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review

Application of Proteomics in Food Technology and Food Biotechnology: Process Development, Quality Control and Product Safety

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Summary

Human food is a very complex biological mixture and food processing and safety are very important and essential disciplines. Proteomics technology using different high-performance separation techniques such as two-dimensional gel electrophoresis, one-dimensional and multidimensional chromatography, combined with high-resolution mass spectrometry has the power to monitor the protein composition of foods and their changes during the production process. The use of proteomics in food technology is presented, especially for characterization and standardization of raw materials, process development, detection of batch-to-batch variations and quality control of the final product. Further attention is paid to the aspects of food safety, especially regarding biological and microbial safety and the use of genetically modified foods.

Key words: proteomics, food proteins and peptides, food quality, food safety

Introduction

The use of proteomics for process development and validation in food technology and food biotechnology as well as corresponding quality control of starting materials and final products was at the beginning rather limited. There were only few presentations in the sections 'Biotechnology perspectives' and 'Proteomics in biotechnology' at HUPO World Congress five years ago, fewer of them really dealing with the application of proteomics (*1*,*2*). In last years it has changed rapidly so proteomics technology is routinely used, and the terms 'industrial process proteomics' (*3*) and 'industrial proteomics' (*4*) are now frequently used (*5*,*6*).

Gupta and Lee (*7*) discuss the use of genomics and proteomic techniques for development, validation and optimization of bioprocesses. Recently, we also have discussed the possibility for the use of this technology for the validation of downstream processing, determination of batch-to-batch variations and quality control of therapeutic proteins (*8*). Proteomics can also be used for validation and control of industrial processes of food products.

In a pioneering review, Piñeiro *et al.* (*9*) discussed the use of proteomics as a tool for the investigation of seafood quality and detection of possible bacterial contamination. The next early use of proteomics in food technology and for quality control was the proof of usage of anabolic steroids in meat and milk products (*10*). By use of 2D electrophoresis and matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spec-

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trometry, Lametsch and Bendixen (*11*) identified several candidates for quality markers for *post mortem* conversion of muscle to pork meat during storage.

The main difficulty in the use of proteomics in the food industry based on processing of plant material is that the complete genome sequence of many plant species is still not known. This situation is now rapidly improving, and the genome of plants such as rice that are important for human and animal nutrition are now either sequenced, or their sequencing is the topic of ongoing projects (*12*). In an analysis of alfalfa (*Medicago sativa* L.) protein pattern during industrial processing, Incamps *et al.* (*3*) demonstrated the use of proteomics for process development and quality control. The genome of this plant was still not sequenced, and the data available from related genomes had to be used. Rice, as the most economically and nutritionally important crop, is the model plant species. Further relevant proteomic analyses have also been performed on industrial plants and plants important for human and animal nutrition such as potato, soybean, wheat and maize (*13*).

In 'classical' fermentation industry, proteomics is also used for bioprocess improvement, validation and quality control (*14*). Microorganisms are important for processing of many food products (*15*), but also as a cause of several side effects such as foulness and food poisoning, and proteomics is increasingly used for their characterization and detection (*16*). Some biofilm-forming microorganisms can resist very aggressive cleaning and sanitation procedures, and can cause serious contamination during the food processing, and the knowledge of their proteome can be useful to detect and to prevent the contamination of food products by these agents (*17*). On the other hand, microbial cells immobilized in natural biofilms can be used in food and beverage fermentation (*18*).

In this paper, the strategy for the use of proteomics in food technology for process validation and optimization, quality control and reduction of batch-to-batch variations of final products is presented. The problem of detection of alternations caused by the use of genetically engineered food of plant origin (*19*), food safety, especially regarding contamination with allergens (*20*) and microorganisms (*16*) is also discussed.

Proteomics as a Tool for Product and Process Validation and Optimization

In a pioneering work, Incamps *et al.* (*3*) performed a systematic proteomic analysis along a plant-scale wet fractionation process of alfalfa biomass. The manufacturing process induces significant changes including chemical modifications, heat-shock protein responses and proteolytic degradation. It was also demonstrated that during biomass processing, especially thermal treatment, a certain level of cellular regulation is still conserved such as induction of heat shock and redox stress proteins. Proteolytic degradation of structural proteins and other changes in meat also start during storage and the first processing step of protein-rich food of animal origin such as porcine meat (*21*).

