

Food Safety and Quality Control: Hints from Proteomics

Angelo D'Alessandro and Lello Zolla*

Department of Ecological and Biological Sciences, DEB, Tuscia University,
IT-01100 Viterbo, Italy

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Summary

Over the last decade, proteomics has been successfully applied to the study of quality control in production processes of food (including meat, wine and beer, transgenic plants and milk) and food safety (screening for food-derived pathogens). Indeed, food quality and safety and their influence on the health of end consumers have growingly become a founding principle in the international agenda of health organizations. The application of proteomics in food science was at first characterized by exploratory analyses of food of various origin (bovine, swine, chicken or lamb meat, but also transgenic food such as genetically modified maize, for example) and beverages (beer, wine), in parallel to the genomic and transcriptomic approaches seeking determination of quantitative trait loci. In the last few years, technical improvements such as microbial biotyping strategies have growingly allowed proteomicists to address the safety issue as well. The newly introduced technical improvements (instrumentation characterized by higher sensitivity such as mass spectrometers) have paved the way for the individuation of food-contaminating pathogens in a fast and efficient workflow which is mandatory in industrial food production chains.

Key words: proteomics, quality control, food safety, nutraceuticals

Introduction – Proteomics in Quality Control

Over the last decades, quality control in all production processes has growingly attracted a great deal of attention. From clinical routine practice (1) to alimentary industry products (2), the individuation of standard quality criteria has become a pivotal step in the agenda of international companies and institutions, both aiming at guaranteeing top quality standards for end users. While early attempts to individuate quality standards through biomolecules mainly relied on rough biochemical parameters, recent advancements in the fields of biochemistry and molecular biology have allowed to widen the horizons, thus including proteins as potential candidate biomarkers for quality testing in various endeavours (3,4). This holds true especially in the field of industrial production processes, in which proteomics, the discipline which focuses on the study of the whole protein complement in a given biological matrix, has growingly con-

tributed valuable insights and relevant pitfalls (the interested reader is referred to Gašo-Sokač *et al.* (2) for further details on this topic). Industrial proteomics (2), especially in the field of the alimentary industry, has already contributed precious developments as far as food quality and safety are concerned. Both these concepts represent funding values in the international community, which continuously strives for the valorisation of local products in each country, although the individuation of shared strict standards for production processes in the alimentary pipeline still remains mandatory. In this view, proteomic research has been focused on the individuation of quality markers of various foods, from meat to vegetables, of biological or genetically engineered origin (2). Once individuated, these long sought-after protein quality biomarkers can be monitored throughout industrial production processes, in like fashion to the actual application of proteomics in other fields of research (5–11).

*Corresponding author; Phone: ++39 0761 357 100; Fax: ++39 0761 357 630; E-mail: zolla@unitus.it

The main goal of this review article is to deliver a panoramic overview of how proteomics has slowly helped gaining insights in the field of quality control and safety in the alimentary industry. From meat and egg quality (12,13) to milk formulas (14,15), from dairy to beef cattle selection (16), beer or wine production (17,18), proteomics is picking up the challenge to become one of the eligible methodologies in quality screening and assessment in the alimentary industry as well. The first step towards the achievement of actual applications of proteomics to quality control processes in the alimentary industry is represented by (i) the awareness of what has already been done, and (ii) the understanding of the main areas which require further improvements. Throughout this review, we will try to explore several topics and conclude with recently introduced proteomic approaches which will be likely applied to monitor specific steps of the food production chain in the industry.

Quality Control in the Alimentary Industry

Recent analyses of healthcare conditions pinpointed the balance between costs and quality (19). As Brezis and Wiist (19) debated in a recent review, 'for-profit health-care industries may increase costs and reduce quality, leading to market failure and contributing to the USA's unflattering position in international comparisons of health-care efficiency'. This trend towards increasing costs at the expense of patients, especially from poorer, sicker or least educated social classes, claims for the need to improve the prevention rather than curing diseases in order to cut costs for medical expenses of the families and institutions in those states where welfare includes a peculiar attention to citizens' health. Because resources are limited, the overuse of costly modalities contributes to expensive health care, which presents a challenge to universal coverage. Again, citing Brezis and Wiist, 'the free market also fosters the proliferation of industries, such as tobacco, food, and chemicals, which externalize costs to maximize profits, seek to unduly influence research by paying experts and universities, and attempt to control the media and regulatory agencies' (19). Over the last ten years, one of the main changes in the mainstream culture has posited that food might be pivotal in the natural prevention of a wide number of diseases (20). This widespread conviction stems from the scientific evidence that numerous clinical, physiopathological and epidemiological studies have underlined the detrimental or beneficial role of nutritional factors in complex inflammation-related disorders such as allergy, asthma, obesity, type 2 diabetes, cardiovascular disease, rheumatoid arthritis and cancer (20). Therefore, physical exercise and food quality have rapidly become a cornerstone in western education seeking to put up a lethal challenge against the above-mentioned diseases and incorrect alimentary habitudes leading to obesity, an actual plague for the new generations especially in the US and Italy (21).

