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Review

Proteomics: general strategies and application to nutritionally relevant proteins

František Kvasnička*

Department of Food Preservation and Meat Technology, Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic

Abstract

Proteomics as a subset of applied genomics technologies will be a key area of biology during the first decade or two of the new Millennium, and that it will have major impact, both directly and indirectly, on nutritional science. The aim of this review is to summarize information about general strategies of proteome and its application to important food proteins (plant, animal, and microbial). Methods are also described for protein separation, identification and determination. This article covers papers published within the last decade.

Keywords: Reviews; Proteomics; Food; Proteins

Contents

1. Introduction

The term ‘‘proteome’’ (PROTEins expressed by a genOME) refers to all proteins produced by a species, much as the genome is the entire set of genes. Unlike the genome, the proteome varies with time and is defined as ‘‘the proteins present in one sample (tissue, organism, cell culture) at a certain point in time’’. There is an increasing interest in proteomics technologies now because deoxyribonucleic acid (DNA) sequence information provides only a static snapshot of the various ways in which the cell might use its proteins whereas the life of the cell is a dynamic process. There are two reasons to study proteomics: (a) the levels of expression of proteins are not particularly well correlated with mRNA levels, and (b) many important regulatory

*Tel.: +420-2-2435-5117; fax: +420-2-311-6284.
E-mail address: kvasnic@vscht.cz (F. Kvasnička).

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signals involve posttranslational changes in proteins (e.g., glycosylation, phosphorylation, oxidation, reduction).

With the sequencing of the human genome in 2000, the genomic age comes to a close and the post-genomic era starts in which proteomics will play an important part. An index of growth of proteomics is the number of publications (see Fig. 1). From three entries in 1995, the list of publications containing the term ''proteome'' or ''proteomics'', retrieved from the Web of Science at beginning of December 2001, has grown to over 800 items [1].

The practical realisation of proteomics depends upon the ability to identify and analyse each of the protein products in a cell or tissue, and this is reliant on the application of several key technologies. Proteomics coined by Wilkins and co-workers some 6 years ago [2,3] can be used to identify the entire protein complement of a cell, tissue, or microorganism at different stages of development, and to examine the integrated response to a particular challenge. The challenge may be hormonal, environmental, or nutritional (in whatever form). As well as revealing the presence of novel proteins, a major application of proteomics is in trying to identify at a protein level early changes involved in degenerative and other diseases, such as in the pancreatic β-cell in diabetes. This may be valuable either to provide insight into the causes of a disease, or in identifying an early marker of the initiation of the disease process. For the nutritional scientist this is clearly of special interest in relation to those diseases where diet plays an important role [4]. The power of the knowledge emerging from the genome is that the identification of the genetic basis of an inherited disease provides logical strategies to treat those afflicted on an individual basis. Beyond its application to diseases with demonstrably genetic causes, however, the direct utility of genomics by itself diminishes. Ultimately, changes in phenotype and not changes in genes are of interest to nutrition and health. The gap between genes and phenotype is spanned by many biochemical steps, each with individual specificities and a sensitivity to diet and the environment. Metabolites are the quantifiable molecules that best reflect phenotype; however, modern biological informatics has yet to embrace the study of metabolites as aggressively as genomics. An understanding of the many factors that contribute to health is necessary and will increase as technologies that quantify metabolites within individuals improve. Technologies that allow the creation of and interaction with accessible annotated databases of metabolite concentrations that reflect individuals with various phenotypes are also needed; fortunately, such technologies are available. In this context, substantial databases of metabolite concentrations will be predictive resources to quantify the relation between metabolites and health. Therefore, metabolite informatics is the logical next step in understanding the role of nutrition in modifying metabolism and ultimately in promoting health [5]. More information about proteome and proteomics one can find elsewhere [6–12].

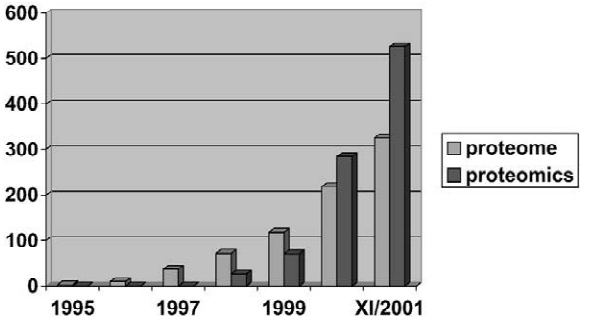

Fig. 1. Number of publications containing the term ''proteome'' or ''proteomics''.