Advances in protocols for food processing have resulted in a reduction of the manufacturing time and optimization of product quality. The increase of production capacity also increases the need for better process control. Software-driven computer control systems, *e.g.* in milk or meat processing industry have made it easier and faster to change parameters during processing and production cycles. Proteins are largely responsible for the characteristics of many food products during the manufacturing process. Physicochemical properties, such as viscosity, thermal conductivity and vapor pressure, but also nutritional and sensory properties of milk, meat and cereal-derived products depend on their protein composition and content (*22*). In wheat flour-derived products the optimal characteristics are determined by gluten proteins; in milk and milk products, the dominating protein is casein. The proteomics-based approach for validation of a process for production of wheat-based foods is shown in Fig. 1. Because of their importance, both proteins/protein groups are well characterized (*23,24*).

Protein compositions of other foods such as meat and meat products, or fruit and vegetables are more complex, and the change of physicochemical properties during processing depends on more than one highly abundant protein (*21,25*). A significant amount of pork and beef is consumed fresh, and meat texture and juiciness are the most important of all organoleptic characteristics contributing to their quality. According to proteomic studies, the meat tenderness in both pork and beef is associated with the structural proteins such as myosin, actin, desmin and tubulin (*26*). In a semi-quantitative comparison, based on the comparison of intensity of different protein/peptide spots in 2D electrophoresis, Laville *et al.* (*27*) identified 14 different proteins that are a kind of 'candidate biomarker' for shear force values of cooked meat. Further studies about the meat texture and drip loss were also performed (*28*). Sayd *et al.* (*29*) also showed that some proteins from sarcoplasmatic reticulum of pig muscle, especially enzymes involved in oxidative metabolism, are responsible for color development which is the next organoleptic characteristic responsible for meat quality. Muscle mitochondria are also highly sensitive to protein carbonylation. By applying a complex labeling strategy, more than 200 carbonylated proteins were detected. Other oxidative modifications such as nitrosylation and hydroxylation were also detected in many carbonylated proteins. This finding provides further evidence of the susceptibility of muscle mitochondrial proteins to oxidative damage (*30*). Storage and treatment during production process are also responsible for changes in fish muscle proteins, again responsible for product properties (*31,32*).

Technological treatment may affect the overall food quality. As demonstrated above, induction of some proteins during the early stages of the process is one of the unexpected changes. Inappropriate heat treatment of milk, meat, cereal products or fruits and vegetables can negatively influence the product quality. The main modifications induced by heat treatment are protein denaturation and the complex series of chemical reactions known as Maillard reaction. An extensive review about Maillard reaction, especially from the proteomic point of

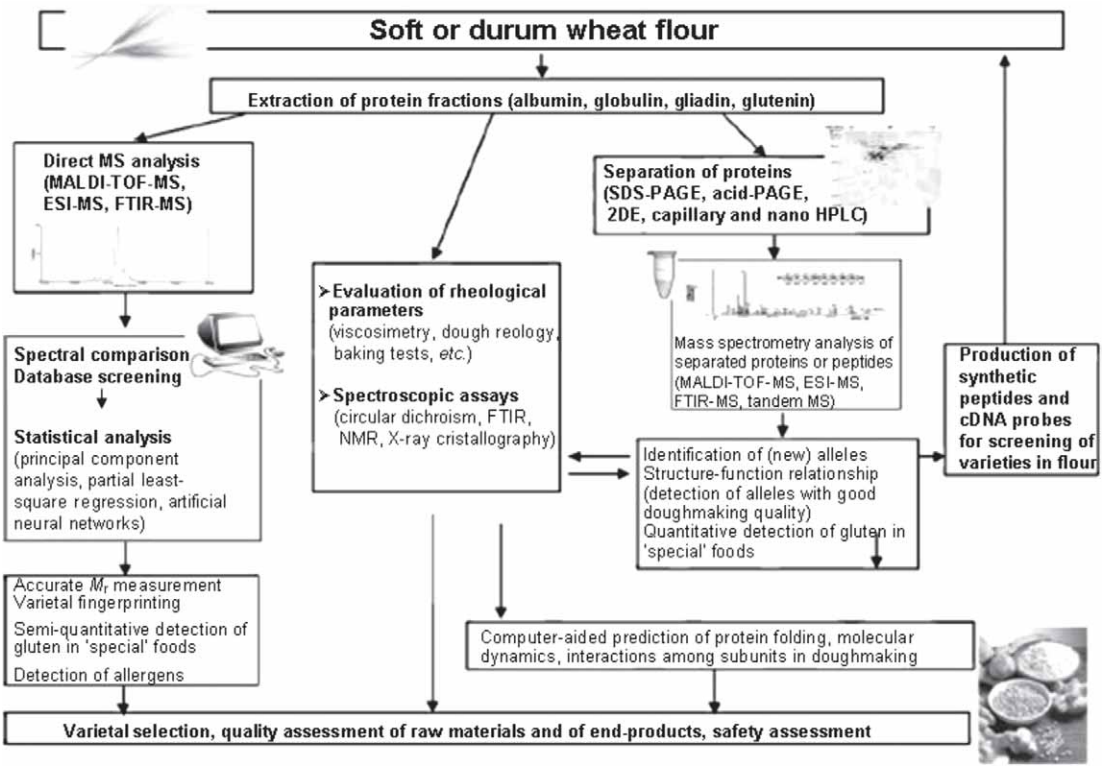

Fig. 1. Proteomics approach to validation of a process for production of wheat-based foods. Reprinted from Mamone *et al.* (*22*) with permission

