NEAR INFRARED SPECTROSCOPY (NIRS) AS FOOD QUALITY AND FOOD FRAUD DETECTOR – APPLIED ON FISH STICKS

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Summary

The aim of this study was to present the professional application of near infrared spectroscopy (NIRs) in the detection of food quality on purchased fish sticks. The measured NIR spectra of samples prepared from fish or squid were related to nutritional labels such as the amount of certain macronutrient and the proportion of the starting material (fish/squid). In the standard procedure, NIRs coupled with chemometric tools such as principal component analysis (PCA) and partial least square regression (PLSR) was used to investigate the qualitative and/or quantitative capabilities in determining food quality. Excellent qualitative differentiation was achieved with PCA, with biplots showing how the explanation of variation increased from 80.12 % when only nutritional information was used in the observation to 96.89% when nutritional information was coupled with the corresponding NIR spectrum. Since higher levels of animal protein in food are associated with an increase in price, the detection probabilities of different protein sources (fish/meat) were tested using PLSR, with 100% of the samples successfully detected. PLSR was also used to detect the correlation of the NIR spectra to the macronutrient contents and the strongest correlation was determined for proteins ($R^2 = 0.99$). The results confirmed the feasibility of using NIRs in the qualitative evaluation of samples where it is possible to determine the predominance of fish or squid, and also to estimate the expected protein content. The protein content is related to the price of the product, since all products containing animal proteins have higher prices that grow proportionally to their share. NIRs is not a qualitative method, but it can help in the selection of products, whose exact composition and possible adulteration can be confirmed by additional laboratory analysis.

Keywords: NIR spectra, fish sticks, chemometrics, qualitative analysis, proteins

Introduction

The European Environment Agency presented the data of fish and seafood supply with the average of 22.6 kg/capita/year as the EU-28 average. Croatia as a Mediterranean country lies under the average with 19.7 kg/Capita/year (EEA, 2016). The overview for the world food supply quantity is presented in Figure 1.

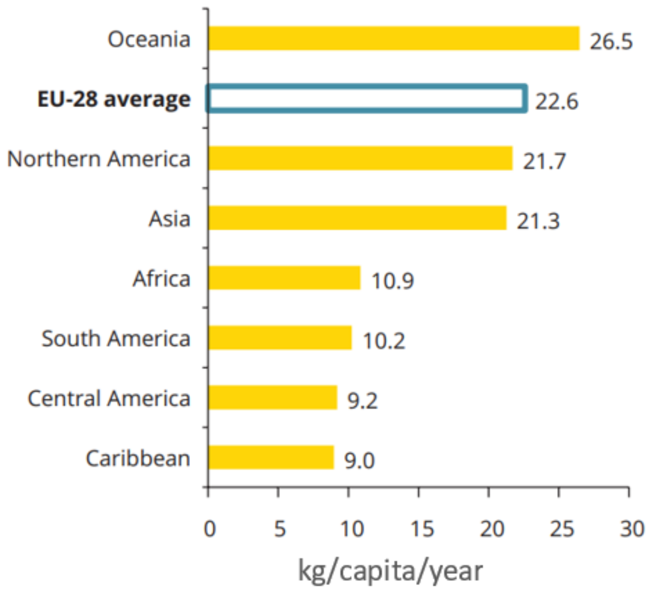

Figure 1. (FAO, 2016, FAO Food Balance Sheets: Food Supply Quantity)

