73 | 0.47 |

data was similar to our findings. On the other hand, common carp in semi-intensive and extensive aquacultures had lower dry matter content than in our study.

**Fat content in fish species.** Fish can be divided into four categories according to their fat content: lean (< 2.5%), low fat (2.5–5.0%), medium fat (5.0–10.0%), and high fat (> 10.0%) (Linhartová et al. 2018). In our study, we found five species to have lower fat content than 2.5%, thus classifying them as lean fish, namely: coalfish (1.03%), Nile perch (1.3%), yellowfin tuna (1.09%), angler (0.062%) and haddock (0.59%). Nile tilapia, rainbow trout, and Greenland halibut can be classified as low-fat fish with fat content in the tissues at 3.38, 4.80, and 4.28%, respectively. The medium fat fish category, with a range of 5.0–10.0%, is represented by European bass (8.58%) and swordfish (9.87%) (Table 3). The highest fat content (over 10.0% in the tissues) was found in five fish species: African sharptooth catfish (11.51%), gilt-head bream (11.33%), Atlantic salmon (11.23%), Atlantic mackerel (15.44%), and common carp (17.14%). Memon et al. (2010) reported the lipid content after the gravimetric determination in freshwater fish from the Indus River to be highly variable between the species (0.85–18.32%). Most of our samples have a fat content in this range. The common carp is the most important species consumed in Slovak Republic. According to observations published, it has a large variability of fat content during the year (Mráz et al. 2012; Zajic et al. 2013). In our study, the fat content (17.14%) was much higher than that reported by Linhartová et al. (2018), those results were measured by gravimetric determination. According to our findings, samples of common carp were highly rich in fat, and the carp could be classified among the high-fat fish (< 10%). Razavi et al. (2014) reported that fat content is highly affected by the overall weight of the fish and can be 20% in bigger specimens. Regarding food habits, the lowest content of fat (≤ 1.092%) was observed in carnivorous species such as coalfish, yellowfin tuna, angler, and haddock. The species African sharptooth catfish, rainbow trout, common carp, and Nile tilapia were investigated in central European conditions in the Czech Republic by Linhartová et al. (2018). Nile tilapia was classified in the same fat-content group as according to our results, but common carp and African sharptooth catfish had a higher fat content in our study. On the other hand, rainbow trout from intensive aquaculture had a more than 6% higher fat content than our results. But in extensive aquaculture, rainbow trout had a lower fat content than our samples. Linhartová et al. (2018) stated that the culture systems highly influenced the content of lipids as well as the composition of fatty acids. The lipid content in fresh material varied between 0.06% in angler and 17.14% in common carp.

**Statistical methods for the evaluation of fish species.** Multidimensional data analyses are statistical methods which give visualisation tools and indices for the explanation of observations. PCA is one such

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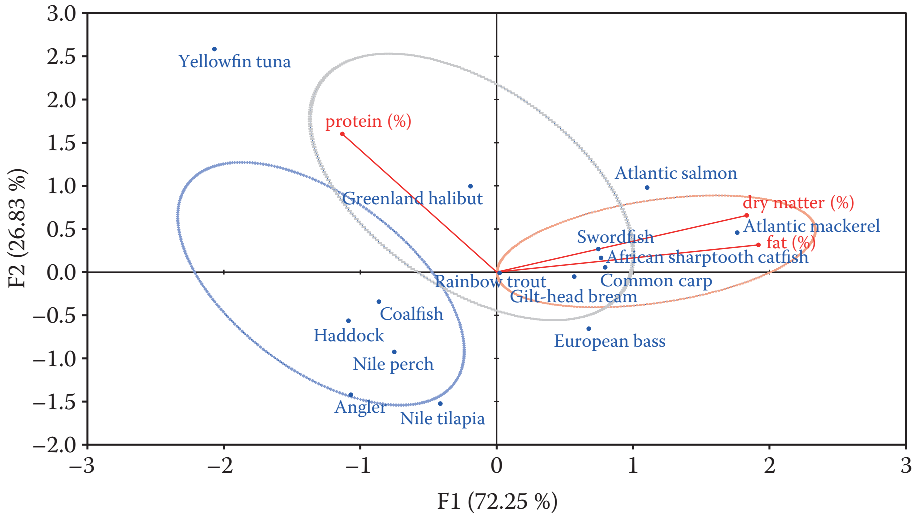


Figure 2. Principal component analysis (PCA) of fish samples (bioplot; axes F1 and F2: 99.07%)

F1 (first component) – presents maximal variance of the objects based on fat content and dry matter content; F2 (second component) – presents the second highest variance based on the protein content; the variance of the first component and the second component is expressed in percentage

method and deals with a data table where a set of individuals is described (Semjon et al. 2018).

