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review

Use of Proteomic Methodology in Optimization of Processing and Quality Control of Food of Animal Origin

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Summary

Food of animal origin, namely meat, seafood, milk and milk products, is the main protein source in human nutrition. These types of food are very complex mixtures that contain proteins and other components, and proteomic techniques enable simultaneous study of several hundred up to several thousand proteins. The use of proteomic methodology for quality control and quality assessment in production as well as for the optimization and development of new manufacturing processes is presented. Newly developed, faster and more selective methods for sample preparation followed by more sensitive mass spectrometry for identification of less abundant proteins are discussed. These techniques will help to understand variations in production, and to find markers for food quality criteria. Furthermore, biologically active peptides in food of animal origin have recently been the focus of proteomic and peptidomic investigations. Isolation and production of biologically active proteins and peptides, including the low abundance ones, will also be a focus of future research. The use of proteomics, peptidomics and metabonomics for the determination of product quality and the detection of adulterations in meat production, seafood identification and in the production of milk and milk products is also discussed.

Key words: proteomics, processing, quality control, food of animal origin

Introduction

The topic of the first part of this review is the application of proteomics in food technology and biotechnology, especially in process development, quality control and the utilization of proteomic methodology in order to insure food safety (*1*). Two previously published reviews on this topic (*2*,*3*) gave an introduction to proteomic methods and discussed their use in order to assess food quality (*2*) and optimize key production parameters (*3*). In a recent review, Pischetsrieder and Baeuerlein (*4*) discussed the successful application of proteomics for the analysis of food composition and quality, safety assessment of genetically modified food, food authentication, identification of food allergens, analysis of the physiological activity of food proteins and peptides, and the analysis of the influence of the production process on the chemical, physicochemical and (possibly) biological properties of food proteins.

In the last 2–3 years, the number of publications dealing with the use of proteomics in food technology and biotechnology has rapidly increased. This research, in turn, has resulted in the publication of the first review articles about the use of proteomics in food production of animal origin, namely meat (*5*), seafood (*6*) and dairy prod-

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ucts (*7*), the role of functional proteomics in lipid research (*8*), and also applications of proteomics in the manufacturing of fermented dairy products (*9*). Recent advances in whole genome sequencing of some plant species that are important for human nutrition have removed the major obstacle for the use of proteomic methods in order to support the optimization of production processes, quality assurance and quality control of food of plant origin (*10–12*).

In fermented food of animal origin such as meat, milk and milk products, changes in the proteome of the substrate (*e.g.* sausages or fermented milk products) and in the starter culture microorganisms (*e.g.* milk bacteria in milk fermentation and cheese production, and bacterial and mixed microflora in fermented meat products) as well as their interaction play a crucial role in the quality of the final product (*9,13,14*).

Finally, organic food of both plant and animal origin plays an increasingly important role in the food market in the developed countries, particularly in the European Union and North America, therefore proteomics is used for the identification and quality assurance of organic food, and the discovery of potentially fake organic products (*15,16*).

Meat and Meat Products

As defined by consumers, tenderness, juiciness, flavour and colour are the most important quality features of meat (*4*). Especially for pork and poultry, water-holding capacity is an additional very important quality trait that is closely related to *postmortem* fluctuations in meat proteome. These changes have recently been investigated by the use of proteomic methods (*4,5,17–20*).

The content and composition of the intramuscular connective tissue is an additional, frequently neglected factor that significantly influences meat quality, especially the tenderness of cooked meat (*21*). The content and relative concentration of different types of collagen vary and are dependent on the type of meat (*22,23*). Proteolysis is responsible for degradation of collagen and other components of intramuscular connective tissue (*e.g.* proteoglycans) during *postmortem* storage of meat. This phenomenon is also partially responsible for increased tenderness of meat after conditioning. Turnover of connective tissue, especially the turnover of different types of collagen seems to be controlled by matrix proteinases and their specific inhibitors (*24,25*). However, the role of these proteins in controlling meat quality still needs to be better explored. In conclusion, connective tissue containing different collagen types, proteoglycans and other matrix proteins is responsible for the so-called 'background' feature of the meat. Consequently, additional identification and quantitation of collagens and collagen proteolysis, also by use of proteomic methods, is necessary.

As discussed above, intramuscular connective tissue is only one of the factors that influence the tenderness and juiciness of meat products. These traits are further influenced by both genetics and environmental factors, and *pre-* and *postmortem* treatments. The knowledge gained by exploring and understanding the variations in the proteome in connection with certain quality and/or processing parameters can be successfully used in order to optimize the production of meat (*5,9,17,20*).

According to Hollung *et al.* (*5*) and Bendixen (*17*), in meat science and technology proteomics can be used for: (*i*) proteome mapping and meat identification; (*ii*) determination of proteome changes due to genetic variations; (*iii*) determination of changes due to pre-slaughter conditions; (*iv*) determination of *postmortem* changes; (*v*) study of changes associated with meat quality traits; (*vi*) peptidomics – study and detection of changes in peptide composition during meat storage and processing (*26*); (*vii*) quality assessment of dry-cured and fermented meat products.

Further topics that will be discussed are meat spoilage, especially during handling and distribution (*27*) and the use of microbial starter cultures for production of fermented and dry-cured meat products (*28,29*). Food safety issues, especially toxicological and microbiological safety of meat and meat products, have already been discussed (*1*), but some additional points regarding the use of proteomics in the identification of recently recognized safety hazards, such as avian and swine flu, will also be mentioned (*30*).

Proteome mapping and meat identification

Proteomic methods have become crucial tools for identification of the meat origin (*5,31*). Because of the economic importance of these products, most proteomic investigations have been performed on beef (*3,5,32*) and pork meat (*5,18,20*). Murgiano *et al.* (*20*) and Hollung *et al.* (*33*) investigated the proteome in meat originating from pigs of different breeds. In this investigation, after extraction from the sample, proteins were separated by 2D electrophoresis and identified by mass spectrometry. Altogether, 1125 protein spots were analyzed, and a total of 63 proteins that were different in two different breeds were identified (*33*). The proteome of bovine skeletal muscle was investigated by Bouley *et al.* (*34*) and changes in the abundance of 13 proteins as a consequence of changes in muscle phenotype were identified. Morzel *et al.* (*35*) gave a thorough investigation of the protein composition of the *longissimus thoracis* of 'Blonde d'Aquitaine' young bulls. They found that the abundance of the enzyme succinate dehydrogenase was the best predictor (or biomarker) of meat tenderness. Ovine muscle proteome was analyzed by Hamelin *et al.* (*36*) in order to investigate muscle hypertrophy, which is important for the increase of the size of muscle cells. A possible role of transferrin and α-1-antitrypsin in myogenic proliferative signalling and yield of muscle proteins was discussed. The proteome of chicken skeletal muscle during growth was investigated by Doherty *et al.* (*37*), and 51 proteins were identified. Unfortunately, some of the low abundance proteins detected by 2D electrophoresis and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry could not be identified because of the lack of chicken genome sequence.

Plant proteins, mostly originating from soybean, are frequently added to processed meat products either for economic reasons or to improve their functional properties. Leitner *et al.* (*38*) used liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem

mass spectrometry (LC-MS/MS) in order to detect soybean proteins in meat products. In all soybean protein-containing meat samples, the plant protein glycinin G4 subunit A4 was identified, and this protein can be used as a target for simpler analytical methods in order to identify the addition of soybean proteins to meat products, and possible adulteration of meat and meat products.

Determination of proteome changes due to genetic variations

Genetic variation in animals, most often due to human selection, causes phenotypic differences that can be studied by use of genomics and proteomics (*5*,*20*). In cattle, breeds selected for milk production are different from meat-producing ones. The Holstein Friesian and Chianina cattle breeds are representative examples of animals that have undergone extreme selection for milk and meat traits, respectively. Quantitative comparison of the proteome in liver samples from these two cattle breeds resulted in thirty-nine differently expressed proteins (about 7 % out of over 550 identified ones) (*39*). Proteomic differences that are dependent on pig breed have also been detected (*20*,*33*). Again, out of over 1200 identified proteins, approx. 8 % were differently expressed when the proteomes of two different pig breeds, Norwegian Landrace and Duroc, were compared. In chicken, significant differences in the skeletal muscle proteome were detected in animals selected for meat production when compared to those selected for egg production (*37*).

Determination of proteome changes due to pre-slaughter conditions

The treatment of animals before slaughter (pre-slaughter conditions) significantly influences meat quality by modulating important parameters, such as water-holding capacity of pork meat and beef tenderness (*5*). Lametsch *et al.* (*40*) and Morzel *et al.* (*41*) detected several differently expressed proteins in the meat of pigs that were exposed to different pre-slaughter conditions. They argue that these proteins are responsible for lower meat quality in inappropriately treated pigs. Roncada *et al.* (*42*) have demonstrated that a blood serum proteome analysis of pigs can be used to monitor animal pre-slaughter treatment, and some proteins, mostly acute-phase proteins such as haptoglobin, annexin A6, apolipoprotein A1 and fetuin, can be used as biomarkers of the immunological and welfare conditions of pigs. By use of nuclear magnetic resonance (NMR)-based metabonomics investigation, Bertram *et al.* (*43*) showed that improper treatment before slaughter causes changes in the plasma metabolite profile in pigs, which could be ascribed primarily to an increased concentration of plasma lactate. A resting period of 1–3 hours prior to slaughter reversed these stress-induced perturbations. The pre-slaughter conditions examined are not identical to improper cattle treatment (*44*) or illegal treatment with antibiotics or performance enhancing agents (*45*), which have been discussed elsewhere (*1*).

Determination of postmortem changes

The study of *postmortem* changes in proteomes has significantly contributed to an increased understanding of the biochemical mechanisms behind the facts about meat quality, such as tenderness and colour of beef and water-holding capacity of pork and poultry meat. For beef and pork meat, a few genetic markers for lower meat quality have been identified, and they have been used to remove susceptible animals from the herd. Some of these markers are linked to aberrant calcium regulation in early *postmortem* muscle (*46*).

Postmortem changes during storage of both beef and pork have been the object of extensive studies, and the calpain system is believed to be important for the degradation of myofibrilar proteins and development of tenderness (*5*). Among the proteins degraded by these proteolytic enzymes are desmin, actin, myosin, troponin, tropomyosin and thioredoxin. Different isoforms of troponin and their fragmentation during beef storage have been the subject of recent studies (*47*,*48*). However, other proteins, such as chaperones and enzymes involved in energy metabolism, also seem to play a role in meat aging and tenderization (*49–51*). In a recent study, Laville *et al.* (*52*) have followed proteomic changes in soluble and insoluble protein fractions during meat aging of both tough and tender beef (Fig. 1).

They found that the solubility of some cytoplasmic proteins, such as heat shock proteins and glycolytic enzymes, decreases. Some enzymes and structural proteins were fragmented, and some full-length proteins, such as actin and troponin, were released from cellular structures like membranes and the myofibrilar network. In tender meat immediately after slaughter, the abundance of protein from the mitochondrial membrane and other apoptosis-linked processes suggested that programmed cellular death had occurred. However, a direct link between the presence of apoptotic markers and meat shear force (tender and tough meat) still has to be confirmed (Fig. 1).

Small heat shock proteins, such as HSP27 (*37*) and HSP20 (*53*), have also been extensively studied as members of a hypothetical underlying cellular mechanism with consequences on tenderness development during meat storage. As discussed above, the additional degradation of collagen and other components of intramuscular connective tissue during *postmortem* storage of meat is responsible for the so-called 'background' feature and increased tenderness of meat after conditioning (*21*).