In this view, proteomics has just revealed itself as a valuable tool to delve into food quality as well (Table 1).

Proteomics for meat safety and quality assessment

Food safety has become a great concern also in the rapidly evolving field of farm animal proteomics (22). Indeed, when it comes to food quality, the last two decades have brought about concerns on meat consumption and its relation to Creutzfeldt-Jakob (mad cow) disease, avian flu and, more recently, to swine flu. However, meat safety and quality are not only a priority in the health-care system agenda, but also for alimentary industries, for which safer and better quality meat might translate into broadening the market and higher incomes. In agricultural sciences as in all other areas of life sciences, the implementation of proteomics and other post-genomic tools is an important step towards more detailed understanding of the complex biological systems that control physiology and pathology of living beings (23). Farm animals are raised in large-scale operations, with the aim to obtain animal products for human consumption. Biological traits have been shown to impact yield and quality of these products. Their individuation and positive selection have been pursued by breeders to the specific end of quality improvement. However, most of the data gathered from experiments on *e.g.* swine and cattle are relevant not only for farm animal sciences, but also for adding depth to our understanding of complex biological mechanisms of health and disease in humans (23).

While one should expect that meat itself (and thus muscles) could be the eligible target in molecular investigation, actually the main goal of most research laboratories has been to characterize the whole animal, especially as far as it concerns biological fluids (including blood and plasma, but also milk as a food itself) and digestive tract (especially gut and liver (16,23)).

Farm animals include cattle (1.3 billion), swine (1 billion), poultry and small ruminants like sheep and goat. In recent years, also fish and prawn have been increasingly produced under farming conditions, and as such widened the concept of livestock and farm animal production (23).

As farm animals represent the most important source of proteins in western countries, the study of the protein fraction in meat has demanded increased attention. In this frame, proteomic studies have been carried on either to determine quality criteria for the improvement of meat or milk quality (12,24,25), or to monitor the health conditions of animals in order to improve their welfare (23, 26).

Genomics has already contributed precious insights in the determination of genes related to production traits (quantitative trait loci) for meat and milk (27). The individuation of these traits has allowed increasing food production, although it created new significant drawbacks including the increased incidence of mastitis in high-yield milk cows or the excess of back fat accumulation in some pig phenotypes such as Mangalica.

The biology of muscle differentiation and growth, carcass composition, and fat deposition patterns have been characterized in detail at the proteome level (28, 29). While intramuscular fat deposition increases the taste and juiciness of meat, the subcutaneous fat deposition was formerly considered a waste in meat production (28). On the other hand, consumer complaints against

