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Differentiation of Four Tuna Species by Two-Dimensional Electrophoresis and Mass Spectrometric Analysis

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1. Introduction

Species belonging to the genus *Thunnus* are pelagic predator fishes, commonly known as tuna. The species within this genus are of commercial value, and six of them are considered the most valued in world trade (D.M., MIPAAF, 31 Gennaio 2008). *Thunnus* species originate from a variety of geographic areas, and for this reason the different species can be characterized by the presence of different biological contaminants and sensory characteristics. The species *Thunnus thynnus* has a higher quality and commercial value due to its excellent organoleptic features.

Tuna species are usually consumed as fillets or processed products. The loss of the external anatomical and morphological features makes the authentication of a fish species difficult or impossible and enables fraudulent substitutions (Marko et al., 2004). Species substitution is very common in fish products, due to the profits resulting from the use of less expensive species. For species of tuna, substitutions have both commercial and health implications (Agusa et al., 2005; Besada et al., 2006; Storelli et al., 2010), thus, analytical techniques to differentiate fish species are essential. The development of suitable analytical methods for fish species identification in prepared and transformed fish products is of great interest to enforcement agencies involved with labelling regulations and the authentication of fish in various products to prevent the substitution of fish species (Mackie et al., 2000; Meyer et al., 1995).

Several biochemical techniques enable the study and identification of fillet or minced fish species. Among these methods, isoelectric focusing (IEF) (Etienne et al., 2000; Rehbein et al., 2000; Renon et al., 2001;), capillary zone electrophoresis (Acuña et al., 2008), and amplification of selected DNA sequences by the polymerase chain reaction (PCR) have been used for the identification of certain groups of fish species (Espiñeira et al., 2008; Hubalkova et al., 2008; Pepe et al., 2005, 2007; Trotta et al., 2005).

Presently, PCR is the most frequently used technique, as DNA is heat-stable and resistant to heat treatments that may be applied to the tuna during processing. However, obtaining an accurate species identification is very difficult if the species show a high degree of homology as *Thunnus* does (Chow & Kishino, 1995; Lopez & Pardo, 2005; Michelini et al., 2007; Pardo

& Begoña, 2004; Terio et al., 2010; Viñas & Tudela, 2009). The sequences usually used as species molecular markers are the DNA mitochondrial fragments especially *cytochome b* (*cyt b*) genes and the ribosomal 16S and 12S subunits (Kochzius et al., 2010; Russo et al., 1996; Zehner et al., 1998). Previous studies demonstrated that these molecular markers are not discriminating for *Thunnus* species, because they have few polymorphisms expressed by point mutations (Bottero et al., 2007).

EU Commission Regulation no. 2065/2001 of 22 October 2001 has established detailed rules for consumer information to be included on labels regarding fish species. Accordingly, it is also necessary to develop new methods to prevent illegal species substitutions in seafood products (EC No 2065/2001). Proteins are playing an increasing role in the international scientific community and proteomics, the large-scale analysis of proteins expressed by a cell or a tissue contributes greatly to the study of gene function (Pandey & Mann, 2000). Recently, proteomics has been applied in the fishing industry with several aims, e.g., to examine the water-soluble muscle proteins from farm and wild fish to show aquaculture effects on seafood quality (Monti et al., 2005) or to elucidate the influence of internal organ colonization by *Moraxella* sp. in internal organs of *Sparus aurata* (Addis et al., 2010). Proteomics has also been considered as a tool for species identification in seafood products with interesting results (Carrera et al., 2006, 2007; Chen et al., 2004; López et al., 2002; Piñeiro et al., 1999, 2001).

The aim of this chapter is to examine the potential of proteomics to identify four tuna species through characterisation of specific sarcoplasmic proteins. We investigated *T. albacares*, *T. alalunga,* and *T. obesus* two dimensional gel electrophesis (2-DE) patterns and also verified the presence of specie-specific proteins for these tuna species. Muscle extracts from four tuna species of the genus *Thunnus* (*T. thynnus, T. alalunga, T. albacares, T. obesus*) were evaluated by both mono and 2-DE and mass spectrometric techniques. In preliminary results (Pepe et al., 2010), proteomics was applied for the identification of a species-specific protein in *T. thynnus* by 2-DE profiles. The analysis of two dimensional gels by ImageMaster™ 2D Platinum software revealed the presence of a protein with a molecular weight of approximately 70 kDa in the *T. thynnus*' 2-DE pattern, which was absent in the other species. This protein, identified as Trioso fosfato isomerasi (gi46909469) through mass spectrometric techniques might be considered a specific marker. The aim of this chapter was to investigate *T. albacares*, *T. alalunga,* and *T. obesus* 2- DE patterns and verify the presence of species-specific proteins for these tuna species.

2. Materials and methods

2.1 Fish samples

In this study, a total of four different tuna species were tested, with three specimens from each species. The whole tuna specimens were identified, according to their anatomical and morphological features, as belonging to *T. thynnus, T. alalunga, T. albacores,* and *T. obesus* species at the Department of Animal Science and Food Inspection, University of Naples, "Federico II". *T. thynnus* and *T. alalunga* specimens were fished in the Mediterranean Sea and supplied by "Pozzuoli fish market", *T. albacares* specimens were fished in the Indian Ocean and supplied by Salerno P.I.F. (Posto di Ispezione Frontaliera), and *T. obesus* specimens were fished in the South East Atlantic Ocean and were obtained from Philadelphia, Pennsylvania, United States. Fish were frozen on board at – 20 ° C and shipped in insulated boxes to the laboratory. Tuna muscle samples were taken and stored at -80 ºC for further analysis.

2.2 Extraction of sarcoplasmic proteins

Raw muscle tissue (3 g) was dipped in 6 mL of 10 mM Tris-HCl buffer at 4 ° C, pH 7.2, supplemented with 5 mM PMSF (phenylmethanesulfonylfluoride). Samples were minced with an "Ultra-turrax" at 4 ° C, for 30 s at 15,000 g to obtain a homogeneus sample of water-soluble proteins. Minced tissues were centrifuged at 15,000 g at 4 ° C for 20 min. The supernatants were then recovered and filtered using Ultrafree CL (0.22 μm) filters, and stored at -20 ° C until analysis by electrophoresis (Carrera et al., 2007). The efficacy and the reproducibility of the extraction protocol of sarcoplasmic proteins was evaluated using *T. alalunga*. The extraction protocol was carried out in triplicate and further checked for quality and quantity by SDS-PAGE.

2.3 SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Protein concentration was measured by the Bradford method (Bradford, 1976) using bovine serum albumin as the standard. Proteins (50 μg) were separated on a 12.5% (w/w) polyacrylamide gel at 25 mA/gel constant current. Gels were stained for 50 min with Coomassie Brilliant Blue R-250 and destained with MilliQ grade water.