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Based on the FAO data, (EEA, 2016; FAO, 2016), since the 1990s, fish supply derived from aquaculture increased since the 1990s from 7 to 44 %. China has played a major part in achieving this increase and currently represents over 60% of world aquaculture production (FAO, 2016; STECF, 2014). It is anticipated that by 2030, over 60% of fish supply for human utilization will be provided by aquaculture (FAO FishStat, 2016). With the increase of food demand, the food system becomes more important. The HLPE (2014) defined a food system as 'all the elements (environment, people, inputs, processes, infrastructures, institutions, etc.) and activities that relate to the production, processing, distribution, preparation and consumption of food, and the outputs of these activities, including socio-economic and environmental outcomes'. Food security is a core purpose of the food system (Maggio et al., 2015), and is seen from the perspective of access to food and its nutritional value, which moves away from the previous paradigm of focusing on the production of food (Ericksen, 2007; Gustafson et al., 2016). Unfortunately, in food access or production, forgery (adulteration) or quality manipulation may occur. Based on the Food Monthly Reports of the European Commission (2021), food quality and its safety must be monitored since cases of unapproved treatment of products regularly occur. Food fraud is also considered a problem, with and as the most common olive oil, milk, honey, saffron, orange juice, apple juice, grape wine, vanilla extract and fish being the most adulterated foods (EC, 2022). Some of the cases from the EC Report from June/2021, related to fish are: (i) Illegal unapproved treatments which include carbon monoxide and injection of nitrates and nitrites, in order to preserve the appearance of freshness given by the red colour, (ii) discovered mislabelled fishes (with the mislabelling rate of 17-36 %) as well as threatened species or (iii) sizing of tons of sea foods without documentation or product traceability requirements, especially red tuna (EC, 2021, p1). Therefore, different organisations are working with the goal of developing trustable methods for food analysis and determination of potential frauds. Such methods have already been developed for several foods (honey, wine, olive oil). For olive oil quality/fraud control, a spectrophotometric method (IOV, 2019) focussing on the quality of fats is used. Fats are characterised by the CH and OH bound and one of the methods for their analysis is investigating absorption of electromagnetic radiation at wavelengths in the near infrared range 780–2500 nm. Furthermore, NIR spectroscopy is a method used in order to investigate foods which comprise of broad bands arising from overlapping absorptions that correspond mainly to overtones and combinations of vibrational modes involving CH, OH and NH chemical bonds (Bøknæs et al., 2002; Valinger et al., 2011).

The aim of this paper was to present a professional aspect of NIR spectroscopy application on available fish sticks made by different producers. The aim was to relate the measured NIR spectra with nutritive labelling and to identify the ability of NIR spectroscopy usage in qualitative differentiation of samples which are prepared from fish or squid.

Materials and methods

Different fish stick products (n=5) were purchased in a local grocery store. Data of the basic nutritional composition as well as the content of fish or seafood (Table 1) were derived from the product labels.

Table 1. Nutritional value per 100 g of product with declared fish and squid content

Fish sticks (abbreviation)	**E** (kcal/kJ)	**Proteins** (g)	**Carbohydrates** (g)	**Fats** (g)	**Fish (squid) share (%)**
Product 1 (P1)	236/987	7	34	8	22 (0)
Product 2 (P2)	198/829	10.4	16.7	9.8	55 (0)
Product 3 (P3)	280/1172	11	30	13	22 (0)
Product 4 (P4)	228.5/956	12.9	21	10.3	0 (61)
Product 5 (P5)	206/866	12.8	16.8	9.8	0 (65)

Fish sticks were left at room temperature for 10 minutes after which they were scanned with a probe connected to the NIR device. The probe was placed on a part of the sample (i) coated for frying (with bread crumbs) and (ii) a cross-section of the stick with no coating. The procedure was repeated 3 times.

NIR spectroscopy was conducted with the NIR spectrometer (NIR – 128 –1.7-USB/6.25/50 mm Control Development Inc., South Bend, USA) with software Spec32. Spectra were recorded in absorbance mode in the wavelength range from 904-1699 nm. For the chemometric analysis raw NIR spectra without any pre-processing were used.

