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Doctoral dissertation: “Application of liquid chromatography-mass spectrometry to the authentication of fish species and fish containing products”

Summary

Food adulteration is currently a major economic and social problem. It not only has economic consequences, it is also often associated with a threat to the health and even the life of consumers, as a result of exposure to allergens and toxins. In the case of fish and fish products, food adulteration includes swapping one species of fish with other, cheaper ones, introducing additives, e.g., phosphates and vegetable proteins, misleading as to the place where the fish has been caught or the farming regime, and modification of the technological treatment methods. Identification of fish species is particularly difficult due to the high number of species and their close phylogenetic relationships. In addition, during technological processing, morphological features, which are usually used to identify the species of the fish (head, tail, dorsal, ventral fins, scales, skin) are removed. In addition, the number of identified proteins reported in fish protein databases is much lower compared to mammalian proteins, making it difficult to identify peptide markers that can be used to differentiate fish.

Food authentication is a means of ensuring food safety and quality, protecting consumers, ensuring that the composition of products conforms to the information on the product label, and ensuring compliance with food laws and regulations. Therefore, there is a need to develop accurate, reliable, and sensitive analytical methods for the identification of fish species, allowing for the detection of counterfeiting and the enforcement of correct labelling of products.

The main objective of this doctoral dissertation was to investigate whether non-targeted proteomic analysis using liquid chromatography coupled with a high-resolution mass spectrometer (LC-MS) can be successfully applied to the authentication of fish species. The research was focused in particular on the search for and identification of peptide markers differentiating fish species, characteristic for a given fish species, including markers resistant to heat treatment processes. Chemometric methods were also used to support the identification of markers, and predictive models were developed. Then, in order to assess the usefulness of the developed methods, commercially available fish products and prepared fish mixtures were analyzed.

Five species of fish were used as research material, which were divided into two groups: hake and pollock, and pangasius, sole, and common dab. The above species were selected because the State Sanitary Inspectorate indicated that in Poland they are most often swapped. In addition, these are the species the sales of which are high in Poland.

As part of the experimental work, trypsin digestion of proteins from frozen and heat-treated fish, fish products and prepared fish mixtures was made. Then, proteomic analyses were performed using high-performance liquid chromatography quadrupole time-of-flight mass spectrometer (LC-QTOF-MS) in the MS and MS/MS scan mode.

Comprehensive processing of the collected data, using, among others, proteomic analysis software and chemometric tools, allowed to identify a number of temperature-resistant peptidomic markers that differentiate fish species. In the case of the studies conducted for pollock and hake, after a detailed verification of the data, 22 pollock-specific differentiating peptides and 17 hake-specific differentiating peptides were identified. On their basis, a database of specific peptide markers was built. Then, the developed database was used to study the authenticity of fish products containing hake or pollock fillets as the main component.

For pangasius, sole, and common dab analyses, a profiling approach was used, where proteomic data were treated as 'features', and then the corresponding statistical models, PCA and OPLS-DA, were developed using a multivariate data analysis. In addition, as for hake and pollock, databases containing differentiating peptidomic markers for sole, pangasius, and common dab have been developed. Analyses of the test samples allowed for a positive verification of the developed statistical models, as well as verification of the correct operation of the developed databases.

In conclusion, it has been shown that untargeted LC-MS proteomic analyses using a QTof as high resolution mass spectrometer can be successfully used to authenticate fish species. Peptide markers specific to hake and pollock, including markers resistant to heat treatment processes, were identified and their suitability for fish authentication was successfully verified. The use of chemometric modeling using PCA and OPLS-DA allowed the development of a predictive model for the authentication of sole, pangasius, and common dab. Differentiating peptides were collected in databases to enable a rapid implementation of the developed method by food control laboratories for routine fish testing.