Study of changes associated with meat quality traits

Oxidation (*54*) and microbial contamination (*30*) are the two leading causes of quality deterioration of meat. Meat colour is one of the most important quality attributes that influences consumer's decisions at the point-of-sale. Carbon monoxide (CO) reacts with myoglobin to form carboxymyoglobin. This reaction provides the stable red colour of fresh meat, similar to oxymyoglobin (*55*). Further oxidation of proteins, lipids and other meat components can have significant negative impacts on meat colour and taste (*54*). By use of a combined immunologic and proteomic approach, Stagsted *et al.* (*56*) identified several reactive water-soluble (α-enolase) and insoluble (actin) proteins in chicken muscle. They also demonstrated differential susceptibility of water-soluble muscle proteins towards oxidation in animals fed with a

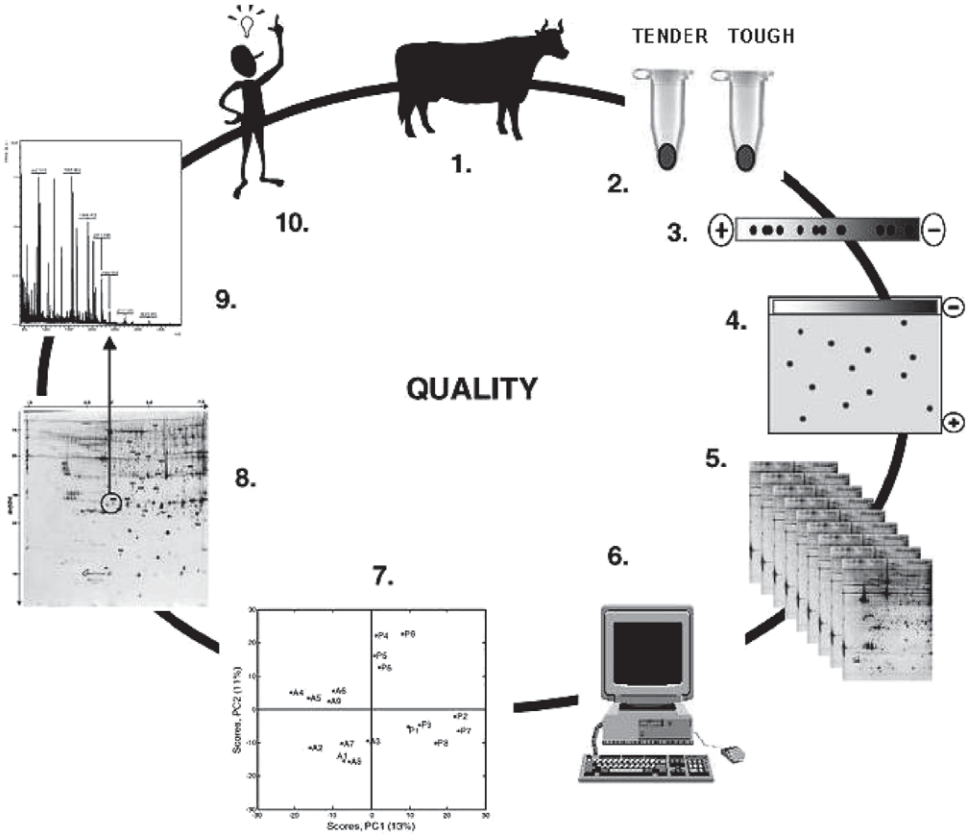

Fig. 1. Workflow in the proteome analysis using 2D electrophoresis or chromatographic separation methods and mass spectrometry: 1. animal or sample chosen for the analysis; 2. sample extraction; 3. isoelectric focusing or first chromatographic step; 4. SDS-PAGE or next chromatographic step as a second dimension; 5. alignments and comparisons of the images; 6. data analysis; 7. data interpretation and selection of significantly changed proteins; 8. extraction of significantly changed protein spots; 9. protein identification by mass spectrometry; 10. interpretation of the results. Reproduced from Hollung *et al.* (*5*) with permission from Elsevier Ltd, copyright 2007

low-antioxidant diet compared to animals fed with a diet supplemented with antioxidant-rich food, such as fruits and vegetables (*56*). Frederiksen *et al.* (*57*) showed that a species of myofibrilar muscle protein myosin from pork can be oxidized by hypervalent myoglobin species. This reaction was followed by cross-linking of myosin. Oxidized myosin in the cell could be visualized by use of fluorescence microscopy. Most of the oxidized protein was localized in plasma membranes (*54*). These data are in contrast to the results of other investigations that were obtained by use of proteomic techniques. According to Marcos *et al.* (*58*) the most labile proteins of *postmortem* muscle are those of the sarcoplasm, and most oxidized proteins were detected in this organelle and in mitochondria. Marcos' group also demonstrated that the protein fraction from the sarcoplasmic reticulum plays a key role in colour development, water-holding capacity and protein solubility of beef (*58*). Newly introduced high-level oxygen packing for fresh meat can also cause protein oxidation and formation of protein aggregates through both covalent and non-covalent intermolecular bonds (*59*) as described above (*57*).

Peptidomics – study and detection of changes in peptide composition during meat storage and processing

Until recently, almost all proteomic studies in meat science were based on a combination of 2D electrophoresis and mass spectrometry (*1,4,5*). Only a few studies used liquid chromatography as a method for sample preparation before protein identification by mass spectrometry (*1,4,5,38*). The use of liquid chromatography enables separation of small peptides generated in fresh and cooked meat and their identification by mass spectrometry (*26*).

Proteolytic degradation of muscle that occurs *postmortem* as part of muscle transformation in meat and during the meat aging process results in the production of protein fragments (*60*). These polypeptides can be further digested, and this process leads to production of smaller peptides and also to the release of some individual amino acids (*61*). Geesink *et al.* (*62,63*) have shown that μ-calpain is responsible for *postmortem* proteolysis, and that this process can be partially inhibited by the specific calpain inhibitor calpastatin.

Unfortunately, there are only a few studies dealing with small peptides in aged and cooked meat (*5,21*). Bauchart *et al.* (*26*) showed that the concentration of some small peptides, such as carnosine and anserine, is relatively constant during meat aging, but they registered a significant decrease of glutathione during storage and cooking. Other small peptides are the result of protein degradation. Collagens I and IV, the main components of the extracellular matrix, are relatively stable during meat aging, but heating above 70 °C promotes peptide bond hydrolysis, and explains the occurrence of collagen breakdown products in cooked meat samples (*21,26*).

Small peptides significantly influence the organoleptic and nutritional value of fresh and cooked meat, and their further characterization should be the next step in the assessment of meat quality.

Quality assessment of dry-cured and fermented meat products

The texture and flavour of dry-cured ham is mainly dependent on the intense proteolytic phenomena during the ripening process. Proteolysis of muscle proteins has been attributed to enzymes originating in the cytosol: lysosome, calpains and cathepsins (*28*). In both dry-cured ham and sausages, proteolysis during processing significantly influences product quality. Proteases of microbial origin also play a role in the development of flavour of both products (*28*,*29*). The role of bacteria and other microorganisms has been investigated more thoroughly in sausages than in ham (*29*,*64*).

As mentioned above, the quality of dry-cured ham depends on the quality of the meat used for processing, especially pork tenderness and water-holding capacity (*5*). Di Luccia *et al.* (*28*) followed the changes in the proteome during ham ripening by use of 2D electrophoresis followed by MALDI-TOF mass spectrometry. They showed that in 12-month-old dry-cured ham, most fibrilar proteins were completely hydrolyzed. During ripening, some sarcoplasmic proteins decreased or totally disappeared. Small peptides, amino acids, as well as some amino acid degradation products, all originating from the hydrolysis of these and other meat proteins, play a crucial role in the development of the flavour of dry-cured ham (*5*,*65*,*66*). The enzymes responsible for this process are proteases, cathepsins and calpains, and different aminopeptidases. Several oligopeptides are products of proteolytic degradation. In order to determine their role in flavour and identify the origin of the dry-cured ham, these peptides have been identified (*65*,*66*), and it was found that some of them are typical for specific products (*67*,*68*). Unfortunately, the role of ham microflora, mainly bacteria, yeast and fungi, has not been investigated sufficiently (*29*,*64*).

Europe is still the major producer of dry-fermented sausages. There is a significant difference in the fermented sausages between Northern and Southern Europe. Northern products have a pH below 5, while the Mediterranean products have a shift towards neutral (pH=5.3–6.2), and this pH difference significantly influences protein degradation in these fermented products. However, the influence of microbial enzymes on the product features seems to be lower than the influence of endogenous meat proteases (*28*,*68*).

Seafood

Similar to meat, from nutritional and technological points of view, proteins are the essential components of seafood. In an early review, Piñeiro *et al.* (*69*) stressed that 'the wide variability of proteins present in seafood and their highly variable composition offer a strong potential to originate a large variety of different products'. However, the complexity of seafood also implies the extreme complexity of seafood proteomes. Consequently, only basic questions can be addressed in this short overview. As expected, proteomic methods have already been applied in order to insure seafood authenticity and quality, and there is a broad use of proteomics for the characterization of allergic components. The use of proteomic methodology for the identification of human pathogens in seafood also started a few years ago, and the first thorough investigations in this field have been published (*70*). Proteomics is also used for the detection and identification of pathogens in different marine organisms (*71*). However, the predicted use of proteomics for the optimization of specific steps in seafood processing, as proposed by Piñeiro *et al.* (*69*), is still infrequent.

There is broad literature data about the use of proteomics for the identification of allergens in seafood, and this topic was already discussed in the first part of this review (*1*). Newly developed methods in mass spectrometry have recently been introduced as a powerful tool for the identification of bacterial pathogens in different seafood products (*72*). Böhme *et al.* (*70*) used MALDI-TOF mass fingerprinting of polypeptides and low-molecular-mass proteins for identification of species of pathogenic Gram-negative bacteria isolated from different types of seafood. In a model investigation, the main 26 bacterial species responsible for seafood spoilage were investigated. For each of them, unique biomarker(s) were identified. However, despite this highly valuable methodical work, where the specific fingerprints for protein biomarkers were obtained and a reference library was created, the bacterial strains were grown in marine broth, and no interactions between the microorganisms and seafood were investigated. Consequently, in order to assess the practicability, this methodology should be applied for the identification of bacterial pathogens in specific sorts of seafood.

Fish

A recently published paper by Forné *et al.* (*73*) gives an excellent overview on fish proteomics. The freshwater teleost zebrafish (*Danio rerio*) has been selected as a model organism for biological research, and the genome of this fish has also been sequenced. From our point of view, there have been interesting model studies about the changes to the skeletal muscle proteome under hypoxia (*74*), the cytosolic proteome of the liver (*75*) and proteomic profiling of single organs of this fish (*76*).

The salmonids, especially Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), comprise one of the most commercially interesting fish groups. Further commercially important freshwater fish species that have been investigated are crucian carp (*Carassius carassius*), channel catfish (*Ictalus punctatus*), and the sea fish cod (*Gadus morhua*) (*73*).

Important proteomic investigations were performed in order to study the influence of different viral (*e.g.* infection with hematopoietic necrosis virus) or bacterial (*e.g.* with the *Renibacterium salmonarum*) diseases. These diseases can cause significant economic loss during fish cultivation (*71*). Low and high susceptibility of the farmed channel catfish (*Ictalus punctatus*) to enteric septicaemia caused by bacteria *Edwardsiella ictaluri* was also investigated in order to identify novel genes associated with disease resistance (*77*). Changes in the proteome and in

posttranslational modifications of muscle proteins of rainbow trout during its life cycle have been documented (*78*). Furthermore, changes induced by stress factors, such as density, anoxia and handling of fish before slaughter, and hence alterations in the quality of this food have been the topic of extensive studies (*76–80*). In cultured fish species, proteomic studies have been performed in order to identify the influence of environmental conditions, food additives, and, recently, feeding with genetically modified crops on food safety and food quality (*6,81–83*). Proteomics has also characterized *postmortem* changes in the fish muscle under different storage conditions (*6,84*). Storage of fresh and frozen fish can cause protein oxidation, reaction with other components, protein aggregation and hydrolysis. Storage conditions also have a significant effect on the taste, flavour and colour of fish muscle (*85–87*).

As predicted by Martinez and Jacobsen Friis (*6*), proteomic methodology has been used successfully for the authentication of fish and fish products. Following the new EU directives for authentication of food products, Mazzeo *et al.* (*88*) used a method based on MALDI-TOF mass spectrometry to identify 25 fish species. Signals generated from polypeptides with molecular mass of about 11 000 were selected as specific biomarkers for identification, and for the rapid detection of fraudulent substitution. Isoelectric focusing and 2D electrophoresis were used as tools to differentiate wild from farmed cod (*89*) and for the characterization of different freshwater fish species (*90,91*).

Other seafood

Most proteomic investigations of other seafood, especially different species of crustaceans, have been focused on the detection of allergens (*92*), and this topic was discussed in our previous review (*1*).