Table 1. Food proteomics

Authors	Ref.	Brief description
D'Alessandro and Zolla	(77)	An overview of basic science studies of proteomics applied to food science.
Gašo-Sokač <i>et al.</i>	(2)	A comprehensive review of the application of proteomic techniques in the field of food quality and safety, which mainly focuses on the latter aspect.
Meat		
Bendixen <i>et al.</i>	(22)	Farm animal proteomics: a review introducing a whole thematic issue devoted to the advancements in the field.
Bjarnadóttir <i>et al.</i>	(32)	The effects of low voltage electrical stimulations of bovine <i>longissimus dorsi</i> investigated through proteomics.
Mora <i>et al.</i>	(50)	Small peptides released from muscle glycolytic enzymes during dry-cured ham processing are exploited to determine the quality of seasoning.
Sentandreu and Sentandreu	(52)	Peptide biomarkers are exploited to determine meat authenticity in meat mixtures.
D'Alessandro <i>et al.</i>	(31)	An integrated proteomic, metabolomic and interactomic investigation which relates the results of omic approaches to standard meat quality indicators (WHC, pH, Minolta values). Breed-specific growing and fat accumulation attitudes in Large White and Casertana are discussed in the light of the integrated results.
Mora <i>et al.</i>	(35)	Intense degradation of myosin light chain isoforms in Spanish dry-cured ham is reported through proteomics.
Lund <i>et al.</i>	(38)	Oxidation of myosin heavy chain promotes meat toughening.
Bjarnadóttir <i>et al.</i>	(34)	Proteome changes in bovine <i>longissimus thoracis</i> muscle during the first 48 h <i>post mortem</i> evidenced shifts in energy status and myofibrillar stability.
Sentandreu <i>et al.</i>	(51)	A proteomic approach to assess the presence of chicken meat in meat mixtures.
Zhang <i>et al.</i>	(33)	A proteomic investigation on fat accumulation in bovine skeletal muscle.
Wimmers <i>et al.</i>	(28)	A study which correlates proteomic profiles of pig muscles to fat deposition tendency and meat juiciness.
Timperio <i>et al.</i>	(16)	Differential proteomic profiles of <i>longissimus dorsi</i> in high fat-depositing Casertana <i>vs.</i> low fat-depositing Large White pigs.
Huff Lonnergan <i>et al.</i>	(30)	Increased levels of glycolytic enzymes end up to exacerbate <i>post-mortem</i> pH and thus influence meat tenderness and flavour.
Guillemin <i>et al.</i>	(42)	Tentative identification of biomarkers of bovine meat tenderness through proteomics. A role is suggested for HSPs.
Laville <i>et al.</i>	(45)	HSP27 might be involved in protecting actin from <i>post-mortem</i> proteolysis.
Morzel <i>et al.</i>	(46)	HSP27 might be one of the main biomarker candidates for beef tenderness in the <i>longissimus thoracis</i> muscle of French Blonde d'Aquitaine bulls.
Kwasiborski <i>et al.</i>	(48)	Proteomic analyses of pig muscles: influences of rearing environment, feeding regimen and metabolic attitudes (Part 1).
Kwasiborski <i>et al.</i>	(49)	Proteomic analyses of pig muscles: influences of rearing environment, feeding regimen and metabolic attitudes (Part 2).
Morzel <i>et al.</i>	(41)	<i>Post-mortem</i> protein oxidation influences meat digestibility (nutritional value).
Lametsch <i>et al.</i>	(37)	<i>Post-mortem</i> protein changes (especially proteolysis phenomena) influence meat tenderness.
Lametsch and Bendixen	(29)	Proteomic correlation to muscle differentiation and growth, carcass composition in pigs.
Davies <i>et al.</i>	(40)	<i>Post-mortem</i> protein oxidation influences protein susceptibility to proteolytic enzymes and thus meat properties (tenderness).
Wolff and Dean	(39)	<i>Post-mortem</i> protein oxidation influences protein solubility, hydrophobicity and thus meat properties (tenderness).
Milk		
D'Alessandro <i>et al.</i>	(15)	Comprehensive proteomics and <i>in silico</i> analysis of bovine milk proteins.
Arena <i>et al.</i>	(63)	Modern proteomic methodologies are described for the characterization of lactosylation protein targets in milk.
D'Alessandro <i>et al.</i>	(13)	Comprehensive proteomics and <i>in silico</i> analysis of human milk proteins. Comparison to bovine milk proteins in order to individuate further fundamental components for milk formulas.
D'Amato <i>et al.</i>	(59)	Proteomics of the whey fraction of bovine milk.
Wilson <i>et al.</i>	(60)	Proteomic analysis of glycosylation patterns in milk proteins in order to individuate potential allergens.
D'Ambrosio <i>et al.</i>	(61)	Proteomic analysis of glycosylation patterns in milk proteins in order to individuate potential allergens.
Reinhardt and Lipolis	(58)	Proteomics of the MFGM fraction of bovine milk.
Fong <i>et al.</i>	(57)	Proteomics of the MFGM fraction of bovine milk.
Johnson and Lucey	(64)	Milk proteomics in order to improve cheese quality and traceability.
Hogarth <i>et al.</i>	(62)	Milk proteomics and mastitis.

Table 1. – continued

Authors	Ref.	Brief description
Wines and beers		
Giribaldi and Giuffrida	(18)	Grapevine proteomics: determination of optimal proteomic biomarkers to improve the knowledge in the field.
Iimure <i>et al.</i>	(71)	Proteomic analysis of 11 different beers: association of the protein profiles with beer-specific quality traits, such as foam stability, and with the relevance of the selection of malt for top quality brewing.
Fasoli <i>et al.</i>	(17)	Beer proteomics through combinatorial peptide ligand libraries for the enrichment of the low-abundance proteome.
Cilindre <i>et al.</i>	(70)	Sparkling wine proteins promote foam stability.
Transgenic food and new technologies in food safety assessment		
Seng <i>et al.</i>	(80)	MALDI-Biotyper allows for microorganism individuation and identification through comparison with database-annotated proteomic fingerprints.
Zolla <i>et al.</i>	(74)	Differential proteomic analysis of maize transgenic seeds.

