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

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.

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.

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

In 'classical' fermentation industry, proteomics is also used for bioprocess improvement, validation and quality control

Microorganisms are important for processing of many food products*, 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*. 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*.

On the other hand, microbial cells immobilized in natural biofilms can be used in food and beverage fermentation

Proteomics as a Tool for Product and Process Validation and Optimization

Soft or durum wheat flour

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Extraction of protein fractions (albumin, globulin, gliadin, glutenin)

Direct MS analysis (MALDI-TOF-MS, ESI-MS, FTIR-MS)

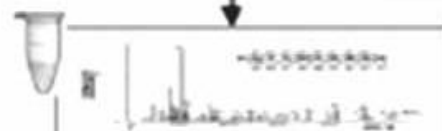**Separation of proteins (SDS-PAGE, acid-PAGE, 2DE, capillary and nano HPLC)**

Spectral comparison Database screening

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Statistical analysis (principal component analysis, partial least-square regression, artificial neural networks)

➢ **Evaluation of rheological parameters** (viscosimetry, dough reology, baking tests, *etc.*)

➢ **Spectroscopic assays** (circular dichroism, FTIR, NMR, X-ray cristallography)

Mass spectrometry analysis of separated proteins or peptides (MALDI-TOF- MS, ESI-MS, FTIR-MS, tandem MS)

Identification of (new) alleles Structure-function relationship (detection of alleles with good doughmaking quality) Quantitative detection of gluten in 'special' foods

Production of synthetic peptides and cDNA probes for screening of varieties in flour

Accurate M_r measurement Varietal fingerprinting

Semi-quantitative detection of gluten in 'special' foods

Detection of allergens

Computer-aided prediction of protein folding, molecular dynamics, interactions among subunits in doughmaking

Varietal selection, quality assessment of raw materials and of end-products, safety assessment

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. *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.

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 *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.

However, some bacteria such as *Bacillus cereus can survive HHP treatment.*
Martinez-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 a-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.

In biofilms, some microorganisms such as sporogen bacterium *Bacillus cereus, the Gram*-positive bacterium *Listeria monocytogens and some* pathogenic *E. coli strains 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.

Other biofilm-forming bacteria, such as *Staphylococcus* species *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, *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 .

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. *Bifidobacteria and lactobacilli* are the most popular microorganisms that are added as live bacteria to food preparations under the generic name of probiotics.

The proteomic map of *Bifidobacterium longum, a strict fermentative anaerobe,* was first performed about five years ago. *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 . *These studies can also be used as a model* for survival of other bacteria under similar conditions.

Prions

All prion diseases or transmissible spongiform encephalopathies (TSEs) are characterized by the deposition of an abnormal conformation (PrPSc) of a normal cellular protein (PrPC) in neural tissues in humans and animals.

Prions

The different protein conformations are associated with different physicochemical properties.

PrPC is relatively soluble and rotease-sensitive, while PrPSc is relatively insoluble and protease-resistant. TSEs include scrapie in sheep and goats, and bovine spongiform ncephalopathy (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.

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.

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.

That is also the reason that most proteomic investigations towards identification and quantification of allergens were performed on food of plant origin.

Milk and milk products, as well as seafood and processed food are other kinds of food that cause allergies.

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'.

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.