Applied genomics technologies (transcriptomics, proteomics, metabolomics, etc. [13]) aim at analysing the components of a cell in its entirety and have, together with the developments in computing, ushered in a new era in scientific methodology for the life sciences. Genomics technologies have mainly been set up and further developed by the pharmaceutical industry, but will also be invaluable to the food industry. Only recently, the first papers about nutrigenomics [14,15] and the application of genomics in nutrition science and food technology have appeared. In these reviews the authors briefly explained the essence of the various applied genomics technologies, and focus on the impact that these technologies will have in the food industry.

The Institute of Food Research in Norwich (UK) has initiated a new programme [16] in nutritional genomics (nutrigenomics) to provide excellence in

scientific research at the intercept of molecular biology and nutrition, by establishing a core intellectual and technical capability in the area of human post-genomic nutrition, exploiting the synergy between our expertise in nutrition, molecular biology, biochemistry, biophysics and genomic science. Recent innovations in genomic technologies will provide nutrition with an exceptionally powerful tool for testing existing diet–health hypotheses, formulating new hypotheses, and defining mechanisms of nutrient action at specified, and varied, cellular levels. There are other institutes dealing with nutrigenomics [17–19]. The 1st International Nutrigenomics Conference was announced and will be held in Netherlands next year [20]. The aim of this review is to summarize information about important food proteins (plant, animal and microbial) and methods for their separation, identification and determination.

2. Description of the proteomics methodology

The critical pathway of proteome research includes [21]:

1. Sample collection, handling and storage.
2. Protein separation (2DE).
3. Protein identification (peptide mass fingerprinting and mass spectrometry).
4. Protein characterisation (amino acid sequencing).
5. Bioinformatics (cross reference of protein informatics with genomic databases).

Mapping the human genome had become possible through a revolution in technology and instrumentation and a similar revolution was now required to enable high-throughput protein analysis. This, however, posed considerable challenges since techniques that had been developed for automated DNA sequencing and analysis could not be easily adapted to protein analysis. The chemical nature of proteins makes them fundamentally different from DNA. Unlike DNA that consists of a string of four chemically similar units (bases), the 20 units (amino acids) that make up proteins have vastly different chemical attributes and some of these units can be modified further, adding to the complexity. The substitution of a single unit in a protein with a different unit can, in some cases, substantially alter a protein's properties to require modifications in the analytical techniques required to study it. Hence, techniques had been tailored to study different classes of proteins. Furthermore, while the human genome contained roughly 30000 genes, estimates of the average number of proteins present in a human cell stood at 500000 to a million. Proteins also varied in abundance and key proteins that were often likely to be drug targets were available only in minute quantities and posed a special challenge for analysis. In addition, unlike the genome of an organism, a proteome was dynamic and changed in response to variations in the cellular environment. Conventional techniques for protein analysis were laborious as the initial mapping of the human genome was being completed. The standard approach to studying proteins en masse started with the preparation of the sample for analysis. Samples such as that from a tissue or cell line had to first be appropriately solubilized. The separation of the proteins in the solubilized sample was then accomplished by a technique called 2-dimensional gel electrophoresis where the proteins were first separated according to their electric charge and then by their size in polymer matrix. Once separated, the arrayed proteins could be seen as a pattern of spots on the polymer matrix (gel) with each spot representing a distinct protein. Individual protein spots then had to be treated by specific enzymes to fragment the protein into ''peptides'' before mass spectrometry could be used for protein identification. If proteins were to be studied in a high-throughput, automated fashion, many concerns would have to be addressed. Sample preparation would first have to be optimised since conventional approaches typically missed many proteins. Conventional 2DE was also considered to be more of a craft since the technique was non-standardized labour intensive, slow, and prone to contamination and, as a result, did not easily generate reproducible data. Similarly, proteins that had extremes in charge or size were also problematic. For example, concerns revolved around the ability of the 2DE method to separate membrane proteins that were often key players in signal transmission between cells and hence candidate drug targets. However, 2DE was still the best technique for looking at all forms of a protein—that is, even

proteins from the same gene that only differed by the attachment of a sugar group, for instance. For these reasons, the technique was considered by many to be a bottleneck in the pursuit of automated protein analysis. It was also widely agreed that commercial information management systems would have to be developed in any proteomics automation effort and such systems were only in their infancy. Software would have to be developed to handle the onslaught of data from the mass spectrometers as well as to allow automated image analysis of the spots on 2DE gels—that is, to archive, compare and determine what differences existed between samples. Others felt that the bottleneck of protein identification and analysis lay at the mass spectrometry stage and large numbers of mass spectrometers would be key in automated analysis.

