

# Food Proteomics: Mapping Modifications

Stefan Clerens<sup>1</sup>, Jeffrey E. Plowman<sup>1</sup> and Jolon M. Dyer<sup>1,2,3</sup>

<sup>1</sup>*Food & Bio-Based Products, AgResearch Lincoln Research Centre*

<sup>2</sup>*Biomolecular Interaction Centre, University of Canterbury*

<sup>3</sup>*Riddet Institute at Massey University, Palmerston North*

*New Zealand*

## 1. Introduction

Proteins are an essential element in the human diet. As food ingredients, they are primarily sourced from plants and animals; important sources include cereals, meat, poultry, fish and dairy. Proteomics offers a powerful new way to characterise the protein component of foods. Proteomics not only reveals which proteins are expressed in each tissue type, it also allows the investigation of differences in the protein composition of different tissues. In addition it has the power to track the proteome of tissues before and after harvest/slaughter, and to evaluate the effect of downstream treatments such as cooking or curing.

The proteomic evaluation of food proteins presents a unique set of challenges and opportunities. Muscle, milk and cereal proteomes are dominated by very abundant proteins, creating a dynamic range problem. In addition to post-translational modifications produced *in vivo*, food proteins are subjected to a wide range of post-harvest/post-slaughter environmental and processing insults prior to consumption. These modifications include side-chain oxidation, cross-link formation and backbone cleavage, and critically influence key food properties such as shelf-life, nutritional value, digestibility and health effects.

A profound understanding of proteomics, protein modifications and redox chemistry has allowed us to pioneer the application of redox proteomics to foods. This has led to the development of a unique proteomics damage scoring system, allowing a direct link between molecular-level understanding to intervention/mitigation at the processing level (Dyer et al., 2010). We anticipate that this ability will be pivotal in the development of next-generation food products.

This chapter outlines current achievements in the field of food proteomics. It deals with the full spectrum of protein-containing foods, including dairy, meat, seafood and cereal proteins. We focus on *ex vivo* protein modifications and their effects on foods. We devote attention to redox proteomics approaches applied to food, and pay special attention to the recent development of advanced redox proteomic-based approaches to evaluate and track food protein modifications. These approaches are illustrated in a case study that compares the protein damage level in a number of commercially available dairy products.

## 2. Proteins and nutrition

Proteins are key functional and structural components of all living cells and are an essential element in the human diet. The human body is capable of synthesising most of the amino

acids from other precursors, but is unable to produce the nine essential amino acids (His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val), which must be supplied from the diet. Another six amino acids (Arg, Cys, Gln, Gly, Pro, Tyr) can be produced by the body, but may need a dietary source when endogenous production cannot meet metabolic requirements. Amino acids also act as precursors for many coenzymes, hormones, nucleic acids and other molecules, and can also be used as a metabolic fuel.

The total amount of protein in the body is fairly static, but individual proteins are constantly being degraded and re-synthesised. The rate of this turnover is affected by stage of life and level of activity. That makes the inclusion of an appropriate quantity of high quality protein in the diet critical for growth and development in children and the maintenance of good health in adults.

Protein can be sourced from plants and animals; important sources include cereals, meat, poultry, fish and dairy foods. These sources differ in the relative bioavailability of protein and, in particular, essential amino acids. In general the digestibility of proteins from vegetable sources is lower than for those of animal origin, being around 78-85% as opposed to 94-97% for meat, dairy and eggs. Animal sources of protein also generally have higher levels of the essential amino acids.

Proteomics, through the application of gel and non-gel approaches, offers a powerful new way to characterise the protein component of foods. Whereas genomics provides information on the total genome of the organism, proteomics reveals which proteins are actually expressed in each tissue type. Furthermore the application of proteomic techniques offers a way to investigate differences in the protein composition of different tissues within a specific animal or vegetable food type, as well as between different varieties of it. In addition it has the power to follow changes in the protein component of various tissues during growth, maturation and post-mortem or post-harvest, as well as downstream treatments such as cooking.

### 3. Food proteomics

This section overviews and summarises the application of classical proteomics in food science, broken down into major food protein groups.

#### 3.1 Dairy

Proteomics has been successfully applied to the study of milk proteins by many research teams, and significant effort has gone into the characterisation of the milk proteome. Also, bioactive milk components are of enormous scientific and commercial interest. Proteomics approaches have been used to compare milk from different species, while proteomic evaluation of other dairy products such as cheese has been a specialist subject that has also received attention.

In general, the dynamic range of proteins in milk poses a challenge to proteomics technologies. This is because the proteins in milk tend to be dominated by the caseins, which make up some 80% of the total protein content. Even when these are removed, the minor components in whey are dominated by one or two proteins; in bovine milk these are  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. Dynamic range issues have been overcome thanks to improvements in mass spectrometer sensitivity, coupled with the application of depletion and/or fractionation techniques. Casein, for example, is easily removed by acid precipitation. Alternatively affinity purification has been successfully used to remove IgA,

lactoferrin,  $\alpha$ -lactalbumin and serum albumin from human colostrum, allowing the identification of 151 proteins, over half of which have not been previously identified in colostrum or in milk (Palmer *et al.*, 2006).

Following fractionation, substantial attention has been given to the identification of minor (including bioactive) components in milk. In one study 2-dimensional electrophoretic (2-DE) proteomic methods were applied to bovine whey after it had been fractionated into acidic, basic and non-bound components by semi-coupled anion and cation exchange chromatography (Fong *et al.*, 2008). Utilising this approach, a large number of minor whey proteins were identified, some of which had not been previously reported in milk; in particular the acidic fraction was found to have a group of osteopontin peptides. Other investigators have used proteomics approaches to compare milk from different species to evaluate their suitability as a substitute for human milk (D'Auria *et al.*, 2005).

Discovery and characterisation of bioactive milk components is of enormous scientific and commercial interest. For example, the host defence proteins in milk and colostrum have the potential to add significant value to the dairy industry, and techniques have been put in place to fractionate and analyse them using proteomics (Smolenski *et al.*, 2007; Stelwagen *et al.*, 2009).

The milk fat globule membrane (MFGM) constitutes another important component of milk. MFGM composition is of interest because it is known to be rich in bioactive components and there is increasing evidence that there are significant health benefits associated with the consumption of MFGM. Vanderghem *et al.* (2008), investigating a simple and rapid approach for the extraction of the MFGM, evaluated a number of different detergents and found that the inclusion of 4% CHAPS resulted in the removal of the highest amount of skim milk proteins as evaluated by 2-DE. Affolter *et al.* (2010) profiled two fractions of the MFGM, a whey protein concentrate (WPC) and a buttermilk protein concentrate (BPC) using three different approaches. Using a LC-MS/MS shotgun proteomics approach, 244 proteins were identified in WPC and 133 in BPC, while label-free profiling was used for semi-quantitative profiling and the determination of protein fingerprints.

Water soluble extracts of Teleme cheeses prepared from ovine, caprine and bovine milk were separated by 2-D gel electrophoresis and analysed by MALDI-MS and by HPLC in conjunction with Edman degradation, MS and tandem MS (Pappa *et al.*, 2008). The 2-DE gels tended to be dominated by the casein and whey proteins, while in the MS analysis of the RP-HPLC-separated peptide fractions a few predominant peptides tended to mask the minor components. Nevertheless, enough differences were observed to enable the source of milk to be identified. Species specific differences were also observed in the tandem MS of peptides originating from casein. The effect of variations in milk protein composition on cheese yield of chymosin-separated sweet whey and casein fractions was examined by 2-D electrophoresis in conjunction with MS and multivariate data analysis (Wedholm, 2008). Using this approach it was possible to identify a range of proteins which had a significant effect on the transfer of proteins from milk to cheese. Included among these was a C-terminal fragment of  $\beta$ -casein, as well as a combination of several other minor fragments of  $\beta$ -,  $\alpha$ s1 and  $\alpha$ s-2 caseins, whose individual effect was relatively low.

### 3.2 Meat

The field of meat proteomics has seen steady growth over the past five years. A large number of studies have been performed, with a few distinct categories emerging. The post mortem conversion of muscle into meat is a significant series of events connected with

protein modification and breakdown, and has received substantial attention. This has been extended to changes occurring in cooked/cured products. Protein marker discovery for various meat quality attributes is another important area. With countless animal breeds all providing different muscle/meat parameters of interest, there is significant potential for exciting discoveries leading to improved quality/value attributes and product differentiation.

Bjarnadottir et al, (2010) examined the insoluble protein fraction of meat (*longissimus thoracis*) from eight male Norwegian Red dual-purpose cattle during the first 48 hour post-mortem period using 2-DE and MALDI-TOF MS/MS and noted significant changes in 35 proteins, divided into three different groups based on predicted function, specifically: metabolism, cellular defence/stress and structural. Of these, most of the metabolic enzymes involved energy metabolism in the cell, while the cellular defence/stress proteins related mainly to the regulation and stabilisation of myofibrillar proteins. Laville et al. (2009) examined proteomic changes during meat aging in tough and tender beef using 2-DE and found that a higher proportion of proteins from the inner and outer membrane of mitochondria were found in the tender group, suggesting that a more extensive degradation of this organelle may be related to the apoptotic process. In another study into relationships between the protein composition of myofibrillar beef muscle proteins and tenderness Zapata et al. (2009) analysed by 1-DE and nano-LC-MS/MS the myofibrillar muscle fraction of *longissimus dorsi* from 22 Angus cross steers that had linearly regressed to shear values. Six bands showed a significant relationship and were found to contain a wide variety of cellular pathways involving structural, metabolic, chaperone and developmental functions. Bouley et al. (2003) applied 2-DE to the separation of proteins from semitendinous muscles from Belgian Blue bulls that were either double-muscled homozygotes or non double muscled homozygotes. With the aim of identifying markers for muscle hypertrophy they found 26 proteins whose expression varied between homozygote types and hence had potential as markers for this trait. Shibata et al. (2009), in contrast, were interested in how the type of feed affected the muscle proteome of Japanese Black Cattle, in particular the difference between grass and grain feeding. The cattle were individually housed in a barn and fed a combination of ad libitum and Italian ryegrass hay until 21 months of age, whereupon half were put onto pasture outdoors until slaughter at 27 months of age. From 2-DE gels, differences were apparent and involved 20 spots from the sarcoplasmic and 9 from the myofibrillar fraction, of which adenylate kinase 1 and myoglobin from the sarcoplasmic fraction and slow twitch myosin light chain 2 from the myofibrillar fraction were significantly higher in the grazing group. All of this was indicative of a change from slow-twitch tissues to fast twitch tissues when the cattle were grazed in the latter fattening period.

