

PROTEOMIC APPLICATIONS IN FOOD CHEMISTRY

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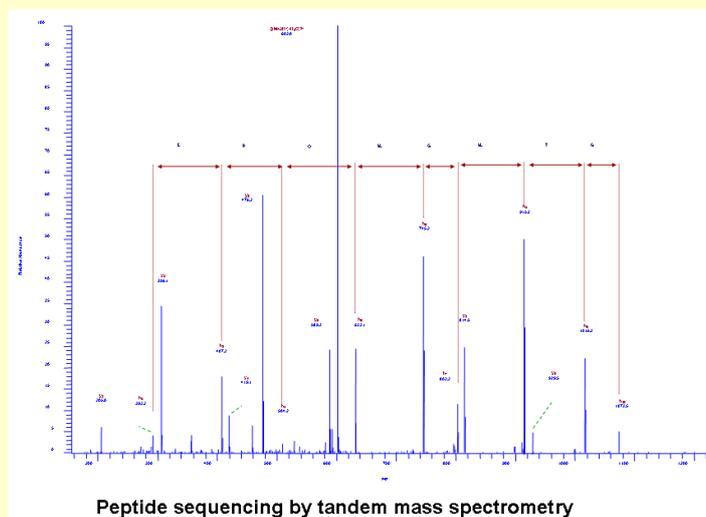
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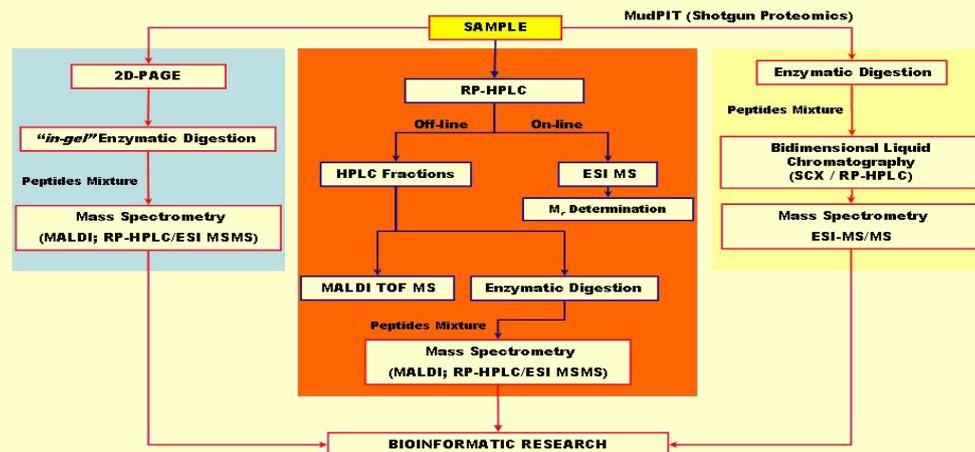
The expertise of the staff of the Laboratory of Organic Mass Spectrometry (LSMO) is in the field of protein chemistry, mass spectrometry (MS) and high performance liquid chromatography (HPLC). Since several years, the activity of the laboratory is mainly devoted to the investigation of proteins by proteomic approaches, with particular emphasis to food proteins (milk proteins, cereal proteins), allergenic proteins and plant proteins.

Keywords: Mass Spectrometry; ESI; MALDI-TOF; HPLC; Proteins

Proteomics is a natural evolution of protein chemistry. It has gained impressive development in recent years principally as a consequence of the introduction of new ionization methods in mass spectrometry, such as Electrospray Ionization (ESI) and Matrix Assisted Laser Desorption/Ionization (MALDI). With the advent of these techniques mass spectrometry has emerged as the method of choice for protein identification and sequencing. Concurrent optimization of the electrophoretic techniques and the availability of efficient methods for gene sequencing, that has resulted in an extensive enlargement of protein sequences databases, have provided an efficient platform for large scale protein investigation.

Current goals of proteomic research are more varied and direct toward the systematic determination of diverse properties of proteins. These include sequence, quantity, state of modification, interaction with other proteins, activity, subcellular distribution and structure.

From an analytical point of view, proteomic investigations are based on the use of highly efficient separation techniques for proteins such as reversed phase high performance liquid chromatography (RP-HPLC) or two dimensional polyacrylamide gel electrophoresis (2D-PAGE) combined with biochemical methods, enzymatic digestion and MALDI end ESI mass spectrometry in order to obtain data suitable for searching, by specific software (Mascot, ProFound, MS-Fit, etc.), genomic, proteins or ESTs (expressed sequence tags) databases. A typical workflow of a proteomic investigation is depicted in the following figure:



Proteomic applications in the field of food chemistry are directed to the investigation of food proteins. The interest for a detailed investigation of the protein content in food is originated by several reasons that include a more precise elucidation of the relationship between protein composition and nutritional and technological properties, the production of methods for food traceability, or even the detection of genetically modified products. Moreover, considering that most of the more consumed foods (milk, eggs, cereals, etc.) may produce allergenic reactions in humans, proteomic investigation may contribute to the detections of allergenic proteins and elucidate the reasons of their allergenic proprieties, thus providing the basis for the production of ipoallergenic or nutraceutical foods (*Pharma-Foods*).

