



New Analytical Tools from Proteomics and Lipidomics

Isabel Medina, M. Pazos, L. Méndez and J. M. Gallardo

Instituto de Investigaciones Marinas

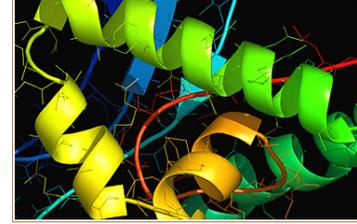
The 3rd “Rendez-vous de Concarneau 30-31 August 2011

Proteomic, Lipidomic and Marine Biotechnology

- ▶ Research areas obviously related to LIPIDS and PROTEINS, respectively and which can be very useful for the optimization of specific biotechnological processes.
- ▶ In the last years, the development of powerful Mass Spectrometers has driven a significant advance in the application of Proteomic to **MARINE BIOTECHNOLOGY** specially coupled to newer **BIOINFORMATIC** tools.
- ▶ **LIPIDOMICS**: Mass Spectrometers and modern NMR use powerful magnets:
High field and High resolution NMR



PROTEOMIC STUDIES



- ▶ **PROTEOME:** Represents the bulk of proteins of an organism, cell, organ or body fluid determined quantitatively. All proteins produced from all genes of a genome.
- ▶ **PROTEOMICS:** Proteome including the processes of protein synthesis, protein-protein interactions, post-translational modifications, and degradation in response to the external environment and ontogenetic events.
- ▶ **Changes** may be directed by factors as growth, differentiation, senescence, environment, genetic manipulation, etc. **PTM** is the chemical modification of a protein after its translation.

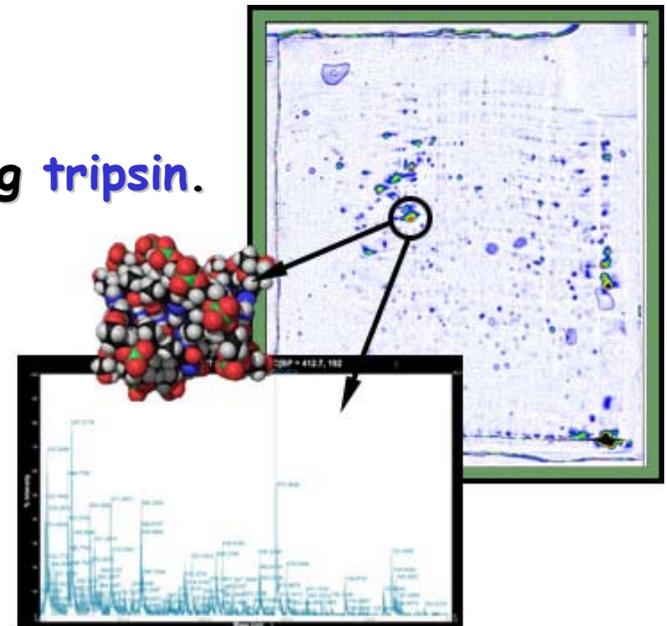
Proteomic Analytical Tools

Proteomic Protocols are based on **PROTEIN ISOLATION** and **MATCH PEPTIDES** to protein identities through the application of MS coupled to database searches.

Different protein isolation protocols and types MS and database searches.

Proteins are digested to peptides by proteases that cut at predetermined sites, commonly using **trypsin**.

- A proteomic project:**
- sample preparation
 - protein separation and analysis with MS
 - data analysis using bioinformatics.