The application of proteomics to following post-mortem changes in porcine muscles has also been of interest. Using a proteomic approach, te Pas et al. (2009) identified several proteins associated with drip loss and shear force in Yorkshire and Duroc pigs but none for cooking loss. In a study of post-mortem changes in *longissimus dorsi* muscles in pigs, Choi et al. (2010) used a 2-DE approach to show that muscles exhibiting a higher degree of protein denaturation not only displayed lower muscle pH, paler surfaces, and higher degrees of fluid loss of exudation, but were also characterised by myofibrillar and metabolic protein degradation. As a result of this work it would appear that myosin, actin, the troponin T 4f isoform and glycogen phosphorylase fragments have potential for explaining variations in

the degree of protein degradation and meat quality. Hwang et al. (2005) also studied post-mortem changes in *longissimus dorsi* muscles of Landrace pigs using a gel-based approach, comparing those that had been fasted for 18 hours before slaughter with those fed on the morning of slaughter. They noted semi-quantitative changes in 27 proteins with muscle ageing, including myosin light chain 1, desmin, troponin T, cofilin 2, F-actin capping protein  $\beta$  subunit, ATP synthase, carbonate dehydrase, triosephosphate isomerase, actin, peroxiredoxin 2,  $\alpha$ -b crystalline and heat shock protein 27 kDa. Lametsch and Benedixen (2001) used 2-DE to follow post-mortem changes in porcine muscles from 4 to 48 hours, paying particular attention to those focusing between 5-200 kDa and pH 4-9, of which 15 were found to show noticeable changes with time. Morzel et al. (2004) used proteomics to monitor the effects of two different pre-slaughter handling techniques on meat aging, specifically mixing of animals from different pens and transport to the abattoir 12 hours before slaughter with no mixing of animals and immediate slaughter after transport. They found significant changes in protein composition with ageing and in animals that were not mixed and slaughtered immediately there was over-expression of mitochondrial ATPase and increased concentration of a protein thought to be a phosphorylated form of myosin light chain 2. Lametsch et al. (2003) also looked at post-mortem changes in pig muscle tissue in relation to tenderness and found significant changes in 103 protein spots, of which 27 of the most prominent were identified. They found significant correlations between shear force and three of the identified actin fragments, the myosin heavy chain, as well as the myosin light chain II and triose phosphate isomerase I. A 2-DE approach was used to study relationships between protein composition and various meat quality traits in the *longissimus dorsi* muscle from Landrace pigs at various times after slaughter (Hwang, 2004). He found that high lightness values (as measured by Hunter L\*) and drip loss coincided with high proteolysis rates. Moreover, 12 proteins appeared to be related to L\* values, including  $\alpha$ -actin, myosin light chain 1, cofilin 2, troponin T and  $\alpha$ -b crystalline chaperone proteins. In addition four proteins were related to drip values, these being troponin T, adenylate cyclase, ATP-dependent proteinase SP-22 and DJ1 protein.

Proteomics has also been applied to the more general area of protein content and meat quality in pork. Kwasiński et al. (2008) related 2-DE spots of the soluble portion from pig *longissimus lumborum* tissue to known meat quality traits using multiple regression analysis and determined that one to two proteins could explain between 25 and 85% of the variability in quality in this tissue. Relationships between the water holding capacity of pork and protein content were examined by van de Wiel and Zhang (2008) using a 2-DE approach. They were able to identify up to eight proteins that appeared to be significantly related to water holding capacity; the most clearly related were creatine phosphokinase M-type (CPK), desmin and a transcription activator. Another proteomic study provided evidence for pig muscles that were darker in colour having more abundant mitochondrial enzymes of the respiratory chain, haemoglobin and chaperone or regulator proteins (Sayd et al., 2006). Lighter coloured meats, in contrast were found to have more glycolytic enzymes. The genotype of the pig was also found to have an effect on meat quality (Laville et al., 2009). They used 2-DE to compare the sarcoplasmic protein profiles of semi-membranous muscles from early post-mortem pigs with different HAL genotypes (RYR1 mutation 1841T/C) and from ANOVA analysis found that 18% of the total matched protein spots were influenced by genotype, while hierarchical clustering analysis identified a further 10% that were coregulated with these proteins. One of the genotypes was found to contain fewer

proteins of the oxidative metabolic pathway, fewer antioxidants and more protein fragments. The effect of the positive influence of compensatory growth on meat tenderness was examined by Lametsch et al. (2006), who divided female pigs into two groups, one of which had free access to the diet, another of which were feed-restricted from day 28 to 80 and were then given free access to the diet. Forty-eight hours post-mortem, proteins affected by compensatory growth were all found to be full length proteins, a result that goes against previous hypotheses that compensatory growth results in increased post-mortem proteolysis.

There has been some interest in applying proteomic techniques to analysing cooked pork products, including ham. Di Luccia et al. (2005) followed the progressive loss by hydrolysis of myofibrillar proteins during the ripening of hams using 2-DE, even to the extent of identifying a novel form of actin in the 2D gel. Sentandreu et al. (2007) used MALDI-TOF MS and MS/MS to identify peptides generated during the processing of ham. Sequence homology analysis indicated that they originated from actin. The protein composition of tough and tender pig meat, as defined by its shear force after cooking, was examined by 2-DE (Laville et al., 2007). They found at least 14 spots that differed significantly in quantity between the two groups, in particular, proteins involved in lipid traffic and control of gene expression were overrepresented in the tender meats, along with proteins involved in protein folding and polymerization.

When it comes to poultry, proteomics has been applied to identifying proteins differentially expressed in Cornish and White Leghorn chickens (Jung et al., 2007). They investigated over 300 spots in their 2-DE gels and found one protein that was differentially expressed in *pectoralis* muscles and four in *peroneus longus* muscles, all of which are assumed to be associated with muscle development, growth stress and movement in chickens. Nakamura et al. (2010) used proteomics to examine the allergenicity of meat from transgenic chickens. Using an allergenome approach they were able to identify five IgE-binding proteins that from 2D-DIGE analysis were found not significantly changed between non-GM and GM chicken. Proteomics also has proved to be useful for detecting chicken in mixed meat preparations. Sentandreu et al (2010) developed a method for the extraction of myofibrillar proteins and the subsequent enrichment of target proteins using OFFGEL isoelectric focusing, which were then identified by LC-MS/MS. Quantitative detection of chicken meat was achieved using AQUA stable isotope peptides and it was possible to detect contaminating chicken down to levels of 0.5%.

### 3.3 Seafood

There are a wide variety of ways that proteomics can be applied to seafood including the identification of species, characterisation of post-mortem changes in the various species of fish, or the effect of additives during the processing of fish muscle (Martinez and Friis, 2004). Gebriel et al. (2010), interested in understanding the effects of environmental, nutritional, biological and industrial factors on fish meat quality, undertook a large scale proteomic approach to first characterise cod muscle tissue composition. Using 1-DE coupled with nanoflow LC and linear trap quadrupole MS they were able to identify 446 unique proteins in cod muscle.

Addis et al. (2010) were interested in how physiological conditions experienced by wild and farmed fish affected muscle proteome of gilthead sea bream. They applied 2-DE and mass spectrometry to systematically characterise the proteome of this sea bream along the

production cycle in four offshore floating cage plants and two repopulation lagoons in different areas of Sardinia. From this they were able to conclude that the protein expression profile of muscle tissue is comparable between the farmed and those found in the wild and therefore that farming in offshore cages would be good for proper muscle development and enable the production of higher quality fish. Veiseth-Kent et al. (2010) were also interested in the effects of fish farming on muscle and blood plasma proteomes but this time in changes induced by crowding. They found that the proteins mainly affected were those involved in secondary and tertiary stress responses and thus provided insight into the mechanisms causing accelerated muscle pH decline and rigor mortis contraction in salmon living under crowded conditions.

The application of proteomics to the study of post-mortem changes of fish muscle has also been of interest. Kjaersgard and Jessen (2003) examined the alterations in cod (*Gadus morhua*) muscle proteins over eight days in storage on ice using 2-DE and noted significant increases in intensity for eight spots over the first two hours, while there were significant decreases in intensity for two other spots over the entire eight days.

### 3.4 Cereal

Given the importance of rice as a staple food, it is not surprising that significant attention has been devoted to its proteome. Komatsu and Tanaka (2004) described the construction of a rice proteome database based around 23 reference 2-DE maps ranging from the cataloguing of its individual proteins to the functional characterization of some its component proteins, including major proteins involved in growth and stress response. As a result of this work, a total of 13,129 proteins are contained within these maps, of which 5092 have been entered into the database. Xue et al (2010) applied proteomics to rice with the view to determining its potential for detecting the unintended effects of genetically modified crops. By examining the total seed protein expression of two strains of transgenic rice with 2-DE they found that some of the seed proteins in the two lines differed in their relative intensities after comparison with their respective control lines. Kim et al. (2009) examined the proteomes of two different cultivars of rice, one high-quality and the other low-quality, and identified 15 proteins that may have important roles in quality determination, regulation of protein stability of imparting disease resistance during grain filing and storage. Ferrari et al. (2009) studied the proteomic profile of rice bran with a view to understanding its functional properties. Through a combination of gel and gel-free approaches they were able to identify 43 proteins with functions ranging from signalling/regulation, enzymic activity, storage, transfer to structural. Kang et al., (2010) were interested in the responses of plants to stress and applied a proteomic approach to determine how rice leaves respond to various environmental factors.

Other cereals that have been examined using proteomics include barley, maize, wheat (Agrawal and Rakwal, 2006) and corn (Ricroch et al., 2011). In the case of wheat, studies have ranged from an examination of the proteome of polyploid wheat cultivars to determine the effect of genome interaction in protein expression (Islam et al., 2003), through to studying allergens in wheat. For instance, Akagawa et al. (2007) undertook a comprehensive characterisation of allergens in wheat using a 2-DE approach coupled with MALDI-TOF MS. As a result of this study they were able to identify nine subunits of low molecular weight glutenins as being the most predominant IgE-binding proteins. The 2-DE maps they were able to generate were also considered to have much potential for the diagnosis of wheat-

allergic patients or the identification of wheat allergens in food. Proteomics has also been applied to identify stress-induced proteins in wheat lines that may have a special role in food science (Horváth-Szanic et al., 2006). Using separation on 2-DE gels coupled with MALDI MS they studied a set of drought-stressed wide-range herbicide resistant transgenic spring wheat lines and noted that a number of inhibitor-like proteins were dominant in the stressed transgenic lines.

Akagawa et al. (2007) undertook a comprehensive characterisation of allergens in wheat using a 2-DE approach coupled with MALDI-TOF MS. As a result of this study they were able to identify nine subunits of low molecular weight glutenins as being the most predominant IgE-binding proteins. The 2-DE maps they were able to generate were also considered to have much potential for the diagnosis of wheat-allergic patients or the identification of wheat allergens in food.