2.1. New proteomic technologies

The utility of the classical approach (2DE–MS) is limited by the inability to reliably monitor proteins that are present in very low abundance in a sample, as well as proteins that are very hydrophobic or that are very acidic or basic. Low copy number proteins might represent key regulatory molecules within cells or signalling molecules in tissues and organs, and the inability to measure changes in expression levels of these important proteins is an important consideration. Hydrophobic proteins that are present in membranes might have key roles in communicating extracellular information to the inside of cells [22]. As proteomics was coming to the forefront of the life sciences, many companies had entered the race to develop new technologies for it. The increasing interest and activity in proteomics is evidenced by a recent conference, which attracted a large attendance and was devoted entirely to proteomic technologies. The 4th IBC Conference on Proteomics was held in Basel, Switzerland on 1–2 March 2001 and covered important recent developments in this field [23]. Some companies were focusing on developing integrated platforms that spanned the length of the protein analysis chain while others were focusing on specific stages of protein analysis. There was, however, no consensus on which stage of protein analysis needed greatest attention or which technology to focus on. While ones had identified the sample preparation and data analysis steps as important bottlenecks that the industry had largely ignored, others viewed the mass spectrometry stage as a key block that could be overcome by the acquisition of tens or hundreds of mass spectrometers. Similarly, others cited the inability of the 2DE protein separation technique to allow adequate relative quantification of the protein levels between samples. These sceptics had developed alternative techniques such as isotope coded affinity tagging that allowed better relative quantitation and concurrent identification. The ICAT technique, however, also suffered limitations since it did not address protein modifications. Some combined the two methods, for instance, planned to dispense completely with 2DE using liquid chromatography (LC) methods for protein separation instead. The potential benefits of using HPLC rather than 2DE are immense [24]. The most prominent are:

1. No long gel runs.
2. No gel removal steps or gel interactions.
3. Fewer size limitations (HPLC can accommodate molecules ranging from small peptides to large proteins).
4. No visualization problems with regard to the need for staining.
5. Greater sensitivity.
6. The ability to act as an immediate portal to subsequent HPLC runs.
7. Multiplicity of kinds of HPLC separation possible.
8. The ability to interface directly with a variety of MS systems.
9. Ease of automation using standard technologies.

Because of its ability to separate and identify molecules with much lower molecular masses, HPLC is the technology of choice for protein identification through peptide analysis, often after a 2DE run isolates the particular protein for tryptic digestion. Where HPLC systems have traditionally lagged, however, is in this initial separation of proteins from complex mixtures, especially because they lack the visualization step that a gel display provides. HPLC systems, however, are catching up to 2DE even in the area of complex protein mixtures.

Multidimensional HPLC for protein analysis primarily relies on tandem or multiple LC combined with tandem MS. Given the sensitivity of the initial LC steps and the fact that everything is done in liquid in-line, such systems are not only easily automated but also capable of separating even the low-abundance and membrane proteins. These proteins cause difficulties in 2DE analysis because of poor yields, difficult visualization, or interference with the gel runs. And, with new informatics tools, analysis of the chromatography data can be presented in more user-friendly displays of multidimensional runs.

LVSEP in capillary electrophoresis is a promising tool for on-line concentration of low-abundance protein samples for proteomics research. An enhancement factor of more than 100 times was achieved compared to the conventional pressure injection method [25].