view, has recently been given (*33*). Specific properties of food products such as color, texture digestibility, and nutritional value can be affected by the Maillard reaction. As a consequence of involving the side amino group of lysine, an essential amino acid, the nutritional value of food can be impaired. Glycation of proteins in meat and meat products is a further change that can affect their quality and nutrition value. It is considered as the first step in Maillard reaction. This reaction can be controlled by modifying food composition, processing and storage conditions (*33*). Furthermore, the Maillard reaction between amino acids, mainly asparagine and reducing sugars such as fructose, galactose, lactose and glucose can lead to formation of harmful acrylamide in food during roasting, toasting and frying processes (*34*). Furthermore, carbonylation of milk proteins such as β-lactoglobulin during industrial treatment can induce allergies against milk products. Carbonylated proteins can be detected by immunoblot and identified by MALDI-TOF MS or electrospray ionization tandem mass spectrometry (ESI-MS/MS) after electrophoretic separation and in-gel digestion (*35*). Scaloni *et al.* (*36*) demonstrated that the protein-bound carbonyl content in heat-treated milk samples was positively correlated with the severity of the treatment. On the other hand, well-controlled Maillard reaction can also be induced to achieve specific benefits like aroma generation in baked product and to improve the physicochemical properties of whey proteins (*22*). Deamidation is further form of chemical degradation of proteins. In this irreversible reaction, glutamine or asparagine are hydrolyzed to glutamic acid or aspartic acid, respectively. Mass spectrometric techniques can also be used for detection of this form of protein degradation (*37*). Posttranslational modifications (PTM) of proteins can cause further modifications during the production process. Heat-susceptible phosphorylated serine and threonine residues can yield dehydroalanine and methyl-dehydroalanine, respectively. Different amino acids can also cross-react and form further artificial products, such as lysinoalanine, lanthionine, and histidinoalanine (*22,38*). The difference in solubility of food proteins, *e.g.* wheat glutenins, largely reflects their ability to form inter- or intramolecular disulfide bonds. The newly developed online liquid chromatography-mass spectrometry (LC-MS) with electron-transfer dissociation is a reliable method for determination of disulfide linkages before and during processing of protein mixtures (*39*). Further changes in PTMs, especially in glycosylation, are a topic of plethora of proteomic studies (*40,41*). Combined with other analytical methods, proteomics gives important information about food quality and safety. Monti *et al.* (*42*) demonstrated the use of proteomic methods, such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by protein identification by LC-MS/MS together with capillary electrophoresis for determination of fatty acids and metal ion content in farmed and wild sea bass. They showed that the growth conditions induce significant biochemical and nutritional differences in food quality. In summary, mass spectrometry and mass spectrometry-based proteomics have largely expanded the knowledge of food components. These analytical technologies enable identification and characterization of food components, mainly proteins, carbohydrates and lipids and their changes during the production process and storage. Isotope labeling techniques for quantitative determination of protein-based

components that have been developed in the last five years can give further, quantitative evaluation and process validation, and determination of batch-to-batch variations (*8,43*).

Proteomics and Food Safety

The role of bacteria in food processing and food safety

Foodborne illnesses result in numbers of hospitalizations and even deaths. Each year in the USA, about 325 000 hospitalizations and 5000 deaths caused by food poisoning are registered. Unfortunately, microorganisms and microbial toxins, especially foodborne ones as weapons of mass destruction still remain a threat. In food technology and biotechnology, careful monitoring of microbial contamination in the final product as well as monitoring of the production process and cleaning and sanitation are one of the most essential factors of the manufacturing process (*44*). The identification, confirmation, and quantification of bacteria and bacterial toxins in food are important analytical problems. The most common bacteria that cause food poisoning are *Staphylococcus aureus*, *Campylobacter jejuni*, some *Salmonella* and *Staphylococcus* species, some *Bacillus* strains and *Escherichia coli* O157:H7 strain. There are well-established and sensitive methods for detection of bacteria and their toxins available, mostly based on immunochemical methods. Proteomics and genomics technologies offer further, more sensitive and specific methods for identification of microbial food contaminants and their toxins, and for monitoring of cleaning and sanitation (*45–48*).

