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New approaches based on comparative proteomics for the assessment of food quality

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During the last decade, the use of integrated 'omics' including genomics, transcriptomics, metabolomics and proteomics has provided a better knowledge of food systems. In fact, technical improvements achieved in mass spectrometry instruments together with the last developments of bioinformatics tools and hardware have allowed proteomics to be the method of choice for food control through the identification of quality biomarkers and the study of the proteome expression data. In this context, modern comparative proteomics is the basis for establishing quantitative differences in the expressed proteome of a certain sample by comparison with a reference through the relative and absolute quantification of proteins and peptides.

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Introduction

The first step towards a better control and characterisation of foods is based on understanding organism systems from a biological point of view. In this regard, the use of 'omics' strategies based on the study of genome through genomics (i.e. identification of mutations), transcriptome through transcriptomics (i.e. elucidating the structure and levels), proteome through proteomics (identifying and quantifying the expressed proteins) and metabolome through metabolomics (i.e. characterising the metabolites present in the system) are necessary for a better knowledge of food systems [1••].

During the last decade, proteomics has been the method of choice to develop methodologies for quality control in food production processes as well as in food safety [2,3]. In fact, the knowledge of structure and function of proteins present in raw food materials and final food products is essential for the optimisation of processing methodologies as proteins play a major role in quality parameters such as texture, colour, crops yield, or changes derived from the response to stress. What is more, technical improvements achieved in mass spectrometry (MS) instruments by increasing their sensitivity and accuracy together with the last developments of potent bioinformatics tools and hardware have allowed the development of more sophisticated methodologies for the identification of quality biomarkers and study the proteome expression data. In this context, comparative proteomics is the basis for establishing quantitative differences in the expressed proteome of a certain sample compared to a control or sample of reference and it allows predicting or identifying potential biomarkers of interest through the relative and absolute quantification of proteins and peptides.

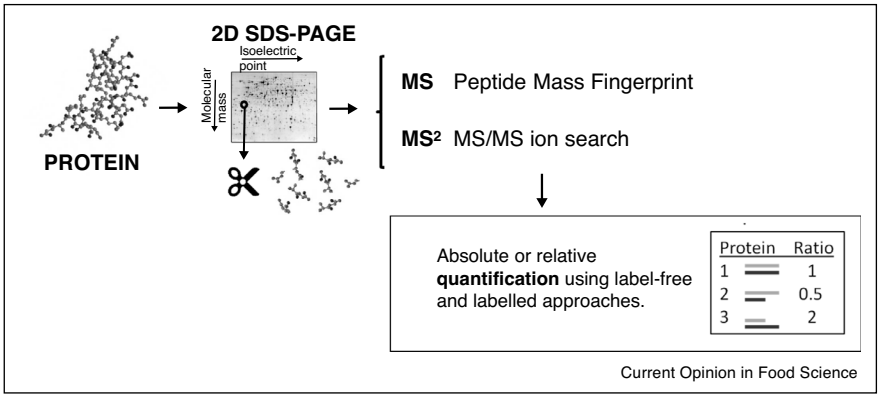
In this review, main methodologies used in comparative proteomics and the latest approaches for the assessment of food control through the characterisation of processes and final product have been described.

Main methodologies used in comparative proteomics

Comparative proteomics can be approached from both a qualitative point of view and under a quantitative perspective. The identification of protein profiles is usually done using Peptide Mass Fingerprint (PMF) strategy that includes a first separation of proteins using two-dimensional gel electrophoresis (2-DE). So, proteins are first separated based on their isoelectric point (pI) and later separated based on their molecular weight. Then, bands containing differential proteins are excised, digested by trypsin, and the generated peptides are extracted and analysed by MS [4] (Figure 1). Numerous software have been specifically developed for the detection of differences between samples in the 2-DE. After separation, the obtained protein mapping can be analysed by comparing the expressed proteins between samples and some of these software even permit the relative quantification of the identified bands.

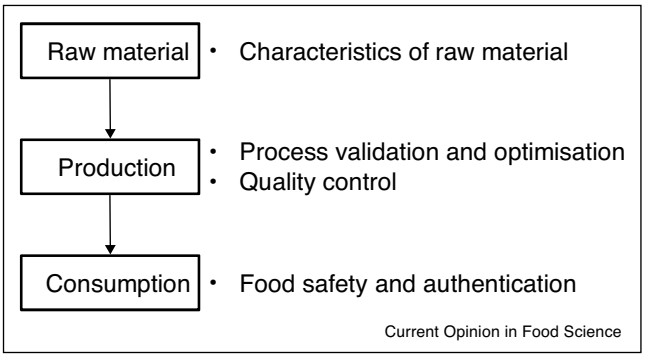
The technical evolution of mass spectrometers occurred during the last decade as well as the development of bioinformatics data analysis platforms have led to a great advance in quantitative proteomics techniques [5], being comparative proteomics one of the main methods of choice used in the assessment of food quality and safety (Figure 2). In this sense, protein/peptide quantitative

Figure 1



Scheme of traditional proteomic approach for the identification of proteins from a food matrix.

Figure 2



Overview of comparative proteomics application to the assessment of food quality and safety.

differences between samples can be measured using two main approaches: the use of labelling techniques that involve stable isotopes and the use of label-free methodologies. Labelling techniques are considered to be the most accurate in quantifying protein abundances whereas label-free methods are considered to be simple as do not require extra sample preparation, versatile as require low amount of sample, less time-consuming, and cost-effective when compared to labelled methodologies [6]. One of the most used label-free approaches is the measurement of the ion areas through the extracted ion chromatograms (EIC) for the relative quantification of proteins and peptides. This methodology has been used in foods to compare protein abundances between two or more sets of samples. On the other hand, multiple reaction monitoring (MRM) is the most used method for the absolute quantification of peptides in complex mixtures. MRM is a very sensitive labelling method that can selectively detect and quantify small and low abundant peptides based on the screening of specific transitions from precursor peptides to product ions.

Characterisation of processes through comparative proteomic approaches

The processing of foods including cooking, pasteurisation, drying, fermentation, or modern techniques such as pulsed electrical fields, microwaves or high pressure treatments influences to a great extent the quality of the final product. In this regard, comparative proteomics has been used to analyse proteome changes that occur in food during its processing [7••].

Bottom-up approaches based on comparative proteomics are the most common methods used for the characterisation of proteome changes as well as amino acid modifications when applying thermal treatments [8,9]. As an example, Figure 3 shows a schematic diagram illustrating the experimental approach used for shotgun proteomic analysis of heat-dependent milk protein modifications. So, cooked meat proteome changes have also been investigated using 2-DE coupled to image analysis and MS to evaluate protein modifications between raw pork meat and cooked ham [10]. Also changes in proteins mainly due to Maillard reactions and redox modifications resulted from the thermal treatment of milk and dairy products have been analysed by MS in tandem. A MRM-based methodology has been recently used to quantify the amount of cross-linked proteins derived via Maillard reaction after heat treatment as well as the glyco-oxidation product Nε-carboxymethyllysine (CML) in a model system [11], showing the efficiency of the MRM method for the quantification of low-abundance post-translational modified proteins.

Differences in proteome expression has also been studied in cereals, fruits and vegetable products during the