Protein samples



2D Gel Electrophoresis

Trypsin Digestion

Trypsin digestion

Liquid chromatography

MALDI/ESI ionisation

MS analysis

MS data



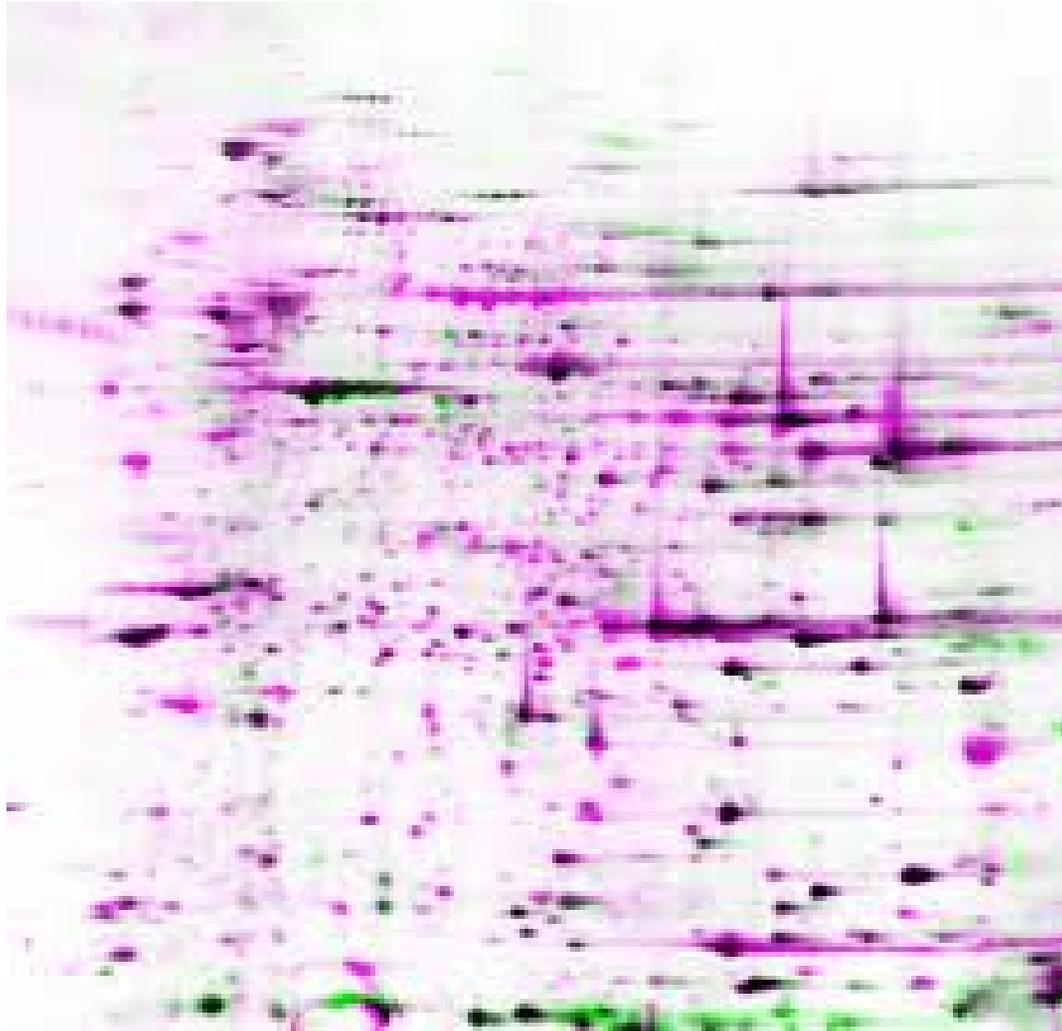
Peptide mass fingerprinting

De novo seq. and homology searches

Plus MS/MS data

Identification of protein and post-translational modification

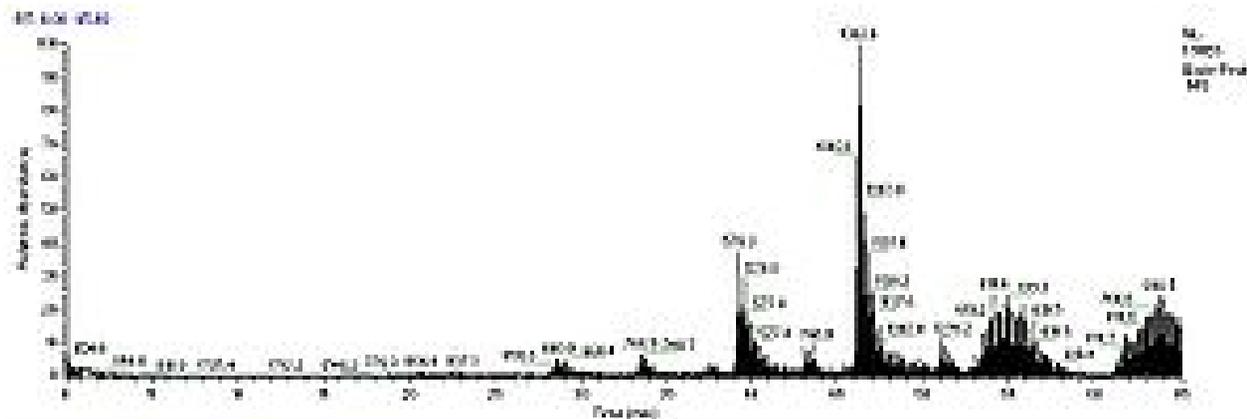
Proteomic Analytical Tools: 2D PAGE



2-D electrophoresis begins with 1-D electrophoresis but then separates the molecules by a second property in a direction 90 degrees from the first

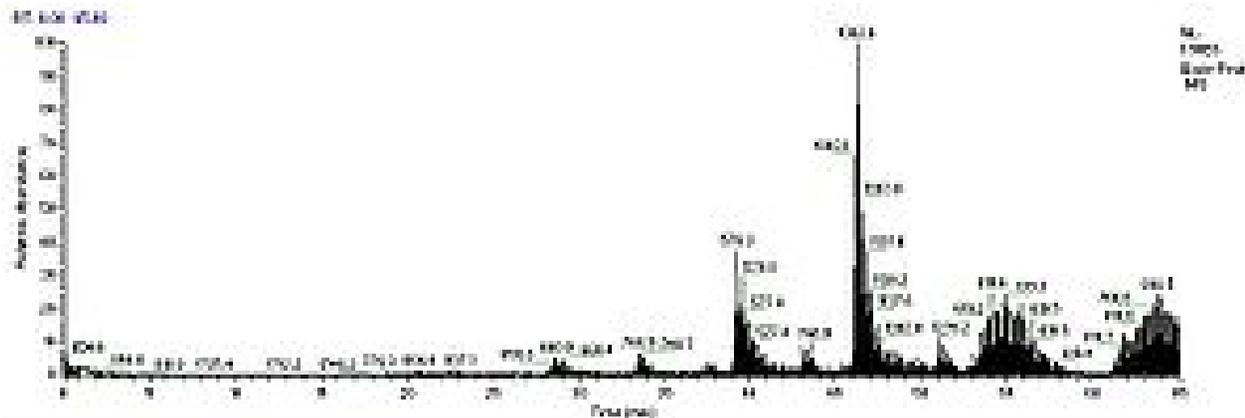
Proteomic Analytical Tools: Mass Spectrometry

1. Masses of peptides, measured as mass (in Da) over charge (m/z), provide a unique **PEPTIDE MASS FINGERPRINT (PMF)**.



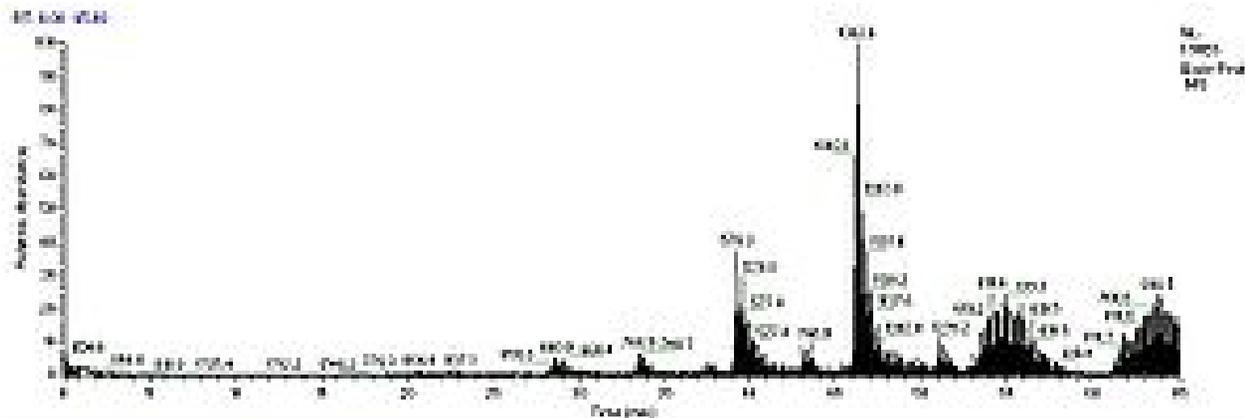
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Proteomic Analytical Tools: Mass Spectrometry

- Using a protein database, the **PMF** can be match to a theoretical digest extracted from all the proteins included in the database.



Proteomic Data Bases

- ▶ It has been impulse: Recent advances in MS leading MS and MS/MS data.
- ▶ However, identifying peptides by interpreting the MS spectra is often difficult, especially for nonmodel organisms, those whose genomes have not been successfully identified.
- ▶ Three types of databases are available: protein databases (**Entrez, Swiss-Prot, and TrEMBL**), genomic databases, and translated expressed sequence tag (EST) datasets.
- ▶ Very few sequences of marine organism are found.
- ▶ Quantification: gel-based (protein spot detection) or gel-free using either stable isotope labeling or label-free spectral counting



Proteomic and Marine Biotechnology: Transgenesis



- ▶ Fish or microorganism transgenesis involves gene integration and transmission steps, the expression of such genes and the subsequent biosynthesis of recombinant proteins.
- ▶ Although the genomes of both wild and transgenic marine organisms are well-defined and not subject to variation, the proteome of organisms may depend on several intracellular and/or extracellular factors and is hence subject to variation, thus being a dynamic state.