2.4 Two dimensional electrophoresis (2-DE)

The first dimensional electrophoresis (isoelectric focusing, IEF) was carried out on non-linear wide-range immobilized pH gradients (pH 3-10; 7 cm long IPG strips; GE Healthcare, Uppsala, Sweden) using the Ettan IPGphor system (GE Healthcare, Uppsala, Sweden). Analytical-run IPG-strips were rehydrated with 50 μg of total proteins in 125 μl of rehydratation buffer and 0.2% (v/v) carrier ampholyte for 12h, at 50 mA, at 20° C. The strips were then focused according to the following electrical conditions at 20°C: 500 V for 30 min, 1000 V for 30 min, 5000 V for 10h, until a total of 15000 V was reached. For preparative gels 100 μg of total proteins were used. After focusing, analytical and preparative IPG strips were equilibrated for 15 min in 6 M urea, 30% (V/V) glycerol, 2% (w/V) SDS, 0.05 M Tris-HCl, pH 6.8, 1% (w/V) DTT, and subsequently for 15 min in the same urea/SDS/Tris buffer solution but substituting the 1% (w/V) DTT with 2.5% (w/V) iodoacetamide. The second dimension was carried out on 12.5% (w/w) polyacrylamide gels (10 cm x 8 cm x 0.75 mm) at 25 mA/gel constant current and 10°C until the dye front reached the bottom of the gel, according to (Hochstrasser et al., 1988) MS-preparative gels were stained for 50 min with Coomassie Brilliant Blue R-250 and destained with MilliQ grade water. The software ImageMaster™ 2D Platinum was used for the analysis of the two dimensional gel images.

2.5 Image analysis

Gels images were acquired with an Epson expression 1680 PRO scanner. Computer-aided 2-D image analysis was carried out using the ImageMaster™ 2D Platinum software. Relative spot volumes (%V) (V=integration of OD over the spot area; %V = V single spot/V total spot) were used for quantitative analysis in order to decrease experimental errors. The normalized intensity of spots on three replicate 2-D gels was averaged and standard deviation was calculated for each condition.

A few initial reference points (landmarks) were affixed for gels alignment, the first step of the image analysis. Landmarks are positions in one gel that correspond to the same position in the other gels. Then, the software automatically detects spots, which represent

the proteins on the gels. The software "matches" the gels, and the corresponding spots are paired. The pair is the association between spots that represent the same protein in different gels. Pairs are automatically determined using ImageMaster powerful gel matching algorithm. The different 2DE images can be compared by synchronized 3-D spots view.

2.6 Protein identification by mass spectrometry

2.6.1 In situ digestion

The analysis was performed on the Comassie blue-stained spots excised from gels. The excised spots were washed first with acetonitrile and then with 0.1M ammonium bicarbonate. Enzymatic digestion was carried out with trypsin (10 ng/µl) in 10mM ammonium bicarbonate pH 8.5 at 4° C for 2 h. The buffer solution was then removed and a new aliquot of the enzyme/buffer solution was added for 16 h at 37° C. A minimum reaction volume, enough for the complete rehydratation of the gel was used. Peptides were then extracted washing the gel particles with 1% formic acid and ACN at room temperature.

2.6.2 MALDI-TOF mass spectrometry

Positive Reflectron MALDI spectra were recorded on a Voyager DE STR instrument (Applied Biosystems, Framingham, MA). The MALDI matrix was prepared by dissolving 10 mg of alpha-cyano-4-hydroxycinnamic acid in 1 mL of acetonitrile / water (90:10 v/v). Typically, 1 µl of matrix was applied to the metallic sample plate, and 1 µl of analyte was then added. Acceleration and reflector voltages were set up as follows: target voltage at 20 kV, first grid at 95% of target voltage, delayed extraction at 600 ns to obtain the best signal-to-noise ratios and the best possible isotopic resolution with multipoint external calibration using a peptide mixture purchased from Applied Biosystems. Each spectrum represents the sum of 1500 laser pulses from randomly chosen spots per sample position. Raw data were analysed using the computer software provided by the manufacturers and are reported as monoisotopic masses. Spectra were manually interpreted, there was no need of any De-isotopic or other post acquisition processing due to the good signal to noise ratio. Peak lists were generated manually and used for proteins identification.

2.6.3 LC-MS/MS analysis

A mixture of peptide solution was analysed by LC-MS/MS analysis using a 4000Q-Trap (Applied Biosystems) coupled to an 1100 nano HPLC system (Agilent Technologies) and Agilent HPLC-Chip/MS. The mixture was loaded on an Agilent reverse-phase pre-column cartridge (Zorbax 300 SB-C18, 5x0.3 mm, 5 µm) at 10 µl/min (A solvent 0.1% formic acid, loading time 5 min). Peptides were separated on a Agilent reverse-phase column (Zorbax 300 SB-C18, 150 mm X 75µm, 3.5 µm), at a flow rate of 0.3 µl/min with a 0% to 65% linear gradient in 60 min (A solvent 0.1% formic acid, 2% ACN in MQ water; B solvent 0.1% formic acid, 2% MQ water in ACN). Nanospray source was used at 2.5 kV with liquid coupling, with a declustering potential of 20 V, using an uncoated silica tip from NewObjectives (O.D. 150 µm, I.D. 20 µm, T.D. 10 µm). Data were acquired in information-dependent acquisition (IDA) mode, in which a full scan mass spectrum was followed by MS/MS of the 5 most abundant ions (2 s each). In particular, spectra acquisition of MS-MS analysis was based on a survey Enhanced MS Scan (EMS) from 400 m/z to 1400 m/z at 4000 amu/sec. This scan

mode was followed by an Enhanced Resolution experiment (ER) for the five most intense ions and then MS^2 spectra (EPI) were acquired using the best collision energy calculated on the bases of m/z values and charge state (rolling collision energy) from 100 m/z to 1400 m/z at 4000 amu/sec. Data were acquired and processed using Analyst software (Applied Biosystems).