Martinez *et al.* (*93*) have investigated *postmortem* muscle degradation during ice-storage of different types of shrimp. They demonstrated changes in several structural proteins, such as myosin and α-actinin, during the storage period. These changes in polypeptide patterns after partial hydrolysis were highly dependent on the investigated shrimp. Degradation of these two proteins was also observed in different animal (*17*) and fish muscles (*6,84*). Some other proteins, such as arginine kinase and polyphenol oxidase, can be used as biomarkers for the identification of commercially relevant shrimp species. Fingerprints of this protein obtained by MALDI-TOF mass spectrometry could be used for the identification of six studied species (*94–96*).

Marine aquaculture of crustaceans can also be hit by infection with different pathogens. Such pathogens like bacteria *Vibrio alginolyticus* can cause great losses during cultivation. Xiong *et al.* (*97*) investigated differentially expressed outer membrane proteins of this bacterium in response to the treatment with six types of antibiotics. These proteins can be potential targets for designing new drugs in order to inhibit multi-resistant bacteria and minimize economic loss. However, similar to the use of antibiotics in animal breeding, the use of these substances in marine aquaculture can support the spread of resistant bacterial species, and have unpredictable consequences for consumers (*1*).

Milk and Dairy Products

Because of their immense importance for human (and also animal) health and nutrition, milk proteins have been studied continuously for over 50 years, and they can be grouped into three major classes: caseins; mucins, a group of glycoproteins related to milk fat globule membrane (MFGM); and whey proteins. During milk separation, which is one of the oldest manufacturing processes in food industry, three fractions can be obtained through the following steps: (*i*) whole milk is centrifuged to obtain the milk fat globule (MFG) and the low fat milk fractions; (*ii*) protein-rich, skimmed milk is ultracentrifuged and separated in two fractions, the whey protein and pellet fraction; and (*iii*) in the last step the casein protein fraction is obtained by washing the pellet from the previous step (*98*). Additionally, fermented milk products such as cheese and yogurt are of enormous nutritional and economic importance, and the genomics and proteomics of milk bacteria, and the mechanism of their interaction with the substrate will also be discussed in this review. As already mentioned, the milk proteome and glycoproteome have been topics of extensive investigations since the creation of the methodology (*9,13,99–101*). However, unlike in meat processing, the use of broad proteomic data for milk production started only a few years ago, and it is still in early development (*21,102–105*). Finally, milk of transgenic animals, primarily cows, goats and sheep, is also used for the production of genetically engineered proteins (*106,107*), but the role of proteomics in the production of these biopolymers is beyond the scope of this review.

In dairy science and technology, proteomics is currently used for: (*i*) the analysis of high-abundance proteins; (*ii*) the analysis of low-abundance proteins; (*iii*) the analysis of proteins in whey; (*iv*) the characterization of milk fat globule (MFG) and milk fat globule membrane (MFGM); and (*v*) the characterization of products containing complex dairy matrices such as cheese and yogurt.

Studies of biologically active peptides in milk and changes in peptide and metabolite composition during storage and processing of milk and milk products are topics of milk peptidomics and metabonomics. There are two main reasons for studying peptides and metabolic products in milk and milk-derived food. Milk components, especially milk proteins, carbohydrates and lipids exert a wide range of nutritional, functional and biological activities. The concentration of urea (*108*), fatty and amino acids (*109*) and some peptides (*110*) can reflect the quality of milk and the way it was handled during transport and storage (*109,110*). Identification of peptides generated from the hydrolysis of bovine milk proteins, mainly caseins and α-lactalbumin, is the main tool for the detection of adulteration of buffalo, caprine and ewe's milk products with the addition of cow's milk (*109,111,112*).

Peptides originating from milk proteins have various health-promoting effects such as: regulation of digestive enzymes and modulation of nutrient absorption (*113*); regulation of the cardiovascular system, *e.g.* anti-hyperten-

sive effects (*114*); regulation of the immune system, *e.g.* enhancing the immune cell functions and stimulation of the phagocytic activities of macrophages (*115*); regulation of the nervous system, *e.g.* by their opoid activity (*116*); antioxidative and other health-promoting activities (*117,118*).

The physiological activity of milk-derived peptides has been the topic of numerous studies during the last ten years (*117*). Experimental evidence exists that bioactive peptides can be released from caseins (see above), α-lactalbumin (α-LA), β-lactoglobulin (β-LG), lactoferrin and serum albumin. Some of these bioactive petides have received special names, such as α- and β-lactorphin, β-lactotensin, serophin, albutensin A, lactoferricin B, lactoferrampin, osteopontin and many others. Their production and biological properties have been the subject of two recently published comprehensive reviews (*113,119*).

Caseins and other high-abundance proteins in milk and milk products

There are only a few highly abundant proteins that are present in milk from all species. In bovine milk, these proteins are casein (CN), β-lactoglobulin (β-LG), α-lactalbumin (α-LA) and bovine serum albumin (BSA) with relative abundances of approx. 80:10:4:1 (*99,100*). Similar to other biological fluids, such as blood plasma, despite the fact that it contains a small number of primary proteins, the milk proteome is still extremely complex. A great deal of this complexity is the consequence of post-translational modifications and the presence of numerous genetic variants of this limited list of proteins (*7*).

2D gels of milk proteins highlight the great complexity of milk proteins. However, despite the presence of hundreds of spots, this study was not able to identify any proteins other than caseins, β-LG, α-LA and cleavage products of these proteins (*120*). Consequently, in order to achieve a deeper analysis of the milk proteome and detect low-abundance proteins, removal of these major proteins and further fractionation are necessary (*7*).

Caseins are a group of unique milk-specific proteins. These proteins represent about 80 % of the total protein in bovine milk. Caseins are a mixture of proteins and sub-classes of proteins. The most abundant caseins are α_{S1}-, α_{S2}-, β- and κ-casein (*121*). Isolation of κ-casein revolutionized the ideas on the structure of the so-called casein micelle in milk, and a realistic model of its structure became possible only after the characterization of the soluble form of this most abundant milk protein (*122*). Many technologically important properties of milk, such as its white colour, stability to heat or ethanol, coagulation by cheese-making enzymes, and gelation characteristics, are due to the properties of casein micelles. It is for these reasons that properties of casein micelles are still a topic of extensive studies (*13,121–123*). It has been known for more than a hundred years that the colloidal caseinate particles contain calcium. Therefore, in nutrition, milk is one of the most important sources of calcium. The nature of the association between the colloidal calcium phosphate and casein, and its effect on casein micelle stability in milk is still not fully understood (*124*). The colloidal stability of milk is in many cases its most important physicochemical property, and it depends on the stability of casein micelles. In good quality milk, the casein micelles are stable in all usually applied technological processes, such as concentration, dehydration, freezing, homogenization and heat treatment. The phosphorylation of caseins and other posttranslational modifications of this protein have a vital role in the interaction with calcium phosphate and the organization of the casein micelle (*125*). The micelles are destabilized by hydrolysis of κ-casein with selected proteinases (rennets), acidification, ethanol, anionic detergents and by high pressure. Proteolysis of κ-casein and acidification are frequently exploited in the manufacturing of cheese, fermented milk and functional caseinate products (*9,13,101,121*). It has to be stressed that the stability of the casein micelles and their size are highly dependent on milk composition, and are also critical for the processing of most dairy products (*101,121,123*). Determination of posttranslational modification of major protein components provides the scientific basis to coagulation and cheese making processes used in dairy production. Further characterization of these proteins is also of fundamental importance for identification of the origin and quality assessment of milk and milk products (*124–128*).

Some physiologically active peptides are natural breakdown products of casein. The most common polypeptide originating from κ-casein during milk clotting is glycomacropeptide (GMP). This glycosylated and phosphorylated polypeptide that contains 64 amino acids is released into whey after specific cleavage of κ-casein by chymosin or pepsin (*129*). Typical concentrations of GMP in whey are between 20 and 25 % (by mass) of all proteins (*130*). Because of its beneficial physiological properties, this phosphorylated glycopeptide has been the subject of extensive investigations for the last ten years. GMP binds bacterial enterotoxins, inhibits bacterial and viral infection, modulates the immune system's response, suppresses gastric secretion, and regulates blood circulation (*129,130*).

The other high-abundance milk proteins, β-LG, α-LA and BSA, are major components of the whey (*131*). The concentration of β-LG in skimmed milk is about 2–4 mg/mL. This protein occurs with high frequency in cows as two genetic variants, variant A and variant B. Because of the different physicochemical characteristics of the two β-LG molecules, the presence of one or the other of these variants significantly affects the properties of the milk. The A variant is expressed at a higher level than the B variant, or the less frequently occurring C variant (*131,132*). β-Lactoglobulin may also be glycosylated, but lactosylation of this protein is a more important chemical modification, which is caused by heating of milk or whey (*133*).

Bovine skimmed milk contains α-LA at a concentration of 1.2 to 1.5 mg/mL. In bovine milk, the mature α-LA is also present in two genetic variants, variants A and B. This protein binds bivalent metals such as zinc and calcium. α-LA is important for normal function of the mammary gland, such as milk secretion and lactose content in milk (*131*).

The addition of less expensive bovine milk to the milk of other species, such as sheep, goat or water buffalo, is of great concern in the field of dairy products

(*109*). This kind of adulteration can be detected by characterizing the high-abundance proteins present in milk or cheese products. Performing MALDI-TOF mass spectrometry analysis on the 'fingerprints' of high abundance proteins, Cozzolino *et al.* (*134*) identified the fraudulent addition of bovine milk to buffalo and ewe products (Fig. 2).

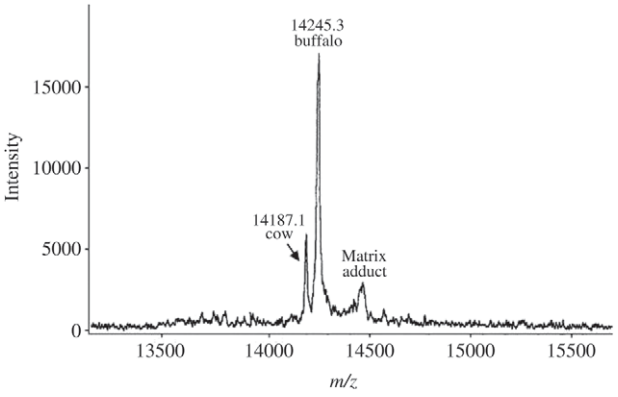

Fig. 2. Detection of the addition of bovine milk to the water buffalo milk in the ratio of 50:50 (by mass); MALDI-TOF mass spectrum of α-lactalbumin obtained from the water-soluble protein fraction from the mozzarella cheese. Mozzarella whey proteins were extracted from the cheese, diluted 1:100 (by volume) with 0.1 % trifluoroacetic acid, and analyzed by MALDI-TOF mass spectrometry. Reproduced from Cozzolino *et al.* (*134*) with permission from John Wiley&Sons Ltd, copyright 2002

Low-abundance proteins

After the removal of high-abundance proteins by selective precipitation and/or size-exclusion or ion-exchange chromatography, 2D electrophoresis and/or additional liquid chromatographic steps can be used for further fractionation of the remaining components. These low-abundance proteins can then be identified by mass spectrometry (*101,106,107*). Blood plasma proteins, such as BSA, serotransferrin and lactoferrin, are only observed in colostrum, which may have special physiological importance for young animals in the early nursing period. Among more than 400 spots in 2D electrophoresis that were separated from bovine milk, identified proteins include β_2-microglobulin, complement components, α_1-antitrypsin, prealbumin, fructose-biphosphate aldolase A, and casein fragments (*135*).

The immunoglobulin fraction accounts for about 1 % (by mass) of total milk protein, and therefore these proteins can be classified as a kind of 'medium-abundance proteins'. In milk, IgG, IgA and IgM have been isolated and characterized. Immunoglobulins in colostrum and milk have a protecting function, especially for the newborn (*131*). Some other proteins that are encoded by genes of the Ig supergene family have also been detected in milk (*136*), but their further classification is beyond the scope of this review.

Lactoferrin is a specific, iron-binding protein that also occurs in milk of most mammary species. The lactoferrin concentration in milk is relatively low, and varies between 20 and 200 mg/L. This protein increases noticeably in response to inflammation or infection. Consequently, lactoferrin plays an important role in the host defense against infection and inflammation (*137*). Antibacterial and antiviral activities of this protein against both DNA and RNA viruses have been detected (*138, 139*), and lactoferrin is now being isolated and purified from cheese whey and commercially utilized in the pharmaceutical and food industries (*140*). Multiple use of this protein and the peptides originating from its partial digestion will be discussed later (*140,141*).