Advanced steps of gene expression follow by
PROTEOMICS



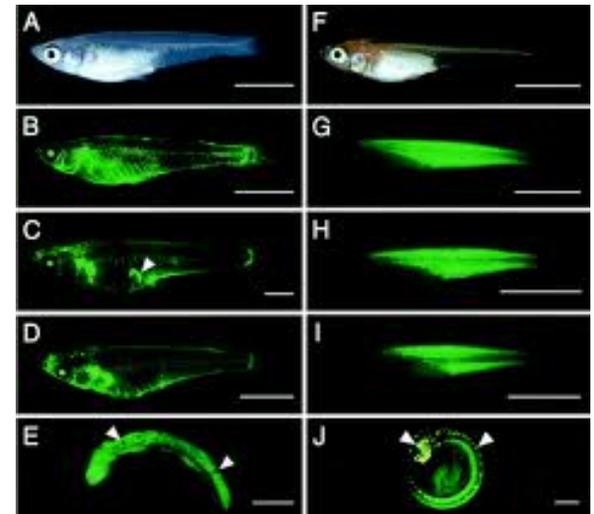
Effect of such factors on the proteome



Optimum levels of a target protein involved in different subjects

Transgenesis & Proteomics: ADAPTATION or GROWTH

- ▶ Aquaculture. Microorganism cultivation.
- ▶ The proteomic changes in wild-type and transgenic radiosensitive fish following exposure to direct irradiation and to X-ray.
- ▶ The proteomic changes reported in transgenic could indicate both immediate protection and longer term adaptation to subsequent radiation exposure.



Transgenesis & Proteomics: GROWTH

- ▶ In AQUACULTURE, studies on the growth rate and in thermo tolerance
- ▶ Growth enhancement strategies: carp, catfish, tilapia and salmon through the incorporation of homologous or heterologous growth hormone genes or cDNAs.
- ▶ Proteomics can be used for studying the cross effect of transgene expression on the biosynthesis of host proteins in genetically improved fish species.



Transgenesis & Proteomics: ALLERGENITY

- ▶ The potential **allergenicity** of proteins in transgenic fish
- ▶ It is important that transgenic fish do not contain new allergens or higher concentrations of known allergens than the same non-*GM* foods
- ▶ In a recent work by Nakamura et al 2010, Regul. Toxicol. Pharmacol., the application of proteomic tools demonstrated that there were no qualitative differences in the allergenic proteins between *GM* and non-*GM*-amago salmons. These results indicate that amago salmon endogenous allergen expression does not seem to be altered by genetic modification.



Proteomics & Antifreezing proteins

- ▶ Both intracellular and extracellular polypeptides involved in the tolerance of Arctic fish at temperatures close to -2°C .
- ▶ Transgenic salmon and transgenic goldfish harboring the gene coding for antifreezing proteins have been developed.
- ▶ Proteomic tools are used for the elucidation of post-translational mechanisms of the processing of antifreeze proteins in this transgenic fish species.
- ▶ Elucidation of **Post-translational mechanism** can permit the biosynthesis of mature antifreezing proteins in a new host.



Marine Biotechnology: Fluorescent proteins



- ▶ **Colorful fluorescent proteins** from marine invertebrates have become highly important: An internal tripeptide sequence that is post-translationally modified to form a cyclized chromophore. Specific and vary practicable form of intrinsic protein fluorescence.
- ▶ The genes of many FPs have been cloned making chimeric proteins in both vivo and in vitro experiments. By using a Proteomic approach it is possible the development of numerous FP variants with different desirable characteristics:
- ▶ Proteomic methodology that combined MS with the sensitivity of FP-based detection methodologies may effectively deal with fundamental structural and functional problems

Proteomic: Environmental Stress

- ▶ Proteomics methods have been also applied to study oxidative stress and to identify protein expression signatures of exposure to marine and estuarine pollutants to understand possible mechanisms of toxicity and xenobiotics in marine ecosystems.
- ▶ Development of Biotechnological Tools: arrays, protein chips...
- ▶ Protein chip technology to analyze the proteomic profile of blue mussel from polluted marine habitats has been applied to discover biomarkers.
- ▶ Changes in sex or sexual maturation



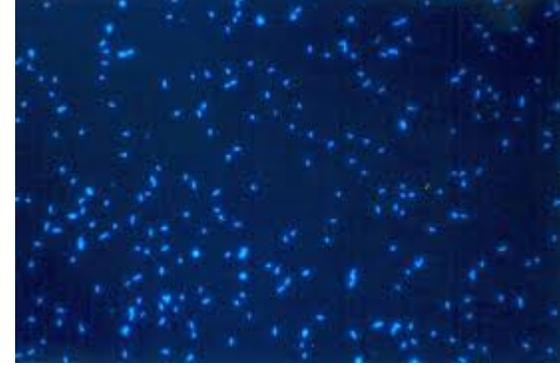
Marine Biotechnology: Molecules of Interest

- ▶ Isolation and characterization of a calponin-like protein from the mussel *Mytilus galloprovincialis*, a polypeptide involved in muscle contraction processes.
- ▶ Likewise, the investigation of mussel adhesive protein which has been reported to harbor an extraordinary biotechnological potential as a biodegradable adhesive material is another promising field for proteomics.
- ▶ There are already companies commercializing recombinant mussel adhesive protein and associated MAP-based smart biomaterials.



Proteomic: Marine bacteria

- ▶ **Marine bacteria: the investigation of marine bacteria by proteomic is still in its early days.**
- ▶ **The protein profiles of thermophilic microorganisms as *Pyrococcus abyssi* investigated when the organism is subjected to different levels of temperature and pressure.**
- ▶ **Studies on the level of protein biosynthesis and expression in *Sphingomonas sp.* cultivated under different conditions.**
- ▶ **Proteome changes related with mechanism of UV protection in cyanobacteria.**
- ▶ **Studies about the relationship between symbiotic bacteria and their hosts. (squid *Euprymna scolopes* due to the presence of the symbiotic marine luminous bacteria *Vibrio fischeri*).**