2.6.4 MASCOT analysis

The mass spectra obtained were then used for protein identification using the MASCOT software that compares peptide masses obtained by MALDI-TOF MS and LC-MS/MS of each spot with the theoretical peptide masses from all the proteins accessible in the databases (Peptide Mass Fingerprinting, PMF). Spectral data were analyzed using Analyst software (version 1.4.1) and MS-MS centroid peak lists were generated using the MASCOT.dll script (version 1.6b9). MS/MS centroid peaks were threshold at 0.1% of the base peak. MS/MS spectra having less than 10 peaks were rejected. MS/MS spectra were searched against NBCI (*National Center for Biotechnology Information*) database, (2006.10.17 version) using the licensed version of Mascot 2.1 version (Matrix Science), after converting the acquired MS/MS spectra in mascot generic file format. The Mascot search parameters were: taxonomy: Animalia; significance threshold: higher than 50 (according to Mascot scoring system, Pappin et al., 1993), allowed number of missed cleavages 3; enzyme trypsin; variable post-translational modifications, methionine oxidation, pyro-glu N-term Q; peptide tolerance 100ppm and MS/MS tolerance 0.5 Da; peptide charge, from +2 to +3 and top 20 protein entries. Spectra with a MASCOT score <25 having low quality were rejected. The score used to evaluate quality of matches for MS/MS data was higher than 30. However, spectral data were manually validated and contained sufficient information to assign peptide sequence.

Little genomic information is available for *Thunnus* genus, so protein identification is limited to a scarce number of tuna sequences deposited in the database. Therefore, once a significant protein match was made, protein sequence data were used for BLAST homology searches against other species in the NCBI database.

3. Results

3.1 SDS-PAGE

The protein extraction protocol developed for *T. alalunga* was used for all examined samples and showed high reproducibility; the extracted proteins were of both good quality and quantity (Figure 1). Protein samples of *T. thynnus, T. albacares*, *T. alalunga,* and *T. obesus* were fractionated by SDS gel electrophoresis as shown in Figure 2. After SDS-PAGE fractionation, some differences could be observed between the different tuna species. SDS-PAGE protein bands, in fact, showed inter-species differences, in particular for proteins with molecular weights lower than 25 kDa.

3.2 Analysis by 2-DE

In order to better elucidate the protein maps of different tuna species, the four samples were subjected to 2D fractionation. Deep analysis of the muscle proteome from the tuna species was undertaken by 2-DE image analysis using the ImageMaster™ 2D Platinum software. The tuna 2-DE images were aligned choosing four landmarks (L1, L2, L3 and L4) in each gel (Figure 3).

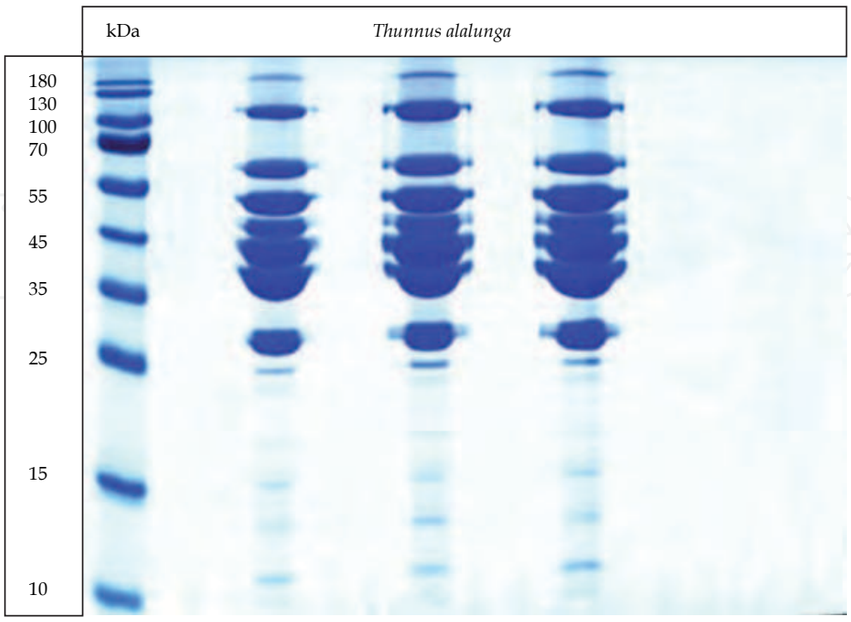

Fig. 1. *T. alalunga* SDS-PAGE. Three different protein samples were compared to verify the reproducibility of the extraction protocol.

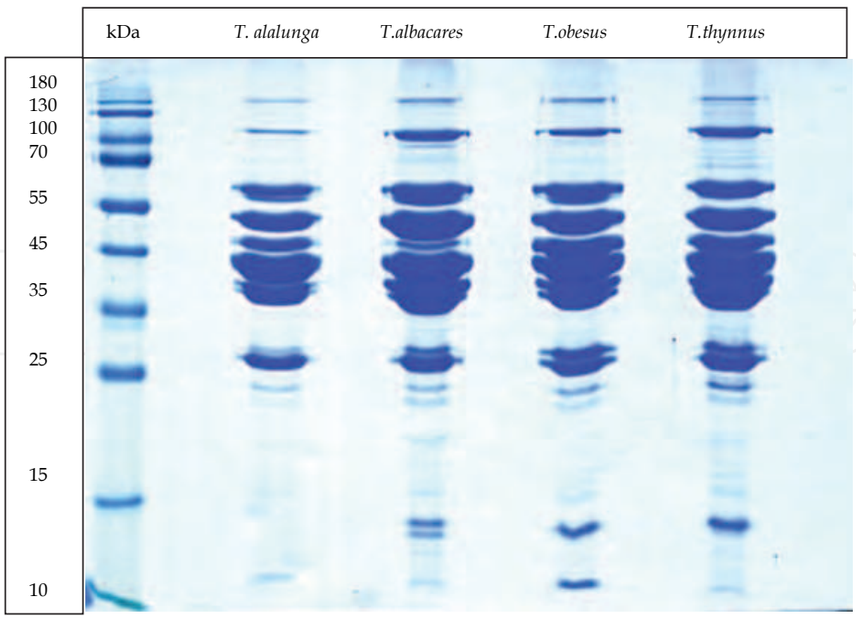

Fig. 2. *T. alalunga, T. albacares, T. obesus,* and *T. thynnus* SDS-PAGE. Proteins with molecular weight lower than 25 kDa are different among species.