### 3.5 Other

Proteomic studies of eggs have ranged from simple exploratory investigations to locating allergenic proteins or more complex interactome studies. Mann *et al.* (2008), using high-throughput mass spectrometry based techniques to search for bioactive compounds in eggs, identified 119 proteins in egg yolk, 78 in egg white and a further 528 proteins in the decalcified egg shell organic matrix. These included some 39 phosphoproteins from the egg shell soluble matrix, 22 of which had not been previously identified as phosphoproteins. D'Alessandro et al. (2010) took this one stage further by taking data from the literature and then regrouping and elaborating them for network and pathway analysis with the view to developing a unified view of the proteomes in egg, specifically those of egg white and yolk. As a result they were able to highlight roles for proteins in cell development or proliferation, cell-to-cell interactions and haematological system development in the egg yolk. The egg white proteins were found to be mainly in pathways involved with cell migration. Lee and Kim (2009) compared immunochemical methods such as ELISA, PCR and a proteomic approach involving MALDI-TOF and LC-tandem quadrupole-TOF MS for their ability to detect some egg allergens. Of these, ELISA proved to be very sensitive and specific; the proteomic approach was not able to detect some egg allergens such as ovomucoid because of its non-denaturing properties with urea and trypsin, while PCR proved unable to distinguish between eggs and chicken meat because it was tissue-non-specific.

Proteomics has also been applied to determine the extent of natural variation in the ripening of tomatoes and ultimately whether it would be possible to set up criteria for ripening at each stage (Kok et al., 2008).

Lei et al. (2011) developed a legume specific protein database incorporating sequences from seven different legume species which, when applied, resulted in a 54% increase in the average protein score and a 50% increase in the average number of matched peptides. When using MALDI-TOF MS data they found their success rate in identifying proteins increased from 19% with the NCBI nr database to 34% when they used the LegProt database.

Legumes are considered an important crop for sustainable agriculture but are relatively poor model systems for genetics and proteomics research, largely because of limited availability of sequence information. This is also the case for soybean as the sequencing of its genome is not yet finished (Komatsu and Ahsan, 2009). Nevertheless, despite the limited information available on soybean protein sequences Krishnan and Nelson (2011) were able to establish that the soybean cultivars with protein contents greater than 45% of the dry seed



weight had a significantly higher content of seed storage proteins than the standard soybean cultivar (Williams 82); the largest difference in higher protein quality was within the 11S storage globulins. Proteomic studies have even been used to back up claims of the health benefits of soy (Erickson, 2005). In a recent study, an isoflavone from soybean has been found to prevent the regulation of proteins that induce apoptosis, cells which are the prime targets of arteriosclerotic stressors such as oxidised low density lipoproteins (Fuchs et al., 2005).

#### 4. Protein modification

Most difficulties encountered in the proteomic evaluation of foods are due to the complexity of food protein modification. Even with advanced proteomic technologies, locating and tracking numerous modifications of proteins and peptides at trace levels adducts can remain a challenge. The need for accurate evaluation of a wide range of low abundance modifications is a differentiating factor associated with the proteomics of *ex vivo* proteins, such as food proteins. This section provides an overview of non-biological (environmental or process-induced) post-translation modifications that affect the performance and quality of food proteins.

Extracellular protein modification, particularly oxidative modification, is implicated in damage and degenerative processes within a diverse range of biological systems, including natural fibres, skin, eyes, pharmaceuticals and notably also in foods and ingredients (O'Sullivan and Kerry, 2009; Østdal et al., 2008). Protein and peptide modification plays a key role in all food quality and value attributes. *Ex vivo* modifications such as amino acid side-chain oxidation, protein-protein cross-linking and backbone cleavage can cause dramatic changes in product properties including shelf life, nutritional value, digestibility, functionality, health benefits and consumer appeal (Kerwin and Remmele, 2007). For instance, heat and/or alkali treatment of foods, feeds or pure proteins is very common in processing and manufacturing steps for a wide range of foods and ingredients, including dairy, meat, cereal and seafood products. However, such treatments can lead to the formation of a series of xenobiotics, including lysinoalanine and ornithoalanine, crosslinked modifications which have been implicated in nutritional damage and adverse health effects (Fay and Brevard, 2005; Gliguen and Birlouez-Aragon, 2005; Rerat et al., 2002; Silvestre et al., 2006).

Protein modification at the primary level is caused by a range of molecular mechanisms, but in food proteins, redox damage and Maillard chemistries are the major causes of modifications.

Reactive oxygen species (ROS) can be generated through exposure to heat and light, in particular, leading to oxidative attack (Dyer et al., 2006a; Grosvenor et al., 2010a; b). Two ROS in particular are implicated in protein oxidative degradation: singlet oxygen and the hydroxyl radical. Singlet oxygen is a highly reactive electrophilic species that reacts with proteins to form hydroperoxides, which decompose to form a variety of secondary oxidation products (Min and Boff, 2002). Where lipids are also present in the food system, initial oxidation to form lipid hydroperoxides can then result in subsequent secondary protein oxidative damage. Such lipid-mediated oxidation is brought about both through the triggering of radical chains and via the by-products of free radical lipid oxidation (Trautinger, 2007). On the other hand, hydroxyl radicals are highly reactive free radical species that can form from the initial generation of hydrogen peroxide and superoxide (Lee et al., 2004). Hydroxyl radicals are capable of reacting with any amino acid residue through

$\alpha$ -hydrogen abstraction or direct residue side chain attack. In particular, they have an affinity for electron rich molecular structures, such as aromatic rings and sulphur-based moieties (Nukuna et al., 2001).

The Maillard reaction is a non-enzymatic process extremely important in the preparation of many food types. It results from initial chemical reaction between an amino acid and a reducing sugar and usually involves heat (Chichester, 1986). Maillard reaction products (MRPs) have a profound influence on food flavour and colour, but also affect all other key food properties. It is known that protein-bound MRPs are present at relatively high levels in common bakery, milk and cooked meat products. However, as any given modification is present in low relative abundance, traditional analytical techniques have had difficulty studying these changes at the molecular level. Proteomic approaches offer a highly sensitive and specific approach to evaluating Maillard products in foods.

The next sub-sections examine in more details the types of protein redox damage generated, followed by an overview of Maillard chemistries.

#### 4.1 Side-chain redox chemistry

For whole proteins, model studies have indicated that protein backbone  $\alpha$ - or  $\beta$ - positions are not the primary site of attack for reactive oxygen species, but that residue side chains are often the first point of ROS attack (Goshe et al., 2000). The protein residues most sensitive to oxidative modification are the aromatic and sulfur-containing amino acids (Berlett and Stadtman, 1997). Model studies on free amino acid oxidation have found that tryptophan, tyrosine, histidine, phenylalanine, and cysteine are the most susceptible to oxidation in solution. This correlates well with observations in whole proteins under oxidative conditions (Asquith et al., 1971; Asquith and Rivett, 1969; Boreen et al., 2008; Davies and Truscott, 2001; Schäfer et al., 1997).

Tryptophan and tyrosine oxidation results in a wide range of modified products, some of which can be used as oxidative markers (Asquith and Rivett, 1971; Davies et al., 1999; Dean et al., 1997; Guedes et al., 2009; Maskos et al., 1992; Simat and Steinhart, 1998; Żegota et al., 2005). Many of these products absorb light in the visible range, such as the tryptophan-derived yellow chromophore hydroxykynurenine, therefore affecting the colour of the protein material.

Additionally, the sulfur-containing residues cysteine and methionine are highly susceptible to oxidative degradation. Within proteins, cysteines are present both as free thiols and within disulfide bonds (cystine). Since disulfide networks play an important role in the structural and mechanical properties of proteins, oxidative modification resulting in non-reversible cleavage of these crosslinks is a critical consideration with respect to food texture, moisture retention and rheology. It is also noteworthy that disulfide bonds can have a protective effect on other residues, due to the same ROS quenching properties that make them vulnerable to oxidation (Li et al., 1992). Methionine oxidation results ultimately in the generation of methionine sulfoxide, which although a reversible oxidative modification *in vivo*, generally accumulates in extracellular proteins (Garner and Spector, 1980).

For proteins with low relative amounts of tryptophan, such as collagen, oxidation of other non-aromatic residues, such as proline, becomes more prevalent. Proline is susceptible to hydroxylation to form hydroxyproline, but other degradation products include glutamic semialdehyde and pyroglutamic acid (Sionkowska and Kaminska, 1999; Stadtman and Levine, 2006a).

Another form of redox modification is side chain scission, with cleavage typically occurring at the  $\beta$ -position (Dean et al., 1997; Stadtman and Levine, 2006a). This scission results in generation of carbonyl containing moieties as side-chain derivatives. For example cleavage of alanine and valine side-chains leads to formation of formaldehyde and acetone respectively. Side chain scission can also result in the introduction of a carbon centred radical into the protein backbone itself (see also sub-section 4.2). Other non-residue specific modifications of note are deamidation and decarboxylation. Deamidation can occur at asparagine and glutamine residues, as well as on peptide and protein *N*-termini. Decarboxylation particularly affects terminal residues (Stadtman and Berlett, 1991).

#### 4.2 Carbonylation and protein backbone cleavage

The term carbonylation refers to the introduction of carbonyl groups into proteins, either on side-chains or on the protein backbone itself, and is a form of modification that has long been associated with protein oxidative damage (Holt and Milligan, 1977; Stadtman, 2006). Large relative quantities of carbonyl groups can be formed within proteins under oxidative conditions, and carbonylation therefore represents the most abundant kind of oxidative damage at the molecular level (Stadtman and Levine, 2006a). Carbonyl moieties are often formed on residue side chains, notably proline, threonine, arginine and lysine (Scaloni, 2006). Protein backbone cleavage can occur when ROS extract  $\alpha$ -hydrogen atoms, leading to the formation of carbon-centred radicals (Dalle-Donne et al., 2006). These radicals can subsequently react with molecular oxygen to form peroxy radicals, then peroxides and alkoxyl derivatives. Protein and peptide bond cleavage occurs via two different pathways; the diamide and  $\alpha$ -amidation pathways (Stadtman and Levine, 2006a). The diamide pathway produces peptide fragments with diamide and isocyanate moieties, while the  $\alpha$ -amidation pathway leads to peptide fragments with amide and  $\alpha$ -ketoacyl moieties.

#### 4.3 Protein crosslinking

Exposure of proteins to heat, alkali and/or oxidative conditions can lead to significant protein-protein inter and intramolecular crosslinking (Lee et al., 2004; Stadtman and Levine, 2006b). Residues with a propensity to form crosslinks include lysine, arginine, tyrosine, serine, cysteine and histidine (Taylor and Wang, 2007).