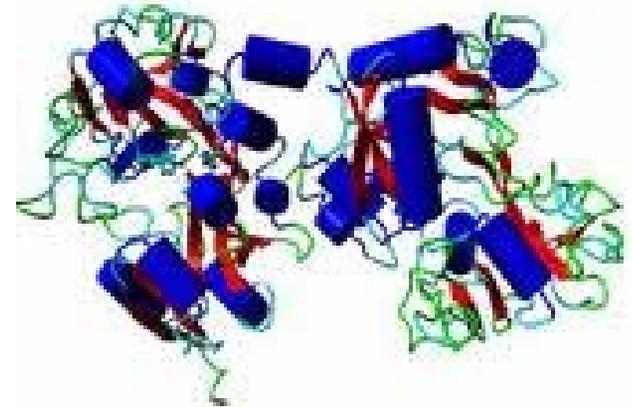
Proteomic: Marine enzymes



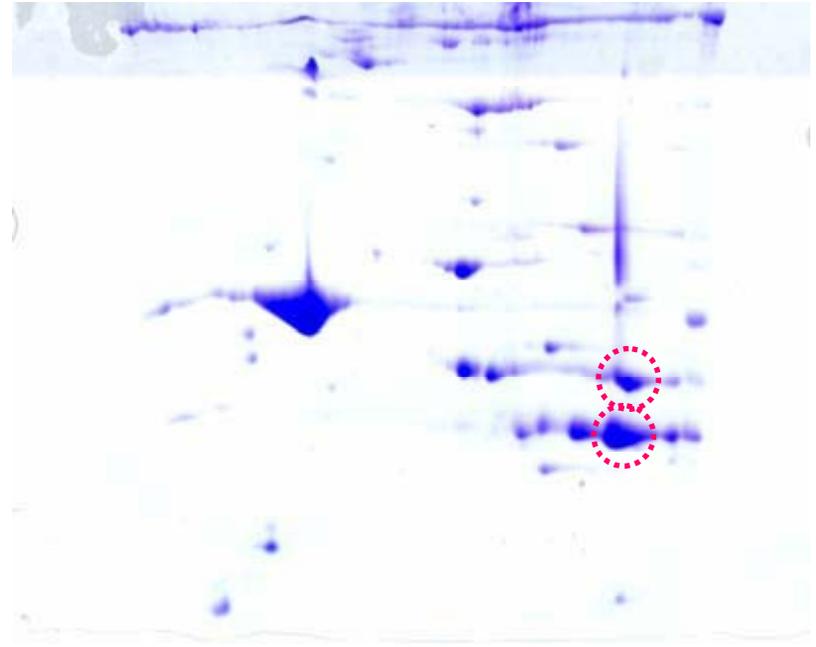
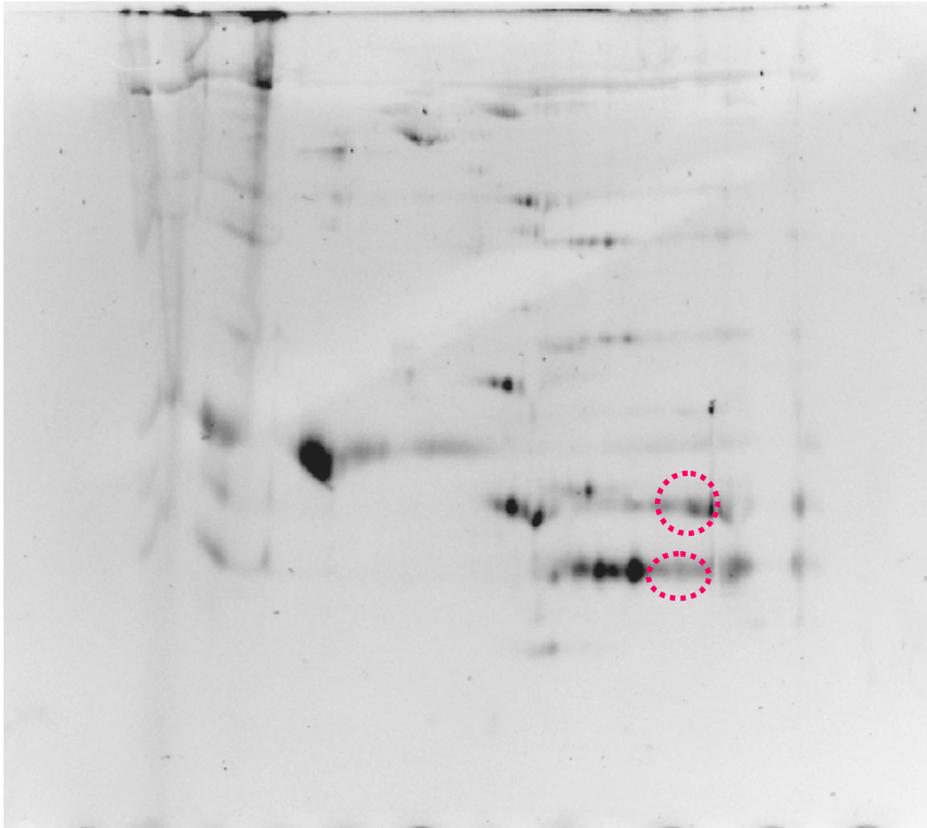
- ▶ Marine enzyme technology is significant focused on the investigation of proteases of industrial interest.
- ▶ Characterization of the peptide sequence of crayfish calciprotein by Fast Atom Bombardment Spectra from the tryptic digests of the protein (Aubagnac et al, 1998).
- ▶ Application of ESI-MS in the investigation of polypeptides in carp.
- ▶ Recent review by Rossano et al, 2011, Digestive Enzymes of the Crustaceans Munida were characterized by Liquid Chromatography and MALDI and then applied in Cheese Manufacturing Source of Digestive Enzymes for Cheese Technology.

Proteomic: Bioactive peptides

- ▶ **Bioactive peptides: MS has been useful for their characterization**
- ▶ **In this field in which several bioactive peptides have been successfully isolated from sources other than marine organisms, the possibility of obtaining such products from marine sources, especially from marine byproducts, is another research domain in which proteomics will surely play a significant role.**
- ▶ **Nutriproteomics: mechanism involved in the effect of bioactives in a given disease: protein expression, oxidation, glycosilation, phosphorilaton,, ...**

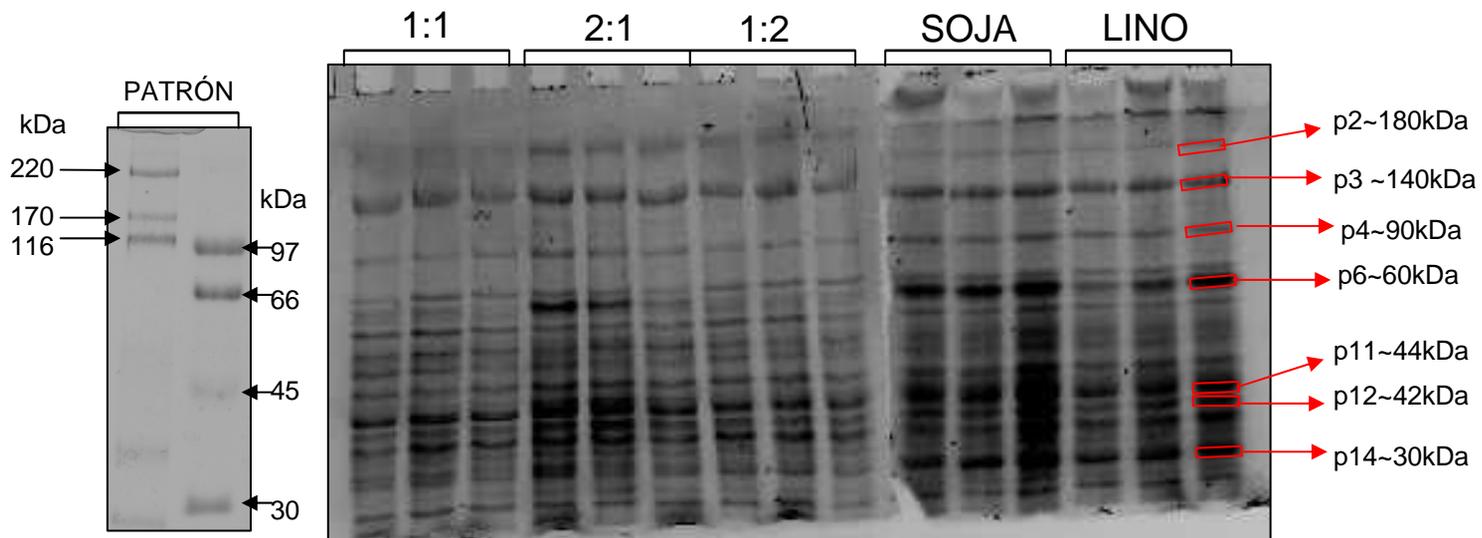
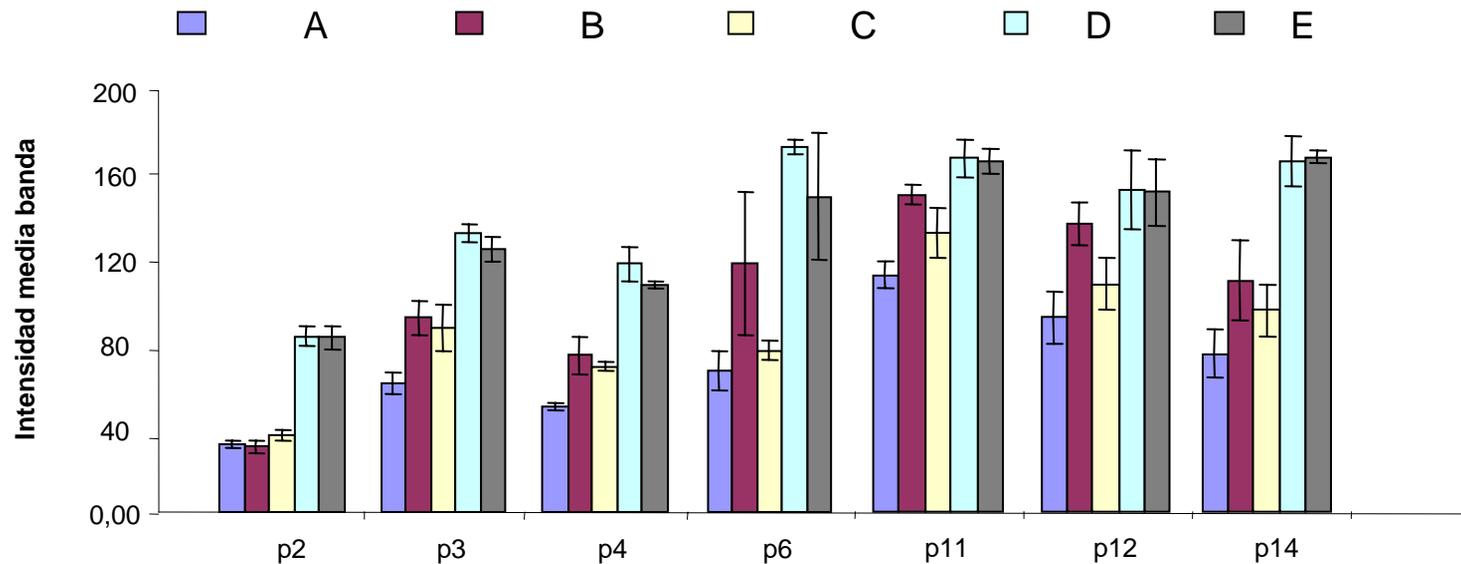