An on-line coupled capillary isotachophoresis (cITP) with capillary zone electrophoresis (CZE) using a two-capillary, three-electrode instrument is another alternative technique for trace analysis [26]. In the first, wider, capillary, the isotachophoretic step ensures stacking of the analytes into sharp zones that are then separated by CZE mode in the second narrower capillary. The first isotachophoretic step enables injection of large sample volumes (up to several hundreds of μl) and concentration of analytes according to the Kohlrausch' regulating function. With respect to the minimal detectable zone an analyte can be up to 10^6 times concentrated during the cITP step. In a complex sample matrix constituents are separated into a stack of zones with trace analytes focused into narrow bands. The matrix constituents can be forced to migrate out of the separation path by proper column switching and the sample is thus cleaned up before its injection into the CZE capillary. The removal of the matrix is well defined and very reproducible because it is based on the signal from the detector of the cITP step.

Low abundance protein can be potentially analysed by carrier ampholyte-free isoelectric focusing technique using the same instrument as in the case of cITP–CZE. The suggested method is based on the principle of both side regulated ionic matrix in CAF-IEF [27]. A sharp step of pH is created in the column filled with a sample dissolved in a background electrolyte by influence of current and solvolytic fluxes. Here, ampholytes are focused upon. The magnitude of the step, its velocity and direction of its movement can be regulated electrically. In this manner, favourable separation properties of the system can be set up, even during the run. This brings several advantages over conventional methods. The principles of the separation can be easily changed, permitting selective pre-concentration (trapping) of minor components by processing large amounts of a sample to be preformed, effective isotachophoresis or IEF pre-separation and final electrophoretic analysis in one run. Advantages of these combinations are discussed together with the right choice of the working electrolyte. A 1000-fold increase in amount of substance in a column can be achieved for both isotachophoresis and capillary zone electrophoresis combined with CAF-IEF preconcentration at reasonable working conditions. It enables a limit of detection at the pmol/ml level with a concentration factor of about 10^7 to be reached.

The CAF-IEF mode in the first wider capillary and CZE mode in the narrower second capillary arrangement is analogical technique to 2DE, but with enhanced sensitivity and selectivity.

The emerging chip-based methods generally fall into two categories: (a) spotted array-based tools, and (b) microfluidic-based tools [22].

In array-based methods, small spots of proteins are immobilized onto silicon-based substrates (typically glass). Such an array can then be used to screen complex protein mixtures for particular binding affinities or other interactions. These arrays potentially address several of the concerns about the existing 2DE–MS approach: the arrays can be produced for relatively little cost, provide consistently reliable and rapid results and are simple to use. Using existing technology, it is possible to array nearly the entire complement of proteins produced from a library of cDNA clones onto a surface and then to probe for small molecule interactions, antibody–antigen specificity or to identify unique proteins from a mixture using fluorescence detection. The ProteinChip System from Ciphergen (Fremont, CA, USA), based on SELDI is a system that can rapidly perform separation, detection and analysis of proteins at femtomole levels directly from biological samples. The ProteinChip ''benchtop'' system and Tandem MS system have several advantages over the

2-D gel method. These include speed of detection (hours versus days), coverage of a broader region of the proteome, small sample requirement (1 μl or 500 cells), and combination of discovery of biomarkers and diagnostic assays in a single system whereas MS is limited to the discovery of biomarkers only. Additional features of the new system include the fact that it is a non-expert, versatile benchtop system (in contrast to other expert, dedicated affinity LC–MS systems) and can be automated for high-throughput compatibility [23].

Microfluidic devices for the analysis of proteins are based on miniaturization which reduces the amount of sample and potentially provides low-cost disposable chips. Liu [28] and Gottschlich [29] both report lab-on-a-chip techniques for separating and detecting protein mixtures. Gottschlich and co-workers have integrated a microreactor, injector and electrophoretic separator and a second reactor for derivatization on a monolithic substrate followed by fluorescence detection. Liu integrates capillary electrophoresis, postcolumn labelling and fluorescence detection on a microfabricated system. Others at the University of Michigan have developed integrated microfluidics systems capable of accurately metering nanoliter drops (0.5–125 nl) in channels using a combination of hydrophobic surface modification and air pressure. Wang and co-workers [30] describe a microfluidic device that integrates an electrospray ionisation source for MS with a protein digestion bed, a capillary electrophoresis channel and an injector on a monolithic substrate. The protein digestion bed incorporates trypsine immobilized onto microbeads to permit faster digestion and to eliminate autodigestion products that might hinder sample characterization. Such a device can rapidly digest, separate and characterize proteins [22].