Lysine can form a range of crosslinks with other residues (Silvestre et al., 2006). Since lysine is an essential amino acid for nutrition, process-induced lysine crosslinking is of concern in the food industry. The best characterised crosslinked modification product is lysinoalanine. Lysinoalanine is formed from initial dehydration of cysteine or serine to form dehydroalanine, which subsequently reacts with proximal lysine residues.

Another key crosslinked modification in proteins is the formation of lanthionine from cystine, a crosslinked derivative which cannot be reductively cleaved (Bessemers et al., 1987; Earland and Raven, 1961). Lanthionine formation is strongly associated with heat and alkali treatment of proteins. For proteins under oxidative conditions, the crosslink dityrosine is formed from two tyrosine residues after ROS attack to form tyrosyl radicals, followed by radical-radical combination (Dyer et al., 2006a).

#### 4.4 Maillard chemistry

Particularly in the case of thermal modification, Maillard chemistries can result in a cascade of complex modifications that can profoundly affect colour, flavour, digestibility and

nutritional value in foods (Gerrard, 2002). The Maillard reaction is a non-enzymatic process extremely important in the preparation of many food types. It results from initial chemical reaction between an amino acid and a reducing sugar and usually involves heat. The sugar carbonyl group reacts with the nucleophilic amino moiety of a free amino acid or protein residue to initially form an N-substitute glycosylamine. This unstable compound subsequently undergoes Amadori re-arrangement and a complex mixture of resultant products can form, many as yet poorly understood. However, these products are responsible for a wide range of flavours and odours in foods, with each food type having distinct patterns of Maillard reaction products (MRP).

Application of proteomics in the area of Maillard chemistry was first performed only within the last decade, and has subsequently been increasingly applied to study protein modification introduced during food processing (Cotham et al., 2003; Gerrard, 2006). Challenges still remain in the areas of quantification and development of procedures with increased sensitivity to locate novel modifications. Research correlating processing parameters to effect on nutritional damage to proteins at the molecular level due to Maillard chemistry has been limited to date.

The effect of MRP on the nutritional value of food is of critical concern and is a topic of considerable interest to the food industry. Processing, packaging and storage conditions can have a profound influence on the nutritional quality and health effects of food products (Ames, 2009; Seiquer et al., 2006). Maillard reactions can lead to a decrease in bio-availability of several essential amino acids (Hewedi et al., 1994; Shin et al., 2003), decreased protein digestibility and formation of some undesirable compounds (Taylor et al., 2003; Uribarri and Tuttle, 2006). The risks and benefits associated with consumption of MRPs remains a controversial issue (Lee and Shibamoto, 2002). Profiling and tracking nutritional quality of proteinaceous foods, such as meat or dairy, throughout processing and storage is of particular importance, due to the continuous development of MRP products, even at reduced temperatures. The enhanced formation of such products associated with oxidation from vitamins, such as ascorbic acid, frequently used as preservatives, is also a concern. MRP formation encompasses a complex array of reactions, starting with the glycation of proteins and progressing to form sugar-derived protein adducts and cross-links also known as advanced glycation end products (AGE). Impaired nutritional value can occur due to changes in protein integrity and function through protein crosslinking mediated by AGE. On the other hand, increased antioxidant activity in food systems can also be attributed to these reactions.

The profile and relative abundance of all these modification products vary between specific proteins and have a significant effect on all critical food quality traits, as discussed in the following sub-section.

#### **4.5 Effects of protein modification on foods**

Progressive generation of modifications at the protein primary level results in changes to secondary, tertiary and higher orders of protein structure through denaturation, protein breakdown and conformational re-ordering, leading to observable changes in the holistic properties of the food (Dean et al., 1997). An overview of the effects on food proteins is here presented based on food type.

##### **4.5.1 Dairy**

The main dairy protein types are caseins, albumins and globulins (Farrell et al., 2004). These proteins impart many of the important nutritional and functional characteristics to dairy

food products. Degradation of milk and dairy products represents a significant area of concern for the dairy industry, due to the development of off-flavours, impaired nutritional quality and function. These problems can appear very rapidly under the right conditions, and create consumer complaints (Havemose et al., 2004; Jung et al., 1998; Mestdagh et al., 2005; Østdal et al., 2008).

Exposure to heat and/or light, in particular, can result in significant degradation in dairy products. For instance, flavour changes become noticeable rapidly in milk exposed to sunlight (Mestdagh et al., 2005). Development of off-flavours in milk and cheeses on light exposure is photosensitised by riboflavin (Becker et al., 2005). Such photo-oxidation increases protein carbonyl levels and leads to the formation of numerous tryptophan, tyrosine, histidine, and methionine oxidative products, as well as crosslink formation, notably dityrosine (Dalsgaard et al., 2007). Oxidation alters the structure of all the major milk proteins, with caseins particularly vulnerable to modification (Dalsgaard et al., 2008). Changes in protein folding, and polymerisation have been noted particularly for lactalbumin and lactoglobulin (Dalsgaard et al., 2007). The hydrolysis of dairy proteins is also affected by oxidation. Impaired accessibility of chymosin to oxidised dairy proteins has been observed, resulting in altered peptide profiles, including lowered total amounts of free N-termini (Dalsgaard and Larsen, 2009). Other notable modifications for dairy products are lysine-derived protein-protein crosslinks. In particular, lysinoalanine is formed from alkali or heat treatment of dairy proteins, and is therefore typically present in all common dairy products and ingredients at varying levels.

#### **4.5.2 Meat and seafood**

Flavour, colour, texture and nutritional value comprise the most essential quality attributes for all varieties of meats, seafoods and their derivative products, with a direct correlation to value and quality (Kerry and Ledward, 2009). These attributes are directly linked to the protein content. Meat typically consists of 10-30% proteins, depending on the species and the cut (Paul and Southgate, 1985). Meat flavour is derived from its profile of proteins, peptides, carbohydrates and lipids (Spanier et al., 2004; Wood et al., 1999). For meat products, lipid oxidation must be considered together with protein modification. Aldehyde-derived secondary lipid oxidation products are associated with the formation of proteinaceous chromophores, and therefore influence colour (Sun et al., 2001). Proteolysis plays an essential role in the developing flavour of meat and seafood products, while protein and lipid oxidation products are inter-related, and influence specific flavour and odour. An important component of meat colour is myoglobin chemistry, along with other proteins such as haemoglobin and cytochrome C (Kerry and Ledward, 2009; Mancini and Hunt, 2005). The texture of meat is directly related to its protein matrix, and is affected by protein backbone cleavage (proteolysis of structural proteins) and crosslinking. In terms of nutritional value, proteins represent a key macronutrient in meat and seafood, while protein-protein and protein-lipid crosslinks, protein cleavage and amino acid damage are associated with the deterioration of nutritional value (Erickson, 1997; Love and Pearson, 1971). Resultant food structural modifications in turn affect properties such as moisture retention, solubility and digestibility (Xiong, 2000).

Meat modification is further influenced by conditions immediately prior to, and after, animal slaughter. Physiological reactions to stress may influence the rate and extent of post mortem pH decline, altering the modification profile and thereby impacting quality

parameters such as tenderness, colour and water-holding capacity (Ferguson et al., 2001; Ferguson and Warner, 2008). Recent findings suggest that pre-slaughter stress may enhance oxidative reactions during ageing (Picard et al., 2010). Oxidative damage is implicated in meat quality deterioration, for example in high O<sub>2</sub> packaging during long-term chilled storage. Protein *ex vivo* modification in seafood products is typically less well studied than meat, however it is of equal concern with respect to the quality and value of the resultant food product (Baron et al., 2007; Kinoshita and Sato, 2007; Kjaersgard and Jessen, 2004).

#### 4.5.3 Grains and cereals

Degradative modification also significantly influences the quality of high protein plant-derived foods, notably grains and cereals. Oxidation of these proteins also influences the formation of off-flavours and the nutritive value (Heinio et al., 2002). Plant proteins are increasingly being used as ingredients and additives and ingredients in processed food, and therefore modifications induced in these proteins during processing and storage and an important consideration for the food as a whole.

### 5. Redox proteomics

The emerging proteomic sub-discipline of redox proteomics is based on the study of key reductive and oxidative chemistries occurring within proteins (Dalle-Donne et al., 2006). In complex biological systems exposed to varied processing and environmental conditions, such as is the case for foods, mapping the vast potential array of protein modifications is particularly challenging. Traditionally, holistic evaluation methodologies have been employed for assessing food proteins, including evaluation of protein extractability and determination of total carbonyl content. However, the advent of advanced proteomic tools and approaches means that mapping and tracking molecular level changes directly within the proteins is now possible. Redox proteomics adopts and customises techniques from more classical shotgun and differential proteomic approaches to provide an unprecedented overview of these molecular changes. Some of the underpinning steps in such an approach are outlined here.

#### 5.1 Characterisation of modifications

The first critical step in mapping food protein modifications is characterisation of the modifications. This can be very challenging as modification patterns can be highly complex, and any given modification, at a specific site in a specific protein, is likely to be present in very low relative abundance. In addition, the induction of protein-protein crosslink networks throughout the substrate further complicates peptide extraction and identification. As a further note, it is critical that any extraction procedure selected for subsequent proteomic analysis does not induce any further protein modification. The protein sample is generally enzymatically digested and analysed by one- or multi-dimensional LC-ESI-MS/MS or LC-MALDI-MS/MS, in a typical MudPIT-type approach (Thomas et al., 2006). Detailed MS/MS evaluation allows specific peptides to be selected and fragmented to provide structural information on modifications, and modification pathways from the key affected amino acid residues can be constructed. Bioinformatic analysis allows the location of modifications within proteins. For food systems where extensive crosslinking inhibits enzymatic digestion, a combined chemical/enzyme approach can be employed. For instance, initial chemical digestion with 2-nitro 5-thiocyanobenzoic acid (NTCB), which

cleaves next to cysteine residues, followed by more standard tryptic digestion, can be effective at digesting crosslinked proteins to produce peptides suitable to mass spectrometric evaluation (Koehn et al., 2009).

## 5.2 Profiling and tracking

Profiling protein modifications allows construction of degradation pathways, while tracking individual or collective modifications provides precise molecular evaluation of processing and/or environmentally-induced changes, as well as validation of any amelioration strategy (Davies et al., 1999; Gracanin et al., 2009).