Biotechnology: In vivo Oxidative Processes



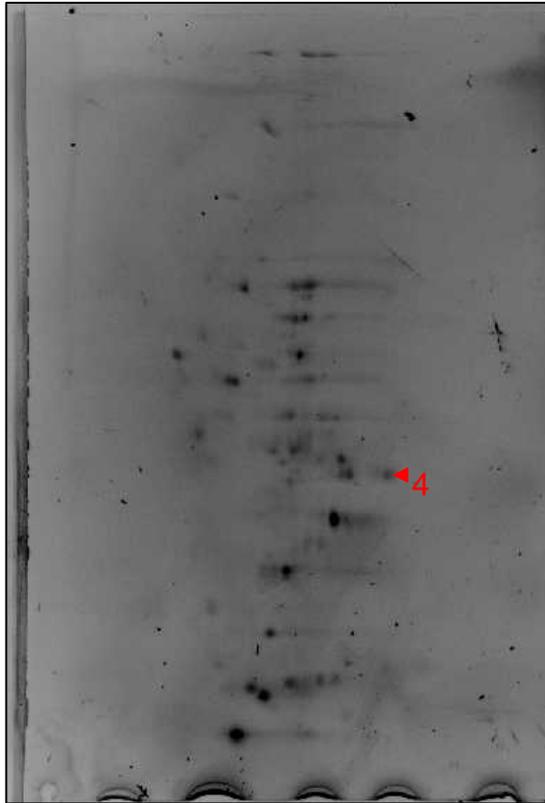
New probes for protein carbonylation: fluorescein-5-thiosemicarbazide (FTSC) and 2-D gel electrophoresis

Oxidation of Liver Cytoplasmatic Proteins WISTAR

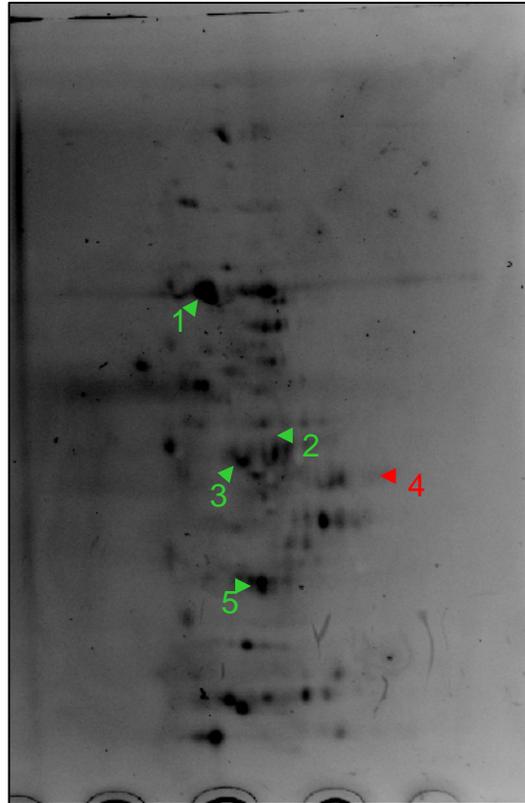


Liver Protein Oxidation

Treatment A



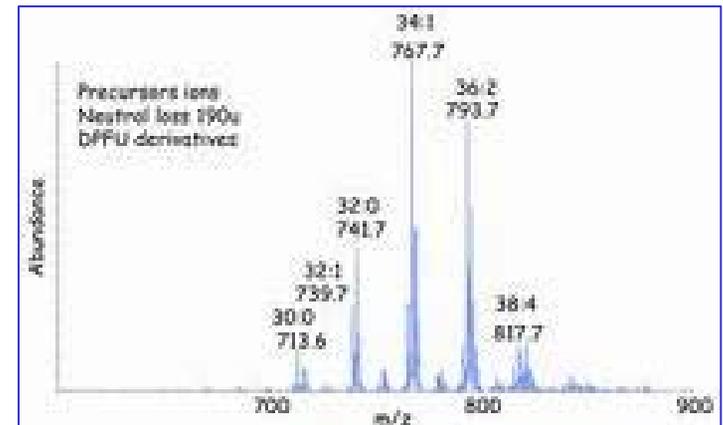
Treatment D



Id no.	Identification
1	ALBÚMINA
2	ARGININOSUCCINATO SINTETASA 1
3	Beta-UREIDOPROPIONASA
	ACIL-CoA DESHIDROGENASA DE CADENA LARGA (ACADL)
4	ASPARTATO AMINOTRANSFERASA
5	3-alfa-HIDROXISTERIODE DESHIDROGENASA