The Xcise platform for integrated spot cutting, digestion and spotting for MALDI-MS as well as the applications of ProteoChip based on Chemical Printer from Proteome Systems (Sydney, Australia), which employs the piezo-electric microvolume liquid dispensing for chemical analysis of the proteins resolved in a 2-D gel was presented by Malcom Pluskal at the 4th IBC conference on Proteomics in Switzerland [23]. The key feature of the Chemical Printer was that it would, ''bring the chemistry to proteins immobilized on membranes, rather than the traditional art of moving proteins for analysis. The technology minimizes sample manipulation, maximizes sensitivity of the analysis and provides an ideal substrate for archiving the sample'', explained Gooley from Proteome Systems Limited [9].

The ''molecular scanner'', recently presented by Hochstrasser et al. [31] is a device that simultaneously digests and electrotransfers proteins and peptides onto a polyvinylidene fluoride (PVDF) membrane. The membrane is then directly scanned using MALDI-TOF-MS to generate peptide mass fingerprints and these data are used to create a fully annotated 2DE map of the initial proteome mixture. Such a device automates several key steps in the 2DE–MS approach to proteome analysis, such as the need to isolate individual spots and perform a series of proteolytic digests on each purified spot. One possible limitation with this device is the reliance on 2DE technology, which does not resolve hydrophobic proteins effectively as discussed previously.

Ultimately, the automation of analytical technologies for simultaneous multiple protein analysis, whether through improved 2DE, variations on HPLC–MS and/or CE–MS, protein arrays or other high-throughput techniques is the only hope for making proteomics a living science. And such a science is the only hope for biological researchers to be able to move into the next and perhaps ultimate ''-omics'': physiomics. The predicted physiomics will be the systemwide informatics science capable of modelling and understanding the biology of the complex temporal and environmental states involved in the life of an individual organism [24].

3. Survey of nutritionally important proteins

Proteins are fundamental and integral food components, both nutritionally and functionally. Nutritionally, they are a source of energy and amino acids, which are essential for growth and maintenance. Functionally, they affect the physicochemical and sensory properties of various proteinaceous foods. In addition, many dietary proteins possess specific biological properties, which make these components potential ingredients of functional or health-promoting foods.

The proteins playing important role in human diet

Nutritionally relevant proteins					
Animal protein		Plant protein		Microbial protein	
Meat and fish		Cereals		Bacterial	
	Myofibrillar (actin, myosin, troponin) *Sarcoplasmic* (enzymes, myoglobin, myoalbumin, myogene A, B, C) *Connective* (collagens, elastins)		*Corn* (zein, zeanin, thiamine-binding protein) *Wheat* (glutenins, gliadins) *Rice* (oryzin, oryzenin) *Barley* (hordein, hordenin) *Rye* (secalin, secalinin) *Oat* (avenine, avenalin)		*Lactobacillus plantarum* (cabbage fermentation, bread dough) *Bifidobacter bifidum* (probiotics, yoghurt) Acetobacter aceti (vinegar) *Salmonella enteritidis* (food decay)
Milk		Legumes		Yeast	
	Caseins (α, β, γ, κ-casein) *Whey proteins* (β-lactoglobulin, α-lactalbumin, immunoglobulines, glycomacropeptide)		*Soy* (glycinin, conglycinin) *Pea* (legumin, vicilin) *Lentil* (lectins, trypsin inhibitors)		*Saccharomyces cerevisiae* (compressed yeast) *Rhodotorula glutinis* (carotenoids, fat) *Pichia pastoris* (decay of beverages)
Egg		Others		Fungi	
	Egg white (ovalbumin, conalbumin, ovomucin, lysozyme, globulins) *Yolk* (lipovitellin, phosvitin, α-livetin)		*Potato* (patatin, amylases, polyphenoloxidases) *Rapeseed* (12S globulin, 2S albumin) *Peanut* (arachin, conarachin, dehydrins) *Walnut* (lipid transfer proteins)		*Penicillium camembertii* (cheese) *Geotrichum candidum* (cheese) *Botrytis cinerea* (cultured mould of grapevine) *Mycoprotein* (hyphae of the organism culture PTA 2684 grown under axenic conditions used as meat, fat or cereal replacer)

Fig. 2. Some nutritionally relevant proteins.

can be divided into three main groups, i.e., animal, plant and microbial proteins. Here I present a survey of some nutritionally relevant proteins (see Fig. 2, Tables 1–3). One can find more information about plant [32,33] and microbial proteomes [34] in recently published articles. The application of MS to food proteins and peptide is reviewed in a paper published in the last year [35].