Within this frame, our laboratory is developing the following line of investigations:

1. Characterization of donkey's milk proteome

The wide use of cow's milk in infant diet has shown that approximately 3% of newborns are allergic to cow's milk proteins (CMP). Therefore, as milk represents the main source of nutrition for infants, research to find a valid alternative has a high priority. Recent clinical studies have demonstrated that, for a large number (83-88%) of the investigated subjects, introducing of donkey milk in the diet of children allergic to CMP represent the unique safe and resolving feeding, especially if compared with classical treatments (infant formulae, hypoallergenic milk, etc.). Although the mechanism of this tolerance has not yet been fully clarified, it is reasonable to hypothesize that the reduced allergenic properties of *Equidae* milk can be related to structural differences of its protein components with respect to bovine milk. However, the characterization at molecular level of the major components of *Equidae* milk is at a relatively early stage, whereas practically nothing is known about the minor protein species. Therefore, with the aim to improve the knowledge about the donkey's milk protein fraction, in the last years we have carried out a detailed characterization of the protein profile of different donkey's milk (DM) samples by typical proteomic approaches. Particularly, our studies allowed the direct characterization of a new variant of β -lactoglobulin II, the direct characterization of lactoferrin, the verification of the gene-derived sequence of serum albumin and, finally, the characterization of the primary structure of the two isoforms of the β -CN and four isoforms of the α_{s1} -CN. Moreover, as nothing is known about the minor or trace protein components of DM, the new goal of this line of research is the investigation of minor and trace protein components in DM (the "hidden" proteome) that normally remain undetected using conventional analytical procedures. This study will be carried out coupling the typical proteomic methods and the ProteoMiner technology, based on the capture of all proteins present in biological samples, and the normalization of their concentration, by a combinatorial library of hexameric peptide ligands (CPLL) used as baits for adsorbing protein species present in solution. In this way, we expect to obtain a substantial enlargement of the knowledge of the protein composition of DM, as well as a more detailed information on the potential allergenic properties of minor protein components.

2. Plant proteomics studies

In the last few years proteomic has become a powerful tool in plant research, in spite of the fact that the available information at genome level, essential for protein identification, is very scarce for many plants. Our works on plant proteomics was differentially focused on cereal and other plant proteomes. The studies on cereal proteome were firstly concerning the structural characterization of gluten proteins. We investigated both High Molecular Weight (HMW) and Low Molecular Weight (LMW) glutenin subunits in durum wheat, bread wheat and in a translocated line. These two classes of proteins are of interest because they are considered the main responsible for the gluten viscoelastic properties. At the same time, in the light of the renewed interest for Farro for human nutrition and as environmentally-friendly cereal crops for the production of niche products, we investigated the antinutritional and allergenic factors in caryopses of Farro. Proteins investigated include non specific lipid transfer protein (nsLTPs), cereal trypsin/R-amylase inhibitor, a family of proteins active against alpha-amylases from several seed parasites and pests and inactive against endogenous alpha-amylases, and puroindolines, a particularly interesting group of proteins linked to starch bound granules and responsible of grain texture. Another recent study performed in our laboratory was focused on the identification of the differentially expressed proteins of flesh in mature orange fruits in order to elucidate their involvement in the anthocyanins biosynthetic process. In details, we performed a comparative investigation on the differentially expressed proteins of the mature fruit flesh proteome of Moro (blood) and Cadenera (common) cultivars by means of 2-DE analyses and RP-HPLC/nESI-MS/MS. This investigation revealed that a notable difference in protein expression occurs in Moro and Cadenera, suggesting that anthocyanins biosynthesis, and all correlated pathways, represent the major difference between blood and common oranges.

Collaborations

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Research Grants

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PRIN 2006 - prot. 2006078987_004 "Studio comparativo delle proteine della cariosside di frumenti normali e transgenici: caratteristiche qualitative-nutrizionali e sicurezza alimentare"

PRIN 2008 - prot. 20087ATS57_004 "Ricerca di allergeni in tracce negli alimenti vegetali e animali attraverso nuovi approcci proteomici e produzione di anticorpi specifici per la loro rivelazione"

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