Chemical compositions were observed to vary among the studied fish species. The results of the PCA of fish samples showed three selected components that explain their total variation in the dataset (Figure 2). The first axis (F1) reflects 72.25% of the variation, the second axis (F2) 26.83% and the third axis (F3) 0.92%. Dimensions F1 and F2 express 99.07% of the total variation in the dataset. The confidence ellipse defines the region that contains 95% of samples from the Gaussian distribution, which is represented in our case by three ellipses in Figure 2. PCA analysis showed three major groups based on basic chemical composition and different mutual ratios of these three components. Coalfish, haddock, angler, and Nile perch belong to the first group in the blue ellipse, represented especially by low fat and dry matter content. The five other species (African sharptooth catfish, guilt-head bream, Atlantic mackerel, swordfish, and common carp) with similarities in lipid content and dry matter form another group. The third group, represented by African sharptooth catfish, guilt-head bream, swordfish, common carp, and Greenland halibut, was based on similar protein content. Two species with low similarity according to protein content were Nile tilapia and yellowfin tuna. European bass was identified as an outlier according to the statistical results, based on its relatively low proteins, medium fat content and dry matter.

A strong linear correlation ($R$ = 0.9566) was observed between fat and dry matter content in the data set of fish species.

On the basis of three variables (protein, fat, dry matter), which were measured by FT-NIR spectroscopy, differences between the species were confirmed by the PLSDA method (Figure 3). Based on these three variables, it is possible to separate individual groups (fish species). According to the confusion matrix for the estimation sample, yellowfin tuna (100%), angler (96.67%), Atlantic mackerel (100%), and Atlantic salmon (100%) are very well discriminated on the factor axes extracted from the original explanatory variables. For other species, the sole use of these variables is insufficient.

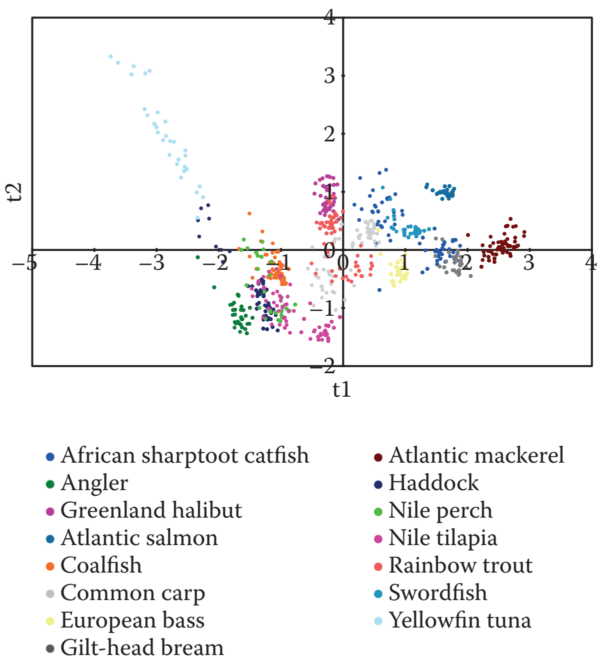


Figure 3. Partial least squares discriminant analysis (PLSDA) of fish species

t1, t2 – indicate the t1 and t2 score vector results from the PLSDA analysis

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## CONCLUSION

The presented FT-NIR spectroscopy analysis in combination with multidimensional data analysis can be considered a useful and powerful tool for the evaluation of fish meat according to its chemical composition. Implementation of FT-NIR in fish meat quality control could be a beneficial method due to simple non-destructive sample preparation, ease of use and fast results. It could be used as an alternative method to conventional laboratory methods for analysis used in the food industry, such as the routinely used screening analyses. Based on the PCA analysis, three major groups were created from the data of basic chemical composition, supported by 95% similarities in the Gaussian distribution. The PLSDA showed a higher percentage of well-classified observations that contributed to the possible discrimination of four fish species based on three chemical components. The basic chemical composition of the meat of common fish species available in retail stores provides valuable information from the nutritional perspective that can promote the incorporation of the fish species into the daily diet as a healthy food.

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