Whey proteins

Whey is considered as a functional milk fraction with a content of proteins and bioactive polypeptides that have a positive effect on the health (*117*), and whey protein fractions are increasingly incorporated as functional ingredients in food, not only in infant formulas, but also for adults (*142*). As ingredients in food, whey proteins can provide antimicrobial activity, immune modulation, improve muscle strength, and may delay and/or ameliorate conditions in different diseases, such as osteoporosis and cardiovascular diseases (*117,143*).

The identification of low-abundance proteins in whey is challenging due to their wide dynamic concentration range. Namely, in comparison with highly abundant proteins, the concentrations of minor protein components vary by at least a factor of 10^6 (*142*). These proteins may play important physiological and eventually therapeutic roles in nutrition and as additives to cosmetic products. Analytical and preparative methods are used for their investigation, such as isoelectric focusing, affinity tagging, immunoabsorption and different chromatographic techniques (*7,135,142,144*). Fong *et al.* (*145*) used a combination of anion- and cation-exchange chromatography and 2D electrophoresis in order to detect both acidic and basic biologically active proteins in bovine whey. By use of this strategy, a large number of low-abundance whey proteins were identified, and a cluster of physiologically active osteopontin peptides was detected in the acidic protein fraction.

Milk fat globule and milk fat globule membrane

Lipid molecules are provided in milk *via* a unique delivery system, milk fat globules (MFG) (*146*). In previous research, the lipid fraction in milk was oversimplified as a relative pure mixture of triacylglycerols (*147*), and the unique structure and composition of MFG was overlooked. In MFG, milk fat globule membrane (MFGM) is the protective coat that surrounds lipid globules. The MFGM prevents flocculation and coalescence of lipid droplets in milk and protects the milk fat against lipolysis (*146*). In order to secrete MFG, portions of the mammary epithelial cell membranes are sacrificed. This process results in a unique structure of MFG, which has been object of extensive studies during the past 20–25 years (*147*). Recent investigations of MFG and MFGM proteome have provided new insights into mammary function and the mechanism of milk secretion (*148*). Mather (*149*) gave an early overview of the proteins found in the MFGM. In that paper, the eight most abundant MFGM proteins, most of them glycosylated, were listed: mucin 1, xantine dehydrogenase/oxidase, periodic acid Schiff III and Schiff 6/7 proteins, CD36, butyrophilin, adipophilin, and fatty-acid binding protein. The four-step protocol for the

isolation of bovine MFGM given in that paper has been modified, but it is still used today for the isolation of MFGM for proteomic analyses (*150*). As shown in Fig. 3 (*151*), MFGM is organized as a trilayer. The inner membrane face is composed of proteins and polar lipids from the endoplasmic reticulum, and the outer bilayer membrane contains the major MFGM proteins listed above, other glycosylated integral membrane proteins and membrane-associated proteins, cholesterol and glycolipids. The outer membrane also contains membrane rafts, which are derived from the apical membrane of the lactating cell (*146*).

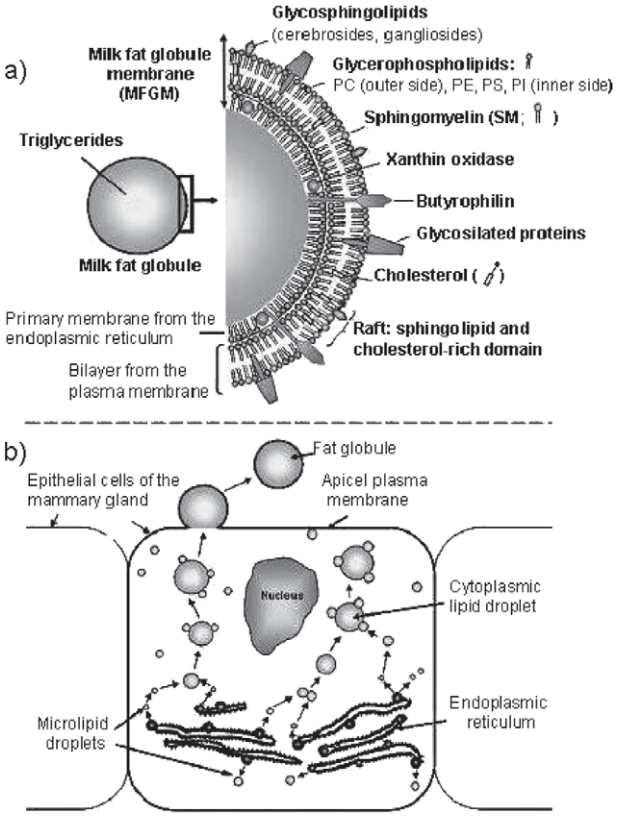

Fig. 3. Milk fat globule membrane. Schematic presentation of: a) the structure of the milk fat globule membrane; and b) the pathways for the synthesis and secretion of milk fat globules. Reproduced from C. Lopez *et al.* (*151*) with permission of the American Chemical Society, copyright 2008

The composition of both lipid and protein changes during lactation, and is modified by diet (*148,151*). Similar to solubilization and analysis of other membrane proteins (*152*), solubilization, sample preparation and identification of hydrophobic, frequently heavily glycosylated MFGM proteins is a challenging task. In pioneering work, Reinhardt and Lippolis (*153*) separated MFGM proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The identification of proteins excised from the 1D gel was performed by high-performance liquid chromatography (HPLC) and mass spectrometry. One hundred and twenty proteins were identified in this study. Most of them (71 %) are classified as membrane proteins, but some cytoplasmic (24 %) and secreted (5 %) proteins were detected. Bianchi *et al.* (*150*) and Vanderghem *et al.* (*154*) used 2D electrophoresis and mass spectrometry in order to establish a reference map of cow MFG. Interestingly, although an optimized solubilization method and 2D electrophoretic separation were used, only a few proteins were detected. These difficulties may have been caused by the analytical methods that were used for protein separation prior to protein identification by mass spectrometry. It is well known that 2D electrophoresis has certain limitations for the analysis of low-abundance, hydrophobic membrane proteins, and therefore alternative methods such as liquid chromatography may have to be used (*152*).

MFGM proteins also have other important functions, such as defense against pathogens (*155,156*). One of the major proteins in MFGM, xantine dehydogenase/oxidase, has a direct antibacterial activity, and inhibits bacterial growth through the formation of hydrogen peroxide or the stimulation of lactoperoxidase in milk (*157*). Additional factors with beneficial health properties, such as cholesterol lowering and inhibition of cancer cell growth, were also documented for MFGM (*158*). A link between MFGM proteins and certain diseases such as multiple sclerosis and coronary heart diseases was also reported (*159*).

These proteins also have an important role in milk processing because the MFG is broken down during heating and homogenization, and subsequent structures and surfaces, different from the native state, are formed (*151, 160,161*). Milk fat globule membrane can also be altered by other factors, such as cooling, drying, separation, and environmental and animal factors. Heat treatment causes denaturation of some milk proteins, and denatured whey components incorporate the MFG and MFGM. Disruption of fat globules by homogenization also increases the surface area of MFG leading to the adsorption of proteins, mainly caseins (*162*). Individual animal-related factors include the changes to the MFGM that depend on the diet, season, and breed, and also changes during lactation (*148,163*). In food technology, MFGM can also be used as a natural emulsifying agent in the preparation of emulsions, infant formulas and reduced-fat products for health-related diets (*151,154,164*).

Cheese and other milk products

Studying the proteins of fermented milk products, especially the enzymes released into complex dairy mixtures, is a challenging task. In cheese, the complexity is due to the simultaneous presence of proteins originating from milk and complex microbial ecosystems containing bacteria, and sometimes also other microorganisms, such as yeast and filamentous fungi (*9,98,101,165,166*). The final content of released proteins as well as peptides and other components is specific to each type of cheese (*98*). The major components of cheese come from milk, and they contain a multiphase matrix that contains not only caseins but also some whey proteins, polypeptides and short peptides, fat, mineral components, organic acids and other low-molecular-size organic components such as urea (*108,167*). Numerous proteins are released through bacterial cell lysis of starter and adventitious microflora in cheese. Proteolysis by bacterial proteases and endogenous proteases coming from milk (*168,169*) is the most important enzymatic process responsible for typical textural and flavour characteristics of cheese (*98,102,170*).

Principally, the statement published in 1984 by Lawrence *et al.* (*171*), and recently discussed by Johnson and Lucey (*13*) is still valid. According to these authors, cheese making is a relatively simple matter: the removal of moisture from rennet coagulum. The three major factors involved are acid production, proportion of fat in the curd and scalding temperature rate. Furthermore, the manufacturing procedures must be as consistent as possible, and the first (and most important) requirement is uniformity of the raw milk. The role of proteomics in cheese production, and production and quality assessment in other fermented dairy products was discussed by Manso *et al.* (*101*) as follows: assessment of the raw milk quality, identification of casein hydrolysis and determination of whey proteins and their truncated forms, monitoring of the appearance of casein degradation products, specificity of microbial proteinases, selectivity of enzymes, study of proteolysis throughout cheese ripening and identification of proteins liberated by microflora.

The clotting of milk is of key importance during the production of cheese and other milk products. Milk quality and the uniformity of the raw material play essential roles in this process and, besides good manufacturing practice, they depend on the cattle breed, lactation stage and cattle diet. The use of genomics and proteomics enables early identification and elimination of cattle that carry genes encoding casein variants with a bad aptitude for milk clotting (*172*). Loss of milk clotting and formation of weak gels can appear during the so-called 'spring flush period' (*173*), and milk composition also changes during lactation (*174*). Such variations in the raw material can be identified by the use of proteomics, glycomics and metabonomics. Proteomic tools also allow characterization of proteolytic pathways that lead to degradation of casein and whey proteins (*175,176*). Wedholm *et al.* (*176*) used 2D electrophoresis and different high-resolution mass spectrometric methods in order to identify different casein and whey protein fragments that have positive effects on the transfer of milk proteins to cheese. Peptide identification also made possible the determination of polypeptides resulting from the first steps in the degradation of the major casein isoforms (*129*). Extraction of water-soluble proteins from cheese is a useful strategy for further detailed proteomic study of the enzymes released during cheese production (*177–179*). This strategy is based on: prefractionation of water-soluble components, protein and peptide separation by 2D electrophoresis or different chromatographic methods, and protein and peptide identification by use of mass spectrometric methods.

Bacterial microflora in cheese manufacturing, their proteomes and genomes, and their interaction with the milk protein matrix have been the topics of recent reviews (*9,98*). Protein modification during milk fermentation, and especially during cheese ripening, is a dynamic process and it still requires further proteomic investigation. The new challenge requires not only characterization of protein components in milk matrices, but also the study of their sequential release during the fermentation process and during cheese ripening. For example, the bacterial peptidase from *Lactobacillus helveticus* was released during the ripening of Emmental cheese, but it may also be partially degraded by other proteinases present in the matrix (*101,180*). Some peptides are highly typical for the cheese type and milk origin. Pappa *et al.* (*102*) have shown that by identification of the peptides specific for milk origin, it is possible to distinguish the milk source used for cheese manufacturing. The manufacture of other processed milk products such as koumiss (*181*), kefir (*182*) and buttermilk (*183*) has also been investigated by the use of proteomics.

Egg Proteomics

Eggs are a valuable source of protein and are important ingredients in many food products. Whole eggs or egg ingredients (egg white and egg yolk) are often used as coagulating, foaming and emulsifying agents, while also contributing nutrients and flavour to different foods (*184,185*). Individual components of eggs have potentially useful biological functions, such as antimicrobial activity, protease inhibitory function, and antigenic or immunogenic characteristics (*186*). Potential allergens in eggs, such as some genetic variants of the ovomucoid from the egg white, are also important from food safety point of view (*187*).