Data Analysis by use of chemometrics

Principal component analysis (PCA) was applied in order to determine the qualitative similarities or differences of the fish sticks samples. The partial least squares regression (PLSR) was applied to investigate the potential of quantitative prediction abilities for all observed parameters given on the product label (Table 1). In order to compare the model results, the coefficient of correlation (R_c), mean square error (MSE), root mean square error (RMSE), Ratio of standard error of performance to standard deviation (RPD) as well as the residual predictive deviation (RPD) were calculated (Fearn, 2002). All calculations were conducted using MS Excel and its *XLStat 2014* add on.

Results and discussion

Environmental conditions at which the samples were recorded (temperature and humidity) were measured using a data logger (Datalogger LOG 32T, Dostmann electronic GmbH, Germany) and average values were 22 °C with 31.6% RH and ± 0.1% deviation.
Measured NIR spectra of fish sticks products are presented in Figure 2. Only one sample, fish stick P2 partially stands out, while other spectra almost overlap and have the same trend. To give better insight where in the spectrum similarities/differences related to the chemical composition of the observed samples can be expected, the basic bands (Okparanma, et al., 2018) are also shown.

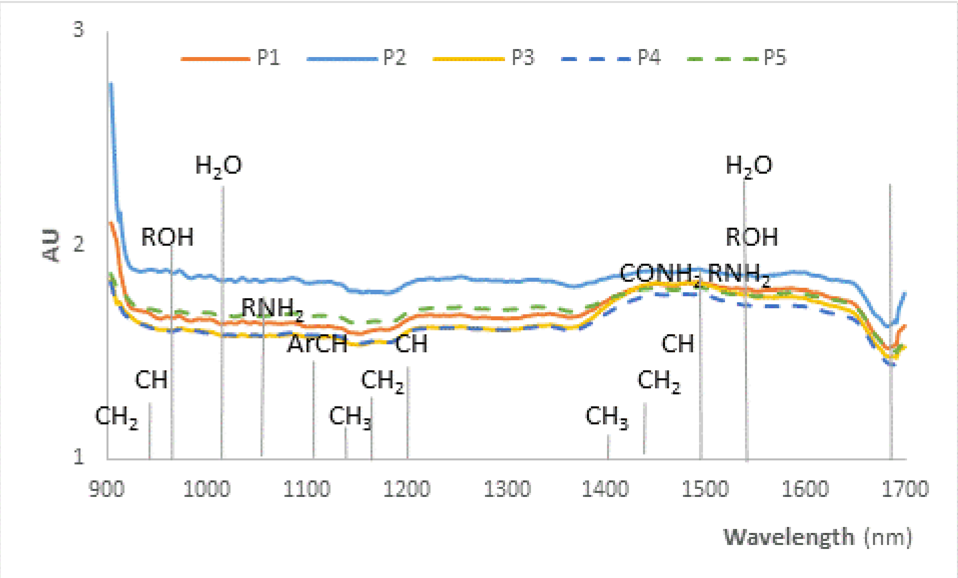

Figure 2. Near infrared scan of fish sticks samples (P1-P3 contain fish, P4 and P5, dashed lines, contain squids)

Due to invisible significant differences in certain parts of the raw NIR spectra, it was necessary to apply chemometric techniques, most commonly PCA and PLSR (Yu, 2021).
To identify qualitative similarities/differences in the observed fish sticks samples, PCA was applied and the obtained results are presented in Figure 3, in a form of biplots. The percentage of the explained variance based on the nutritional composition (Figure 3A) was 80.12%, while the percentage of explained variations in the observed data set increased to 96.89% when the NIR scans were associated with the nutritional composition (Figure 3B). Based on the Biplot in Fig. 3B, samples of fish fingers positioned in the upper part of the score plot, in the 1st & 2nd quadrant (P1 and P2). For those samples content of carbohydrates (CHO) is higher than the content of proteins and fats. The products P4 and P5, which contain squid are positioned in the same quadrant as variable "% squid", in the fourth quadrant.