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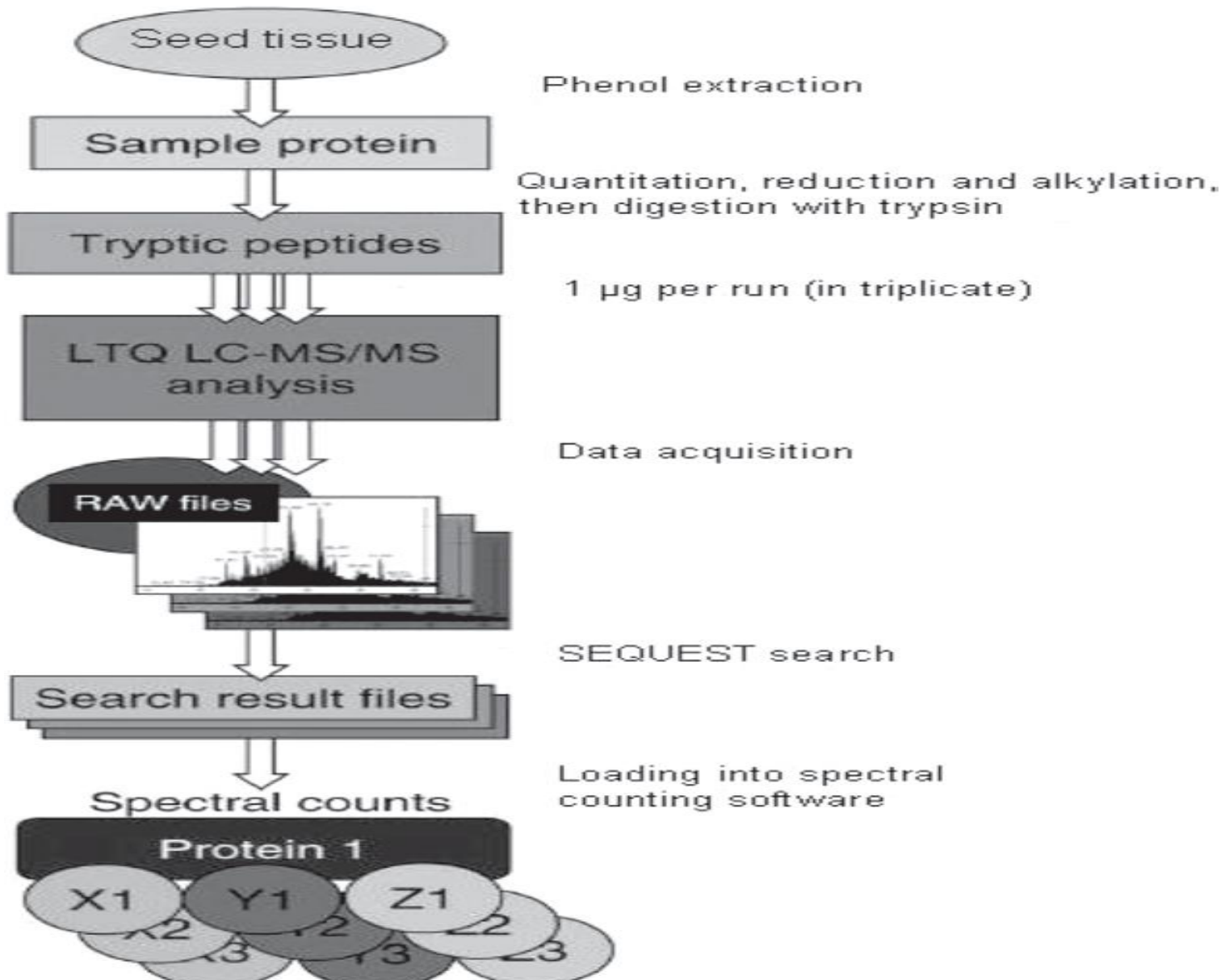
Workflow for biological sample preparation and LCMS/ MS analysis of proteome using in-solution digestion and label-free quantitative analysis. Reprinted from Stevenson *et al.* (*82) with permission*

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. *In order to improve the quality, in GM* food of plant origin, some harmful or allergenic proteins can also be removed. *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.

The simplest proof of GM in food is the detection of foreign DNA derived from genetically modified organisms. *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. *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.

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. compared proteomes of GM and non*-GM seeds of the model plant *Arabidopsis thaliana. Analytical* validation of the method comparative 2D electrophoresis, *and assessments of both natural variability and unintended effects were performed.*

These studies can be used as fundaments for further quality ssessment of GM crops, although faster and more effective methods such as differential in-gel electrophoresis (DIGE)*, isotope labeling techniques,* and gel-free, label-free quantitative approaches *have* recently been developed.

Proteomic techniques are increasingly used for assessment of raw materials and final products as well as for control, optimization and development of new rocesses in food technology and biotechnology.

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 *and label-free quantitative* proteomics *are scarcely used. The use of* these methods combined with the already developed validation strategies *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.