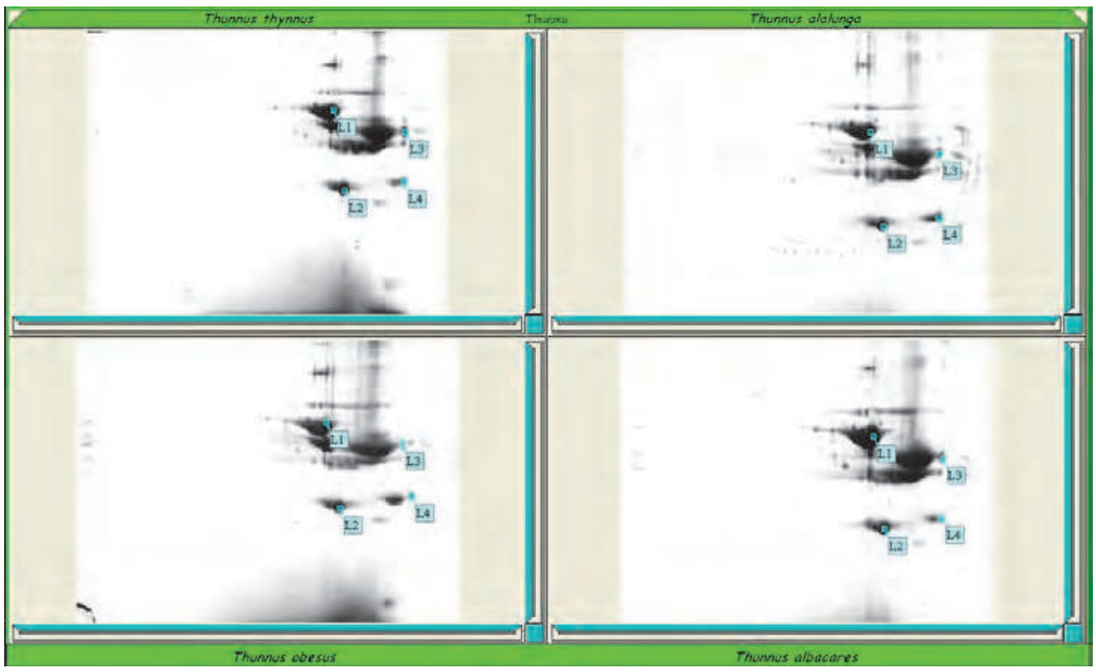

Fig. 3. 2DE gel images alignment: landmarks affixing (L1, L2, L3 and L4).

The software correctly detected and aligned spots between the four tuna 2-DE gel images, as reported in Figure 4. The ImageMaster™ 2D Platinum found: 107 total spots on *T. thynnus* 2-DE gel, 93 total spots on the 2-DE gel of *T. alalunga*, 115 total spots on *T. albacares* 2-DE gel and 123 total spots on the 2-DE gel of *T. obesus*.

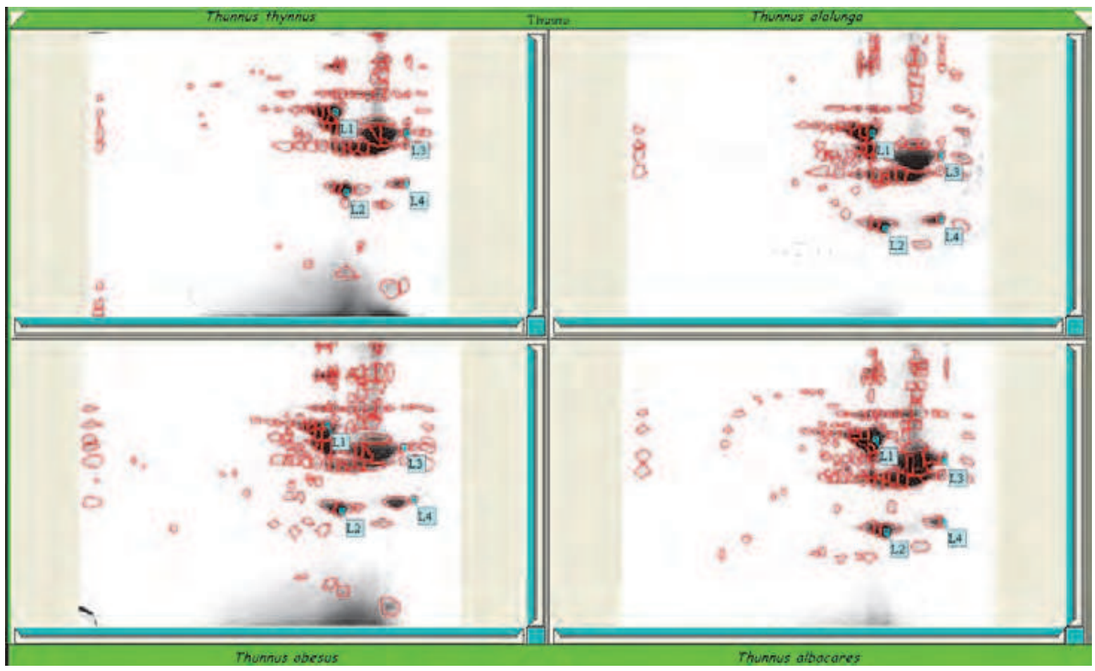

Fig. 4. Spot detection. Spots from the 2-DE arrayed samples representing proteins are circled in red.

Gel matching of tuna 2-DE images indicated the presence of spots that were both common to the four species, and the presence of spots that were specific for each species (Fig. 5). The software detected 28 specific spots on *T. thynnus* 2-DE gel, 48 specific spots on the 2-DE gel of *T. alalunga* , 65 specific spots on *T. albacares* 2-DE gel and 60 specific spots on the 2-DE gel of *T. obesus*.