Raikos *et al.* (*186*) analyzed the chicken egg proteome by the use of SDS-PAGE and 2D electrophoresis followed by MALDI-TOF mass spectrometry. Unfortunately, the high-abundance egg proteins ovalbumin and conalbumin were not separated during sample preparation, and very few proteins were identified. Because of better sample preparation, Guérin-Dubiard *et al.* (*188*) identified sixteen proteins in egg white, two of them for the first time from this source. Again, a very high level of polymorphism was found for some high-abundance proteins, such as ovotransferrin and ovomucoid. Because of its technological properties, especially foaming and gelling, egg white represents an important raw material, and its proteomic characterization is a valuable contribution towards the understanding of these processes. Egg yolk contains proteins with a very broad pI range, and it is commonly used for production of oil-water emulsions. Different egg yolk proteins, such as yolk plasma glycoproteins (YPG) and phosvitin, which play a major role in emulsification, have been identified by mass spectrometry after separation by 2D electrophoresis (*189,190*). Further thorough proteomic analyses of egg yolk (*191, 192*) and egg white (*193,194*) have been performed. By the use of combinatorial ligand libraries in order to enrich the understanding of low-abundance proteins, D'Ambrosio *et al.* (*192*), Farinazzo *et al.* (*194*) and D'Alessandro *et al.* (*195*) listed 260 distinct gene products in egg yolk, and 148 in egg white. New specific antimicrobial proteins playing a crucial role in antimicrobial responses, and vitamin-binding proteins were detected in egg yolk. Although the chicken egg proteome is still not complete, some of the already identified proteins may be of biopharmaceutical interest as physiologically active substances.

Conclusions and Future Outlook

Only a few proteins, and often only the high-abundance ones, can be studied if 'traditional' methods are used for quality control and quality assessment of raw

material and final products in food manufacturing, or are used for monitoring the optimization and development of new processes in food technology and biotechnology. Proteomic techniques enable simultaneous study of several hundred up to several thousand proteins. As already discussed (*1*), the most commonly used methods for proteomic analysis of food of animal origin are 2D electrophoresis followed by mass spectrometry, most frequently MALDI-TOF. The use of faster and more sensitive methods for sample preparation followed by quantitative mass spectrometry (*196*) will direct future investigations towards understanding variations and finding markers (or 'biomarkers') for food quality criteria. Utilizing these new tools is especially important for investigations of complex biological mixtures, such as meat and meat products, seafood, and milk and milk products.

In fermented meat and milk products, complex interactions between proteins (and other mixture components) and the microorganisms involved in fermentation process can be followed by proteomics. The use of proteomics has also resulted in the establishment of reference maps in order to detect microbial stain-to-strain variation and *in vivo* adaptation to environmental conditions (*8*). However, the complexity of microbial flora in fermented foods is still not fully understood.

Biologically active peptides and proteins have been found in foods of animal origin, especially in milk and milk products (*119*), and recently also in eggs (*195*). Their isolation and use in food and food additives should be the focus of the future research. The detection and investigation of these peptides in meat is still in the embryonic stage (*26*). Further development of this field is a very interesting topic, but its results and directions are difficult to predict.

Methods in proteomics, peptidomics and metabonomics are already very powerful tools for the determination of product quality and the detection of adulterations in meat production (*38*), seafood identification (*88*,*95*), and in the production of milk and dairy products (*109*). The optimization of high-throughput sample preparation together with the optimization of mass spectrometric methods towards the design and production of simpler, faster and less cost- and work-intensive instruments and methods shall in the near future further support and enhance this development.

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References

1. D. Gašo-Sokač, S. Kovač, Dj. Josić, Application of proteomics in food technology and food biotechnology: Process development, quality control and product safety, *Food Technol. Biotechnol. 48* (2010) 284–295.
2. M. Carbonaro, Proteomics: Present and future in food quality and evaluation, *Trends Food Sci. Technol. 15* (2004) 209–216.
3. J.Z. Han, Y.B. Wang, Proteomics: Present and future in food science and technology, *Trends Food Sci. Technol. 19* (2008) 26–30.
4. M. Pischetsrieder, R. Baeuerlein, Proteome research in food science, *Chem. Soc. Rev. 38* (2009) 2600–2608.
5. K. Hollung, E. Veseith, X. Jia, E.M. Færgestad, K.I. Hildrum, Application of proteomics to understand the molecular mechanisms behind meat quality, *Meat Sci. 77* (2007) 97–104.
6. I. Martinez, T. Jacobsen Friis, Application of proteome analysis to seafood authentication, *Proteomics, 4* (2004) 347–354.
7. B. Casado, M. Affolter, M. Kussman, OMICS-rooted studies of milk proteins, oligosaccharides and lipids, *J. Proteomics, 73* (2009) 196–208.
8. M. Schittmayer, R. Birner-Gruenberger, Functional proteomics in lipid research: Lipases, lipid droplets and lipoproteins, *J. Proteomics, 72* (2009) 1006–1018.
9. V. Gagnaire, J. Jardin, G. Jan, S. Lortal, Proteomics of milk and bacteria used in fermented dairy products: From qualitative to quantitative advances, *J. Dairy Sci. 92* (2009) 811–825.
10. M. Rossignol, J.B. Peltier, H.P. Mock, A. Matros, A.M. Maldonado, J.V. Jorrín, Plant proteome analysis: A 2004–2006 update, *Proteomics, 6* (2006) 5529–5548.
11. J.V. Jorrín, A.M. Maldonado, M.A. Castillejo, Plant proteome analysis: A 2006 update, *Proteomics, 7* (2007) 2947–2962.
12. J.V. Jorrín-Novo, A.M. Maldonado, S. Echevarría-Zomeño, L. Valledor, M.A. Castillejo, M. Curto *et al.*, Plant proteomics update (2007–2008): Second generation proteomics techniques, an appropriate experimental design, and data analysis to fulfill MIAPE standards, increase plant proteome coverage and expand biological knowledge, *J. Proteomics, 72* (2009) 285–314.
13. M.E. Johnson, J.A. Lucey, Major technological advances and trends in cheese, *J. Dairy Sci. 89* (2006) 1174–1178.
14. T. Dambrouck, R. Marchal, L. Marchal-Delhaut, M. Parmentier, A. Maujean, P. Jeandet, Immunodetection of proteins from grapes and yeast in a white wine, *J. Agric. Food Chem. 51* (2003) 2727–2732.
15. C. Zörb, G. Langenkämper, T. Betsche, K. Niehaus, A. Barsch, Metabolite profiling of wheat grains (*Triticum aestivum* L.) from organic and conventional agriculture, *J. Agric. Food Chem. 54* (2006) 8301–8306.
16. C. Zörb, T. Betsche, G. Langenkämper, Search for diagnostic proteins to prove authenticity of organic wheat grains (*Triticum aestivum* L.), *J. Agric. Food Chem. 54* (2009) 2932–2937.
17. E. Bendixen, The use of proteomics in meat science, *Meat Sci. 71* (2005) 138–149.
18. D.F.M. van de Wiel, W.L. Zhang, Identification of pork quality by proteomics, *Meat Sci. 77* (2007) 46–54.
19. B. Picard, C. Berri, L. Lefaucheur, C. Molette, T. Sayd, C. Terlouw, Skeletal muscle proteomics in livestock production, *Briefings Funct. Genom. 9* (2010) 259–278.
20. L. Murgiano, A. D'Alessandro, M.G. Egidi, A. Crisa, G. Prosperini, A.M. Timperio *et al.*, Proteomics and transcriptomics investigation on *longissimus* muscles in Large White and Casertana pig breeds, *J. Proteome Res.* (2010) 6450–6466.
21. P.P. Purslow, Intramuscular connective tissue and its role in meat quality, *Meat Sci. 70* (2005) 435–447.
22. A. Listrat, C. Lethias, J.F. Hocquette, G. Renand, F. Ménissier, Y. Geay, B. Picard, Age-related changes and location of types I, III, XII and XIV collagen during development of skeletal muscles from genetically different animals, *J. Mol. Histol. 32* (2000) 349–356.
23. M.A. Lawson, P.P. Purslow, Development of components of the extracellular matrix, basal lamina and sarcomere in chick quadriceps and pectoralis muscles, *Br. Poultry Sci. 42* (2001) 315–320.
24. D. Belcerzak, L. Querengesser, W.T. Dixon, V.E. Baracos, Coordinate expression of matrix-degrading proteinases and

their activators and inhibitors in bovine skeletal muscle, *J. Anim. Sci. 79* (2001) 94–107.

25. P.P. Purslow, The structure and functional significance of variations in the connective tissue within muscle, *Comp. Biochem. Physiol. A, 133* (2002) 947–966.

26. C. Bauchart, D. Rémond, C. Chambon, P. Patureau Mirand, I. Savary-Auzeloux, C. Reynès, M. Morzel, Small peptides (<5 kDa) found in ready-to-eat beef meat, *Meat Sci. 74* (2006) 658–666.

27. G.J.E. Nychas, P.N. Skandamis, C.C. Tassou, K.P. Koutsoumanis, Meat spoilage during distribution, *Meat Sci. 78* (2008) 77–89.

28. A. Di Luccia, G. Picariello, G. Cacace, A. Scaloni, M. Faccia, V. Liuzzi *et al.*, Proteomic analysis of water soluble and myofibrillar protein changes occurring in dry-cured hams, *Meat Sci. 69* (2005) 479–491.

29. M.L. Stecchini, M. Spaziani, M. Del Torre, G. Lippe, Protein breakdown and volatile profile of low-acid sausages from crossbreed pigs, *Vet. Res. Commun.* (Suppl. 1), *33* (2009) 261–263.

30. M.P. Doyle, M.C. Erickson, Emerging microbiological food safety issues related to meat, *Meat Sci. 74* (2006) 98–112.

31. A.M. Mullen, P.C. Stapleton, D. Corcoran, R.M. Hamill, A. White, Understanding meat quality through the application of genomic and proteomic approaches, *Meat Sci. 74* (2006) 3–16.

32. M. Cagnazzo, M.F. te Pas, J. Priem, A.A. de Witt, M.H. Pool, R. Davoli, V. Russo, Comparison of prenatal muscle tissue expression profiles of two pig breeds differing in muscle characteristics, *J. Anim. Sci. 84* (2006) 1–10.

33. K. Hollung, H. Grove, E.M. Færgestad, M.S. Sidhu, P. Berg, Comparison of muscle proteome profiles in pure breeds of Norwegian Landrace and Duroc at three different ages, *Meat Sci. 81* (2009) 487–492.

34. J. Bouley, B. Meunier, C. Chambon, S. De Smet, J.F. Hocquette, B. Picard, Proteomic analysis of bovine skeletal muscle hypertrophy, *Proteomics, 5* (2005) 490–500.

35. M. Morzel, C. Terlouw, C. Chambon, D. Micol, B. Picard, Muscle proteome and meat eating qualities of *Longissimus thoracis* of 'Blonde d'Aquitaine' young bulls: A central role of HSP27 isoforms, *Meat Sci. 78* (2008) 297–304.

36. M. Hamelin, T. Sayd, C. Chambon, J. Bouix, D. Milenkovic, H. Leveziel *et al.*, Proteomic analysis of ovine muscle hypertrophy, *J. Anim. Sci. 84* (2006) 3266–3276.

37. M.K. Doherty, L. McLean, J.R. Hayter, J.M. Pratt, D.H.L. Robertson, A. El-Shafei *et al.*, The proteome of chicken skeletal muscle: Changes in soluble protein expression during growth in a layer strain, *Proteomics*, *4* (2004) 2082–2093.

38. A. Leitner, F. Castro-Rubio, M.L. Marina, W. Lindner, Identification of marker proteins for the adulteration of meat products with soybean proteins by multidimensional liquid chromatography–tandem mass spectrometry, *J. Proteome Res. 5* (2006) 2424–2430.

39. A.M. Timperio, A. D'Alessandro, L. Pariset, G.M. D'Amici, A. Valentini, L. Zolla, Comparative proteomics and transcriptomics analyses of livers from two different *Bos taurus* breeds: 'Cianina and Holstein Friesian', *J. Proteomics*, *73* (2009) 309–322.

40. R. Lametsch, L. Kristensen, M.R. Larsen, M. Therkildsen, N. Oksbjerg, P. Ertbjerg, Changes in the muscle proteome after compensatory growth in pigs, *J. Anim. Sci. 84* (2006) 918–924.

41. M. Morzel, C. Chambon, M. Hamelin, V. Santé-Lhoutellier, T. Sayd, G. Monin, Proteome changes during pork meat ageing following use of two different pre-slaughter handling procedures, *Meat Sci. 67* (2004) 689–696.