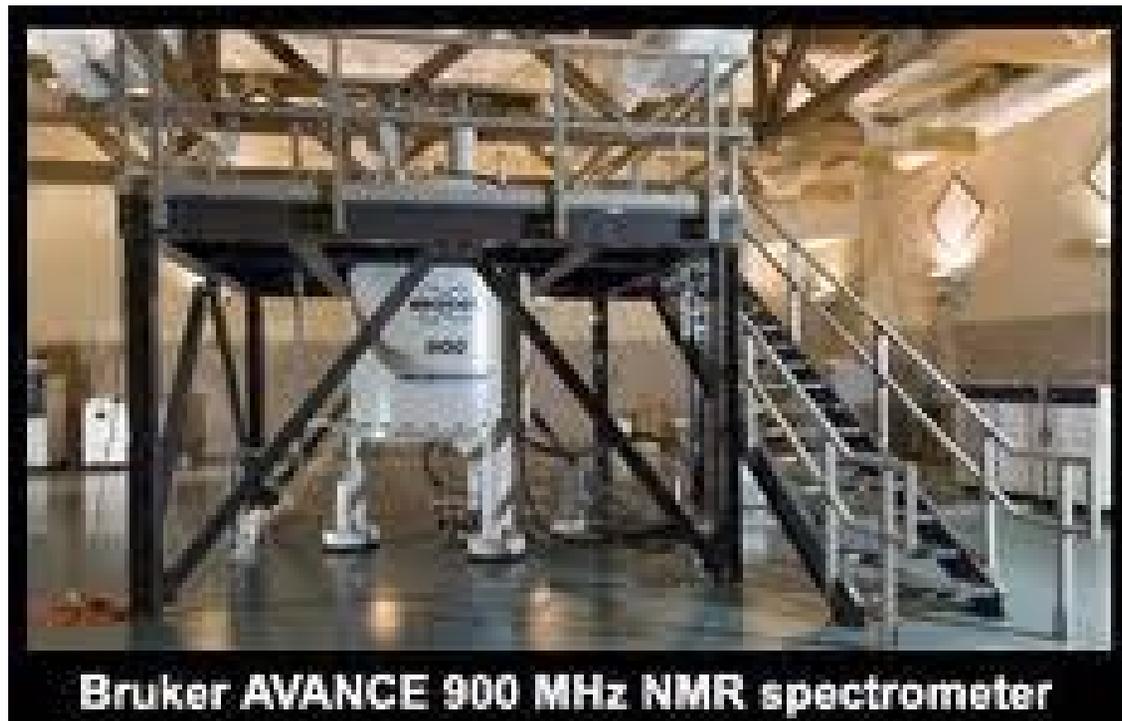
Lipidomic and Marine Biotechnology

- ▶ It is a research field dedicated to the understanding of lipid function through the complete qualitative and quantitative analysis and profiling of lipid species, elucidation of lipid-lipid and lipid-protein interactions, and lipid mediated signalling networks in biological systems.
- ▶ Improvements in analytical techniques have proved highly efficient for the characterization and quantification of molecular lipid species in total lipid extracts



Lipidomic

- ▶ Basically, a lipidome is the comprehensive and quantitative description of a set of lipid species present in an organism. This approach is profiling by high-field NMR, HPLC, MS/MS and LC/MS, GC.