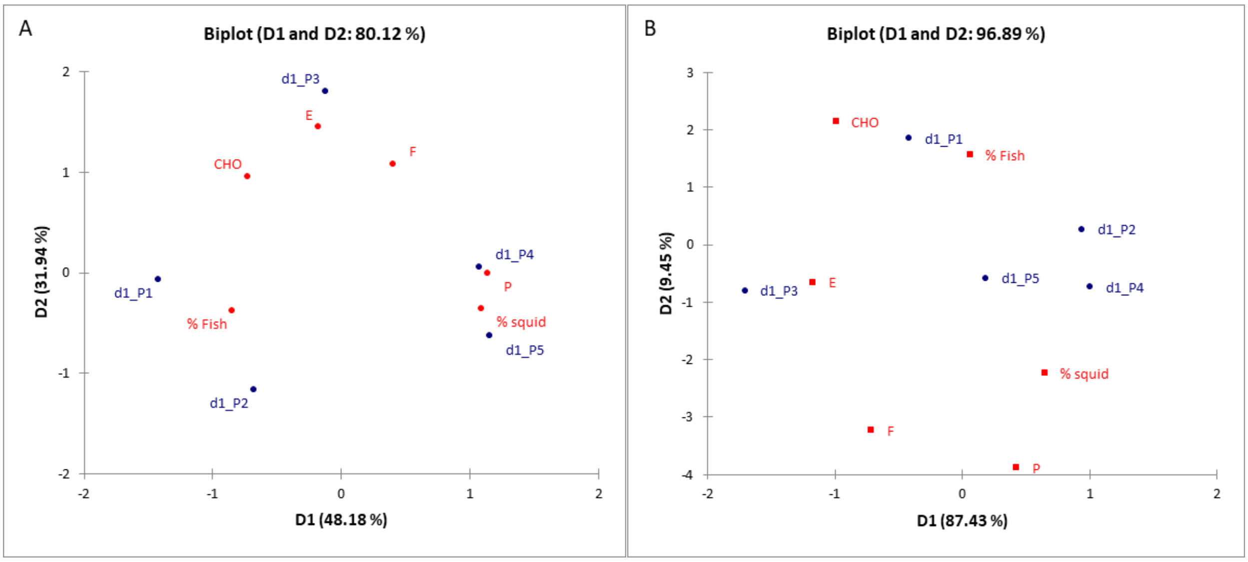

Figure 3. Distribution of the fish stick samples based on nutritional composition (A) and the NIR scan related to the nutritional composition (B)

The qualitative differentiation is confirmed to be successful when the NIR scan was included in the PCA observation (Figure 3B vs Figure 3A). These findings created the predispositions for the next step, which is the quantitative assessment, i.e. the possibility of potential prediction of the nutritional composition of fish sticks based on NIR spectra (Cozzolino et al., 2005; Nilsen and Esaiassen, 2005). To implement this, NIR spectra were used as input data related to currently available nutritional data from the packaging (as outcome variables). The relationship of the mentioned input/output data was presented by following parameters: R^2, SD, MSE, RMSE, RER and the RPD (Birkić et al., 2022). The trends that are desirable are the lowest errors with as little scatter as possible and the highest possible values of the correlation coefficient, RER and RPD (Balbino et al., 2022). The ratio between the standard deviation of the population and the standard error of cross-validation, RPD value > 3 is considered as a value and such models are recommended for screenings, while RPD > 5 is considered as a good value and such models are suitable for quality control. Also, it is expected that the range error ratio (RER) is larger than the RPD values (Fearn, 2002). The strongest correlation between the NIR spectra information and the information from the product label was determined for proteins ($R^2 \approx 0.99$) and all RER values were, as expected, higher that the RPD values. It can be seen from Table 2 that almost all parameters could be screened by NIRs. Content of proteins and fish share in the products are even suitable to be screened using NIRs. The reason for this are the vibrations of certain molecule bands of the illuminated sample which absorbed light selectively and generated specific NIR spectra (Downey, 1996; Folkestad et al., 2008; Mathiassen et al., 2011). In this case, the NIR spectra shows the overtone and combination bands of N-H, O-H, and C-H groups (Balbino et al., 2022, Yu, 2021).