42. P. Roncada, B. Begni, M. Amadori, S. Cristoni, I.L. Archetti, C. Boldetti *et al.*, Blood serum proteome for welfare evaluation in pigs, *Veterinary Res. Comm.* (Suppl. 1), *31* (2007) 321–325.

43. H.C. Bertram, N. Oksbjerg, J.F. Young, NMR-based metabonomics reveals relationship between pre-slaughter exercise stress, the plasma metabolite profile at time of slaughter, and water-holding capacity in pigs, *Meat Sci. 84* (2010) 108–113.

44. C. Xu, Z. Wang, Comparative proteomic analysis of livers from ketotic cows, *Vet. Res. Commun. 32* (2008) 263–273.

45. C. Nebbia, G. Gardini, A. Urbani, The proteomic approach as a tool to detect the illegal treatment of cattle with performance enhancing agents, *Vet. Res. Commun.* (Suppl. 1), *32* (2006) 121–125.

46. S. Barbut, A.A. Sosnicki, S.M. Lonergan, T. Knapp, D.C. Ciobanu, L.J. Gatcliffe *et al.*, Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat, *Meat Sci. 79* (2008) 46–63.

47. S. Muroya, M. Ohnishi-Kameyama, M. Oe, I. Nakajima, K. Chikuni, Postmortem changes in bovine troponin T isoforms in two-dimensional electrophoretic gel analyzed using mass spectrometry and western blotting: The limited fragmentation into basic polypeptides, *Meat Sci. 75* (2007) 506–514.

48. S. Muroya, M. Oe, I. Nakajima, M. Shibata, K. Chikuni, A non-destructive method to monitor changes in a troponin T peptide in beef drip with a monoclonal antibody, *Meat Sci. 83* (2009) 155–160.

49. X. Jia, K.I. Hildrum, F. Westad, E. Kummen, L. Aass, K. Hollung, Changes in enzymes associated with energy metabolism during the early post mortem period in *longissimus thoracis* bovine muscle analyzed by proteomics, *J. Proteome Res. 5* (2006) 1763–1769.

50. X. Jia, M. Ekman, H. Grove, E.M. Færgestad, L. Aass, K.I. Hildrun, K. Hollung, Proteome changes in bovine *longissimus thoracis* muscle during the early postmortem storage period, *J. Proteome Res. 6* (2007) 2720–2731.

51. M.F.W. te Pas, J. Jansen, K.C.J.A. Broekman, H. Reimert, H.C.M. Heuven, Postmortem proteome degradation profiles of *longissimus* muscle in Yorkshire and Duroc pigs and their relationship with pork quality traits, *Meat Sci. 83* (2009) 744–751.

52. E. Laville, T. Sayd, M. Morzel, S. Blinet, C. Chambon, J. Lepetit *et al.*, Proteome changes during meat aging in tough and tender beef suggest the importance of apoptosis and protein solubility for beef aging and tenderization, *J. Agric. Food Chem. 57* (2009) 10755–10764.

53. D.J. Pulford, S. Fraga Vazquez, D.F. Frost, E. Fraser-Smith, P. Dobbie, K. Rosenvold, The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH, *Meat Sci. 79* (2008) 623–630.

54. T. Astruc, P. Marinova R. Labas, P. Gatellier, V. Santé-Lhoutellier, Detection and localization of oxidized proteins in muscle cells by fluorescence microscopy, *J. Agric. Food Chem. 55* (2007) 9554–9558.

55. P. Joseph, S.P. Suman, R.A. Mancini, C.M. Beach, Mass spectrometric evidence of aldehyde adduction in carboxymyoglobin, *Meat Sci. 83* (2009) 339–344.

56. J. Stagsted, E. Bendixen, H.J. Andersen, Identification of specific oxidatively modified proteins in chicken muscles using a combined immunologic and proteomic approach, *J. Agric. Food Chem. 52* (2004) 3967–3974.

57. A.M. Frederiksen, M.N. Lund, M.L. Andersen, L.H. Skibsted, Oxidation of porcine myosin by hypervalent myoglobin: The role of thiol groups, *J. Agric. Food Chem. 56* (2008) 3297–3304.

58. B. Marcos, J.P. Kerry, A.M. Mullen, High pressure induces changes on sarcoplasmic protein fraction and quality indicators, *Meat Sci. 85* (2010) 115–120.

59. M.N. Lund, R. Lametsch, M.S. Hviid, O.N. Jensen, L.H. Skibsted, High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage, *Meat Sci. 77* (2007) 295–303.

60. G.H. Geesink, M. Koohmaraie, Postmortem proteolysis an calpain/calpastatin activity in callipyge and normal lamb biceps femoris during extended postmortem storage, *J. Anim. Sci. 77* (1999) 1490–1501.

61. A.M. Mullen, S. Stoeva, K. Laib, G. Gruebler, W. Voelter, D.J. Troy, Preliminary analysis of amino acids at various locations along the *M. longissimus dorsi* in aged beef, *Food Chem. 69* (2000) 461–465.

62. G.H. Geesink, R.G. Taylor, M. Koomaraie, Calpain 3/p94 is not involved in postmortem proteolysis, *J. Anim. Sci. 83* (2005) 1646–1652.

63. G.H. Geesink, S. Kuchay, A.H. Chishti, M. Koomaraie, μ-Calpain is essential for postmortem proteolysis of muscle proteins, *J. Anim. Sci. 84* (2006) 2834–2840.

64. R. Talon, S. Leroy, I. Lebert, Microbial ecosystems of traditional fermented meat products: The importance of indigenous starters, *Meat Sci. 77* (2007) 55–62.

65. F. Toldrá, M.C. Aristoy, M. Flores, Contribution of muscle aminopeptidases to flavor development of dry-cured ham, *Food Res. Int. 33* (2000) 181–185.

66. M.A. Sentandreu, S. Stoeva, M.C. Aristoy, K. Laib, W. Voelter, F. Toldrá, Identification of small peptides generated in Spanish dry-cured ham, *J. Food Sci. 68* (2003) 64–69.

67. S. Sforza, A. Pagazzani, M. Motti, C. Porta, R. Virgili, R. Galaverna, A. Dossena, R. Marchelli, Oligopeptides and free amino acids in Parma hams of known cathepsin B activity, *Food Chem. 75* (2001) 267–273.

68. M.A. Sentandreu, M. Armenteros, J.J. Calvette, A. Ouali, M.C. Aristoy, F. Toldrá, Proteomic identification of actin-derived oligopeptides in dry-cured ham, *J. Agric. Food Chem. 55* (2007) 3613–3619.

69. C. Piñeiro, J. Barros-Velázquez, J. Velázquez, A. Figueras, J.M. Gallardo, Proteomics as a tool for the investigation of seafood and other marine products, *J. Proteome Res. 2* (2003) 127–135.

70. K. Böhme, I.C. Fernández-No, J. Barros-Velázquez, J.M. Gallardo, P. Calo-Mata, B. Cañas, Species differentiation of seafood spoilage and pathogenic Gram-negative bacteria by MALDI TOF mass fingerprinting, *J. Proteome Res. 9* (2010) 3169–3183.

71. A.T. Booy, J.D. Haddow, L.B. Ohlund, D.B. Hardie, R.W. Olafson, Application of isotope coded affinity tag (ICAT) analysis for the identification of differentially expressed proteins following infection of Atlantic salmon (*Salmon salar*) with infectious hematopoietic necrosis virus (IHNV) or *Renibacterium salmoninarum* (BKD), *J. Proteome Res. 4* (2005) 325–334.

72. S.C. Russel, Microorganism characterization by single particle mass spectrometry, *Mass Spectrom. Rev. 28* (2009) 376–387.

73. I. Forné, J. Abián, J. Cerdà, Fish proteome analysis: Model organisms and non-sequenced species, *Proteomics, 10* (2010) 858–872.

74. C.A. Bosworth, C.W. Chou, R.B. Cole, B.B. Rees, Protein expression patterns in zebrafish skeletal muscle: Initial characterization and the effects of hypoxic exposure, *Proteomics, 5* (2005) 1362–1371.

75. N. Wang, L. MacKenzie, A.G. De Souza, H. Zhong, G. Goss, L. Li, Proteome profile of cytosolic component of zebrafish liver generated by LC-ESI MS/MS combined with trypsin digestion and microwave-assisted acid hydrolysis, *J. Proteome Res. 6* (2007) 263–272.

76. A. Abramsson, A. Westman-Bringkmalm, J. Pannee, M. Gustavsson, M. von Otter, K. Blennow *et al.*, Proteomics profiling of single organs from individual adult zebrafish, *Zebrafish, 7* (2010) 161–168.

77. N.J. Booth, A.L. Bilodeau-Bourgeois, Proteomic analysis of head kidney tissue from high and low susceptibility families of channel catfish following challenge with *Edwardsiella ictaluri, Fish Shellfish Immunol. 26* (2009) 193–196.

78. M. Salem, P.B. Kenney, C.E. Rexroad III, J. Yao, Proteomic signature of muscle atrophy in rainbow trout, *J. Proteomics, 73* (2010) 778–789.

79. F. Provan, A. Bjørnstad, D.M. Pampanian, E. Lyng, R. Fontanillas, O.K. Andersen *et al.*, Mass spectrometric profiling – A diagnostic tool in fish?, *Mar. Environ. Res.* (Suppl. 1), *62* (2006) 105–108.

80. X. Liu, L. Alfonso, E. Altman, S. Johnson, L. Brown, J. Li, O-acetylation of sialic acids in *N*-glycans of Atlantic salmon (*Salmo salar*) serum is altered by handling stress, *Proteomics, 8* (2008) 2849–2857.

81. L. McLean, I.S. Young, M.K. Doherty, D.H.L. Robertson, A.R. Cossins, A.Y. Gracey *et al.*, Global cooling: Cold acclimation and the expression of soluble proteins in carp skeletal muscle, *Proteomics, 7* (2007) 2667–2681.

82. G. Monti, L. De Napoli, P. Mainolfi, R. Barone, M. Guida, G. Marino, A. Amoresano, Monitoring the food quality by microfuidic electrophoresis, gas chromatography, and mass spectrometry techniques: Effects of aquaculture on the sea bass (*Dicentrarchus labrax*), *Anal. Chem. 77* (2006) 2587–2594.

83. N.H. Sissener, S.A.M. Martin, P. Cash, E.M. Hevrøy, M. Sanden, G.I. Hemre, Proteomic profiling of liver from Atlantic salmon (*Salmo salar)* fed genetically modified soy compared to the near-isogenic non-GM line, *Mar. Biotechnol. 12* (2010) 273–281.

84. I.V.H. Kjærsgård, M.R. Nørrelykke, F. Jessen, Changes in cod muscle proteins during frozen storage revealed by proteome analysis and multivariate data analysis, *Proteomics, 6* (2006) 1606–1618.

85. M.P. Richards, M.A. Dettmann, E.W. Grunwald, Pro-oxidative characteristics of trout hemoglobin and myoglobin: A role for released heme in oxidation of lipids, *J. Agric. Food Chem. 53* (2005) 10231–10238.

86. C.P. Baron, I.V.H. Kjærsgård, F. Jessen, C. Jacobsen, Protein and lipid oxidation during frozen storage of rainbow trout (*Oncorhynchus mykiss*), *J. Agric. Food Chem. 55* (2007) 8118–8125.

87. B. Egelandsdal, L.P. Ren, Y.S. Gong, M. Greaser, M.P. Richards, Factors affecting solubilization and oxidation of proteins during equine metmyoglobin-mediated lipid oxidation in extensively washed cod muscle, *Food Chem. 122* (2010) 1102–1110.

88. M.F. Mazzeo, B.D. Giulio, G. Guerriero, G. Ciarcia, A. Malorni, G.L. Russo, R.A. Siciliano, Fish authentication by MALDI-TOF mass spectrometry, *J. Agric. Food Chem. 56* (2008) 11071–11076.

89. I. Martinez, R. Slizyte, E. Dauksas, High resolution two-dimensional electrophoresis as a tool to differentiate wild from farmed cod (*Gadus morhua*) and to assess the protein composition of klipfish, *Food Chem. 102* (2007) 504–510.

90. A. Berrini, V. Tepedino, V. Barromeo, C. Secchi, Identification from freshwater fish commercially labeled perch by isoelectric focusing and two-dimensional electrophoresis, *Food Chem. 96* (2006) 163–168.

