Trends in Analytical Chemistry 52 (2013) 135–141

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Review

Proteomics and its applications for food authentication and food-technology research

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ARTICLE INFO

Keywords:
Food authentication
Foodomics
Food processing
Food technology
High-intensity focused ultrasound (HIFU)
Mass spectrometry (MS)
Microorganism detection
Pathogen
Proteomics
Selected MS/MS ion monitoring (SMIM)

ABSTRACT

This review is a critical overview of advances in proteomics applied in food technology, which may be classified into two main topics: (i) authentication of food components as a tool to comply with food-labeling regulations and policies; and, (ii) food-technology research, mainly for the development of fast, reliable methods to detect and to identify spoilage and/or pathogenic microorganisms in food and for the study of changes in food components as a consequence of food processing.

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1. Introduction

Technological advances in the food industry offer substantial benefits to consumers in the global food market. Consumers demand products that meet their nutritional preferences and are extremely vulnerable to food-safety issues. Both academia and the food-science industry face a new challenge: the need to develop strategies and products that are not only safe but also contribute to the maintenance of good health and that may even prevent the development of specific disease-risk factors. In light of this, the recent successes of proteomics methodologies make them a promising strategy to address these concerns.

Proteomics is defined as the large-scale analysis of proteins in a particular biological system at a certain time [1]. Proteomics includes not only the structural and functional knowledge of

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http://dx.doi.org/10.1016/j.trac.2013.05.019

proteins but also the study of their modifications, interactions, localization and quantification. Proteomics is emerging as a powerful tool for food-technology research [2,3] because it is helping to address the major challenges faced by food analysts and researchers:

- the development of simple, fast methodologies for routine use;
- the analysis of complex or highly processed food matrices; and,
- the quantification of trace levels of analytes with a high degree of selectivity.

This review illustrates up-to-date applications for and the new trends in proteomics methodologies for food-technology research, classified into two main topics:

(1) the authentication of food components, where proteomics is used as a tool to comply with food-labeling regulations and policies; and,
(2) the food-technology area, mainly in the development of fast, reliable methods to detect and to identify spoilage and/or pathogenic microorganisms in foods and in the study of the changes in food components as a consequence of food processing.

2. Proteomics

Proteomics studies are usually divided in three main areas, as follows.

2.1. Protein identification and characterization

Mass spectrometry (MS) is the method of choice for characterization and identification of proteins. The analysis of a proteome usually relies on one or several separation steps followed by MS analysis. The general approach consists of comparing MS experimental data with calculated mass values obtained from a sequence database using a search engine, such as Mascot [4].

In an approach known as peptide-mass fingerprinting (PMF) [5], a technique, such as two-dimensional gel electrophoresis (2-DE), is used to isolate an unknown protein, which is then enzymatically digested into peptides and subjected to MS. Another approach, usually known as peptide-fragmentation fingerprinting (PFF), uses tandem MS (MS/MS) to produce fragment-ion data from one or more peptides from the protein to identify the protein unambiguously [6]. Alternatively, MS/MS fragment-ion data from the entire protein can be used in a similar way for an approach known as top-down proteomics. For all these approaches, it is necessary for the corresponding protein to be present in the database. If the unknown protein is not present in the database, the best match will probably be the entry with the closest homology, usually a related protein from a related species. If the sequence similarity within protein databases is too low, peptides must be sequenced *de novo* [7], meaning that the MS/MS spectrum must be interpreted manually or through computer-assisted identification of the fragment ions with mass differences corresponding to the masses of the individual amino acids (AAs).

More than 300 different types of post-translational modification (PTM) occur on proteins in response to a wide range of intracellular and extracellular signals [8]. PTMs play crucial roles in protein function because they may alter protein activity, localization or stability. The mass shift in the modified AA with respect to the unmodified residue is the basis of the detection and the characterization of PTMs by MS. However, because PTMs are usually present in very low stoichiometry, modification-specific enrichment techniques are needed [8].

2.2. Differential proteomics

Quantitative information at the protein level, such as the relative abundance of a specific protein among different samples or the absolute amount of the protein, is very helpful when determining differences between different conditions (control *vs.* case). Relative quantification can be achieved with different methodologies, which may be classified as gel-based, label-based, and label-free approaches.

Gel-based methods consist of comparing the signal of an electrophoretically-isolated spot among different samples.

For label-based methods, proteins or peptides are labeled using a mass tag that is introduced metabolically, enzymatically or chemically, and relative quantification is obtained from the MS read-out. Quantification is based on the ratio of heavy/light peptide pairs.

Label-free approaches avoid the use of labeling with stable isotopes. The protein amount is calculated based on the MS-derived ion-current signal of the peptides or proteins or on the number of identified MS/MS spectra (spectral counts) for the protein.

However, for the absolute quantification of proteins, isotopically-labeled synthetic peptides are needed as internal standards for each target protein. For a more detailed description of all these quantitative proteomics approaches, we refer the reader to Panchaud et al. [9].

2.3. Functional proteomics

Most proteins function closely with other proteins. Functional proteomics studies address the integrated analysis of the functional interactions among different proteins and the networks thereof. In this sense, interactomics is defined as the study of the interactions between a specific protein and others and the consequences of these interactions [10–12]. For this transition from structural to functional proteomics, different platforms are currently being developed, but they are not necessarily MS-based; some of these techniques include affinity purification, yeast two-hybrid assays, protein microarrays, activity-based proteomics, phage display and capture-compound MS.

2.4. Proteomics workflows

A challenge for MS technology is the complex nature and large dynamic range of proteomes. Partial purification, depletion of high-abundance proteins, and selective enrichment are some of the methods used when working with complex samples [13]. After one of these techniques, further separation is performed at the protein and/or peptide level, typically based on gel electrophoresis and/or liquid chromatography (LC), before analysis by MS. Depending on how proteins will be analyzed in the mass spectrometer, two different proteomics workflows can be followed: bottom-up or top-down approaches (Fig. 1).

In the most common workflow, referred as a bottom-up or peptide-based approach, the protein/s of interest are converted into peptides using enzymes, such as trypsin, and the resulting peptide fragments are then analyzed by MS [1]. Bottom-up approaches can be further divided depending on whether the fractionation step is performed before (at the protein level) or after the enzymatic digestion (at the peptide level). A typical method for the former strategy utilizes a 2-DE gel-based approach, wherein proteins are separated based on their isoelectric point (p*I*) and molecular weight (*Mr*), so they can be individually excised from the gel and digested into peptides that are analyzed by MS. In the latter approach, also referred as shotgun proteomics, the protein mixture is enzymatically digested without prior fractionation, and the resulting peptides are analyzed by LC–MS. When the peak capacity