ABSTRACT OF THE THESIS

Title:CHEMICAL-MOLECULAR STUDY OF NUTRITIONAL PROFILES FOR IDENTIFICATION OF FISH SPECIES

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The fishery products have been considered a good food for their nutritional characteristics and they are an alternative to the consumption of the meat of animals hunted or bred on firm land. In recent years, the market has tried to satisfy the huge demand for fisheries products also through the expansion of trade and the following import and export all over the world.

According to a study, during 2015 [1], conducted by ISMEA (Institute for Agricultural and Food Market Services), the total production of fish is expected to grow by 2.6%. Consumer demand for fish remains strong; we think that direct human consumption, which accounts for over 85% of all fish uses, is estimated to grow by 2%, to 147.5 million tons.

This resulted in a slight increase *pro-capite* intake of fish, from 20.0 kg in 2014 to 20.1 kg in 2015.

The research project developed during the period 2014-2016 and its purpose is the improvement of information and knowledge of identifying nutrient profiles of different fish species. We use an approach that combines chemical and genetic-molecular analysis.

The aim of the work was to translate all data of research into useful information for inclusion in a public database: ITTIOBASE. This project is a collaboration between the Department of Chemical and Pharmaceutical Sciences of the University of Ferrara and the Istitute Zooprofilattico Sperimentale delle Venezie - CSI Adria.

The first part of research has been facing a preliminary study of the most representative species of the Italian fisheries and aquaculture. Information on the fish species was collected from local market and we founded 13 different species of fish.

For each species, we used ten animals to be analyzed; each specimen was morphologically identified, classified and photographed.

The second part of the project involved the genetic analysis. We analyzed 118 samples.

Molecular analyzes provided three steps: (1) extraction of nucleic acids from muscle tissue samples, (2) the quantification of DNA (ng/µL) and (3) amplification with PCR end-point. The third step was mase using primers universal (CoiFishF1 and CoiFishR1) of a stretch of about 700bp of the mitochondrial gene coding for the Cytochrome Oxidase First (COI). COI is gene target for Barcoding analysis.[2,3]

The amplification product of each sample was quantified and purified to be able to give a sequence. Finally the sequences were inserted in BLAST to create genetic alignments and confirm genus and species of each sample. The identity (genus and species) for each sample was detected through biomolecular analysis and the data confirm the preliminary results of morphological identification. Sequences were used in studying genetic variability; we observe how this parameter could be present in a highly conserved gene as the cytochrome oxidase I. A clear variability can be noticed in some different species, the one with greater variability was the *Sardina pilchardus*; followed by frigate *Auxis rochei* and *Lepidopus caudatus*. In agreement with other published studies[4] we can highlight absence of variability in the *Thunnus thynnus*.

This lack of variability of a mitochondrial gene leads to the difficulty of the technique barcoding going to correctly identify the species of the genus *Thunnus*.

In the third part of the project, chemical analysis were performed on frozen muscle tissue to assess the lipid profile of the different species. Initially we extracted the lipid component and we continued with the transesterification of triglycerides. The fatty acid methyl esters are separated with GC-MS analysis. Experimental data were evaluated and compared, we can find a possible quantitative differences in lipid profiles. The results are very interesting: in many species such as *Sardina pilchardus*, *Auxis rochei*, *Thunnus alalunga* and *Scomber scombrus*, the DHA is included in a range between 19% and 22%. It is in agreement with the results of many studies than include these categories of fish in the diet for proper intake of omega-3.

Another interesting and not expected data is the high content (range between 2% and 8%) of linolenic acid. This is the precursor of EPA and DHA, in the fresh water species such as *Telestes muticellus, Alosa fallax lacustris* and *Thymallus thymallus*. This suggests a greater enhancement of these fish species in the diet, through an increasing information to the final consumer.

We proceeded with the determination of the PCA; for this analysis we used the data obtained by GC-MS. The PCA of the main components show the formation of well-defined and characteristic clusters for each species. For some species such as *Thymallus thymallus*, *Scomber scombrus* and *Sardina pilchardus,* the clusters are well defined and compact, for other species like *Thunnus thynnus,* the cluster becomes more widely distributed; this can be linked to the presence of different distribution areas of fishing and otherwise homogeneous size for some species.

In the fourth part of the project, the samples of pelagic species are been analyzed in a preliminary study. *Scomber scombrus, Thunnus alalunga, Thunnus thynnus, Auxis rochei*, belonging to the family Scombridae, are used in techniques of stable isotopes of carbon and nitrogen.

The aim was to study how the combination of fatty acid profiles and isotopic measurements could be a new tool for tracking and characterization of these species. The Barcoding can't be discriminatory for the genus *Thunnus* and the application of these techniques, combination of stable isotopes and fatty acid profiles, can bridge the gap of genetic analysis.

In fact the integrated analytical approach, in this work, showed good discrimination of species and a spatial geographic separation in the two largest predators *Thunnus alalunga* and *Thunnus thynnus*.

All information, derived from different techniques, were included in ITTIOBASE database. This information is addressed to a broad audience of consumers, the information is free and easily accessible to everyone; this allows to extend the scientific results of this study directly in an practical and versatile tool that can be used by qualified personnel and the final consumer.

The last part of the project was based on frauds.

In the food sector frauds are considered illegal conduct which affect the legal and commercial rights (contractual/equity) of the consumer. The crime of fraud on the market is achieved regardless of an actual and concrete injury heritage.

It is an act that is configured in a decrease in the value of the goods, economic or nutritional.[5]

When it comes to food fraud it refers to the production, processing, distribution and so on trade in foods does not comply with current legislation.[6,7] The most frequent cases of food fraud to the detriment of consumers are accomplished through false declarations of origin, quality, composition, characteristics of a food.

The purpose of this part was the development of biomolecular methods to investigate the most frequent species replacements; with particular attention to three major families Scombridae, Gadidae-Merlucciidae and Octopodidae.

Techniques HRMA (High Resolution Melt Analysis) and Real-time PCR has been successful for a correct identification of fresh fish products, processed and mixtures.

In particular the case of the identification of *Octopus vulgaris*, with fast and reliable method, fromotheroctopus.

All developed molecular techniques have practical implications through market surveys that have led in some cases to find out the non-compliance in labeling or even the species substitutions.

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