Table 2. Relation of nutritive information and NIR spectra calculated by partial linear regression

Parameter	R^2	SD	MSE	RMSE	RER	RPD
Fish (yes/no)	0.917	0.315	0.020	0.141	7.088	3.882
Energy (kcal)	0.825	26.940	145.153	12.048	6.806	2.670
Proteins (g)	0.988	0.537	0.058	0.240	24.553	9.989
CHO (g)	0.906	4.833	4.672	2.161	8.006	3.659
Fat (g)	0.925	0.990	0.196	0.443	11.289	4.072
Fish (%)	0.948	10.300	21.150	4.600	11.957	4.901
Squid (%)	0.894	22.540	101.630	10.080	6.448	3.426

The proteins are related to the N-H band vibrations (Pérez-Marín et al., 2006) which correspond to the vibration in the range of 1400-1600 nm (second overtone region) as well as in the range of 900-1100 nm (third overtone region) (Eldin, 2011) which is in the range of the used device, 900-1700 nm.

Conclusions

The results confirmed the possibility of applying NIRs in the qualitative assessment of fish sticks composition for which it is possible to determine whether it has dominant share of fish or squid. NIR is also capable of protein content estimation, which can prove to be important in determining the price of the products. Namely, all products with animal protein content have higher prices, which grow in proportion to the share of proteins they contain. Consequently, NIR screening can provide an insight whether the price of the product is justified based on the protein content. Although NIRs is not a qualitative method, it can help in the screening of food product composition, with the condition that this composition is later confirmed by additional analytical methods.

References

Balbino, S., Vincek, D., Trtanj, I., Egređija, D., Gajdoš-Kljusurić, J., Kraljić, K., Obranović, M., Škevin, D. (2022): Assessment of Pumpkin Seed Oil Adulteration Supported by Multivariate Analysis: Comparison of GC-MS, Colourimetry and NIR Spectroscopy Data, *Foods* 11(6), 835.

Birkić, A., Valinger, D., Jurinjak Tušek, A., Jurina, T., Gajdoš Kljusurić, J., Benković, M. (2022): Evaluation of the Adsorption and Desorption Dynamics of Beet Juice Red Food Colour on Alginate Microbeads, *Gels* 8(1), 13.

Bøknæs, N., Jensen, K.N., Andersen, C.M., Martens, H. (2002): Freshness Assessment of Thawed and Chilled Cod Fillets Packed in Modified Atmosphere Using Near-infrared Spectroscopy, *LWT - Food Sci. Tech.* 35(7), 628-634.

Cozzolino, D., Murray, I., Chree, A., Scaife, J.R. (2005): Scaife Multivariate determination of free fatty acids and moisture in fish oils by partial least-squares regression and near-infrared spectroscopy, *LWT - Food Sci. Tech.* 38(8), 821-828.

Downey G. (1996) Non-invasive and non-destructive percutaneous analysis of farmed salmon flesh by near infra-red spectroscopy, *Food Chem* 55(3), 305-311.

EC (2021): Monthly Summary of Articles on Food Fraud and Adulteration, Food and Fraud Cases. 2021. https://knowledge4policy.ec.europa.eu/node/47003_hr

EC (2022): Supporting policy with scientific evidence: Food Fraud. https://knowledge4policy.ec.europa.eu/node/221_hr

EEA Report (2016): Seafood in Europe, A Food system approach for sustainability. Report No 25/2016.

Eldin AIB (2011): Near Infra-Red Spectroscopy. In: Wide Spectra of Quality Control (ed. Akyar, I). Intech.

Ericksen, P.J. (2007): 'Conceptualizing food systems for global environmental change research', *Global Environmental Change* 18(1), 234–245.