the blandness of modern lean meat and the frequent reference to the more strongly flavoured meat that was available years ago have prompted reconsideration of high-fat-depositing typical pig breeds. Comparative proteomic analyses have been conducted for example on *longissimus dorsi* muscles of lean meat pig Large White in comparison with high-fat-depositing typical Casertana pig breed (16). As a result, proteomic analyses followed by interactomic *in silico* elaboration evidenced a wide series of metabolic alterations including an increased oxidative metabolism in lean meat of Large White, or an inefficient metabolism relying on glycolysis for energy production in Casertana pigs. On the other hand, increased levels of glycolytic enzymes end up to exacerbate *post-mortem* pH drop, thus resulting in the variation of organoleptic properties of the meat, such as tenderness and flavour (30), as it has been further assessed in Casertana and Large White pigs through the integration of results from different omics approaches (proteomics, metabolomics, interactomics) to standard meat quality indicators (such as Minolta values, pH drop, dressing percentage, back fat thickness, water-holding capacity; 31). Higher levels of glycolytic enzymes and lactate accumulation were related to slow pH drop in Casertana pigs, albeit not to rapid pH lowering in Large White counterparts. On the other hand, the individuation of pyruvate kinase M1 and tropomyosin levels in Large White were related to water-holding capacity and Minolta values at 24 h after slaughter. Bioinformatic analyses strengthened the correlation between over-expression of structural proteins in Large White and more accentuated growth aptitude in this breed. Conversely, enzymes taking part in branching glycolytic reactions, such as glycerol 3-phosphate and creatine kinase M, were related to accentuated lipogenesis and slower albeit prolonged glycolytic rate in Casertana, respectively. Breed-specific differences at the protein level were not only related to growth performances and fat accumulation tendency *in vivo*, but they also affected *post-mortem* performances through a direct influence on the forcedly anaerobic behaviour of pig muscles after slaughter (31).

Meat tenderness is a highly valued consumer trait and thus a definition of multiple processes that influence meat tenderness will provide clues towards improving meat quality and value (Fig. 1). Changes occurring

in the muscle *post mortem* tend to influence tenderness values (32–47). In the study by Lametsch *et al.* (37), muscle samples were taken at slaughter and 72 h *post mortem* and the changes in the proteome registered by two-dimensional electrophoresis were related to Warner-Bratzler shear force. One hundred and three spots were found to change significantly ($p < 0.01$) over time, and of these the 27 most pronounced changes were identified. Eleven out of the 27 changes were fragments of actin. Other identified myofibril proteins or fragments included myosin heavy chain, titin, myosin light chain I, myosin light chain II, CapZ, and cofilin. Correlation analysis revealed significant correlations of three of the identified actin fragments and the myosin heavy chain fragment with shear force. Moreover, myosin light chain II and triose phosphate isomerase I were also found to correlate significantly with shear force (29).

Besides, oxidation of myosin heavy chain, a predominant protein in the myofibril, is known to promote aggregation and toughening of meat (32,34,38). On the other hand, degradation of structural proteins, *viz.* desmin, filamin, dystrophin, and talin (all located at the periphery of the Z-line) may disrupt the lateral register and integrity of myofibrils themselves as well as the attachments of the peripheral layer of myofibril to the sarcolemma (30,32). Degradation of proteins within the myofibril that are associated with the thick and thin filaments may allow lateral movement or breaks to occur within the sarcomeres of *post-mortem* aged samples. Titin, nebulin, and troponin T, by their ability to directly interact with, or modulate the interaction between, major proteins of the thick and thin filaments and (or) the Z-line, play key roles in muscle cell integrity (30,32). Disruption of these proteins, especially titin and nebulin, could initiate further physicochemical and structural changes that result in myofibril fragmentation and loss of muscle cell integrity, and ultimately in tenderization of the muscle.

Alterations of the gel electrophoretic pattern of muscle proteins have been documented, although recent advancements in proteomic technologies have allowed to add further pieces to the puzzle of modifications occurring upon sacrifice of the animal. One of the main changes which have been reported so far is the alteration of the phosphorylation pattern of muscle proteins in a species-specific way (16,31). However, the most promising results will be likely obtained through the introduction of