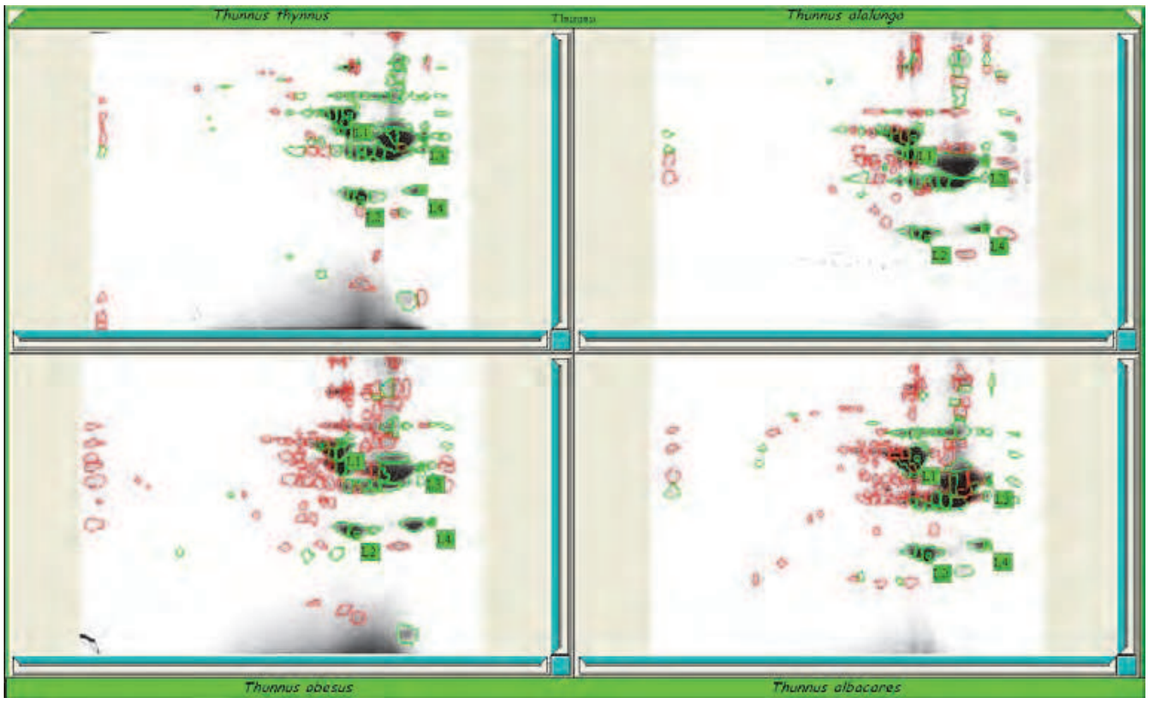

Fig. 5. Gel matching: spots circled in green are common to the four tuna species, spots circled in red are not paired and therefore specific for each species.

3.3 Identification of non-paired/specie-specific spots

The comparison of the 3-D view of the "not paired" spots in the four 2-DE gel images makes it possible to find the most interesting spots for the characterization of the four tuna species (Fig 6-13). These proteins were considered species-specific markers.

3.4 Protein identification

Protein spots were excised from the gel and reduced, alkylated, and in-gel digested with trypsin. The resulting peptide mixtures were analyzed directly by MALDI-TOF MS and/or LC MS/MS. The MS/MS spectra were used to search for a non-redundant match using the in-house MASCOT software, thus taking advantage of the specificity of trypsin and of the taxonomic category of the samples. NCBInr database updates are regularly uploaded to in house version of MASCOT. We filtered identifications restricting to Animalia taxonomy.
Molecular weights values that matched within the given mass accuracy of 100 ppm were recorded and the proteins that had the highest number of peptide matches were examined. Protein identification is limited to a scarce number of tuna sequences deposited in the database. Therefore, for proteins identified with low MASCOT score, protein sequence data were used for BLAST homology searches against other species in the NCBI database. The

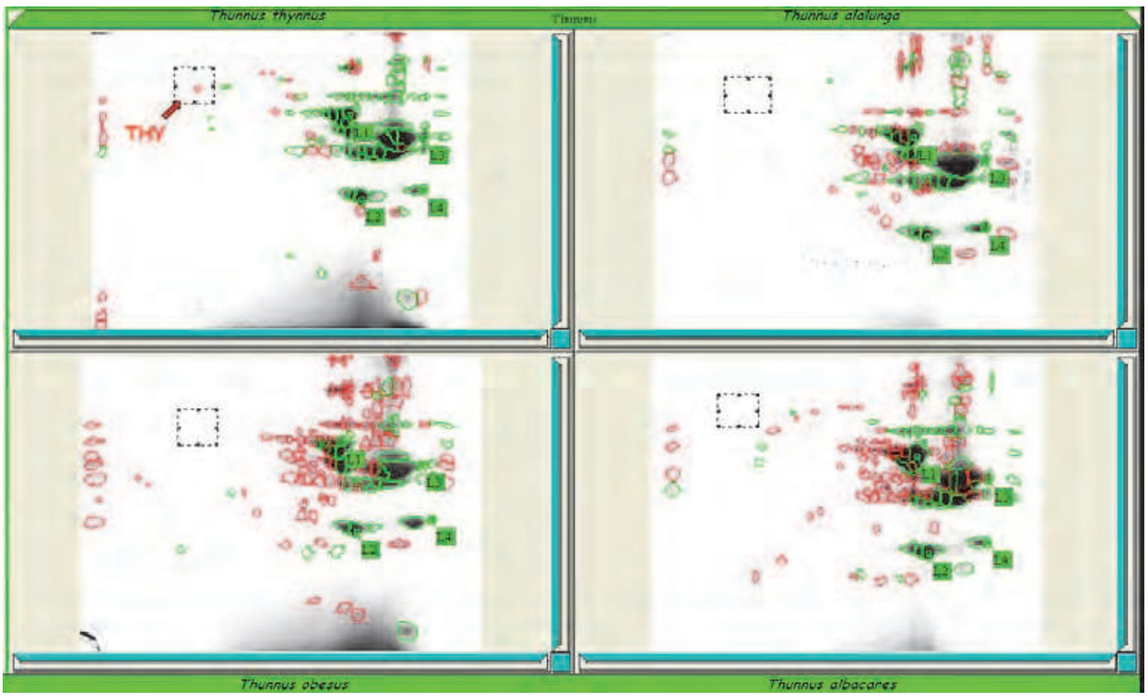

Fig. 6. An example of a *T. thynnus* spot that might be a specific marker (labeled THY). Equivalent areas on all gels highlighted with a box.

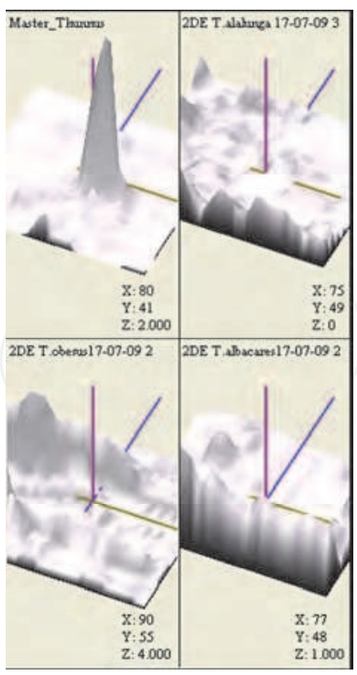

Fig. 7. 3-D view of the *T. thynnus* species-specific spots and 3-D view of the same area from the other species gels. No spot/protein is present in the gel arrayed samples from the other species.