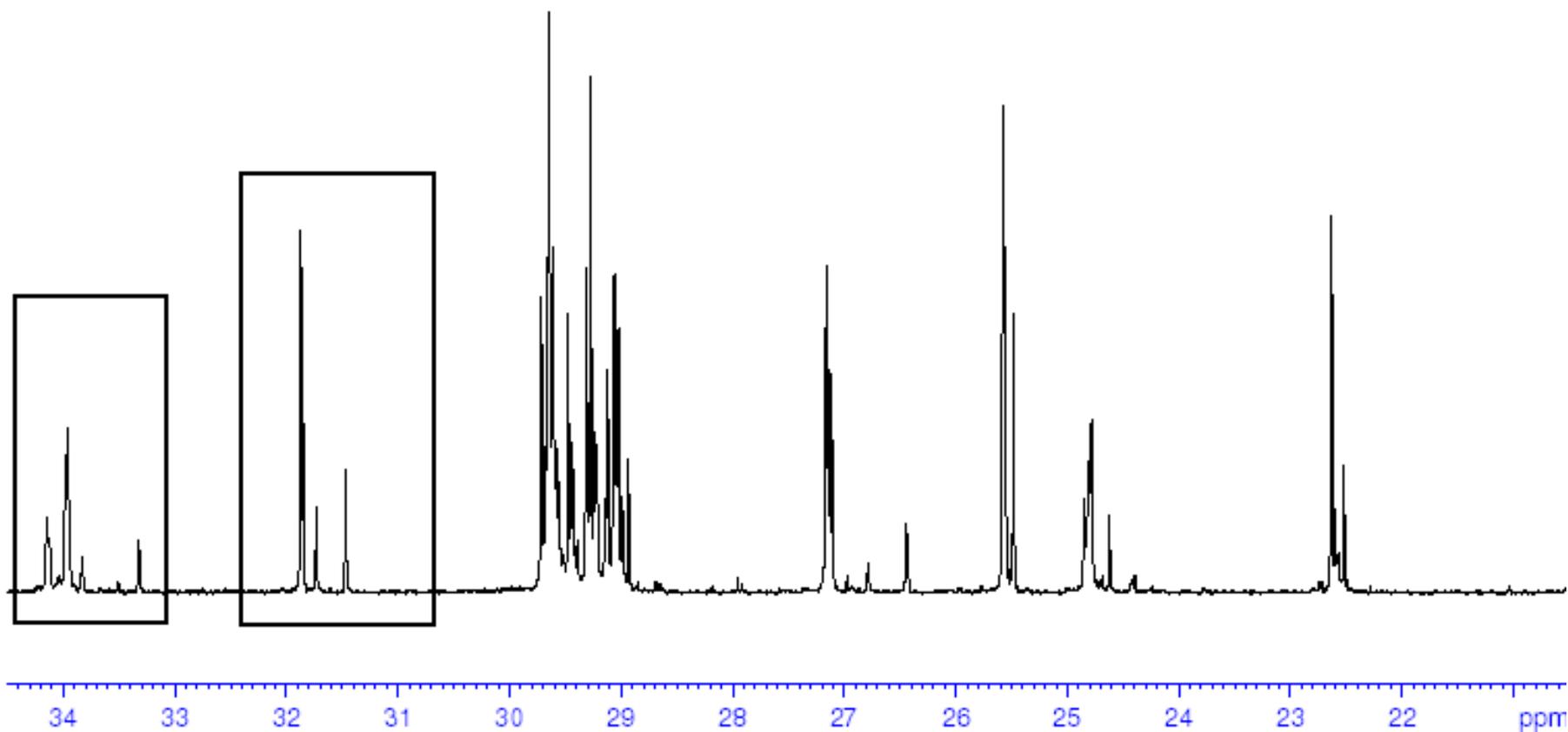


Bruker AVANCE 900 MHz NMR spectrometer

Lipidomic: Improvements in NMR and MS

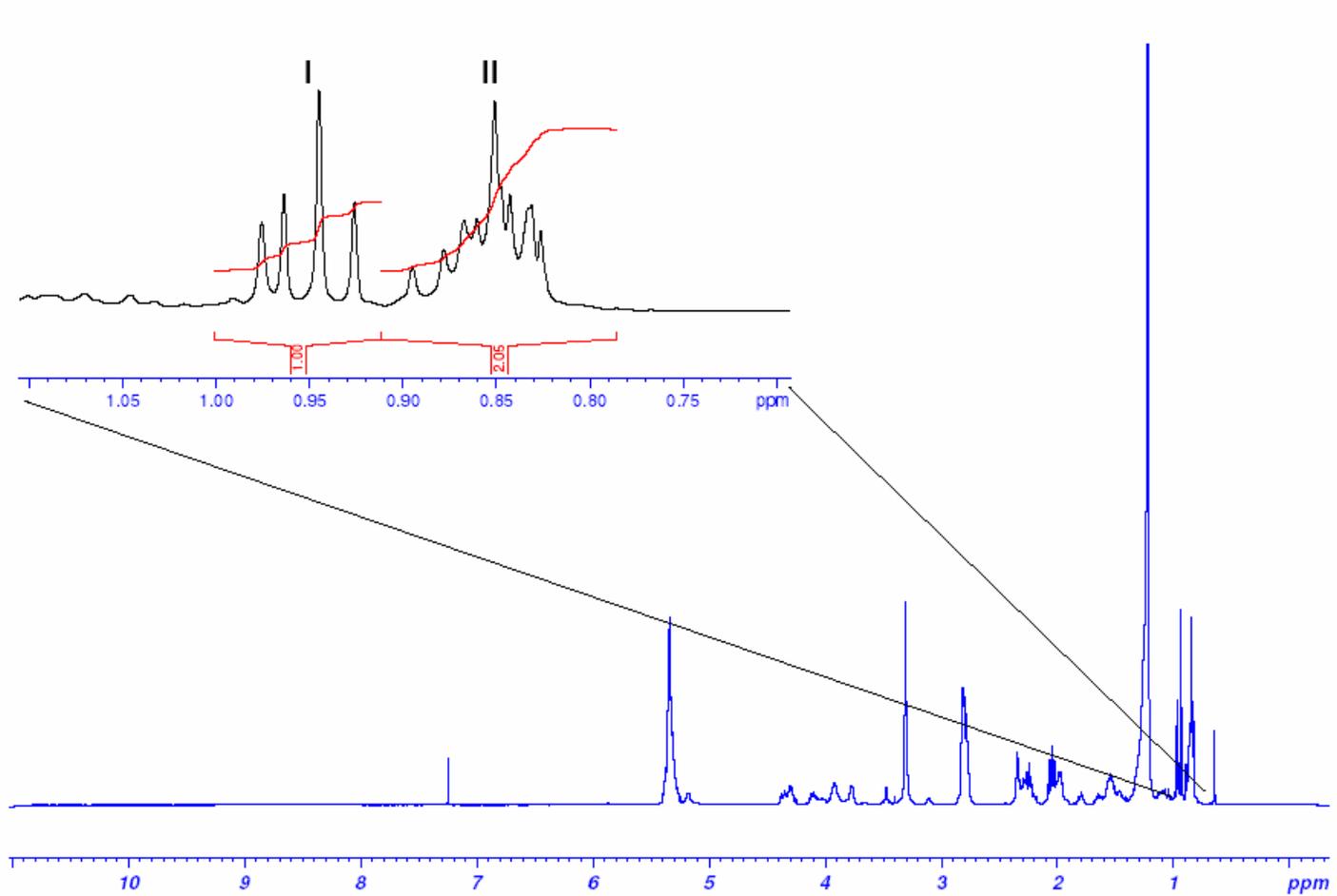
- ▶ **Global Lipidomic Analysis:** Identify and quantify hundreds to thousands of cellular lipid species via a high throughput basis.
- ▶ **Targeted Lipidomic Analysis:** Identification of one or a few lipid classes of interest.
- ▶ **Novel Lipid Discovery:** Directed towards the discovery of novel lipid classes and molecular species.

Lipidomic: Improvements in NMR and MS



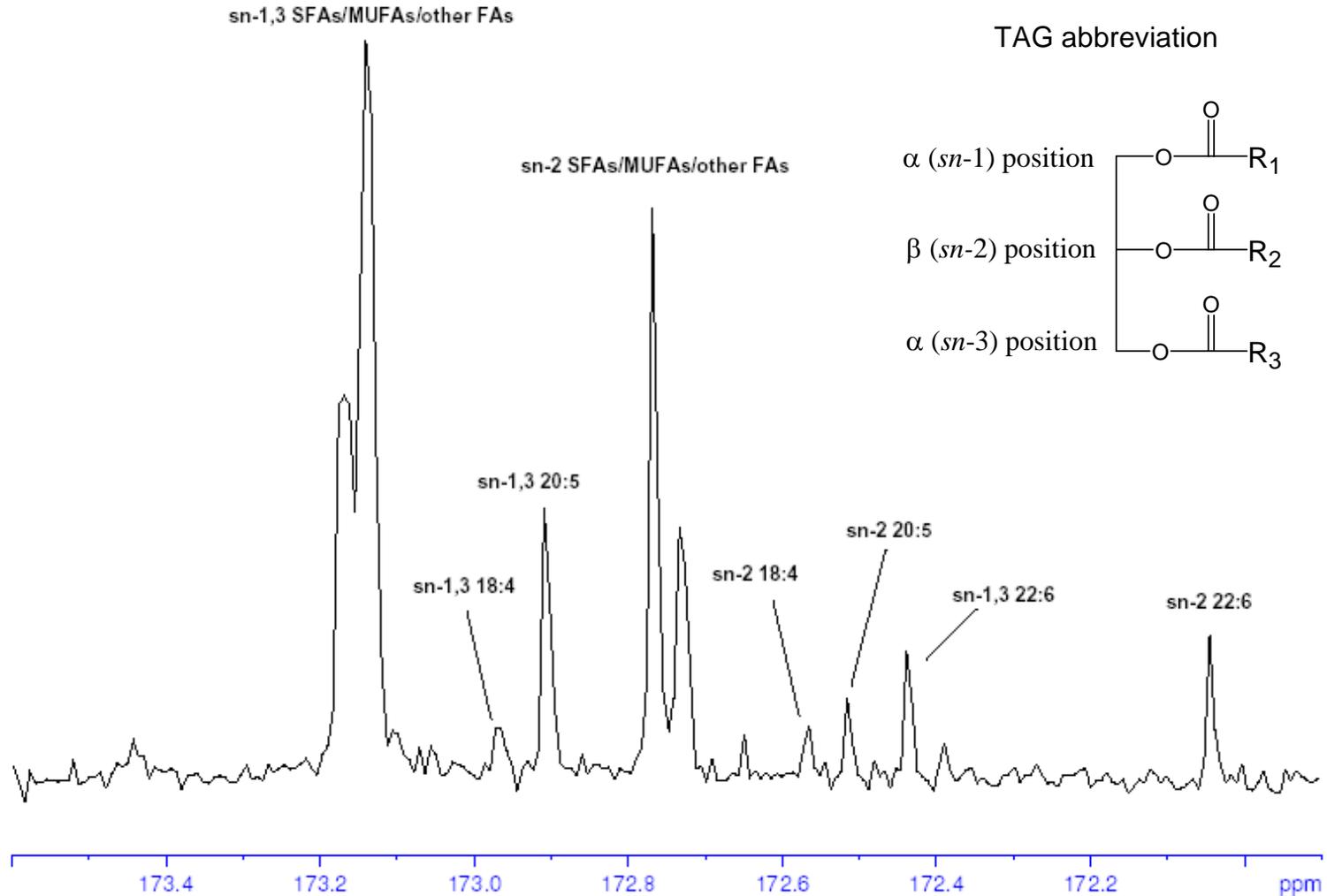
Full mapping of lipid signals. High Resolution and Quantitation