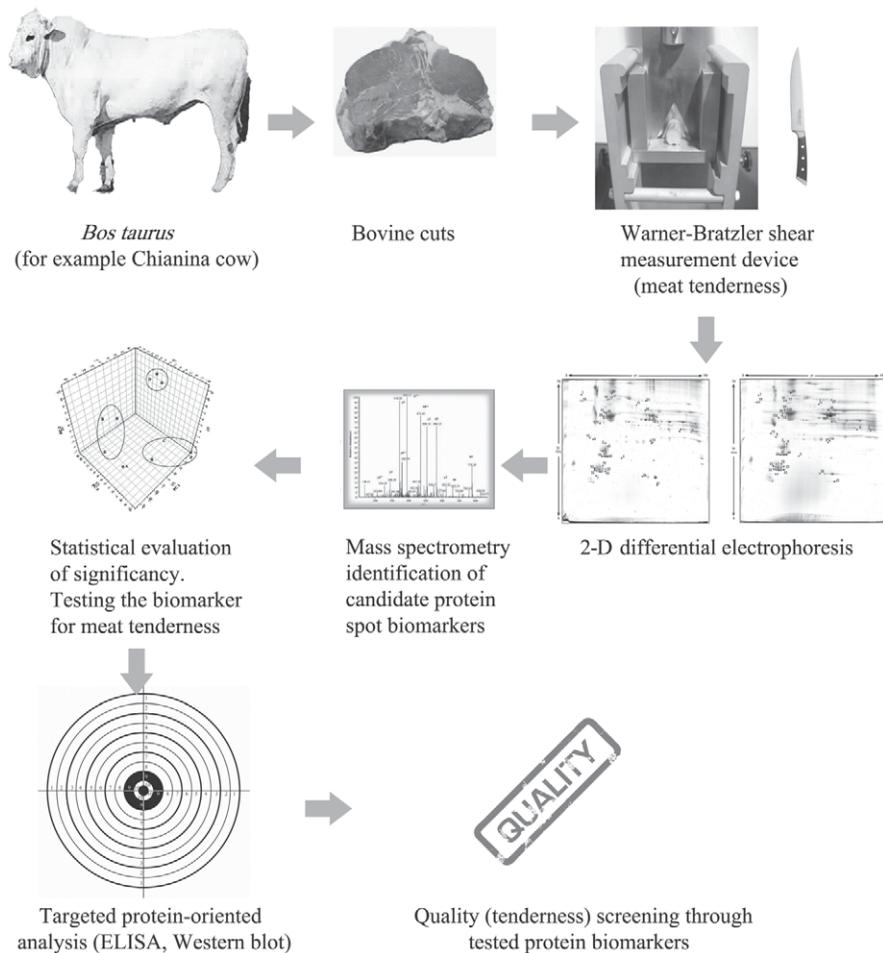


Fig. 1. An experimental workflow illustrating how proteomics can be applied to the individuation of protein candidate biomarkers for meat tenderness. Bovine meat samples are collected after sacrificing the animal and tested for meat tenderness through the Warner-Bratzler shear measurement device, in order to test shear force properties of the muscle. These data are thus compared with differential 2-D electrophoretic profiles of protein extracts from muscles showing opposite shear force trends. Candidate protein spot biomarkers are then digested and identified through mass spectrometry and finally statistically tested for significance in the model. They could be exploited eventually for targeted screening through antibody-based approaches, such as ELISA and Western blot. Finally, they could be adopted for rapid selection of individuals based on the presence/absence of quality-related (tenderness-related, in this case) markers

redox proteomics approaches in the study of meat quality, as protein oxidation appears to contribute significantly to the tenderness of the meat through triggering the formation of both protein fragments and aggregates (32,38).

Oxidation of proteins may cause changes in protein hydrophobicity, conformation, and solubility and alter susceptibility of protein substrates to proteolytic enzymes (36,39,40). This has been regarded as a major cause for the low digestibility and hence, lesser nutritional value of oxidized proteins (41). However, so far early attempts to determine the redox profile of *post-mortem* muscle proteins have been hitherto limited to the individuation of carbonylated proteins through the fluorimetric assays involving 2,4-dinitrophenylhydrazine (DNPH) (38). This implies that there is still plenty of room for improvement in the field of redox proteomics applied to meat quality.

Beef tenderness exhibits a high and uncontrolled variability, which is a reason for the consumer's dissatisfaction. Moreover, the beef industry does not have pre-

dictive tools to measure meat tenderness. During the last few years, the impact of muscular characteristics such as collagen, lipids, proteolytic systems and fibres on meat tenderness has been studied (42–44).

Therefore, it is small wonder that other research groups have focused their attention on the identification of suitable biomarkers to be adopted as indicators of meat quality and, in particular, of meat tenderness. The observations of Laville *et al.* (45) through 2-D electrophoresis (2-DE) on tender *vs.* tough meat from young Charolais bulls resulted in the postulation of a role for apoptosis in the linkage between animal slaughter and meat physicochemical properties. In that study, two extreme groups of *longissimus thoracis* muscle samples from young Charolais bulls, classified according to Warner-Bratzler shear force (WBSF) of meat grilled at 55 °C, were analyzed by 2-DE. As a result, higher quantity of proteins of the inner and outer membrane of mitochondria was found in the tender group, suggesting a more extensive degradation of mitochondria that may be related to the apoptotic process (45).

Other groups pursue the identification of actual biomarkers related to meat tenderness, like Guillemain *et al.* (42,43), who reported the individuation of 24 likely proteins whose quantitation paralleled beef tenderness in relation to muscle and animal type. Analogously, Morzel *et al.* (46) pinpointed the small heat shock protein HSP27 as one of the main biomarker candidates for beef tenderness in the *longissimus thoracis* muscle of French Blonde d'Aquitaine bulls. This protein is known to play a role in mitochondrial apoptotic pathway and might be well related to the observations by Laville *et al.* (45), or rather be involved in the protection of downstream proteins (structural proteins such as actin; 47) from protease- or ROS-induced fragmentation, due to its chaperone activity.

Finally, the rearing environment has been shown to play a role in meat proteomic signatures, as outdoor or indoor rearing resulted in dramatically different proteomic patterns in pig meat organoleptic properties, including back fat accumulation (48,49).