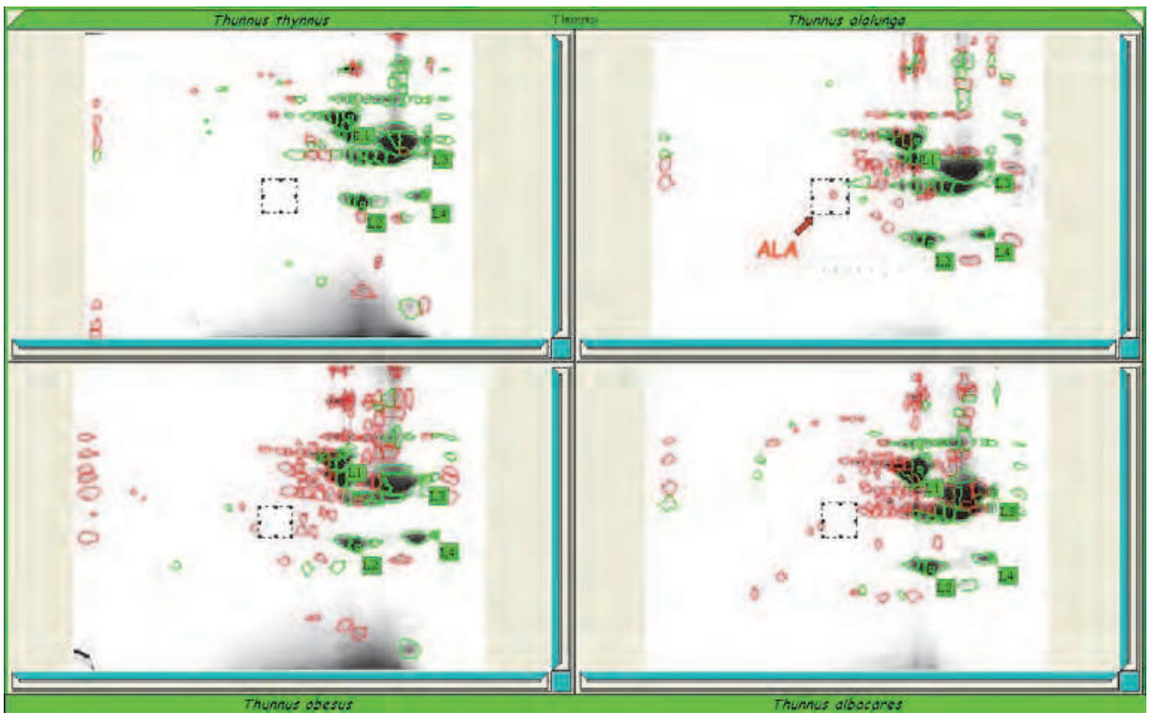

Fig. 8. An example of a *T. alalunga* spot that might be a specific marker (labeled ALA). Equivalent areas on all gels highlighted with a box.

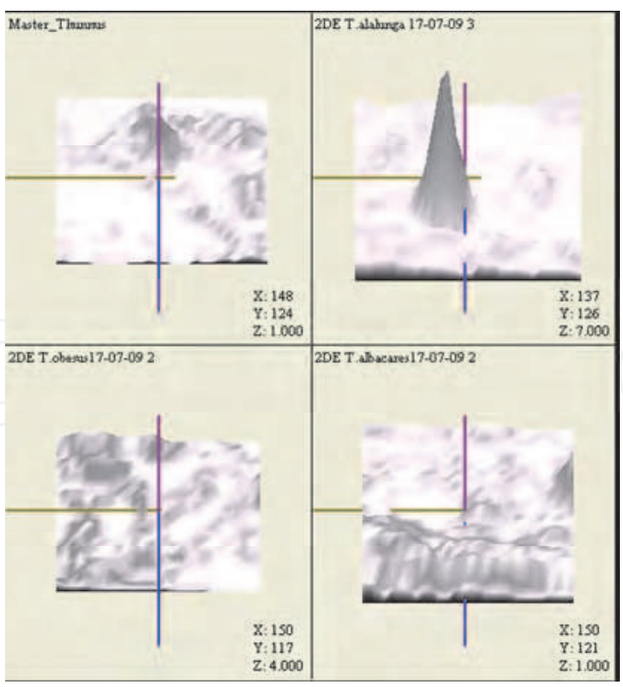

Fig. 9. 3-D view of the *T. alalunga* species-specific spots and 3-D view of the same area from the other species gels. No spot/protein is present in the gel arrayed with samples from the other species.

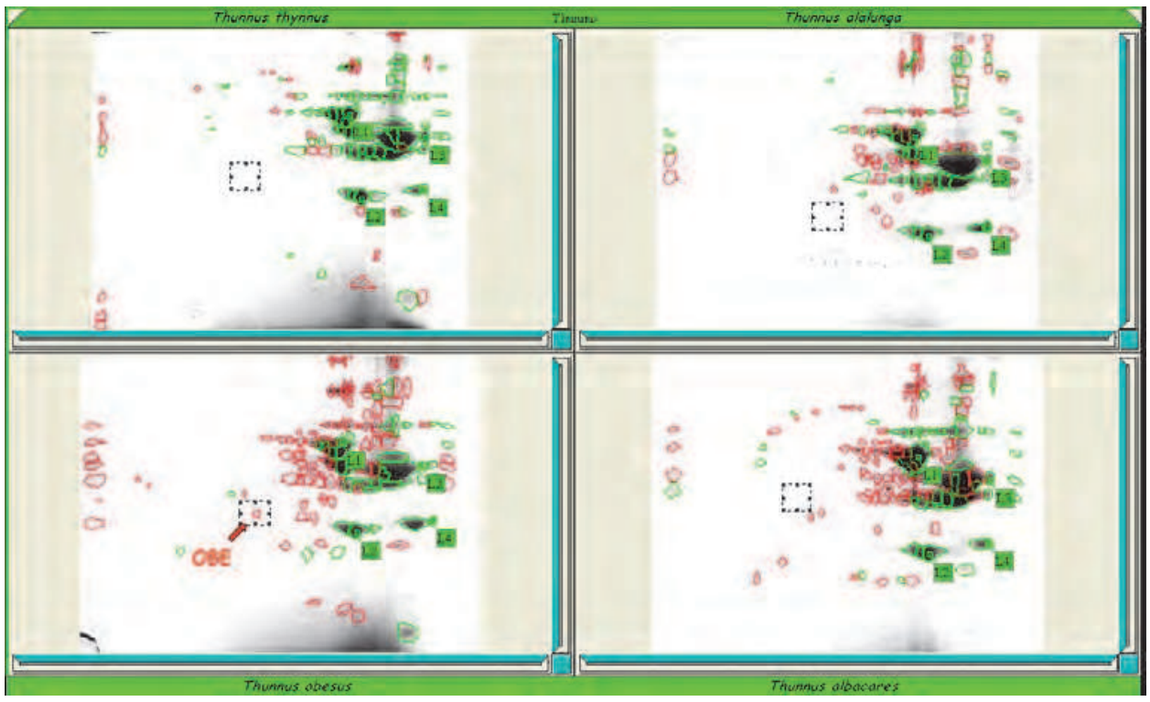

Fig. 10. An example of a *T. obesus* spot that might be a specific marker (labeled OBE). Equivalent areas on all gels highlighted with a box.