There are only few investigations that follow changes of proteomics of contaminating bacteria during food processing and equipment sanitation. The use of high hydrostatic pressure (HHP) technology is a new method for food preservation. Proteins are known to be the most important target of high pressure in living organisms (*49*) and HHP inhibits the growth of microorganisms by inactivating key enzymes that are involved in DNA replication and transcription enzymes and modifying both microbial cell walls and membranes (*50*). However, some bacteria such as *Bacillus cereus* can survive HHP treatment. Martínez-Gomariz *et al*. (*51*) analyzed changes in the proteome of this model organism during the HHP treatment. They found quantitative differences and identified some of differently expressed proteins. As expected, the expression of some proteins involved in nucleotide metabolic process was changed, but some other proteins such as those involved in carbohydrate catabolic process and transport, refolding, amino acid biosynthesis and bacterial ciliary and flagellar motility were also differentially expressed.

In a remarkable study, Boehmer *et al*. (*52*) follow proteomic changes in whey samples from a group of cows before and 18 h after the infection with *E. coli.* Due to decreased milk production and quality, discarded milk and cattle mortality, such infections can cause mastitis, which is the most costly disease that affects the dairy industry. The aim of that study was the identification of biomarkers for evaluation of the efficacy of adjunctive therapies in decreasing inflammation associated with mastitis. Higher expression of some acute phase proteins such as transthyretin and complement C3 were found in whey samples 18 h after bacterial infection, but also some antimicrobial peptides and further acute phase α-1-acid glycoprotein were also detected. These proteins are candidate biomarkers for future research into the effect of bacterial inflammation during mastitis.

As mentioned above, biofilm formation is an important fact that has to be taken into consideration during design of cleaning of stainless containers and other surfaces in food processing facilities. This problem has already been discussed in a review paper about microbial proteomics (*5*). In biofilms, some microorganisms such as sporogen bacterium *Bacillus cereus* (*53–55*), the Gram-positive bacterium *Listeria monocytogens* (*56*) and some pathogenic *E. coli* strains (*57*) can survive on the surface of stainless steel containers and other surfaces in the manufacturing facility, even under cleaning and sanitizing conditions. Better knowledge of biofilm formation and conditions that cause its degradation is necessary to prevent contamination by the above listed bacteria (*58*). Other biofilm-forming bacteria, such as *Staphylococcus* species (*59*) can survive food processing and cause human and animal infection. Incorporation of microorganisms is a kind of the natural way for their immobilization, and the high density of biofilms gives them better ability to survive aggressive treatment, but also a substantial biocatalytic potential. The use of immobilized bacterial cells and bacterial biofilms for biosensors for food quality analysis and fermentation process control has been discussed elsewhere (*5,18,60*), and use of immobilized yeasts in brewing and winemaking processes will be presented later. In summary, in addition to physiological and genomic analyses, proteomic analysis of biofilm-forming microbial cells gives valuable information about their behavior during food processing and storage, symbiosis, possible infection and potential food poisoning, their defense against antimicrobial agents, and the potential to survive the cleaning and sanitation process (*5,18,58*).

The health-promoting properties of some bacterial species that colonize the human gastrointestinal tract have been documented in clinical trials and they are gaining popularity as food additives (*61*). Bifidobacteria and lactobacilli are the most popular microorganisms that are added as live bacteria to food preparations under the generic name of probiotics (*61–63*). The proteomic map of *Bifidobacterium longum*, a strict fermentative anaerobe, was first performed about five years ago (*64,65*). The topics of the following investigations included the survival mechanisms of this bacterium, focused on altered protein expression following bile salt, heat or osmotic shock, which these bacteria are exposed to in the human gastrointestinal tract and during the food manufacturing process (*66–69*). These studies can also be used as a model for survival of other bacteria under similar conditions (*69,70*).

Prions

All prion diseases or transmissible spongiform encephalopathies (TSEs) are characterized by the deposition of an abnormal conformation (PrP^{Sc}) of a normal cellular protein (PrP^{C}) in neural tissues in humans and animals. The different protein conformations are associated with