Both redox and Maillard chemistries generate a range of products in susceptible protein regions, and it is the type and abundance profile of these multiple products that results in the overall food properties. Changes in relative abundance in mass spectrometric analysis can be used to track the progressive loss of native peptides and proteins of parent peptides, and the corresponding formation of modification products within those proteins and peptides (Grosvenor et al., 2009). This can be used to evaluate differing protein processing or treatment parameters, as well as monitor variation under a range of environmental conditions. However, the effectiveness of MS ion abundance data to track modification is limited by factors such as variation between analyses.

A related approach to evaluating molecular level is the characterisation and tracking of marker peptides. An initial analysis of the protein substrate is performed to identify peptides which fulfil selected criteria, such as reproducible extraction and a unique mass within the MS profile of the sample (Grosvenor et al., 2009). Individual marker peptides are then monitored and tracked. Another emerging approach to profile protein modifications is to utilise a scoring system, where different modifications are assigned a score and the range of modifications observed at a certain MS/MS identification threshold contribute to the overall score (Dyer et al., 2010). The data can be broken down to evaluate selected modification types, and scores between samples allow effective comparison and contrast.

A recent, novel approach to tracking protein modifications is to utilise stable isotope tags, such as iTRAQ (Wiese et al., 2007; Wu et al., 2006). Peptides from protein samples subjected to a range of conditions, such as differing hydrothermal processing times, are each tagged with a different iTRAQ label after enzymatic digestion and subsequently combined into one sample. MS/MS analysis of individual peptides generates reporter ions which allow direct tracking of the relative abundance of specific native or modified peptides across the conditions. Proof-of-concept for this approach has been demonstrated in model peptides, but its utility in more complex samples, such as whole protein foods, is yet to be fully explored (Grosvenor et al., 2010a; b; 2011).

## 5.3 Mapping carbonylation

As discussed earlier in this chapter, holistic evaluation of protein carbonyl content is a relatively good indicator of overall oxidative degradation (Thomas et al., 2006). However, to locate where such carbonylation is occurring, more advanced proteomic strategies are required.

Characterisation and mapping of carbonylation sites within the proteome can be achieved by affinity purification coupled to LC-MS/MS. Typically, in this approach reaction of biocytin hydrazide to tag and trap the carbonyl groups results in hydrazone conjugate formation (Thomas et al., 2006). Streptavidin immobilised on agarose is then used to select

out the carbonylated proteins or peptides, and then evaluation of the proteins is performed using a standard MudPIT approach. Mapping of carbonylation within food proteins has been very limited to date, but promises to further advance our understanding of food protein molecular changes under oxidative stress.

#### **5.4 Crosslink characterisation**

Protein-protein crosslinks are very difficult to characterise and locate proteomically. Specific crosslinks are typically present in very low abundance within the protein substrate. In addition, protein digestion techniques must leave crosslinked peptides intact during extraction. To add to the challenge, software for identifying and sequencing crosslinked peptides are highly limited in their utility. However, more recently new approaches are emerging which may progressively make comprehensive elucidation of crosslinks in complex food protein mixtures possible.

Many crosslink evaluation strategies developed to date are based initially on partial digestion of the protein substrate, often followed by isotopic labelling (Back et al., 2002; Chen et al., 1999). The most common isotopic labelling approach is based around the action of trypsin, which adds an oxygen atom to the C-terminus at its point of protein cleavage (Mirza et al., 2008). Since crosslinked peptides have at least two C-termini, tryptic digestion in  $^{18}\text{O}$  isotopically enriched water results in two or more  $^{18}\text{O}$  atoms being incorporated where a crosslink is present. A parallel digestion is performed in regular water. Subsequent proteomic analysis of the resultant peptides can then select for crosslinked peptides through identification of a characteristic mass shift of 8 amu relative to the unlabelled peptide in the case of a single crosslink (Back et al., 2002).

### **6. Redox proteomics case study**

#### **6.1 Introduction**

In this case study we present the concept and practical application of an advanced damage scoring framework to characterise the oxidative and non-oxidative damage occurring in UVB-irradiated protein fractions and in commercially available dairy products.

As established in the previous section, redox proteomics is an essential component of the molecular-level characterisation of food. In particular, it can provide strong evidence for protein damage due to food processing and packaging, incurred during retail display or during preparation. Since nearly all food product quality parameters correlate directly or indirectly with protein quality and function, molecular-level characterisation of protein modification and damage is of crucial importance for the food industry. Understanding of protein damage at the amino acid residue level and correlation with processing parameters will ultimately allow process improvement and mitigation/repair of protein damage.

We have previously used a damage scoring system for the characterisation of UVB-induced photo-oxidation in wool (Dyer 2010). This system allowed us to assign an absolute numerical value to the degree of damage in the samples under investigation.

In this case study, we describe a significantly refined version of our damage scoring system, allowing us to perform an advanced evaluation of redox and non-redox protein modifications, with precise weighting and thresholding functionality. This is achieved in a state-of-the-art redox proteomics context, based on LC-MS/MS data, ensuring representative coverage across all key proteins in a sample.



First, in a proof of concept approach, we show how damage scoring helps to quantify and characterise the damage in two model proteins as induced by a redox event. Then, we demonstrate the application of our damage scoring system to commercially available dairy products, sourced from a local supermarket.

### 6.1.1 Proof of concept – lactoferrin and $\beta$ -lactoglobulin

Our previous work has allowed us to develop an advanced understanding of the photochemistry underpinning oxidative modification of proteins subjected to UVB irradiation (Dyer et al., 2006a; Dyer et al., 2006b; Dyer et al., 2009; Dyer et al., 2010; Grosvenor et al., 2010a). Therefore, UVB irradiation was chosen as the method to reliably induce oxidative modifications in dairy proteins. The aim of this proof of concept preamble was to establish that our damage scoring system is able to detect redox proteomic differences between control and treated samples.

Bovine lactoferrin and  $\beta$ -lactoglobulin were chosen as the model proteins. Lactoglobulin is the major protein in whey and is extremely well characterised, including its potential post-translational modifications. Lactoferrin is a high value milk protein that encodes a number of antimicrobial peptides, however its potential modifications are less well characterised (Arseneault et al., 2010; Murdock et al., 2010; Stelwagen et al., 2009).

The proteins, at a concentration of 2.4 mg/ml in PBS, were subjected to UVB light for 40 minutes, while controls were left untreated. This procedure was expected to induce a significant amount of oxidative modifications compared to the control.

After reduction, alkylation and trypsin digestion of the proteins, the peptide samples were analysed using nanoLC-MS/MS. This allowed the acquisition of MS/MS information for a large number of peptides. This MS/MS data was then utilised for peptide/protein identification and characterisation of modifications. Specifically, data files were converted to peak lists, imported in the ProteinScape data warehousing software, and serial Mascot searching of the data using specific sets of variable modifications was initiated. A number of specific searches including a selection of variable modifications were conducted. Each search focused on a specific set of modifications, e.g. non-oxidative modifications such as methylation, deamidation, ubiquitylation, or on progressive sets of oxidative modifications such as 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> stage oxidation events of tryptophan. Searching was performed in the NCBI nr database, querying approximately 40,000 *Bos taurus* sequences. A peptide homology threshold FDR of 6% was obtained. The various search results were then compiled per sample and exported to Excel. A collection of Visual Basic scripts was developed to remove redundancy, count, weight, score and sum the different damage modifications. Essentially, each targeted modification is given a factor reflecting its severity, based on its rank in the modification pathway (Dyer et al., 2010). The number of occurrences of each modification (multiplied by the modification factor) is then weighted against the number of observed susceptible residues. The sum of weighted scores constitutes the damage score. In a scenario-based approach, the trade-off between selectivity and sensitivity as determined by the peptide score acceptance threshold was explored. A score threshold of 35 was chosen as the best compromise. Figure 1 shows the damage scores obtained for the control and irradiated lactoferrin and  $\beta$ -lactoglobulin samples.

The current version of our scoring model provides a non-oxidative damage score (NODS) comprising 6 modifications, and an oxidative damage score (ODS) comprising 12 modifications. Together they make up the total damage score (TDS).

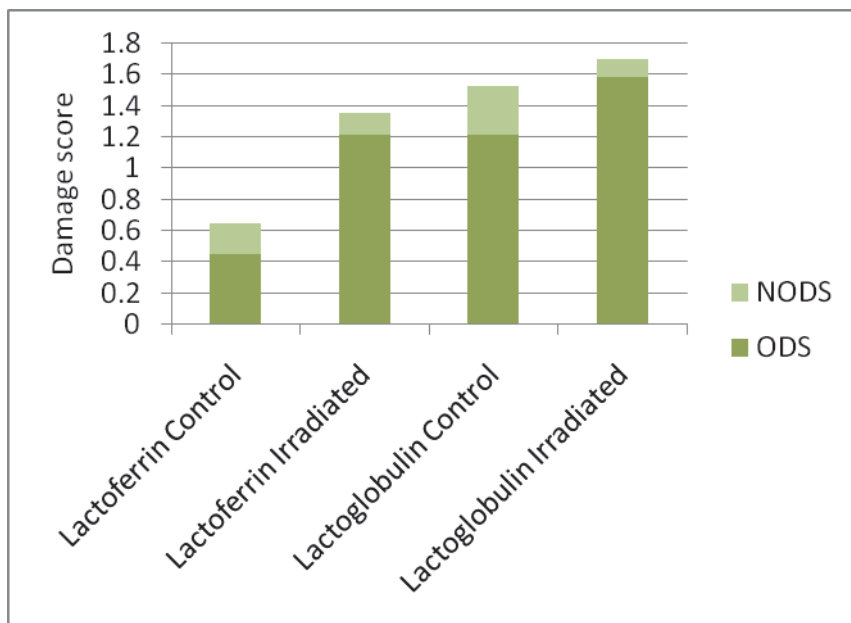


Fig. 1. Damage scores obtained for the control and irradiated lactoferrin and  $\beta$ -lactoglobulin samples. NODS, non-oxidative damage score; ODS, oxidative damage score.

Lactoferrin starts off with a relatively low TDS, made up of approximately 70% ODS and 30% NODS. After UV irradiation, the lactoferrin TDS more than doubles, with oxidative damage representing approximately 90% of the TDS.

Lactoglobulin has a much higher base damage level compared to lactoferrin - close to the damage level of irradiated lactoferrin. The reason for this is unknown, but it is possible that the process used to produce lactoglobulin includes a step that introduces more damage, compared to the process for lactoferrin. This finding highlights an additional strength of this scoring system: unexpected or unsuspected protein damage is revealed. In lactoglobulin, oxidative damage is responsible for approximately 80% of the TDS in the control sample. After irradiation, the TDS increases by 11%, with the oxidative damage component seeing a 30% increase.