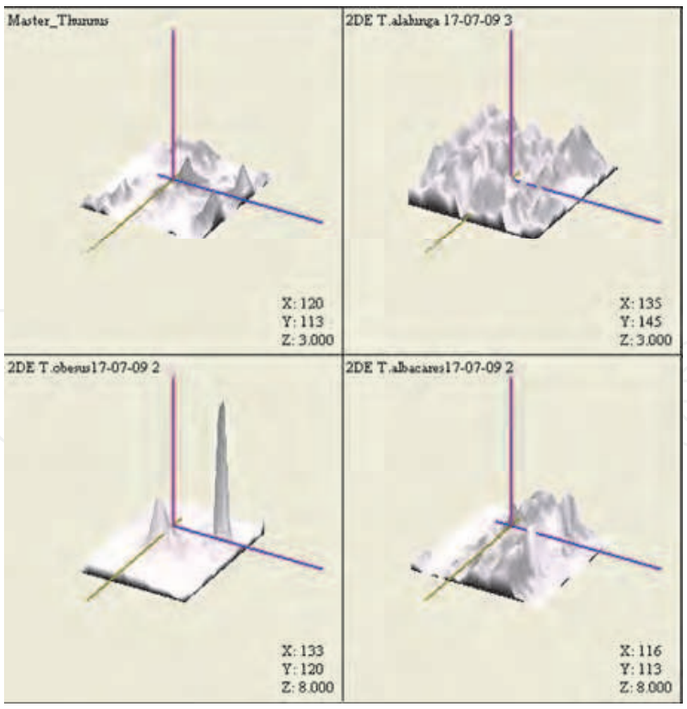

Fig. 11. 3-D view of the *T. obesus* species-specific spots and 3-D view of the same area from the other species gels. No spot/protein is present in the gel arrayed with samples from the other species.

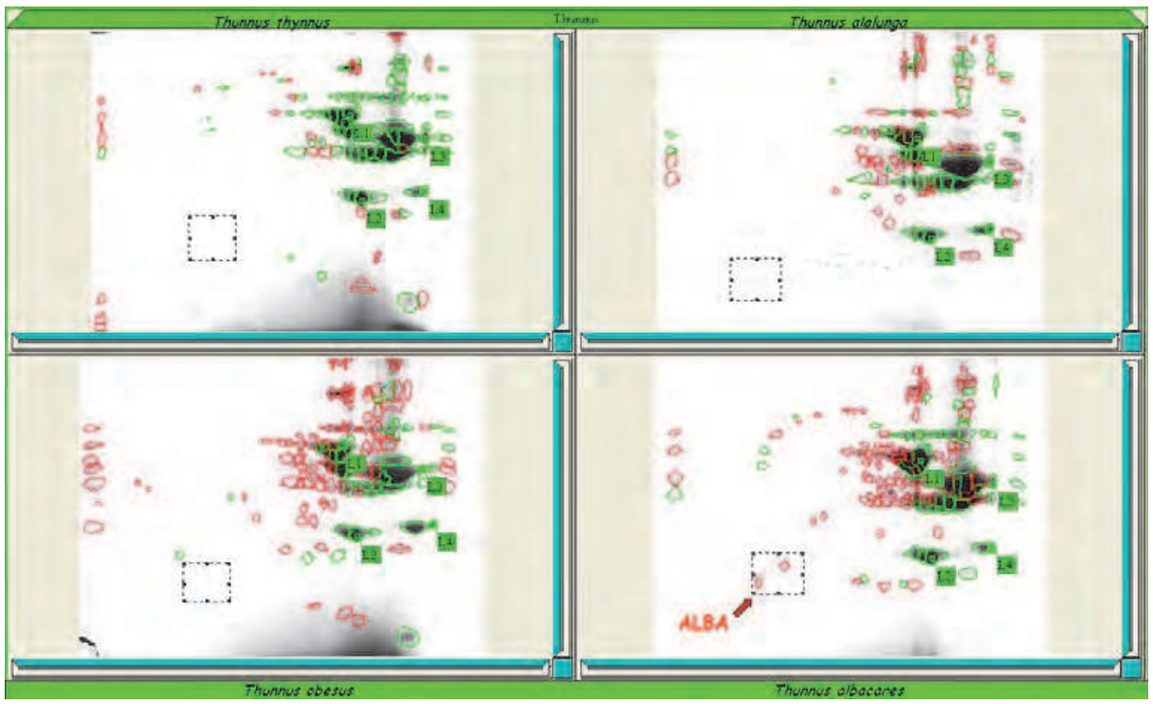

Fig. 12. An example of *T. albacares* spots that might be a specific marker (labeled ALBA). Equivalent areas on all gels highlighted with a box.

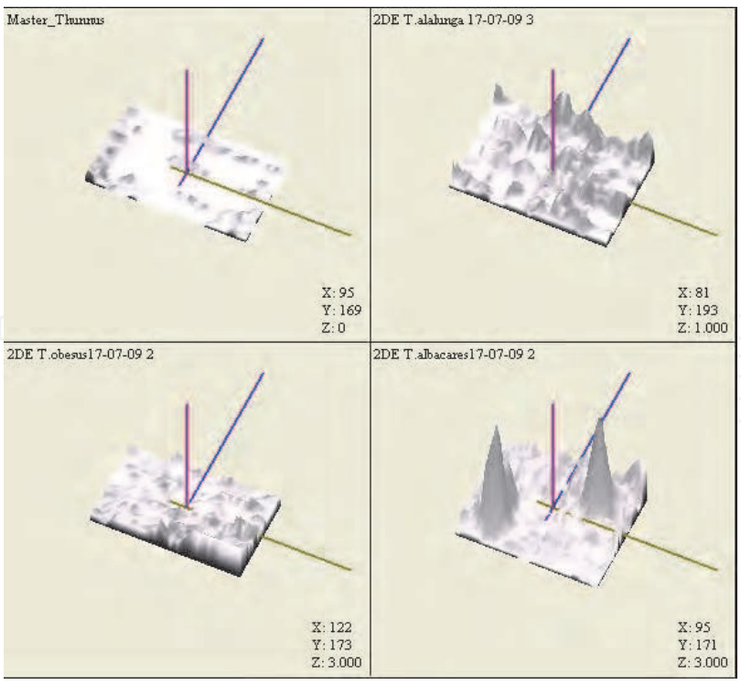

Fig. 13. 3-D view of the *T. albacares* species-specific spots and 3-D view of the same area from the other species gels. No spot/protein is present in the gel arrayed with samples from the other species.

BLAST alignment was done for: Triosephosphate isomerase (THY), Pyruvate kinase (ALA) and Fast skeletal muscle troponin T (ALBA). The results of mass spectrometric analysis of the species-specific markers are shown in Table 1.