91. C. Infante, G. Catanese, M. Ponce, M. Manchado, Novel method for the authentication of frigate tunas (*Auxis thazard* and *Auxis rochei*) in commercial canned products, *J. Agric. Food Chem. 52* (2004) 7435–7443.

92. K. Motoyama, Y. Suma, S. Ishizaki, Y. Nagashima, K. Shiomi, Molecular cloning of tropomiosins identified as allergens in six species of crustaceans, *J. Agric. Food Chem. 55* (2007) 985–991.

93. I. Martinez, T. Jakobsen Friis, M. Careche, *Post mortem* muscle protein degradation during ice-storage of Arctic (*Pandalus borealis*) and tropical (*Penaeus japonicus* and *Penaeus monodon*) shrimps: A comparative electrophoretic and immunological study, *J. Sci. Food Agric. 81* (2001) 1199–1208.

94. I. Ortea, B. Cañas, J.M. Gallardo, Mass spectrometry characterization of species-specific peptides from arginine kinase for the identification of commercially relevant shrimp species, *J. Proteome Res. 8* (2009) 5356–5362.

95. I. Ortea, B. Cañas, P. Calo-Mata, J. Barros-Velázquez, J.M. Gallardo, Arginine kinase peptide mass fingerprinting as a proteomic approach for species identification and taxonomic analysis of commercially relevant shrimp species, *J. Agric. Food Chem. 57* (2009) 5665–5672.

96. J.P. Zamorano, O. Martínez-Álvarez, P. Montero, M. del Carmen Gómez-Guillén, Characterisation and tissue distribution of polyphenol oxidase of deepwater shrimp (*Parapenaeus longirostris*), *Food Chem. 112* (2009) 104–111.

97. X.P. Xiong, C. Wang, M.Z. Ye, T.C. Yang, X.X. Peng, H. Li, Differentially expressed outer membrane proteins of *Vibrio alginolyticus* in response to six types of antibiotics, *Marine Biotechnol. 12* (2010) 686–695.

98. T. Pogačić, N. Kelava, Š. Zaberlin, I. Dolenčić-Špehar, D. Samaržija, Methods for culture-independent identification of lactic acid bacteria in dairy products, *Food Technol. Biotechnol. 48* (2010) 3–10.

99. A. Conti, M.G. Giuffrida, M. Cavaletto: Proteomics of Human Milk. In: *Proteomics of Human Body Fluids*, V. Thongboonkerd (Ed.), Human Press, Totowa, NJ, USA (2007) pp. 437–451.

100. R. O'Donnell, J.W. Holland, H.C. Deeth, P. Alewood, Milk proteomics, *Int. Dairy J. 14* (2004) 1013–1023.

101. M.A. Manso, J. Léonil, G. Jan, V. Gagnaire, Application of proteomics for characterisation of milk and dairy products, *Int. Dairy J. 15* (2005) 845–855.

102. E.C. Pappa, J.A. Robertson, N.M. Rigby, F. Mellon, I. Kandarakis, E.N.C. Mills, Application of proteomic techniques to protein and peptide profiling of Teleme cheese made from different types of milk, *Int. Dairy J. 18* (2008) 605–614.

103. J. Meltretter, A. Schmidt, A. Humeny, C.M. Becker, M. Pischetsrieder, Analysis of the peptide profile of milk and changes during thermal treatment and storage, *J. Agric. Food Chem. 56* (2008) 2899–2906.

104. F. Chevalier, C. Hirtz, N. Sommerer, A.L. Kelly, Use of reducing/nonreducing two-dimensional electrophoresis for the study of disulfide-mediated interactions between proteins in raw and heated bovine milk, *J. Agric. Food Chem. 57* (2009) 5948–5955.

105. F. Fenaille, V. Parisod, J.C. Tabet, P.A. Guy, Carbonylation of milk powder proteins as a consequence of processing conditions, *Proteomics, 5* (2005) 3097–3104.

106. L. Pampel, R. Boushaba, M. Udell, M. Turner, N. Titchener-Hooker, The influence of major components on the direct chromatographic recovery of a protein from transgenic milk, *J. Chromatogr. A, 1142* (2007) 137–147.

107. M.A. Al-Ghobashy, M.A.K. Williams, B. Brophy, G. Laible, D.R.K. Harding, On-line casein micelle distruption for downstream purification of recombinant human myelin basic protein produced in the milk of transgenic cows, *J. Chromatogr. B, 877* (2009) 1667–1677.

108. X. Dai, X. Fang, F. Su, M. Yang, H. Li, J. Zhou, R. Xu, Accurate analysis of urea in milk and milk powder by isotope dilution gas chromatography-mass spectrometry, *J. Chromatogr. B, 19* (2010) 1634–1638.

109. P.A. Guy, F. Fenaille, Contribution of mass spectrometry to assess quality of milk-based products, *Mass Spectrom. Rev. 25* (2006) 290–326.

110. A. Di Luccia, G. Picariello, A. Trani, G. Alviti, P. Loizzo, M. Faccia, F. Addeo, Occurrence of β-casein fragments in cold-stored and curdled river buffalo (*Bubalus bubalis* L.) milk, *J. Dairy Sci. 92* (2009) 1319–1329.

111. C. D'Ambrosio, S. Arena, A.M. Salzano, G. Renzone, L. Ledda, A. Scaloni, A proteomic characterization of water buffalo milk fraction describing PTM of major species and the identification of minor components involved in nutrient delivery and defense against pathogens, *Proteomics*, *8* (2008) 3657–3666.

112. R.K. Chen, L.W. Chang, Y.Y. Chung, M.H. Lee, Y.C. Ling, Quantification of cow milk adulteration in goat milk using high-performance liquid chromatography with electrospray ionization mass spectrometry, *Rapid Commun. Mass Spectrom. 18* (2004) 1167–1171.

113. H. Korhonen, A. Pihlanto, Bioactive peptides: Production and functionality, *Int. Dairy J. 16* (2006) 945–960.

114. J.J. Pins, J.M. Keenan, Effects of whey peptides on cardiovascular disease risk factors, *J. Clin. Hypertens. 8* (2006) 775–782.

115. H. Meisel, R.J. FitzGerald, Biofunctional peptides from milk proteins: Mineral binding and cytomodulatory effects, *Curr. Pharm. Des. 9* (2003) 1289–1295.

116. R. Yamauchi, H. Usui, J. Yunden, Y. Takenaka, F. Tani, M. Yoshikawa, Characterization of β-lactotensin, a bioactive peptide derived from bovine β-lactoglobulin, as a neurotensin agonist, *Biosci. Biotechnol. Biochem. 67* (2003) 940–943.

117. A.R. Madureira, C.I. Pereira, A.M.P. Gomes, M.E. Pintado, F.X. Malcata, Bovine whey proteins – Overview on their main biological properties, *Food Res. Int. 40* (2007) 1197–1211.

118. S.M. Lam, P.J. Moughan, A. Awati, H.R. Morton, The influence of whey protein and glycomacropeptide on satiety in adult humans, *Physiol. Behav. 96* (2009) 162–168.

119. A.D. Madureira, T. Tavares, A.M. Gomes, M.E. Pintado, F.X. Malcata, Physiological properties of bioactive peptides obtained from whey proteins, *J. Dairy Sci. 93* (2010) 437–455.

120. M. Galvani, M. Hamdan, P.G. Righetti, Two-dimensional gel electrophoresis/matrix-assisted laser desorption/ionisation mass spectrometry of commercial bovine milk, *Rapid Commun. Mass Spectrom. 15* (2001) 258–264.

121. P.F. Fox, A. Brodkorb, The casein micelle: Historical aspects, current concepts and significance, *Int. Dairy J. 18* (2008) 677–684.

122. D.F. Waugh, L.K. Creamer, C.W. Slattery, G.W. Dresdner, Core polymers of casein micelles, *Biochemistry*, *9* (1970) 786–795.

123. M. Glantz, T.G. Devold, G.E. Vegarud, H. Lindmark Månsson, H. Stålhammar, M. Paulsson, Importance of casein micelle size and milk composition for milk gelation, *J. Dairy Sci. 93* (2010) 1444–1451.

124. C. Holt, Casein micelle substructure and calcium phosphate interactions studied by Sephacryl column chromatography, *J. Dairy Sci. 81* (1998) 2994–3003.

125. E.S. Sørensen, L. Møller, M. Vinther, T.E. Petersen, L.K. Rasmussen, The phosphorylation pattern of human α_{s1}-casein is markedly different from the ruminant species, *Eur. J. Biochem. 270* (2003) 3651–3655.

126. L.A. Di Luccia, G. Picariello, A. Trani, G. Alviti, P. Liozzo, M. Faccia, F. Addeo, Occurrence of β-casein fragments in cold-stored and curdled river buffalo (*Bubalus bubalus* L.) milk, *J. Dairy Sci. 92* (2009) 1319–1329.

127. A. Matéos, J.M. Girardet, D. Mollé, A. Dary, L. Miclo, J.L. Gaillard, Two-dimensional cartography of equine β-casein variants achieved by isolation of phosphorylation iso-

forms and control of the deamidation phenomenon, *J. Dairy Sci. 92* (2009) 2389–2399.

128. P. Roncada, A. Gaviraghi, S. Liberatori, B. Canas, L. Bini, G.F. Greppi, Identification of caseins in goat milk, *Proteomics*, *2* (2002) 723–726.

129. M.E. Farías, M.J. Martinez, A.M.R. Pilosof, Casein glycomacropeptide pH-dependent self-assembly and cold gelation, *Int. Dairy J. 20* (2010) 79–88.

130. C. Thomä, I. Krause, U. Kulozik, Precipitation behaviour of caseinomacropeptides and their simultaneous determination with whey proteins by RP-HPLC, *Int. Dairy J. 16* (2006) 285–293.

131. H.M. Farrell Jr., R. Jimenez-Flores, G.T. Bleck, E.M. Brown, J.E. Butler, L.K. Craemer *et al.*, Nomenclature of the proteins of cow's milk – Sixth revision, *J. Dairy Sci. 87* (2004) 1641–1674.

132. K.F. Ng-Kwai-Hang, F. Grosclaude: Genetic Polymorphism of Milk Proteins. In: *Advanced Dairy Chemistry, Vol. 1: Proteins, Part B*, P.F. Fox, P.L.H. McSweeney (Eds.), Kluwer Academic/Plenum, New York, NY, USA (2003) pp. 739–816.

133. F. Morgan, S. Bouhallab, D. Mollé, G. Henry, J.L. Maubois, J. Léonil, Lactolation of β-lactoglobulin monitored by electrospray ionisation mass spectrometry, *Int. Dairy J. 8* (1998) 95–98.

134. R. Cozzolino, S. Passalacqua, S. Salemi, D. Garozzo, Identification of adulteration in water buffalo mozzarella and in ewe cheese by using whey proteins as biomarkers by matrix-assisted laser desorption/ionization mass spectrometry, *J. Mass Spectrom. 37* (2002) 985–991.

135. M. Yamada, K. Murakami, J.C. Wallingfold, Y. Yuki, Identification of low-abundance proteins of bovine colostral and mature milk using two-dimensional electrophoresis followed by microsequence and mass spectrometry, *Electrophoresis*, *23* (2002) 1153–1160.

136. W.N. Eigel, J.E. Butler, C.A. Ernstrom, H.M. Farrell, Jr., V.R. Harwalkar, R. Jennes, R.McL. Whitney, Nomenclature of the proteins of cow's milk – Fifth revision, *J. Dairy Sci. 67* (1984) 1599–1631.

137. P.P. Ward, S. Uribe-Luna, O.M. Conneely, Lactoferrin and host defense, *Biochem. Cell Biol. 80* (2002) 95–102.

138. H.J. Vogel, D.J. Schibli, W. Jing, E.M. Lohmeier-Vogel, R.F. Epand, R.M. Epand, Towards a structure-function analysis of bovine lactoferricin and related tryptophan- and arginine-containing peptides, *Biochem. Cell Biol. 80* (2002) 49–63.

139. B.W.A. van der Strate, L. Beljaars, G. Molema, M.C. Harmsen, D.K.F. Meijer, Antiviral activities of lactoferrin, *Antiviral Res. 52* (2001) 225–239.

140. B.S. Horton, Commercial utilization of minor milk components in the health and food industries, *J. Dairy Sci. 78* (1995) 2584–2589.