different physicochemical properties (*71*). PrP^{C} is relatively soluble and protease-sensitive, while PrP^{Sc} is relatively insoluble and protease-resistant. TSEs include scrapie in sheep and goats, and bovine spongiform encephalopathy (mad cow disease or BSE). Human form of this disease is infectious Creutzfeldt-Jakob disease (CJD) caused by the consumption of meat and meat products of prion-infected animals (*71,72*). The outbreaks of BSE and infectious variant CJD have prompted the need for reliable screening methods for prion infections as part of the safety control for meat and meat products. Identification of prion proteins is usually a time-consuming process and includes immunoaffinity techniques, combined with one- and two-dimensional electrophoresis and mass spectrometry (*73,74*). Although intensive studies have been performed, it is still long way to identifying reliable biomarkers for prion infection. Detection of prion--binding proteins did not give further revealing information about the biology of prions and the pathogenesis of TSE (*72,74–76*). One of potential biomarker candidates is ubiquitin. This protein could be identified in the cerebrospinal fluid of CJD patients (*77*). However, this recent study has been performed only with a small number of samples, and ubiquitin as a highly abundant protein cannot be taken in consideration as a reliable biomarker. Herbst *et al*. (*78*) used a multidisciplinary approach to identify *ante mortem* markers for prion disease. This rather complex strategy combines matrix--assisted laser desorption/ionization Fourier transform mass spectrometry (MALDI-FTMS), mass fingerprinting and bioinformatics for identification of candidate biomarkers in infected animals. Again, results of this study are still rather limited, and true positive rate was relatively low. More promising is recently published study by Nomura *et al*. (*79*). This group reported detection of autoantibodies in the sera of cattle with bovine spongiform encephalopathy. These autoantibodies were directed against glial fibrilary acidic proteins, and could be detected only in the serum of TSE-infected animals.

Tsiroulnikov *et al*. (*80*) presented a method for decontamination of meat and bone meal by use of bacterial proteolytic enzymes. Nattokinase from *Bacillus subtilis* that has been used for fermentation of boiled soybeans is also able to degrade prion proteins and potentially prevent prion infection (*81*). However, it is still a safety risk, if such contaminated animal food is used, and prion detection and elimination of diseased animals and contaminated meat (*74–79*) is a much safer way to prevent these kinds of foodborne diseases.

Allergens and toxic components

Proteins are responsible for many allergic reactions. The most threatening allergic reaction, anaphylaxis, is most frequently caused by peanuts or tree nuts (*82*). That is also the reason that most proteomic investigations towards identification and quantification of allergens were performed on food of plant origin (*83*). Milk and milk products, as well as seafood and processed food are other kinds of food that cause allergies (*35*). However, there are only few investigations of animal proteins involved in these adverse reactions.

Proteomic strategies used in order to achieve more detailed and comprehensive characterization of food allergens are referred as 'allergenomics' (*84*). The common procedure for detection of proteins involved in allergic reactions is protein extraction (*e.g.* with 8 M urea and 4 % CHAPS, buffered with 40 mM TrisHCl, pH=7–8), electrophoretic separation (SDS-PAGE or 2D electrophoresis), and detection of IgE binding proteins by immunoblotting. After tryptic digestion, the IgE binding proteins as potential allergens can be identified by mass spectrometry (*84,85*). This very effective, but also time--consuming method is similar to the method presented in Fig. 2. Stevenson *et al*. (*82*) use gel-free, label-free quantitative approach for identification of peanut allergens. Quantitative evaluation was achieved by peak integration and spectral counting in comparison with a protein standard. The workflow of this analytical procedure is shown in Fig. 2. In the future, this method could be useful for high-throughput profiling of proteins, including seed allergens. However, more standardization and validation are still necessary.

Most allergies in the USA are caused by peanuts and peanut-containing food products (*82*), and peanut proteins that may cause allergies are well characterized. Chassaigne *et al.* (*86*) use 2D electrophoresis, immuno-