Spot ID	Protein	Accession number	Species	M W	pI	Analysis Method	Score
THY	**Triosephosphate isomerase** [Priapulus caudatus]	gi46909469	***T. thynnus***	22.9 kDa	6.51	LC-MS/MS	MASCOT 83 BLAST 421
ALA	**Pyruvate kinase muscle** [Danio rerio]	gi40786398	***T. alalunga***	58.6 kDa	6.54	LC-MS/MS	MASCOT 93 BLAST 1052
ALBA	**Fast skeletal muscle troponin T Subunits** [Gadus morhua]	gi20386541	***T. albacares***	27.2 kDa	9.48	LC-MS/MS	MASCOT 92 BLAST 269
OBE	**Beta-enolase** [Epinephelus coioides]	gi295792264	***T. obesus***	47.5 kDa	6.29	MALDI	MASCOT 159

Table 1. Identification of potential species-marker proteins from 2-DE arrays of the four *Thunnus* species.

4. Discussion

The specific proteins have important metabolic functions. Pyruvate kinase identified in *T. alalunga* is an enzyme involved in glycolysis. This protein catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, yielding pyruvate and ATP. The specific protein identified in *T. Thynnus* is triose phosphate isomerase (TPI), a glycolytic enzyme which catalyzes the interconversion of dihydroxyacetone phosphate (DHAP) with D-glyceraldehyde-3-phosphate. TPI plays an important role in glycolysis and is essential for efficient energy production. Beta-enolase was identified in *T. obesus* and is a muscle-specific enolase (MSE) and is an enzyme of the lyase class that catalyzes the dehydration of 2-phosphoglycerate to form phosphoenolpyruvate. It appears to have an important function in striated muscle development and regeneration. The species-specific *T. albacares* protein is troponin T, fast skeletal muscle subtype. Troponin T (also symbolized TNTF) is the tropomyosin-binding subunit of troponin, the thin filament regulatory complex which confers calcium-sensitivity to striated muscle actomyosin ATPase activity.
Therefore, all the identified species-specific proteins have an important metabolic function. For this reason it is not reasonable to think that these proteins do not exist in the other *Thunnus* species. But the ImageMaster™ 2D Platinum software did not find these proteins in the same localization of the other species 2D gels, which means that these proteins have a different isoelectric point and molecular weight in the other analysed species. The image analysis was correct for species identification, and it was confirmed by the 3-D view; the different spots are proteins and not artifacts caused by aberrant staining of the gel. So, the presence of the species-specific spot in a different area of the gel could indicate (e. g. pyruvate kinase) a higher rate for glycolysis in *T. alalunga.* It is important to continue the studies to enhance the knowledge of the identified species-specific spots and to identify other spots that could have species-specific function.

5. Conclusion

Proteomics has been demonstrated to be a useful method to increase scientific knowledge on animals and plants (Pandey & Mann, 2000). The progress in proteomic analytical techniques has enabled more accurate and reliable information for determining species differences (Tyers & Mann, 2003). The realization of a unique fingerprint for a given species is possible through the separation and subsequent identification of specific proteins.

In this study, a proteomic assay for the identification of species-specific markers of commercially important species of the genus *Thunnus* was undertaken. The proteomic fingerprinting of four species of the genus *Thunnus* was obtained using two dimensional electrophoresis followed by protein identification using mass spectrometry. The analysis of the 2-DE images revealed significant differences between the four tuna species investigated.

The gel matching (Figure 5) shows that there are several different spots between the species, circled in red. The number of species-specific spots identified by the software is substantial for each *Thunnus* species (28 out of 107 total spots for *T. thhynnus*; 48 out of 93 total spots for *T. alalunga*; 65 out of No 115 total spots for *T. albacares* and 60 out of No 123 total spots for *T. obesus*).

The 3-D view of the gels revealed the presence of some red circled spots absent in the same areas from the other species gels. These spots were chosen as species-specific.

The occurrence of species-specific protein spots may be due to differentially expressed proteins only present at low levels or absent in the other species. Thus, 2-DE analysis helped us to identify species-specific proteins, which could be used as specific markers to delineate each species.

The value of a proteomics approach to differentiate tuna species relies on both the ability to obtain the visualization of different protein spots in a 2D map but also the unique identification of the protein candidate by using mass spectral and bioinformatics procedures. Analyses were further enhanced through morphological visualization by 3-D reconstruction of differential spots from the four tuna species. In this way, it was possible to enhance differences and identify highly unique proteins from the *Thunnus* species. This second phase of study further validated the proteomic analysis technique as it confirmed that spots found in different locations and morphology on the 2D gels also corresponded to different proteins.

We have demonstrated that proteomics could be employed to differentiate species when they show contain high degrees of genetic homology (e.g. *Thunnus*). The DNA sequences normally used as species molecular markers are not discriminating for *Thunnus* species (Bottero et al., 2007). Moreover, without the option of a proteomic investigation, it would be necessary to further investigate the genome of each species, to identify genes that may differ between the species. This study shows how the use of proteomics tools is important for species identification.

The future developments of this study should be the identification of other species-specific proteins with metabolic functions characteristic of each species, to then identify species-specific genes. Primers can be subsequently designed for routine molecular biology methods to identify raw and processed fish products by PCR. In fact, PCR is currently routinely used for species identification and maintains this role due to practical attributes such as speed and cost. However, proteomics can provide an immediate and unambiguous identification of protein biomarkers, and in cases where the genomes are similar between species, the analysis of the proteome has a decisive advantage.

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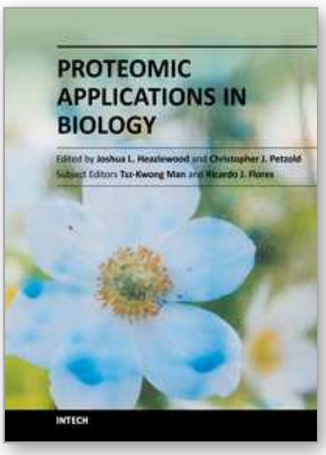

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The past decade has seen the field of proteomics expand from a highly technical endeavor to a widely utilized technique. The objective of this book is to highlight the ways in which proteomics is currently being employed to address issues in the biological sciences. Although there have been significant advances in techniques involving the utilization of proteomics in biology, fundamental approaches involving basic sample visualization and protein identification still represent the principle techniques used by the vast majority of researchers to solve problems in biology. The work presented in this book extends from overviews of proteomics in specific biological subject areas to novel studies that have employed a proteomics-based approach. Collectively they demonstrate the power of established and developing proteomic techniques to characterize complex biological systems.

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