Applied genomics technologies (transcriptomics, proteomics, metabolomics, nutrigenomics, etc.) will contribute to following research areas of the nutritional sciences and food technology [14]:

1. Screening for novel functional bioactives.
   - Availability of new rapid screening methods for detection of bioactivity.
2. Safety evaluation of food ingredients.
   - Evaluation of absorption, body distribution and metabolism of food ingredients.
3. Detection and control of food spoilage or pathogenic microorganisms.
   - Identification of biomarkers (genes, proteins, metabolites) representative for specific food spoilage and/or pathogenic microorganisms.
4. Efficacy testing of bioactive functional food ingredients.
   - Changes in gene expression and proteome relevant to the states or treatment of certain diseases.
5. Food allergy.
   - Identification of allergic proteins through sophisticated proteomics based on recognition of specific posttranslational modification and digestion-resistant peptide features.
6. Quality and authenticity of foods.
   - Proteome of certain food (wheat, wine, fish) can be used to authenticate food origin or food quality.

Table 1
Survey of animal proteins

Protein	Purpose of the analysis	Method or combination of methods applied	Ref.
Egg proteins			
Protein profiles	Characterization of major proteins in chicken eggs (white and yolk) and cow's milk (casein and whey) whole egg in raw or cooked minced meat	CE	[36]
		IEF	[37]
Conalbumin	Alkalisation treatment	GE, DSC, SEM	[38]
Globulin	Distinguishing between irradiated and unirradiated egg	CE	[40]
Ovoglobulin G2, G3, G4	Washing and oiling of eggs	1D-PAGE	[39]
Lysozyme	Distinguishing between irradiated and unirradiated egg fresh and stored eggs egg white allergens	CE, reversed-phase HPLC, SDS–PAGE, CZE	[40,42]
Ovoalbumin, ovalbumin A1	Distinguishing between irradiated and unirradiated egg egg white allergens Alkalisation treatment		
	Washing and oiling of eggs	CE, CZE, GE, DSC, SEM, 1D-PAGE	[38–41]
Ovomucoid	Egg white allergens	CZE	[41]
Ovotransferrine	Distinguishing between irradiated and unirradiated egg egg white allergens	CE, CZE	[40,41]
Vitelline	Fresh and stored eggs	Reversed-phase HPLC, SDS–PAGE	[42]
Meat proteins	Detection of mechanically recovered meat Authenticating commercial flatfish Identify meat species		
	Dhub (lizard) meat	IEF, CE, ultra thin-layer IEF, horizontal SDS gradient 8-18 electrophoresis, vertical SDS electrophoresis	[43–46]
	Identification of animal material in food (which can be applied to ruminant feed), analysis of BSE and BSE-related tissue	SDS–PAGE, ELISA, PCR	[47]
	Flatfish species identification and differentiation	CZE	[48]
	Separation and quantification of fish and squid myofibrillar proteins	SDS–CGE	[49]
	Detection of turkey meat in beef and pork	ELISA	[50]
	Detection of chicken meat in raw meat mixtures (burgers, sausages)	ELISA	[51]
Milk proteins	Genetic variants of milk proteins from different species	CE	[52]
	Protein profiles, adulteration of fresh milk	CE	[53]
	Whey protein/casein ratio in mares' colostrum and milk	SDS–PAGE, CE	[54]
	Whey protein degradation during heat treatment of cheesemaking milk	CE	[55]
	Whey protein fraction of milk powders (monitoring storage conditions)	CE	[56]
	Determination of α-lactalbumin and β-lactoglobulins of cow's milk	ELISA	[57]
	Analysis of milk proteins and protein polymorphism; evaluation of heat treatment of milk; adulteration detection in dairy products; and monitoring of proteolysis in cheese and milk	CE	[58]
	protein changes in stored ultra-high-temperature-treated milks	CE, cation-exchange HPLC	[59]
Bovine β lactoglobulin B (β-LgB)			
Lactoglobulin A (β-LgA)	Cow milk in ewe milk and cheese, separation of milk proteins, heat-treated milk, raw and UHT milk	CE, CZE	[60–63,71]
Casein, α, β, κ	Separation of milk proteins	CE	[61,63]
Casein	Casein fractions in goats milk	CE	[64]
	Casein degradation in Cheddar cheese	CZE	[65]
	Study of proteolysis during ripening of Gouda-type cheese	CE	[66]
	Identification of bovine milk caseins	CE, SDS–PAGE	[67]
β-Casein	Transgenic modification of milk protein structure	FPLC, 1D-PAGE, CZE	[68]
α-Lactalbumin	Separation of milk proteins Heat-treated milk	CE, CZE	[61,63,71]
β-Lactoglobulin	Glycosylation of bovine β-lactoglobulin	MS (ESI), CE, reversed-phase HPLC	[69]
Caprine	Polymorphism	CE	[70]
Bovine serum albumin (BSA)	Heat-treated milk	CZE	[61,71]