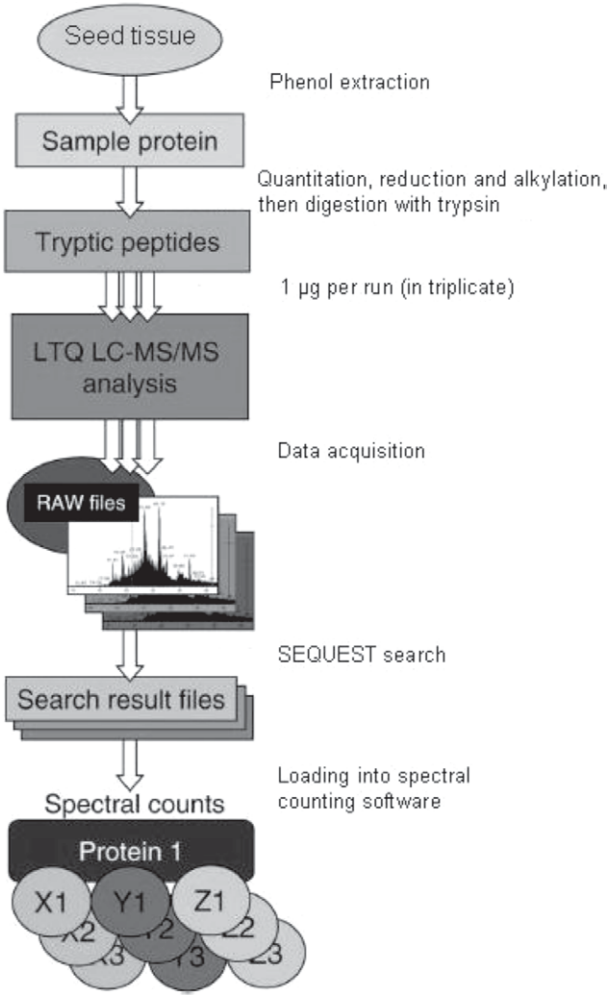

Fig. 2. Workflow for biological sample preparation and LC-MS/MS analysis of proteome using in-solution digestion and label-free quantitative analysis. Reprinted from Stevenson *et al.* (*82*) with permission

blotting and high-resolution mass spectrometry for allergen detection in peanut seeds. They detected several isoforms of main allergens: Ara h 1, Ara h 2 and Ara h 3/4. Proteomic analyses show different contents of these allergens in different peanut varieties, and also the presence of several fragments of these proteins (*87*). As shown by Stevenson *et al.* (*82*), these proteins are absent from genetically engineered peanut seeds.

Bässler *et al*. (*88*) used a multidimensional protein fractionation strategy and LC-MS/MS for the molecular characterization of tomato seed allergens. In subsequent *in silico* modeling, high homology between epitopes of known allergens from walnut (*89*), cashew nut (*90*) and buckwheat (*91*) was found. Further proteomic analyses of plant proteomes were performed to detect allergens in wheat flour (*83*), maize (*92*) and sesame seeds (*93*).

By use of sophisticated quantitative proteomics technology, Chassaigne *et al*. (*86*) showed that genetically engineered peanut seeds contained significantly reduced amount of main allergens. Genetically modified (GM) tomato and soybean plants are approved for food use by the US Food and Drug Administration. During the assessment procedure, the allergic properties of the gene donor and the recipient organisms are considered in order to determinate the appropriate testing strategy. The amino acid sequence of the encoded protein was compared to all known allergens to assess whether the protein is a known allergen (*88*), and to indicate a probability of allergic cross-reactivity and formation of neo--allergens. Further risk of food allergenicity is the stability of the protein in acidic environment in the presence of stomach protease pepsin. These tests were followed by *in vitro* and *in vivo* binding assays to human IgE, and no adverse reactions were found (*94*). However, some residual risk after long-term consumation of such food still remains, and further studies regarding allergenic potential of GM plants were performed. In subsequent proteomic study, GM *versus* non-modified soybean samples were compared, and 2 new potential allergens were indeed identified. In a short-term study, none of the individuals tested reacted differently to the GM *versus* non-modified samples (*95*). After this study, a residual risk of allergies after long-term consumption of GM crops still remains.

Food of animal origin, especially seafood and milk products, can also cause allergies. However, proteomics tools have only been sparingly applied in the investigation of allergens in these products. It is well known that changes in the main milk protein casein such as carbonylation (*36*) or forming of covalent complexes between casein micelles and β-lactoglobulin (*96*) and modification of other proteins (*97*) during the production process, mainly heating, can cause induction of allergies to milk products, but a thorough proteomic and 'allergenomic' investigation has still to be performed. In their review about the use of proteomics as a tool for the investigation of seafood and other marine products, Piñeiro *et al.* (*9*) recommend the use of proteomics for detection of allergens in food of this origin. However, there are still only few studies in this field. Taka *et al*. (*98*) characterized an allergenic parvalbumin from frog by the use of LC-ESI-MS. The main crustacean allergens are proteins tropomyosin and arginine kinase (*99,100*). Tropomyosin is a myofibrillar protein of 35–38 kDa, and proteins from six species of crustaceans have also been cloned (*101*). Arginine kinase from some commercially relevant shrimp species was characterized by use of proteomic methods (*102*). Some additional shrimp allergens such as sarcoplasmatic calcium binding protein (SCP) have also been detected (*103,104*). Interestingly, this protein was previously detected as allergen in crayfish *Procambarus clarkii* (*105*). This finding further confirms the thesis of Bässler *et al*. (*88*) about shared epitopes in allergens of different origin.