141. M.B. Strøm, B.E. Haug, O. Rekdal, M.L. Skar, W. Stensen, J.S. Svendsen, Important structural features of 15-residue lactoferrin derivates and methods for improvement of antimicrobial activity, *Biochem. Cell Biol. 80* (2002) 65–74.

142. A. Panchaud, M. Kussmann, M. Affolter, Rapid enrichment of bioactive milk proteins and iterative, consolidated protein identification by multidimensional protein identification technology, *Proteomics*, *5* (2005) 3836–3846.

143. K. Marshall, Therapeutic application of whey protein, *Altern. Med. Rev. 9* (2004) 136–156.

144. D. Mollé, J. Jardin, M. Piot, M. Pasco, J. Léonil, V. Gagnaire, Comparision of electrospray and matrix-assisted laser desorption ionization on the same hybrid quadrupole time-of-flight tandem mass spectrometer: Application to bidimensional liquid chromatography of proteins from bovine milk fraction, *J. Chromatogr. A, 1216* (2009) 2424–2432.

145. B.Y. Fong, C.S. Norris, K.P. Palmano, Fractionation of bovine whey proteins and characterisation by proteomic techniques, *Int. Dairy J. 18* (2008) 23–46.

146. N. Argov, D.G. Lemay, J.B. German, Milk fat globule structure and function: Nanoscience comes to milk production, *Trends Food Sci. Technol. 19* (2008) 617–623.

147. H. Timmen, S. Patton, Milk fat globules: Fatty acid composition, size and *in vivo* regulation of fat liquidity, *Lipids*, *23* (1988) 685–689.

148. T.A. Reinhardt, J.D. Lipolis, Developmental changes in the milk fat globule membrane proteome during transition from colostrum to milk, *J. Dairy Sci. 91* (2008) 2307–2318.

149. I.H. Mather, A review and proposed nomenclature for major proteins of the milk-fat globule membrane, *J. Dairy Sci. 83* (2000) 203–247.

150. L. Bianchi, M. Puglia, C. Landi, S. Matteoni, D. Perini, A. Armini *et al.*, Solubilization methods and reference 2-DE map of cow milk fat globules, *J. Proteomics*, *72* (2009) 853–864.

151. C. Lopez, V. Briard-Bion, O. Menard, F. Rousseau, P. Pradel, J.M. Besle, Phospholipid, sphingolipid, and fatty acid compositions of the milk fat globule membrane are modified by diet, *J. Agric. Food Chem. 56* (2008) 5226–5236.

152. Dj. Josic, J.G. Clifton, Mammalian plasma membrane proteomics, *Proteomics*, *7* (2007) 3010–3029.

153. T.A. Reinhardt, J.D. Lippolis, Bovine milk fat globule membrane proteome, *J. Dairy Res. 73* (2006) 406–416.

154. C. Vanderghem, C. Blecker, S. Danthine, C. Deroanne, E. Haubruge, F. Guillonneau *et al.*, Proteome analysis of the bovine milk fat globule: Enhancement of membrane purification, *Int. Dairy J. 18* (2008) 885–893.

155. L. Sando, R. Pearson, C. Gray, P. Parker, R. Hawken, P.C. Thompson *et al.*, Bovine *Muc1* is a highly polymorphic gene encoding an extensively glycosylated mucin that binds bacteria, *J. Dairy Sci. 92* (2009) 5276–5291.

156. G. Smolenski, S. Haines, F.Y. Kwan, J. Bond, V. Farr, S.R. Devis *et al.*, Characterisation of host defence proteins in milk using proteomic approach, *J. Proteome Res. 6* (2007) 207–215.

157. H.M. Martin, J.T. Hancock, V. Salisbury, R. Harrison, Role of xanthine oxidoreductase as an antimicrobial agent, *Infect. Immun. 72* (2004) 4933–4939.

158. K. Dewettinck, R. Rombaut, N. Thienpont, T.T. Le, K. Massens, J. Van Camp, Nutritional and technological aspects of milk fat globule membrane material, *Int. Dairy J. 18* (2008) 436–457.

159. P. Riccio, The proteins of the milk fat globule in the balance, *Trends Food Sci. Technol. 15* (2004) 458–461.

160. B.Y. Fong, C.S. Norris, Quantification of milk fat globule membrane proteins using selected reaction monitoring mass spectrometry, *J. Agric. Food Chem. 57* (2009) 6021–6028.

161. S. Gallier, D. Gragson, R. Jaminéz-Flores, D. Everett, Using confocal laser scanning microscopy to probe the milk fat globule membrane and associated proteins, *J. Agric. Food Chem. 14* (2010) 4250–4257.

162. C. Lopez, Focus on the supramolecular structure of milk fat in dairy products, *Reprod. Nutr. Dev. 45* (2005) 497–511.

163. E.L.F. Graves, A.D. Beaulieu, J.K. Drackley, Factors affecting the concentration of sphingomyelin in bovine milk, *J. Dairy Sci. 90* (2007) 706–715.

164. M. Corredig, R.R. Roesch, D.G. Dalgleisch, Production of a novel ingredient from buttermilk, *J. Dairy Sci. 86* (2003) 2744–2750.

165. Dj. Josic, S. Kovac, Application of proteomics in biotechnology – Microbial proteomics, *Biotechnol. J. 3* (2008) 496–509.

166. S. Mansour, J. Bailly, J. Delettre, P. Bonnarme, A proteomic and transcriptomic view of amino acid catabolism in the yeast *Yarrowia lipolytica, Proteomics*, *9* (2009) 4714–4725.

167. H. Schlichterle-Cerny, M. Affolter, C. Cerny, Hydrophylic interaction liquid chromatography coupled to electrospray mass spectrometry of small polar compounds in food analysis, *Anal. Chem. 75* (2003) 2349–2354.

168. A.L. Kelly, F. O'Flaherty, P.F. Fox, Indigenous proteolytic enzymes in milk: A brief overview of the present state of knowledge, *Int. Dairy J. 16* (2006) 563–572.

169. A. Santillo, A.L. Kelly, C. Palermo, A. Sevi, M. Albenzio, Role of indigenous enzymes in proteolysis of casein in caprine milk, *Int. Dairy J. 19* (2009) 655–660.

170. G. Mucchetti, R. Ghiglietti, F. Locci, S. Francolino, B. Bonvini, M. C. Remagni *et al.*, Technological, microbiological and chemical characteristics of Pannerone, a traditional Italian raw milk cheese, *Dairy Sci. Technol. 89* (2009) 419–436.

171. R.C. Lawrence, H.A. Haep, J. Gilles, A controlled approach to cheese technology, *J. Dairy Sci. 67* (1984) 1632–1645.

172. I.J. Giambra, L. Chianese, P. Ferranti, G. Erhardt, Genomics and proteomics of deleted ovine *CSN1S1*I*, *Int. Dairy J. 20* (2010) 195–202.

173. H. Grimley, A. Grandison, M. Lewis, Changes in milk composition and processing properties during the spring flush period, *Dairy Sci. Technol. 89* (2009) 405–416.

174. N. Tao, E.J. DePeters, J.B. German, R. Grimm, C.B. Lebrilla, Variations in bovine milk oligosaccharides during early and middle lactation stages analyzed by high-performance liquid chromatography-chip/mass spectrometry, *J. Dairy Sci. 92* (2009) 2991–3001.

175. J.S. Lillard, D.A. Clare, C.R. Daubert, Glycosylation and expanded utility of a modified whey protein ingredient *via* carbohydrate conjugation at low pH, *J. Dairy Sci. 92* (2009) 35–48.

176. A. Wedholm, H.S. Møller, A. Stensballe, H. Lindmark-Månsson, A.H. Karlsson, R. Andersson *et al.*, Effect of minor milk proteins in chymosin separated whey and casein fractions on cheese yield as determined by proteomics and multivariate data analysis, *J. Dairy Sci. 91* (2008) 3787–3797.

177. C. Dupas, I. Adt, A. Cottaz, R. Boutrou, D. Molle, J. Jardin *et al.*, A chromatographic procedure for semi-quantitative evaluation of caseinophosphopeptides in cheese, *Dairy Sci. Technol. 89* (2009) 519–529.

178. M. Lund, Y. Ardö, Purification and identification of water-soluble phosphopeptides from cheese using Fe(III) affinity chromatography and mass spectrometry, *J. Agric. Food Chem. 52* (2004) 6616–6622.

179. V. Gagnaire, M. Piot, B. Camier, J.P.C. Vissers, G. Jan, J. Léonil, Survey of bacterial proteins released in cheese: A proteomic approach, *Int. J. Food Microbiol. 94* (2004) 185–201.

180. R. Wu, W. Wang, D. Yu, W. Zhang, Y. Li, Z. Sun *et al.*, Proteomics analysis of *Lactobacillus casei* Zhang, a new probiotic bacterium isolated from traditional home-made koumiss in Inner Mongolia of China, *Mol. Cell. Proteomics*, *8* (2009) 2321–2338.

181. I.M.P.L.V.O. Ferreira, O. Pinho, D. Monteiro, S. Faria, S. Cruz, A. Perreira *et al.*, Effect of kefir grains on proteolysis of major milk proteins, *J. Dairy Sci. 93* (2010) 27–31.

182. P. Morin, R. Jiménez-Flores, Y. Pouliot, Effect of processing on the composition and microstructure of buttermilk and its milk fat globule membranes, *Int. Dairy J. 17* (2007) 1179–1187.

183. C. Thomä-Worringer, J. Sørensen, R. López Fandiño, Health effect and technological features of caseinomacropeptide, *Int. Dairy J. 16* (2006) 1324–1333.

184. L. Campbell, V. Raikos, S.R. Euston, Modification of functional properties of egg- white proteins, *Nahrung/Food, 47* (2003) 369–376.

185. V. Kiosseoglou, Egg yolk protein gel and emulsions, *Curr. Opin. Colloid Interface Sci. 8* (2003) 365–370.

186. V. Raikos, R. Hansen, L. Campbell, S.R. Euston, Separation and identification of hen egg protein isoforms using SDS-PAGE and 2D gel electrophoresis with MALDI-TOF mass spectrometry, *Food Chem. 99* (2006) 702–710.

187. P. Rupa, Y. Mine, Molecular allergology of egg white ovomucoid, *ACS Symposium Series, Vol. 1001* (2008) pp. 382–399.

188. C. Guérin-Dubiard, M. Pasco, D. Mollé, C. Désert, T. Croguennec, F. Nau, Proteomic analysis of hen egg white, *J. Agric. Food Chem. 54* (2006) 3901–3910.

189. L. Nilsson, P. Osmark, C. Fernandez, M. Andersson, B. Bergenståhl, Competitive adsorption of water soluble plasma proteins from egg yolk at the oil/water interface, *J. Agric. Food Chem. 54* (2006) 6881–6887.

190. L. Nilsson, P. Osmark, C. Fernandez, B. Bergenståhl, Competitive adsorption of proteins from total hen egg yolk during emulsification, *J. Agric. Food Chem. 55* (2007) 6746–6753.

191. K. Mann, The chicken egg white proteome, *Proteomics*, *7* (2007) 3558–3568.

192. C. D'Ambrosio, S. Arena, A. Scaloni, L. Guerrier, E. Boschetti, M.E. Mendieta *et al.*, Exploring the chicken egg white proteome with combinatorial peptide libraries, *J. Proteome Res. 7* (2008) 3461–3474.

193. K. Mann, M. Mann, The chicken egg yolk plasma and granule proteomes, *Proteomics*, *8* (2008) 178–191.

194. A. Farinazzo, U. Rastuccia, A. Bachi, L. Guerrier, F. Fortis, E. Fasoli *et al.*, Chicken egg yolk cytoplasmatic proteome, mined via combinatorial ligand libraries, *J. Chromatogr. A, 1216* (2009) 1241–1252.

195. A. D'Alessandro, P.G. Righetti, E. Fasoli, L. Zolla, The egg white and yolk interactomes as gleaned from extensive proteomic data, *J. Proteomics*, *73* (2010) 1028–1042.

196. D.L. Tabb, L. Vega-Montoto, P.A. Rudnick, A.M. Variyath, A.J.L. Ham, D.M. Bunk *et al.*, Repeatability and reproducibility in proteomic identifications by liquid chromatography-tandem mass spectrometry, *J. Proteome Res. 9* (2010) 761–766.