Table 2
Survey of plant proteins

Source	Proteins	Purpose of the analysis	Method or combination of methods applied	Ref.
Cereal proteins	Prolamine, gluteline	Separation of cereal protein (wheat, oats, rice, barley, and rye) and cultivars differentiation (wheat)	CZE	[72]
	Storage proteins	Characterization of maize and sorghum proteins	CZE	[73]
	Storage proteins	Differentiation, characterization of proteins and protein classes, classification, quality prediction in breeding	HPCE	[74]
	Grain protein	Identification wheat–rye chromosomal translocations, detection of flour or grain derived from 1AL.1RS and 1BL.1RS wheat	HPCE	[75]
	Gluten	advances in the analysis of gluten structure and quality	HPCE, reversed-phase HPLC, SDS–PAGE, PCR	[76]
	Prolamine	identification of cereal protein (wheat, oats, rice)	CE	[77]
	Wheat albumins, globulins, gliadins and glutenins	Characterisation of Hard red winter wheat (Shawnee) protein fractions	Reversed-phase HPLC, HPCE, 1D-PAGE, SDS–PAGE	[78]
	Wheat albumins, globulins, gliadins and glutenins	Monitoring of wheat maturation process	HPCE	[79]
	Casein, gliadin, zein	Separation of tryptic digests of β-casein, of gliadins from various of durum and bread wheat, and of zeins extracted from corn	CZE, cIEF	[80]
	High molecular mass glutenin subunits	Wheat proteins separation by SDS–CE and SDS–PAGE	SDS–CE, SDS–PAGE	[81]
	Gliadins	Wheat varieties	GE	[82]
	Gliadin subclasses	Preparative fractionation of gliadins	A-PAGE	[83]
	Wheat gliadins	Initial studies and application to varietal identification, wheat variety identification, classification and for predicting wheat quality	GE, CE	[84]
	High molecular mass glutenin subunits	Glutenin subunit composition vs. breadmakimg properties	SDS–PAGE, CZE	[85]
	High molecular mass glutenin subunits	Variation among Glu-1 high molecular mass glutenin subunits in New Zealand, Australian and Canadian wheat	CE, SDS–PAGE, reversed-phase HPLC	[86]
	High molecular mass glutenin subunits	Separation of low and high molecular mass glutenin subunits	CE, SDS–PAGE, reversed-phase HPLC	[87]
	Gliadins and glutenins	Ultrastructure of gliadin and glutenin	EM, HPCE	[88]
	Gliadins and glutenins	Gliadins and glutenins from four hard red winter wheat separated by a novel 2-dimensional (2D) technique	Reversed-phase HPLC, CZE	[89]
	Gliadins and glutenins	Wheat varietal identification	CE	[90]
	Zein	Quantitative analysis of zein proteins in corn	MEKC	[91]
	Hordein	Separation and characterization of barley (*Hordeum vulgare* L.) hordeins	CZE, two-dimensional reversed-phase HPLC and CZE	[92]
Peanut		Separation of peanut-seed and peanut-leaves proteins	CZE	[93]
	Acid soluble and acid insoluble peanut proteins	Identification of heat sensitive proteins during roasting of peanut seed (*Arachis hypogaea* L.)	CE, PAGE	[94]
	Peptide maps	Identification of peptides of peanut protein digest as potential indicators of peanut maturity	CZE, reversed-phase HPLC	[95]
		Characterization of methanol-soluble and methanol-insoluble proteins from developing peanut seed	SDS–PAGE, CE	[96]