It is presently unclear why the NODS appears to decrease between the control and irradiated samples. The fact that this is observed both for lactoferrin and lactoglobulin suggests that it is a direct or indirect effect of the irradiation. It is possible that some non-oxidative modifications remain undetected in the irradiated samples because of severe damage (e.g. crosslinking). Replicate analyses, detailed analysis of the modifications involved, and comparison with other proteins may help elucidate this further.

Using our scoring system, we observed an approximate doubling of the damage score for lactoferrin, and a substantial increase for lactoglobulin after irradiation. The ODS, in particular, increased significantly in the irradiated samples - in accordance with the experimental design, which strongly favoured oxidative modification.

This provides unequivocal proof that our system is able to detect differences in damage between control and treated samples using proteomics data.

### 6.1.2 Application – Commercial dairy products

With proof of concept established, we sought to apply our scoring system to commercially available dairy products, and investigate how various processing treatments influenced the damage score compared to fresh farm-sourced milk, expected to return the lowest damage score.

Nine commercially available dairy products were sourced from a local supermarket. Milk sourced directly from a local farm was included in our analysis as an expected low damage control.

The following samples were included:

- Farm sourced milk
- Standard fat milk
- Standard fat milk powder
- Standard fat milk UHT
- Skim milk UHT
- Skim milk powder
- Evaporated milk
- Low fat evaporated milk
- Lactose free UHT
- Skim calcium enriched UHT

Because of challenges associated with the protein concentration dynamic range in dairy, whey fractions were obtained from all samples in order to eliminate casein.

These fractions were loaded on anion exchange cartridges, and the unretained fraction was selected for further analysis. This fraction, among many other proteins, contains lactoferrin.

Samples were analysed as described in the previous section. Briefly, samples were reduced, alkylated, trypsin digested and analysed using LC-MS/MS. Data was imported in ProteinScape, repeatedly searched using specific sets of variable modifications, and combined search results were exported to Excel for scoring. As before, a score threshold of 35 was chosen. Figure 2 shows the damage scores obtained for the dairy samples.

Farm sourced milk and standard fat milk have very similar damage levels, although the NODS for farm sourced milk is slightly higher. In general, the levels of non-oxidative damage are in the same range in all samples, whereas the ODS differs substantially between samples, and gives rise to dramatic differences in TDS.

The TDS in standard fat milk powder is increased compared to standard fat milk, likely reflecting the additional processing step the former has undergone. UHT treatment dramatically increases the TDS for this product family, giving the highest damage score in this test. The other UHT-treated products also return some of the highest damage scores, underscoring the potential of UHT to induce hydrothermal damage, measured primarily as oxidative side chain modifications.

When heat-treated standard fat products are compared with heat-treated low fat products, substantially lower overall damage is recorded in the latter. Both standard fat milk UHT compared to skim milk UHT, and evaporated milk compared to low fat evaporated milk support this observation. Observations in a range of proteinaceous systems indicate that lipid oxidation has a synergistic effect with protein oxidation during damaging processing treatments or environmental insult. Consequently, products with lower lipid levels would be expected in many damaging conditions to sustain lower oxidative damage. Intriguingly however, this trend is not observed in powder products, with skim milk powder returning a

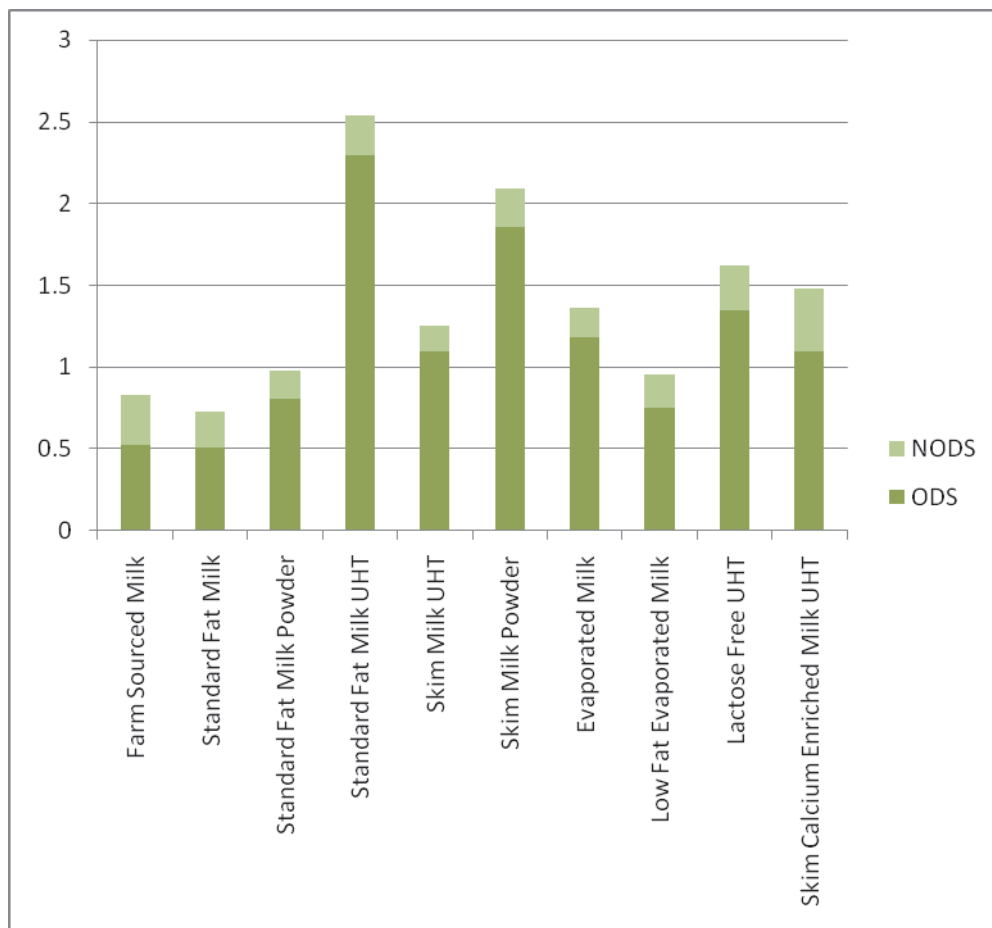


Fig. 2. Damage scores obtained for nine off-the-shelf dairy products and one farm-sourced milk sample. NODS, non-oxidative damage score; ODS, oxidative damage score.

very high TDS compared to standard fat milk powder. The molecular damage processes induced during skimming and during food protein spray drying processes therefore require further investigation.

## 6.2 Redox proteomics conclusions

In a proof of concept study, we showed that it is possible to detect molecular-level damage in a redox proteomics context. Specifically, we investigated control and UVB irradiated lactoferrin and lactoglobulin. This revealed substantial differences in the amount of oxidative modification and hence damage score between control and treated samples.

We then applied our damage scoring system to commercially available dairy products. This represented a significant increase in complexity, with analyses scaled up from individual proteins in the proof of concept, to the proteome level in the commercial samples. Using LC-

MS/MS approaches and automated data analysis routines, we were able to successfully acquire and process data, allowing damage scoring at the sample proteome level.

Scores returned for the dairy samples revealed that differently treated products can harbour substantial amounts of molecular-level damage. Farm-sourced and standard fat milk had the lowest levels of damage, while samples that had undergone processing treatments such as UHT or spray drying, showed elevated damage scores.

The importance of detailed protein damage scoring at the molecular level in food systems, with direct correlation and implications to holistic product quality parameters, cannot be overstated. Indeed, the scoring system we have developed is equally applicable to other protein foods such as meat, seafood, cereal or soy. Global trends indicate growing consumer awareness of correlations between excessive processing and decreased product quality. Consumers also increasingly demand pure, untreated, clean and healthy foods. Product quality parameters such as flavour, odour, eating quality, nutritional value and digestibility are directly correlated with protein modification and molecular level damage. Therefore, understanding and evaluating protein quality and function is of crucial importance for the food industry. It will allow food producers to track and evaluate improvements to processing parameters, will allow substantiation of marketing claims to increasingly discerning and knowledgeable consumers, and will ultimately provide the world with healthier, more nutritious food.

## 7. Future directions

The application and modification of proteomic approaches to analyse the complexity of food protein modification is anticipated to become increasingly important in the area of general food science, quality assurance and product differentiation. In addition, we anticipate further application of these approaches to understanding food protein modification with respect to subsequent digestive processes, and to actually tracking the molecular modification and truncation of proteins during human consumption and digestion. Until now, this area has been limited by the lack of sensitive and specific technologies to track molecular level damage mechanisms. However, development of the enabling proteomic technologies summarised in this chapter indicates that the goal of mapping the complex process of food protein digestion is attainable. This will facilitate the correlation of modification and digestion profiles with food quality traits, particularly nutritional and health effects.

Another key area of future development is in the development of advanced food quality and function. Future step-change advances in meat and dairy biotechnologies, such as advanced phenotyping for quality traits and predictive selection for resistance to specific oxidative stresses, will require integrated evaluation at the molecular level. In addition, to truly understand and control these phenotypes and product attributes, it is critical that proteomics is linked to lipidomics, as protein and lipid modification are correlated. These approaches have the potential to open up considerable new areas, enabling study of the networks involving the functional interactions and pathways of food proteins both *in vivo* and *ex vivo*.

## 8. References

- Addis M.F., Cappuccinelli R., Tedde V., Pagnozzi D., Porcu M.C., Bonaglini E., Roggio T., Uzzau S. 2010. Proteomic analysis of muscle tissue from gilthead sea bream (*Sparus aurata*, L.) farmed in offshore floating cages. *Aquaculture* 309, 245-252.