In the light of this accumulating body of literature on meat quality and proteomics, recent investigations of Toldrà and Sentandreu's group have been performed to exploit proteomics as an actual tool to be readily adoptable in the field of meat quality assessment (50–52). Proteomics has been exploited either to monitor small peptides from glycolytic proteins released during processing of dry cured ham (50), which could determine qualitative signatures of seasoning progress, or rather to investigate and determine the presence of protein mixes from different animal species in order to discover meat adulteration cases (51,52). These examples represent brilliant demonstration of the rapid shift of proteomic knowledge in the field of meat science to actual applications in the quality control processes of the food production chain.

Milk and milk formulas: Hints from proteomics

Milk is one of the richest physiological liquids, whose function has always been tied to the provision of nutrients to offspring.

In recent decades, milk has been growingly suggested to contribute to post-natal development of the newborn, through stimulation of their anatomical growth, maturation of their immune system, completion of their digestive tract, and the establishment of symbiotic microflora. While analytical studies revealed qualitative and quantitative differences in milk composition for major proteins, lipids and carbohydrates from various mammals, comparative genomics and bioinformatic studies suggested a general conservation of milk and mammary genes/proteins among mammalian species, proposing species-specific milk variations associated with gene duplication and transcriptional/translational regulative events (53–55).

As the protein fraction constitutes perhaps the most biologically relevant component in milk, a long list of proteomic investigations targeting milk proteins have been carried out so far, especially on bovine milk (for further details the interested reader is referred to D'Alessandro *et al.* (13,15)). This joint effort culminated in the identification and classification of over 573 non-redundant fully annotated and referenced proteins (15), al-

though the list is still under expansion, especially as far as other farm animal species are concerned (56).

However, bovine milk has the longest history and the broadest commercial interest. Indeed, bovine milk consumption is of extreme importance to humans, as it has been observed that lactase persistency and cattle domestication have almost convergently evolved in most human populations (57).

Bovine milk proteins, present at a concentration of 32 g/L, are generally classified as caseins (80 % of the total milk protein content), whey proteins (16 %), peptones/low molecular mass peptides (3 %) and milk fat globule membrane (MFGM) proteins (1 %) (15). Similarly to other biological fluids, milk proteome complexity is dramatically exacerbated by the wide dynamic range of concentration of its proteins. In fact, a whole hidden proteome exists in each milk fraction, whose proteins likely exert physiological functions yet unknown. Due to the dramatic heterogeneity of milk protein concentrations, approaches targeting protein fractions have been so far conducted upon preliminary fractionation, which allowed discrimination between MFGM (58,59) and whey proteins (60).

Qualitative proteomic profiles have also been expanded with the inclusion of post-translational modifications and, in particular, with the analysis of glycosylation (O- and N-glycosylation) and lysine lactosylation differential patterns (61–63). Taken together, all these data have helped configuring a detailed scenario from which researchers can derive precious information on milk quality (alterations of these profiles upon mastitis; 64) and exploit this knowledge background to improve cheese production quality (65). Mastitis in dairy cows occurs when leukocytes are released into the mammary gland, usually in response to an invasion of the teat canal by bacteria. There is a long list of mastitis-provoking bacteria, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* or *Escherichia coli*.

On the other hand, the individuation of the differential proteomic profiles of human and bovine milk could be exploited to ameliorate milk formulas for infants, thus guaranteeing products of improved quality while excluding the likelihood of insurgence of adverse immune reactions (13). In fact, as milk represents the main source of nutrition for infants, the question of an effective human milk substitute becomes mandatory when a formula-fed baby is allergic to cow's milk proteins (66). In this case, formulas containing extensively hydrolysed milk proteins should be preferred, but even such a formula may cause allergic reactions in highly sensitive patients. If there is evidence of allergy to cow's milk with IgE-associated symptoms, after 6 months of age, a soybean formula may be recommended only when tolerance to soy protein has been established by clinical challenge. In infants with allergic reactions to cow's milk proteins, even after extensive hydrolyzation, proteomic techniques coupled with immunological methods may make it possible to select other milk products that do not contain the same allergens as ordinary cow's milk. In this paper, evidence will be presented that proteomic evaluation of proteins from different mammalian species may be a suitable method of testing whether proteins from the milk of different mammalian species may be used as a substitute for un-

treated bovine milk. Proteomic evaluation of milk from different mammalian species may not only be of help when recommending suitable feeding in cases of cow's milk allergy but it also gives new insight into the background of allergic reactions caused by milk proteins (66).