Table 2. Continued

Source	Proteins	Purpose of the analysis	Method or combination of methods applied	Ref.
Potato	Patatin	Isolation and characterization of patatin isoforms	IEC, IEF, 1D-PAGE, CE	[97]
	Patatin	Effect of storage of whole potatoes on the patatin and protease inhibitor content	CE–MALDI-TOF-MS	[98]
Soy		Characterization of soy protein and alkaline protease hydrolysates of soy protein	CE	[99]
	Soybean seed protein	Seed quality vs. protein composition	CGE	[100]
		Detection of soy proteins in dairy products	SDS–PAGE, IEF, gel-permeation chromatography	[101]
Miscellaneous	Protein of royal jelly	Characterization of protein in royal jelly	SDS–PAGE, CE	[102]
	Wine protein	Identification of the grape, winemaking technique	CZE	[103]
			CZE, 1D-PAGE	[104]

7. Production of food ingredients.
    - The yield of bioprocesses (production of amino acids, carbohydrates, etc.) may be controlled through metabolome/proteome of microorganisms used for such production.
8. Food processing.
    - Proteome and/or metabolome of starter culture of fermentation processes (beer, cheese, sausage, etc.) can be used to predict the quality of the fermented end-product.

Combining proteomic technologies with genetics, molecular biology, protein biochemistry and/or biophysics and bioinformatics will result in accelerated discovery of protein functional information. Mycoprotein is an example of results of application of genomics technologies in food industry. As a new food ingredient, mycoprotein, is about to be introduced into the market in the United States, which has both physical and clinical functional properties. The mycoprotein is the generic name given to the ribonucleic acid-reduced biomass comprising the hyphae (cells) of the organism culture PTA 2684 grown under axenic conditions (i.e., without adventitious microbial growth) in a continuous fermentation process. Mycoprotein can be used as muscle fibre replacer, fat replacer or cereal replacer. More information can be found on a web site [109] or in a special report [110]. Korhonen et al. [111] reviewed impact of processing on bioactive proteins and peptides.

It is expected that the application of the integrated nutrigenomics (transcriptomics, proteomics, metabolomic) approach in nutritional sciences, will allow for accelerated implementation of mechanistic knowledge in food design. In food technology it will reduce research and development times, thereby reducing costs and shortening time-to-market [14].

Table 3
Survey of microbial proteins

Proteins	Purpose of the analysis	Method or combination of methods applied	Ref.
Protein profiles	Characterization of *Enterococcus* species	SDS–PAGE	[105]
Protein profiles	Whole-cell proteins of *Lactobacillus delbrueckii*	CGE, PAGE	[106]
	Analysis of a recombinant DNA protein in [*Escherichia coli*] fermentation broth matrix	CE	[107]
Mannoprotein α, β	*Saccharomyces cerevisiae*, emulsification properties	CE	[108]

4. Nomenclature

2DE	Two-dimensional gel electrophoresis
A-PAGE	Acidic polyacrylamide gel electrophoresis
CAF-IEF	Carrier ampholyte-free isoelectric focusing
CE	Capillary electrophoresis
CEC	Capillary electrochromatography
CE–SDS	Sodium dodecyl sulfate (SDS) polymer-filled capillary gel electrophoresis
CGE	Capillary gel electrophoresis
cIEF	Capillary isoelectric focusing
cITP–CZE	On-line coupled capillary isotachophoresis with capillary zone electrophoresis
CZE	Capillary zone electrophoresis
DSC	Differential scanning calorimetry
ELISA	Enzyme-linked immunosorbent assay
EM	Electron microscopy
FPLC	Fast protein liquid chromatography
GE	Gel electrophoresis
ICAT	Isotope coded affinity tagging
IEC	Ion-exchange chromatography
IEF	Isoelectric focusing
JPR	*Journal of Proteome Research*
HPLC	High-performance (pressure) liquid chromatography
HPCE	High-performance capillary electrophoresis
LVSEP	Large volume sample stacking using electroosmotic flow pump
MALDI-TOF-MS	Matrix assisted laser desorption/ionization time-of-flight mass spectrometry
MEKC	Micellar electrokinetic chromatography
1D-PAGE	One-dimensional polyacrylamide gel electrophoresis
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PROTEOME	PROTEins expressed by a genOME
SDS–PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SELDI	Surface-enhanced laser desorption/ionization
SEM	Scanning electron microscopy

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