If not inactivated or degraded during processing, some food components such as plant lectins constitute a possible risk, since consumption of raw or incorrectly processed beans can cause outbreaks of gastroenteritis, nausea, diarrhoea, and even more severe side reactions. Most plant lectins are secretory proteins. After secretion, they accumulate either in vacuoles or in the cell wall and intercellular spaces, mostly in seeds. Lectins such as concanavalin A, phytohemagglutinin, pea lectin and flavin are present in quite high levels and accumulate in vacuoles in cotyledons (*106,107*). Most lectins show high specificity to distinct sugars, but they also have an extensive homology in primary structure, also from unrelated species. On the other hand, a plant species such as castor bean may contain structurally related lectins with different toxicity. Castor bean lectin ricin shows relatively weak agglutination, but very high toxicity for humans and animals; *Ricinus communis* agglutinin is a weakly toxic, but strong agglutinin (*106*).

Ricin and *Phaseolus vulgaris* lectin are two most common lectins that cause food poisoning (*106,108*). In humans, consumption of other raw beans can also cause gastroenteritis, nausea and diarrhoea (*109*). On the other hand, bean extracts enriched with lectins or lectin-related amylase inhibitors are used as active ingredients of so--called 'weight-blockers' in dietetic preparations (*110,111*). Proteomic strategies to quantitative analysis of potentially harmful lectins in raw and processed food in dietary preparations include the use of chromatographic or electrophoretic strategies combined with mass spectrometry (LC-MS/MS, MALDI-TOF MS or MALDI-TOF/TOF MS). Affinity chromatography with immobilized glycoproteins or oligosaccharides can be used for enrichment of lectins. Lectin-enriched fraction can be further separated, *e.g.* by cation-exchange chromatography, followed by tryptic digestion and protein identification by mass spectrometry (*106*). However, these methods are still complex, expensive and time consuming. After detection of these potentially harmful components by proteomic methods, specific, 'food based' protocols, *e.g.* ELISA or other simple and fast protocols for their detection and quantitative determination can be developed.

In order to increase muscle accretion and reduce fat deposition, cattle are treated by anabolic steroids (*112*). All biochemical events that are caused by steroid use are oriented towards anabolic metabolism, resulting in a lower tyrosine aminotransferase as a marker of catabolism and a higher muscle building (*113*). Use of steroids can be detected by genomic or proteomic methods (*114–116*). In a study performed on calves, differential expres-

sion of adenosin kinase and reticulocalbin in the liver of calves treated with anabolic compound was found (*116*). It was also shown that metabonomics can be effectively used to study the different disruptive metabolic response in cattle after the use of anabolic steroids (*10,112*). Several biomarkers such as trimethylamine-*N*-oxide, dimethylamine, hippurate, creatine and creatinine were detected in urine of cattle treated with anabolic steroids. These urinary biomarkers characterize the biological fingerprint of anabolic treatment.

Pharmacological practices that are used to increase protein production in livestock can be detected by metabonomic and proteomic techniques that can be used as alternative techniques for screening analysis of veterinary drugs in animal products (*116,117*). Long-time and low-dose treatment administration of antibiotics, mainly tetracyclines, has also been used as a growth promoter in livestock production. This use is banned today in the European Union (*118*). One of the main reasons is that systematic antibiotic use promotes the development of resistant bacterial population (*119*). As already discussed above, the use of proteomics to elucidate molecular mechanisms of meat quality is well established (*28*). Gratacós-Cubarsí *et al*. (*120*) demonstrated that after administration, tetracyclines are rapidly degraded, but in the muscle of pigs treated with tetracyclines, several differentially expressed proteins were detected. Five spots in 2D electrophoresis that belong to differentially expressed proteins and candidate biomarkers for tetracycline treatment were identified as enzymes involved in muscle metabolism and two novel porcine proteins (*120*). Similar differences were also observed in the composition of egg proteins from treated and non-treated chicken (*121*).

Consequences of Genetic Modifications

Exogen DNA fragments can be inserted into the genome of the host organism, mostly the plant, in order to improve productiveness, enhance tolerance to herbicides, or induce production of new substances not present prior to GM (*122*). In order to improve the quality, in GM food of plant origin, some harmful or allergenic proteins can also be removed (*86,123*). However, proteins in the living cell are in permanent interaction, and introduction of a foreign gene product, change in concentration or complete removal of another cellular protein can induce complex and possibly unexpected changes in complete cellular proteome (*121,124*).