- Affolter M., Grass L., Vanrobaeys F., Casado B., Kussmann M. 2010. Qualitative and quantitative profiling of the bovine milk fat globule membrane proteome. *J Proteomics* 73, 1079-1088.
- Agrawal G.K., Rakwal R. 2006. Rice proteomics: A cornerstone for cereal food crop proteomes. *Mass Spec Rev* 25, 1-53.
- Akagawa M., Handoyo T., Ishii T., Kumazawa S., Morita N., Suyama K. 2007. Proteomic analysis of wheat flour allergens. *J Agric Food Chem* 55, 6863-6870.
- Ames J.M. 2009. Dietary Maillard reaction products: implications for human health and disease. *Czech Journal of Food Sciences* 27 (Special Issue), S66-S69.
- Arseneault M., Bédard S., Boulet-Audet M., Pézolet M. 2010. Study of the interaction of lactoferricin B with phospholipid monolayers and bilayers. *Langmuir* 26, 3468-3478.
- Asquith R.S., Hirst L., Rivett D.E. 1971. Effects of ultraviolet radiation as related to the yellowing of wool. *Applied Polymer Symposium* 18, 333-335.
- Asquith R.S., Rivett D.E. 1969. The photolysis of tyrosine and its possible relationship to the yellowing of wool. *Text Res J* 39, 633-637.
- Asquith R.S., Rivett D.E. 1971. Studies on the photooxidation of tryptophan. *Biochim Biophys Acta* 252, 111-116.
- Back J.W., Notenboom V., De Koning L.J., Muijsers A.O., Sixma T.K., De Koster C.G., De Jong L. 2002. Identification of cross-linked peptides for protein interaction studies using mass spectrometry and  $^{18}\text{O}$  labeling. *Anal Chem* 74, 4417-4422.
- Baron C.P., Kjærsgård I.V.H., Jessen F., Jacobsen C. 2007. Protein and lipid oxidation during frozen storage of rainbow trout (*Oncorhynchus mykiss*). *J Agric Food Chem* 55, 8118-8125.
- Becker E.M., Cardoso D.R., Skibsted L.H. 2005. Deactivation of riboflavin triplet-excited state by phenolic antioxidants: mechanism behind protective effects in photooxidation of milk-based beverages. *Eu Food Res Tech* 221, 382-386.
- Berlett B.S., Stadtman E.R. 1997. Protein oxidation in aging, disease, and oxidative stress. *J Biol Chem* 272, 20313-20316.
- Bessems G.J., Rennen H.J., Hoenders H.J. 1987. Lanthionine, a protein cross-link in cataractous human lenses. *Exp Eye Res* 44, 691-695.
- Bjarnadóttir S.G., Hollung K., Færgestad E.M., Veiseth-Kent E. 2010. Proteome changes in bovine longissimus thoracis muscle during the first 48 h postmortem: Shifts in energy status and myofibrillar stability. *J Agric Food Chem* 58, 7408-7414.
- Boreen A.L., Edhlund B.L., Cotner J.B., McNeill K.M. 2008. Indirect photodegradation of dissolved free amino acids: The contribution of singlet oxygen and the differential reactivity of DOM from various sources. *Environ Sci Technol* 42, 5492-5498.
- Bouley J., Chambon C., Picard B. 2003. Proteome analysis applied to the study of muscle development and sensorial qualities of bovine meat. *Sciences des Ailments* 23, 75-78.
- Chen X., Chen Y.H., Anderson V.E. 1999. Protein cross-links: Universal isolation and characterization by isotopic derivatization and electrospray ionization mass spectrometry. *Anal Biochem* 273, 192-203.
- Chichester C.O. 1986. *Advances in food research (advances in food and nutrition research)*. Academic Press. Boston.

- Choi Y.M., Lee S.H., Choe J.H., Rhee M.S., Lee S.K., Joo S.T., Kim B.C. 2010. Protein solubility is related to myosin isoforms, muscle fiber types, meat quality traits, and postmortem protein changes in porcine longissimus dorsi muscle. 127, 183-191.
- Cotham W.E., Hinton D.J., Metz T.O., Brock J.W., Thorpe S.R., Baynes J.W., Ames J.M., Tanaka S. 2003. Mass spectrometric analysis of glucose-modified ribonuclease. *Biochem Soc Trans* 31, 1426-1427.
- D'Alessandro A., Righetti P.G., Fasoli E., Zolla L. 2010. The egg white and yolk interactomes as gleaned from extensive proteomic data. *J Proteomics* 73, 1028-1042.
- D'Auria E., Agostoni C., Giovannini M., Riva E., Zetterström R., Fortin R., Franco Greppi G., Bonizzi L., Roncada P. 2005. Proteomic evaluation of milk from different mammalian species as a substitute for breast milk. *Acta Pædiatrica* 94, 1708-1713.
- Dalle-Donne I., Scaloni A., Butterfield D.A., editors. 2006. *Redox Proteomics - From protein modifications to cellular dysfunctions and diseases*. John Wiley and Sons, Hoboken.
- Dalsgaard T.K., Heegaard C.W., Larsen L.B. 2008. Plasmin digestion of photooxidized milk proteins. *J Dairy Sci* 91, 2175-2183.
- Dalsgaard T.K., Larsen L.B. 2009. Effect of photo-oxidation of major milk proteins on protein structure and hydrolysis by chymosin. *Int Dairy J* 19, 362-371.
- Dalsgaard T.K., Otzen D., Nielsen J., Larsen L.B. 2007. Changes in structures of milk proteins upon photo-oxidation. *J Agric Food Chem* 55, 10968-10976.
- Davies M.J., Fu S., Wang H., Dean R.T. 1999. Stable markers of oxidant damage to proteins and their application in the study of human disease. *Free Rad Biol Med* 27, 1151-1163.
- Davies M.J., Truscott R.J.W. 2001. Photo-oxidation of proteins and its role in cataractogenesis. *J Photochem Photobiol B* 63, 114-125.
- Dean R.T., Fu S., Stocker R., Davies M.J. 1997. Biochemistry and pathology of radical-mediated protein oxidation. *Biochem J* 324, 1-18.
- Di Luccia A., Picariello G., Cacace G., Scaloni A., Faccia M., Liuzzi V., Alviti G., Musso S.S. 2005. Proteomic analysis of water soluble and myofibrillar protein changes occurring in dry-cured hams. *Meat Sci* 69, 479-491.
- Dyer J.M., Bringans S., Bryson W.G. 2006a. Characterisation of photo-oxidation products within photoyellowed wool proteins: tryptophan and tyrosine derived chromophores. *Photochem Photobiol Sci* 5, 698-706.
- Dyer J.M., Bringans S.D., Bryson W.G. 2006b. Determination of photo-oxidation products within photoyellowed bleached wool proteins. *Photochem Photobiol* 82, 551-557.
- Dyer J.M., Clerens S., Cornellison C.D., Murphy C.J., Maurdev G., Millington K.R. 2009. Photoproducts formed in the photoyellowing of collagen in the presence of a fluorescent whitening agent. *Photochem Photobiol* 85, 1314-1321.
- Dyer J.M., Plowman J., Krsinic G., Deb-Choudhury S., Koehn H., Millington K., Clerens S. 2010. Proteomic evaluation and location of UVB-induced photomodification in wool. *Photochem Photobiol B Biol* 98, 118-127.
- Earland C., Raven D.J. 1961. Lanthionine formation in keratin. *Nature* 191, 384-384.
- Erickson B.E. 2005. Proteomics data back up soy health claims. *J Proteome Res* 4, 219.
- Erickson M.C. 1997. Lipid oxidation: Flavor and nutritional quality deterioration in frozen foods. In: Erickson M.P., Hung Y.C., eds). *Quality in Frozen Food*. Springer, New York, pp 141-173.

- Farrell H.M., Jr., Jimenez-Flores R., Bleck G.T., Brown E.M., Butler J.E., Creamer L.K., Hicks C.L., Hollar C.M., Ng-Kwai-Hang K.F., Swaisgood H.E. 2004. Nomenclature of the proteins of cows' milk - Sixth revision. *J Dairy Sci* 87, 1641-1674.
- Fay L.B., Brevard H. 2005. Contribution of mass spectrometry to the study of the Maillard reaction in food. *Mass Spec Rev* 24, 487-507.
- Ferguson D.M., Bruce H.L., Thompson J.M., Egan A.F., Perry D., Shorthose W.R. 2001. Factors affecting beef palatability – farmgate to chilled carcass. *Aust J Exp Agr* 41, 879-891.
- Ferguson D.M., Warner R.D. 2008. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants. *Meat Sci* 80, 12-19.
- Ferrari F., Fumagalli M., Profumo A., Viglio S., Sala A., Dolcini L., Temporini C., Nicolis S., Merli D., Corana F., Casado B., Iadarola P. 2009. Deciphering the proteomic profile of rice (*Oryza sativa*) bran: A pilot study. *Electrophoresis* 30, 4083-4094.
- Fong B.Y., Norris C.S., Palmano K.P. 2008. Fractionation of bovine whey proteins and characterisation by proteomic techniques. *Int Dairy J* 18, 23-46.
- Fuchs D., Erhard P., Turner R., Rimbach G., Daniel H., Wenzel U. 2005. Genistein reverses changes of the proteome induced by oxidized-LDL in EA.hy 926 human endothelial cells. *J Proteome Res* 4, 369-376.
- Garner M.H., Spector A. 1980. Selective oxidation of cysteine and methionine in normal and senile cataractous lenses. *Proc Nat Acad Sci USA* 77, 1274-1277.
- Gebriel M., Uleberg K.E., Larssen E., Hjelle B.A., Sivertsvik M., Møller S.G. 2010. Cod (*Gadus morhua*) muscle proteome cataloging using 1D-PAGE protein separation, nano-liquid chromatography peptide fractionation, and linear trap quadrupole (LTQ) mass spectrometry. *Journal of Agriculture & Food Chemistry* 58, 12307-12312.
- Gerrard J.A. 2002. New aspects of an AGEing Chemistry – recent developments concerning the Maillard reaction. *ChemInform* 33, 243-243.
- Gerrard J.A. 2006. The Maillard reaction in food: Progress made, challenges ahead. *Trends Food Sci Technol* 17, 324-330.
- Gliguem H., Birlouez-Aragon I. 2005. Effects of sterilization, packaging, and storage on vitamin C degradation, protein denaturation, and glycation in fortified milks. *J Dairy Sci* 88, 891-899.
- Goshe M.B., Chen Y.H., Anderson V.E. 2000. Identification of the sites of hydroxyl radical reaction with peptides by hydrogen/deuterium exchange: prevalence of reactions with the side chains. *Biochem* 39, 1761-1770.
- Gracanin M., Hawkins C.L., Pattison D.I., Davies M.J. 2009. Singlet-oxygen-mediated amino acid and protein oxidation: Formation of tryptophan peroxides and decomposition products. *Free Rad Biol Med* 47, 92-102.
- Grosvenor A.J., Morton J.D., Dyer J.M. 2009. Profiling of residue-level photo-oxidative damage in peptides. *Peptides In preparation*.
- Grosvenor A.J., Morton J.D., Dyer J.M. 2010a. Isobaric labelling approach to the tracking and relative quantitation of peptide damage at the primary structural level. *J Agric Food Chem* 58, 12672-12677.
- Grosvenor A.J., Morton J.D., Dyer J.M. 2010b. Profiling of residue-level photo-oxidative damage in peptides. *Amino Acids* 39, 285-296.