Proteins and quality of wines and beers

Grapevine (*Vitis* spp.) is at present considered the most important fruit plant throughout the world, both due to its economic importance and to its role as a non-climacteric model species. The relevance of the studies devoted to the dissection of grapevine biology and biochemistry underlines the great amount of attention that this plant has attracted over the last decade (18). During the last decade, unexpected detrimental effects of wine on human health have been reported, in particular in France, where red wine consumption has fuelled a debate about the so-called French paradox. The French paradox stemmed from the observation that French people suffering from coronary heart disease represented but a minority percentage, despite following a diet relatively rich in saturated fats (67), albeit rich in (red) wine consumption. As wine might hold both commercial and health benefits (when consumed responsibly), the investigation of the protein fraction has attracted a great deal of interest, both in the developing plant and its components and the end-product, the wine (68,69). Some grape berry proteins are relevant for the production quality as they are known to resist fermentation and to cause turbidity in wines. As brilliantly synthesized by Giribaldi and Giuffrida (18), a detailed knowledge of the protein content and characteristics of grape berries and juices is important for winemakers, since protein precipitation is a major cause of haze formation in wines, and especially in white wines. The denaturation and subsequent aggregation of proteins can lead to amorphous sediment or flocculation, causing turbidity. A haze or deposit in bottled wine indicates that the product is unstable, has a low commercial value and is therefore unacceptable for sale, and winemakers usually perform some kind of fining, such as bentonite absorption, to avoid this defect. Other than representing a likely production quality-limiting factor, some of the proteins detected in both wine and grape berries, such as chitinase and lipid transfer protein (LTP), are known to be allergenic (70). In this respect, a further burden is represented by treatments with fining agents, which have been traditionally used in winemaking despite being also allergenic (e.g. albumin or casein). Conversely, proteins could also be considered as important constituents in wines, for example in the sparkling wine industry, because they promote foam formation and stability (70).

Proteomics has also been applied for the quality control in production processes of other widespread alcoholic drinks, such as beers (17,71). It is easier to understand the relevance of beer when considering that it is the world's oldest and most widely consumed alcoholic beverage and the third most popular drink overall after water and tea. It is produced by brewing and fermentation of starch, mainly derived from cereal grains, typically malted barley. In a recent study, Imure *et al.* (71) performed 2-D gel electrophoretic analyses of 11 different commercial beers and through mass spectrometry iden-

tified 85 out of 199 protein spots. These results allowed them to classify and link proteome profiles to beer-specific quality traits, such as foam stability, and to confirm the relevance of the selection of malt for top quality brewing.

In a second study (17), the authors investigated the beer proteome *via* prior capture with combinatorial peptide ligand libraries (ProteoMiner, Bio-Rad, Hercules, CA, USA, as well as a homemade library of reduced polydispersity) at three different pH (4.0, 7.0 and 9.3) values. This preliminary fractionation method allowed the investigators to handle large volumes of beer from which they were able to enrich the protein fraction specifically, equalize protein abundances and thus reveal very low-abundance protein fractions. *Via* mass spectrometry analysis of the recovered fractions, after elution of the captured populations in 4 % boiling sodium dodecyl sulphate (SDS), Fasoli *et al.* (17) were able to categorize such species in 20 different barley protein families and 2 maize proteins, the only ones that had survived the brewing process (the most abundant ones being Z-type serpins and lipid-transfer proteins). Besides, 40 unique gene products from *Saccharomyces cerevisiae*, one Z-type from *S. bayanus* and one from *S. pastorianus* were identified, which are known to be routinely used in the malting process for lager beer. As stressed by the authors themselves, the knowledge of the residual proteome in beer might help brewers in selecting proper proteinaceous components that might enrich beer flavour and texture and thus have evident benefits for the production and quality control processes of beer.

Quality control of transgenic food

Quality control becomes a fundamental principle in the alimentary industry when it comes to genetically modified organisms. Over recent years, it has become clear that food and feed plants carry an inherent risk of contaminating our food supply (72). The current procedures include the investigation of possible unintended effects to assess the safety of food and feeds derived from modern biotechnology. To improve the probability of detecting unintended effects, profiling techniques such as proteomics are currently tested as complementary analytical tools to the existing safety assessment. These tools include electrophoretic techniques along with mass spectrometry-based identification of newly expressed or over-expressed proteins upon genetic engineering of the plant (73,74). In order to understand the importance of these tools in quality control of food from either wild type or recombinant plants, it could be worthwhile to recall the concept of the substantial equivalence, according to which a novel food (for example, genetically modified foods) should be considered the same as and as safe as a conventional food if it demonstrates the same characteristics and composition as the conventional food (75). In this view, the comparison of proteomic profiles between wild type and seeds from genetically-modified maize plants have revealed valuable information about the equivalence of these foods. In their 2008 article, Zolla *et al.* (74) analyzed the proteomic profiles of one transgenic maize variety (event MON 810) in two subsequent generations (T05 and T06) with their respective isogenic controls (WT05 and WT06). Thus, by comparing the prote-

omic profiles of WT05 with WT06 the authors were able to determine the environmental effects, while the comparison between WT06 and T06 seeds from plants grown under controlled conditions enabled the investigation of the effects of DNA manipulation. Finally, by comparison of T05 with T06 seed proteomes, the authors were able to trace some similarities and differences between the adaptations of transgenic and isogenic plants and the same strictly controlled growth environment. Approximately 100 total proteins were differentially modulated at the expression level as a consequence of the environmental influence (WT06 *vs.* WT05), whereas 43 proteins were up- or down-regulated in transgenic seeds with respect to their controls (T06 *vs.* WT06), which could be specifically related to the insertion of a single gene into a maize genome by particle bombardment. Transgenic seeds responded differently to the same environment as compared to their respective isogenic controls, as a result of the genome rearrangement derived from gene insertion (74).