FAO (2016): The state of world fisheries and aquaculture. Contributing to food security and nutrition for all, Food and Agriculture Organization of the United Nations, Rome.

FAO FishStat (2016): 'Marine fish + crustaceans' (http:// faostat3.fao.org/home/E) accessed 8 August 2016.

Fearn, T. (2002): Assessing Calibrations: SEP, RPD, RER and R2, *NIR News* 13, 12.

Folkestad, A., Wold, J.P., Rørvik, K-A., Tschudi, J., Haugholt, K.H., Kolstad, K., Mørkøre, T. (2008): Rapid and non-invasive measurements of fat and pigment concentrations in live and slaughtered Atlantic salmon (Salmo salar L.), *Aquaculture* 280(1-4), 129-135.

Gustafson, D., Gutman, A., Leet, W., Drewnowski, A., Fanzo, J. and Ingram, J. (2016): 'Seven food system metrics of sustainable nutrition security', *Sustainability* 8, 196.

HLPE (2014): Food losses and waste in the context of sustainable food systems, High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security, Rome.

IOV, international olive council (2019): Method of analysis – Spectrophotometric investigation in the ultraviolet. https://www.internationaloliveoil.org/wp-content/uploads/2019/11/Method-COI-T.20-Doc.-No-19-Rev.-5-2019-2.pdf

Lin, M., Cavinato, A.G., Huang, Y., Rasco, B.A. (2003): Predicting sodium chloride content in commercial king (Oncorhynchus tshawytscha) and chum (O. keta) hot smoked salmon fillet portions by short-wavelength near-infrared (SW-NIR) spectroscopy, *Food Res. Int.* 36(8), 761-766.

Maggio, A., Van Criekinge, T., and Malingreau, J. P. (2015): Global food security 2030: Assessing trends with a view to guiding future EU policies, JRC Science and Policy Reports — Foresight series, European Commission, Joint Research Centre, Publications Office of the European Union, Luxembourg.

Mathiassen, J.R. Misimi, E., Bondø, M., Veliyulin, E., Østvik, S.O. (2011): Trends in application of imaging technologies to inspection of fish and fish products, *Trends Food Sci. Tech.* 22(6), 257-275.

Nilsen, H., Esaiassen M. (2005): Predicting sensory score of cod (Gadus morhua) from visible spectroscopy, *LWT - Food Sci. Tech.* 38(1), 95-99.

Okparanma, R. N., Araka, P. P., Ayotamuno, J. M., & Mouazen, A. (2018): Towards enhancing sustainable reuse of pre-treated drill cuttings for construction purposes by near-infrared analysis : a review, *JCECT* 9(3), 19–39.

Pérez-Marín, D., Fearn, T., Guerrero, J.E., Garrido-Varo, A. (2009): A methodology based on NIR-microscopy for the detection of animal protein by-products, *Talanta* 80(1), 48-53.

STECF (2014): The economic performance of the EU aquaculture sector (STECF-14-18), JRC Science and Policy Reports, European Commission; Scientific, Technical and Economic Committee for Fisheries, Publications Office of the European Union, Luxembourg, EUR 27033 EN, JRC 93169, 451.

Uddin, M., Okazaki, E., Ahmad, M.U., Fukada, Y.,Tanaka, M. (2006): NIR spectroscopy: A non-destructive fast technique to verify heat treatment of fish-meat gel, *Food Control* 17(8), 660-664. https://doi.org/10.1016/j.foodcont.2005.04.009

Valinger, D., Gajdoš Kljusurić, J., Kovačević, D., Kurtanjek, Ž. (2011): Application of NIR spectroscopy for monitoring quality of surimi. Proceedings of 13th Ružička days, Today Science – Tomorrow industry, Vukovar, 16-17. September 2011. (ed. D. Šubarić), pp. 431– 438.

Yu, W. (2021): Applications of near infrared spectroscopy for fish and fish products quality: a review, *IOP Conference Series: Earth and Environmental Science* 657, 012115.