The simplest proof of GM in food is the detection of foreign DNA derived from genetically modified organisms (*125*). The comparison between GM and non-GM crops comprises agronomic and phenotypic characteristics that are very sensitive indices of alterations and also robust indicators of equivalence. Feed performance studies with rapidly growing animals are also sensitive bioassays in the level of nutritional value of GM food (*126,127*). The GM food has been in use worldwide for over 10 years and until now no verifiable unintended toxic or nutritional effects as a result of consumption of GM products have been registered (*128,129*). However, the above-mentioned complex changes in proteome as a consequence of GM can be detected only by use of proteomics technology. In a very extensive series of studies, Ruebelt *et al*. (*19,130,131*) compared proteomes of GM and non-GM seeds of the model plant *Arabidopsis thaliana*. Analytical validation of the method (comparative 2D electrophoresis, *19*) and assessments of both natural variability (*130,132*) and unintended effects (*131*) were performed. These studies can be used as fundaments for further quality assessment of GM crops, although faster and more effective methods such as differential in-gel electrophoresis (DIGE) (*51*), isotope labeling techniques (*43*), and gel-free, label-free quantitative approaches (*82*) have recently been developed.

GM crops, especially maize (*133*), tomato (*88*) and soybean (*95,134*) were the topics of further, intensive proteomic studies. Erny *et al*. (*133*) studied alcohol-soluble endosperm proteins, so-called zein proteins from corn of GM and non-GM maize by the use of capillary electrophoresis followed by mass spectrometry. Proteomic fingerprints of different maize lines including the transgenic one were analyzed. Unfortunately, only the analytical method was demonstrated and no further conclusions regarding differences between GM and non-GM seeds were documented. Comparative 2D electrophoresis was used for the analysis of GM and non-GM soybean seeds and eight differently expressed proteins were identified. One of them is Gly m Bd 28k fragment, already known as an allergen (*134*). Allergens were already identified in GM soybean seeds (*95*), and further careful monitoring of these foods is still necessary.

Conclusions

Proteomic techniques are increasingly used for assessment of raw materials and final products as well as for control, optimization and development of new processes in food technology and biotechnology. The ways for possible use of proteomics in food processing and for quality control and safety assessment of final products are illustrated in Fig. 3.

However, most proteomic analyses are performed by the use of comparative 2D electrophoresis, and recently developed, faster and more effective methods such as quantitative isotope labeling (*8,51*) and label-free quantitative proteomics (*82*) are scarcely used. The use of these methods combined with the already developed validation strategies (*19,130,131*) will enable better in-process control and characterization of batch-to-batch variations, as well as increasing use of proteomics for answering some key questions in food science – detection of food contaminants and allergens, and further assessment of safety of GM foods.

There are some papers discussing the potential of proteomics and its use to assess food quality (*135*) and technology (*136*). However, an overview about the use of this promising technique for the characterization of the complete production process in food manufacturing, biological and microbial safety and quality control of the final product (Fig. 3) is still missing. This review shall give further information and enable better understanding of this technique towards better collaboration with researchers engaged in food science and industry.

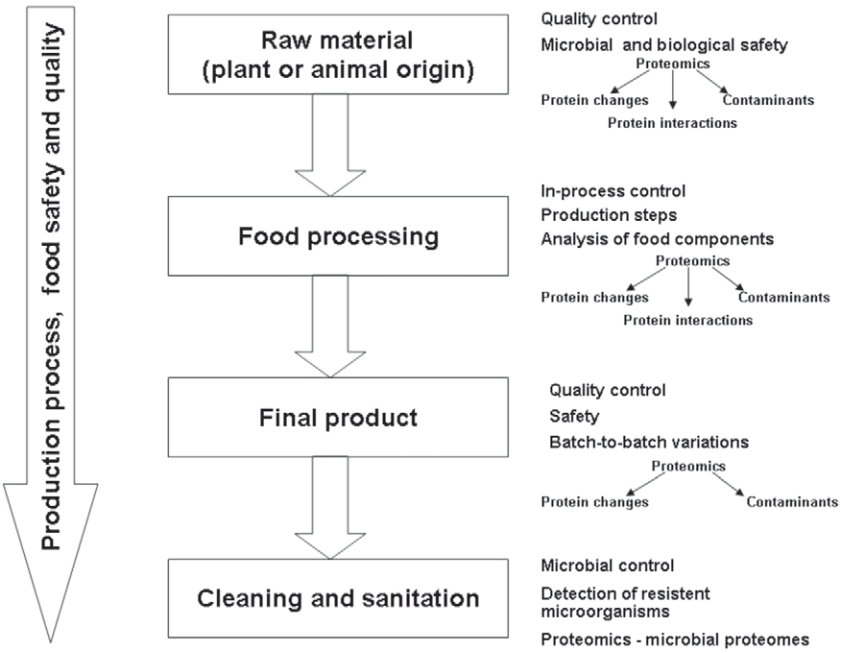

Fig. 3. Use of proteomics in the development pathway for food production, and assessing of food safety and quality

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