Proteomic assessment of food safety in the field of transgenic maize has also been complemented by metabolomic approaches (76). The metabolic profiles of seeds from the transgenic maize variety 33P67 and of the corresponding traditional variety were also investigated using one- and two-dimensional NMR techniques (76). About 40 water-soluble metabolites in the maize seed extracts were identified. The ¹H spectra of transgenic and non-transgenic maize seed samples turned out to be conservative, showing the same signals and therefore the same metabolites, allowing one to assess that no significant differences in metabolic profile exist between transgenic maize and traditional variety (76).

Food Safety: New Proteomic Technologies and Future Perspectives

For the foreseeable future, the individuation of qualitative and quantitative protein biomarkers might become pivotal also in food testing to determine food safety and authenticity (77). As both these concepts are somehow intimately intertwined, proteomics might provide valuable shortcuts to determine, for example, which milk has been used to produce a specific cheese, and thus identify the production origin of an aliment of certified and guaranteed 'controlled origin'. In practice, LC-MS-based methods have been established for example to assess the addition of porcine or bovine gelling agents to porcine, bovine, lamb or chicken meat, which is of clear commercial interest, especially in the light of religious issues on certain meat consumption (78).

Analogously, MS-based approaches have been proposed to screen bovine sera to seek for traces of performance-enhancing agents, which are illegally used in cattle (especially in young veals) and other meat-producing species to increase food conversion and lean meat production (79).

On the other hand, food safety is not only a matter of determining the origin of a product, but also to evaluate its edibility through biochemical assessment of product purity, both from a chemical and microbiological standpoint. As for the latter, the recent epidemy of mu-

tant *E. coli*, which has involved the north of Germany and almost paralyzed vegetable commerce within Europe at the beginning of June 2011, might represent a warning sign. Nonetheless, big strides in the field of the application of mass spectrometry to microbiology are still defining an ongoing revolution which might spread its benefits to the food safety endeavour as well: the introduction of Bruker Daltonics' MALDI-Biotyper (80). This technology allows for rapid and >99 % accurate identification of bacteria and microorganisms (and region-specific sub-strains) cultured from routine clinical samples through the identification of species-specific proteomics profiles (Fig. 2). Its application to the field of food safety might result in something more than a suggestive perspective, contributing to diminishing the likelihood of untoward risks rising from the assumption of unsafe food.

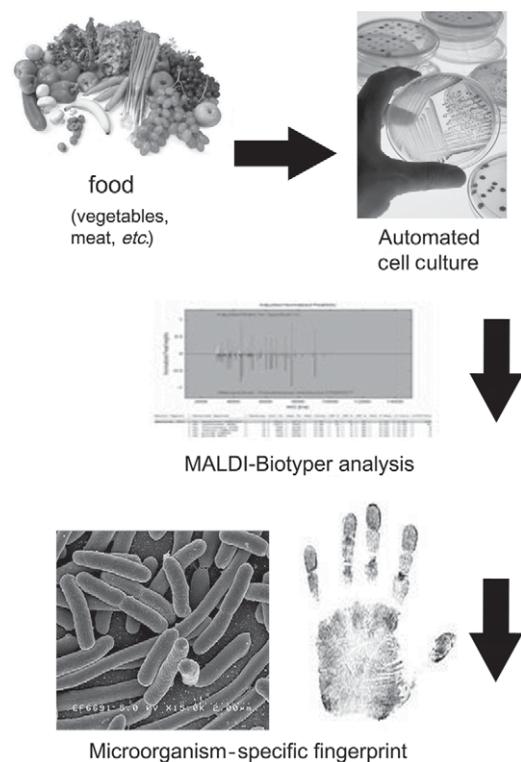


Fig. 2. The mechanisms which are used by Bruker MALDI-Biotyper for the identification of microorganisms. This innovative technology might be applied not only in the clinical setting, but also for the identification of pathogens which might end up compromising food safety

Conclusions

An extensive range of applications for proteomics in the field of quality control is hereby reported and reviewed. In recent years, quality control has partially shifted towards the assessment of the high-end standardization of production processes in the alimentary industry and, reasonably enough, this represented a first step towards personalized medicine (curing people through nutraceuticals) and personalized drugs (theranostics; 81).

In all these fields proteomics has already proved its worth and will possibly expand its influence in the upcoming years, with the introduction of new investigative

technologies, such as the ones enabling the assessment of quantitation, post-translational modification, *post hoc in silico* elaborations and tissue analysis through imaging mass spectrometry (81).

In parallel, as food quality and safety represent two key points in the European and international agenda for the foreseeable future, the application of proteomics in these research endeavours will continue to contribute relevant basic science and applied/translational insights.

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