Lipidomic: Proton NMR spectra



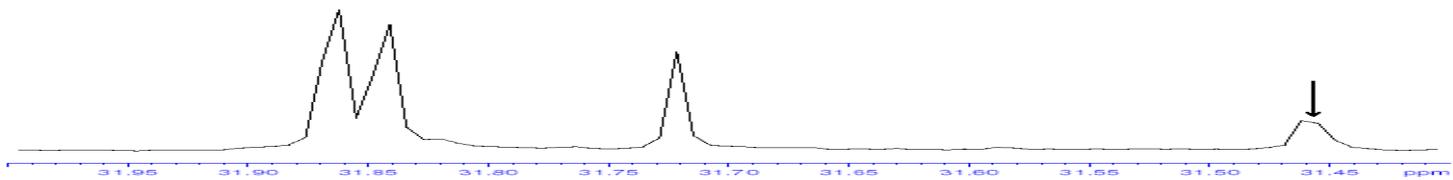
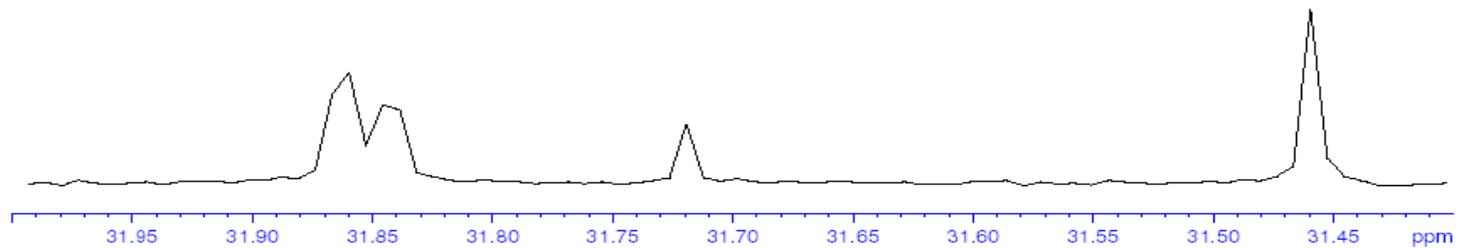
Studies of Biotechnological Processes: incorporation of omega 3 PUFA

Lipidomic: ^{13}C NMR spectra



Studies of Biotechnological Processes: transesterification

Lipidomic: ^{13}C NMR spectra

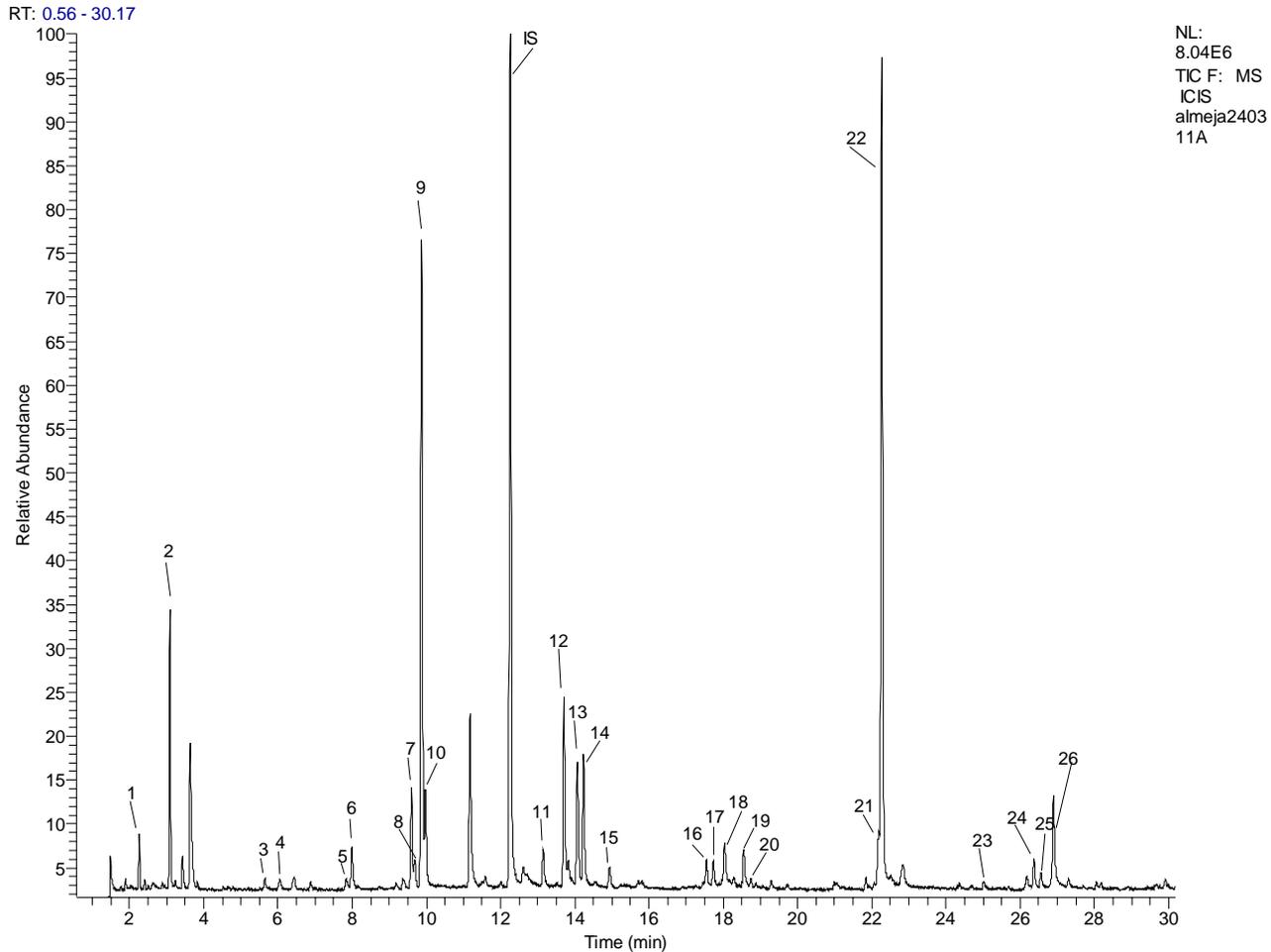


Studies of Biotechnological Processes: minor lipids

Medina et al, 2011.

Lipidomic: Lipid oxidation by-products

- ▶ Hydroperoxides, aldehydes coming from oxidation of EPA, DHA, ... Mechanism involved in bioactive effect of fatty acids, Flavors, Enzymatic reactions...



Conclusions

- ▶ Both, Proteomic and Lipidomic are Research Areas with a great potential in Marine Biotechnology.
- ▶ Modified organism, production of compounds, mechanism involved in biochemical reactions, health,...
- ▶ The development is driving by:
 - ▶ New and powerful MS spectrometers
 - ▶ High field and high resolution NMR
 - ▶ Biodatabases
 - ▶ Quantitative analysis

