

## Proteomics analysis for the identification of three species of *Thunnus*

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**Abstract** The genus *Thunnus* comprises many species, some of higher quality and commercial value for their excellent organoleptic features, while others of lower quality and value. Consequently, these species are subjected to frequent fraudulent substitution. Increasing trade in fillet and minced fish makes the identification of external anatomical and morphological features of fish impossible. Proteomics was used for the identification of three *Thunnus* species. Muscle extracts were evaluated by both mono- and two-dimensional electrophoresis and mass spectrometric techniques. Preliminary results demonstrate that the tested species displays a high degree of polymorphism, making possible an accurate identification.

**Keywords** Proteomics species identification · *Thunnus* genus ·  
Two-dimensional electrophoresis

### Abbreviations

2DE	two-dimensional electrophoresis
IEF	isoelectric focusing
PCR	polymerase chain reaction
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
SDS-PAGE	sodium dodecylsulfate-polyacrylamide gel electrophoresis

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## Introduction

Species belonging to the *Thunnus* genus often have different qualities and commercial values. The increasing trade of tuna fillets makes fish species identification difficult because their morphological characteristics are partially or completely lost during processing. Several analytical techniques may be used to study and identify prepared fish products. Among these methods, capillary zone electrophoresis (Acuña et al. 2008), isoelectric focusing (IEF) of sarcoplasmic proteins (Rehbein et al. 2000), and amplification of specific DNA fragments, using polymerase chain reaction (PCR) appear to be the most suitable techniques (Pepe et al. 2007).

However, these methods are unable to identify closely related species (e.g., *Thunnus*). Proteomics analysis has been applied to fish products to examine water-soluble muscle proteins from farm and wild fish to show aquaculture effects on seafood quality (Monti et al. 2005). Only recently, proteomics was considered for species identification in seafood products (Carrera et al. 2007).

A classical proteomics approach based on the analysis of muscle proteins by two-dimensional electrophoresis (2DE) allows more accurate fish protein characterization than IEF. Such analyses characterize sarcoplasmic proteins with both their isoelectric points and molecular weights (Piñeiro et al. 1999). In this study, we applied proteomics analyses, for the first time, to the identification of three tuna species (i.e., *T. thynnus*, *T. alalunga*, and *T. albacares*). Sarcoplasmic proteins were studied by 1- and 2DE, and the most significant proteins for the characterization of the three species were analyzed subsequently by mass spectrometry.

## Materials and methods

In this study, six tuna specimens were analyzed, two *T. thynnus* fished in the Mediterranean Sea and supplied by the “Pozzuoli fish market,” two *T. albacares* from Ecuador, and two *T. alalunga* from Palermo. Total sarcoplasmic proteins were extracted from fish muscle (3 g) according to the method of Carrera et al. (2007). Total protein content was measured by the BioRad method. From each tuna species, two different protein aliquots (50 and 10 µg) were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). IEF was performed using 7-cm gel strips in the non-linear pH 3–10 range. In the second dimension (SDS-PAGE), the proteins were separated on a 12.5% (w/w) polyacrylamide gel, at a constant current of 25 mA. Gels were stained with Coomassie Brilliant Blue R-250 dye. ImageMaster™ 2D Platinum software was used for the analysis of the 2D gel images.

The most significant spots for species identification, revealed by the software, were excised from the SDS gel and subjected to an in situ protein hydrolysis protocol. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectra of the peptide mixtures were recorded using a Voyager DE-STR MALDI-TOF instrument (Applied Biosystem) operating in reflector mode. The mass spectra obtained were used for protein identification using the Mascot software. The software compares peptide masses obtained by MALDI-TOF for each spot with the theoretical peptide masses from all proteins included in the peptide mass fingerprinting databases. The National Center for Biotechnology Information (NCBI) databank was accessed, restricting analysis to the pertinent taxonomies. The scarce number of tuna sequences deposited in the database limited the identification of proteins. Therefore, the obtained protein sequences were analyzed for homologies with other fish species in the NCBI database using BLAST.

## Results

SDS-PAGE allowed an evaluation of the efficacy of the extraction protocol and revealed certain differences among the protein patterns of the analyzed tuna species. Protein extracts showed qualitative, inter-species differences, in particular for protein bands with molecular weights less than 10 kDa.

The results obtained by 2DE demonstrate noticeable inter-species differences from the assignment and distribution of the electrophoretic spots, even after visual observation. The analysis of the 2D gel revealed the presence of a protein with a molecular weight of approximately 70 kDa in the *T. thynnus* 2DE pattern, which was absent in the other species. This protein, identified as Trioso fosfato isomerasi (gi46909469) by mass spectrometry might be considered a specific marker. Other proteins in the *T. albacares* and *T. alalunga* 2DE patterns were revealed as species-specific, and similar analyses are currently underway.

## Discussion

Proteomics appears to be a useful method to increase our knowledge on proteins from food-producing animals (Pandey and Mann 2000). The preliminary results from both 1- and 2DE show significant inter-species differences. In fact, proteomic fingerprinting of three species from the *Thunnus* genus was obtained. For *T. Thynnus*, analysis revealed the presence of species-specific proteins that might be used as specific markers. This study offers a new tool for species identification of raw and minced fish. From species-specific proteins, it might be possible to identify species-specific genes and design primers for routine molecular biology methods for processed fish products.

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