- Grosvenor A.J., Morton J.D., Dyer J.M. 2011. Proteomic characterisation of hydrothermal redox damage. *J Sci Food Agric*, Early View.
- Guedes S., Vitorino R., Domingues R., Amado F., Domingues P. 2009. Oxidation of bovine serum albumin: identification of oxidation products and structural modifications. *Rap Comm Mass Spec* 23, 2307-2315.
- Havemose M.S., Weisbjerg M.R., Bredie W.L.P., Nielsen J.H. 2004. Influence of feeding different types of roughage on the oxidative stability of milk. *Int Dairy J* 14, 563-570.
- Heinio R.-L., Lehtinen P., Oksman-Caldentey K.-M., Poutanen K. 2002. Differences between sensory profiles and development of rancidity during long-term storage of native and processed oat. *Cereal Chem* 79, 367.
- Hewedi M.M., Kiesner C., Meissner K., Hartkopf J., Erbersdobler H.J. 1994. Effects of UHT heating of milk in an experimental plant on several indicators of heat treatment. *J Dairy Res* 61, 305-309.
- Holt L.A., Milligan B. 1977. The formation of carbonyl groups during irradiation of wool and its relevance to photoyellowing. *Text Res J* 47, 620-624.
- Horváth-Szancics E., Szabó Z., Janáky T., Pauk J., Hajós G. 2006. Proteomics as an emergent tool for identification of stress-induced proteins in control and genetically modified wheat lines. *Chromatographia* 63, S143-S147.
- Hwang I. 2004. Proteomics approach in meat science: a model study for Hunter L\* value and drip loss. *Food Science and Biotechnology* 13, 208-214.
- Hwang I.H., Park B.Y., Kim J.H., Cho S.H., Lee J.M. 2005. Assessment of postmortem proteolysis by gel-based proteome analysis and its relationship to meat quality traits in pig longissimus. *Meat Sci* 69, 79-91.
- Islam N., Tsujimoto H., Hirano H. 2003. Proteome analysis of diploid, tetraploid and hexaploid wheat: towards understanding genome interaction in protein expression. *Proteomics* 3, 549-557.
- Jung K.C., Jung W.Y., Lee Y.J., Yu S.L., Choi K.D., Jang B.G., Jeon J.T., Lee J.H. 2007. Comparisons of chicken muscles between layer and broiler breeds using proteomics. *AJAS* 20, 307-312.
- Jung M.Y., Yoon S.H., Lee H.O., Min D.B. 1998. Singlet oxygen and ascorbic acid effects on dimethyl disulfide and off-flavor in skim milk exposed to light. *J Food Sci* 63, 408-412.
- Kang S., Chen S., Dai S. 2010. Proteomics characteristics of rice leaves in response to environmental factors. *Frontiers in Biology* 5, 246-254.
- Kerry J.P., Ledward D., editors. 2009. Improving the sensory and nutritional quality of fresh meat. Woodhead Publishing Ltd, Cambridge, UK.
- Kerwin B.A., Remmele R.L.J. 2007. Protect from light: Photodegradation and protein biologics. *J Pharm Sci* 96, 1468-1479.
- Kim Y.J., Choi S.H., Park B.S., Song J.T., Kim M.C., Koh H.J., Seo H.S. 2009. Proteomic analysis of the rice seed for quality improvement. *Plant Breeding* 128, 541-550.
- Kinoshita Y., Sato T. 2007. Proteomic studies on protein oxidation in bonito (*Katsuwonus pelamis*) muscle. *Food Science and Technology Research* 13, 133-138.
- Kjaersgard I.V.H., Jessen F. 2004. Two-dimensional gel electrophoresis detection of protein oxidation in fresh and tainted rainbow trout muscle. *J Agric Food Chem* 52, 7101-7107.

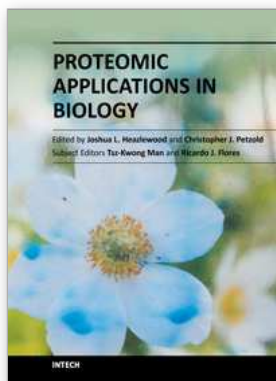
- Kjærsgård I.V.H., Jessen F. 2003. Proteome analysis elucidating post-mortem changes in cod (*Gadus morhua*) muscle proteins. *J Agric Food Chem* 51, 3985-3991.
- Koehn H., Clerens S., Deb-Choudhury S., Morton J., Dyer J.M., Plowman J.E. 2009. Higher sequence coverage and improved confidence in the identification of cysteine-rich proteins from the wool cuticle using combined chemical and enzymatic digestion *J Proteomics* 73, 323-330.
- Kok E.J., Lehesranta S.J., van Dijk J.P., Helsdingen J.R., Dijkma W.T.P., Van Hoef A.M.A., Koistinen K.M., Karenlampi S.O., Kuiper H.A., Keijer J. 2008. Changes in gene and protein expression during tomato ripening - consequences for the safety assessment of new crop plant varieties. *Food Science and Technology International* 14, 503-518.
- Komatsu S., Ahsan N. 2009. Soybean proteomics and its application to functional analysis. *J Proteomics* 72, 325-336.
- Komatsu S., Tanaka N. 2004. Rice proteome analysis: A step toward functional analysis of the rice genome. *Proteomics* 5, 938-949.
- Krishnan H.B., Nelson R.L. 2011. Proteomic analysis of high protein soybean (*Glycine max*) accessions demonstrates the contribution of novel glycinin subunits. *J Agric Food Chem* 59, 2432-2439.
- Kwasiborski A., Sayd T., Chambon C., Santé-Lhoutellier V., Rocha D., Terlouw C. 2008. Pig *Longissimus lumborum* proteome: Part II: Relationships between protein content and meat quality. *Meat Sci* 80, 982-996.
- Lametsch R., Bendixen E. 2001. Proteome analysis applied to meat science: Characterizing post mortem changes in porcine muscle. *J Agric Food Chem* 49, 4531-4537.
- Lametsch R., Karlsson A., Rosenvold K., Andersen H.J., Roepstorff P., Bendixen E. 2003. Postmortem proteome changes of porcine muscle related to tenderness. *J Agric Food Chem* 51, 6992-6997.
- Lametsch R., Kristensen L., Larsen M.R., Therkildsen M., Oksbjerg N., Ertbjerg P. 2006. Changes in the muscle proteome after compensatory growth in pigs. *J Anim Sci* 84, 918-924.
- Laville E., Sayd T., Morzel M., Blinet S., Chambon C., Lepetit J., Renand G., Hocquette H.F. 2009. 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, 10755-10764.
- Laville E., Sayd T., Terlouw C., Chambon C., Damon M., Larzul C., Leroy P., Glénisson J., Chérel P. 2007. Comparison of sarcoplasmic proteomes between two groups of pig muscles selected for shear force of cooked meat. *J Agric Food Chem* 55, 5834-5841.
- Lee J.-Y., Kim C.J. 2009. Determination of allergenic egg proteins in food by protein-, mass spectrometry-, and DNA-based methods. *Journal of AOAC International* 93, 462-477.
- Lee J., Koo N., Min D.B. 2004. Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comp Rev Food Sci Food Safety* 3, 21-33.
- Lee K.G., Shibamoto T. 2002. Toxicology and antioxidant activities of non-enzymatic browning reaction products: Review. *Food Reviews International* 18, 151-175.
- Lei Z., Dai X., Watson B.S., Zhao P.X., Sumner L.W. 2011. A legume specific protein database (LegProt) improves the number of identified peptides, confidence scores and overall protein identification success rates for legume proteomics. *Photochem* 72, 1020-1027.

- Li Z., Bruce A., Galley W.C. 1992. Temperature dependence of the disulfide perturbation to the triplet state of tryptophan. *Biophys J* 61, 1364-1371.
- Love J.D., Pearson A.M. 1971. Lipid oxidation in meat and meat products - a review. *J Am Oil Chem Soc* 48, 547-549.
- Mancini R.A., Hunt M.C. 2005. Current research in meat color. *Meat Sci* 71, 100-121.
- Mann K., Olsen J.V., Mačėk B., Gnad F., Mann M. 2008. Identification of new chicken egg proteins by mass spectrometry-based proteomic analysis. *World's Poultry Science Journal* 64, 209-218.
- Martinez I., Friis T.J. 2004. Application of proteome analysis to seafood authentication. *Proteomics* 4, 347-354.
- Maskos J., Rush J.D., Koppenol W.H. 1992. The hydroxylation of tryptophan. *Arch Biochem Biophys* 296, 514-520.
- Mestdagh F., De Meulenaer B., De Clippeleer J., Devlieghere F., Huyghebaert A. 2005. Protective influence of several packaging materials on light oxidation of milk. *J Dairy Sci* 88, 499-510.
- Min D.B., Boff J.M. 2002. Chemistry and reaction of singlet oxygen in foods. *Comp Rev Food Sci Food Safety* 1, 58-72.
- Mirza S.P., Greene A.S., Olivier M. 2008. <sup>18</sup>O labeling over a coffee break: A rapid strategy for quantitative proteomics. *J Proteome Res* 7, 3042-3048.
- Morzell M., Chambon C., Hamelin M., Sante-Lhoutellier V., Sayd T., Monin G. 2004. Proteome changes during pork meat ageing following use of two different pre-slaughter handling procedures. *Meat Sci* 67, 689-696.
- Murdock C., Chikindas M.L., Matthews K.R. 2010. The pepsin hydrolysate of bovine lactoferrin causes a collapse of the membrane potential in *Escherichia coli* O157:H7. *Probiotics and Antimicrobial Proteins* 2, 112-119.
- Nakamura R., Nakamura R., Nakano M., Arisawa K., Ezaki R., Horiuchi H., Teshima R. 2010. Allergenicity study of EGFP-transgenic chicken meat by serological and 2D-DIGE analysis. *Food and Chemical Toxicology* 48, 1302-1310.
- Nukuna B.N., Goshe M.B., Anderson V.E. 2001. Sites of hydroxyl radical reaction with amino acids identified by 2H NMR detection of induced 1H/2H exchange. *J Am Chem Soc* 123, 1208-1214.
- O'Sullivan M.G., Kerry J.P. 2009. Sensory and quality properties of packaged meat. In: Kerry J.P., Ledward D., (eds). *Improving the Sensory and Nutritional Quality of Fresh Meat*. Woodhead Publishing Limited. Cambridge. pp 595-597.
- Østdal H., Weisbjerg M.R., Skibsted L.H., Nielsen J.H. 2008. Protection against photooxidation of milk by high urate content. *Milchwissenschaft* 63, 119-122.
- Palmer D.J., Kelly V.C., Smit A.M., Kuy S., Knight C.G., Cooper G.J. 2006. Human colostrum: Identification of minor proteins in the aqueous phase by proteomics. *Proteomics* 6, 2208-2216.
- Pappa E.C., Robertson J.A., Rigby N.M., Mellon F., Kandarakis I., Mills E.N.C. 2008. Application of proteomic techniques to protein and peptide profiling of Teleme cheese made from different types of milk. *Int Dairy J* 18, 605-614.
- Paul A.A., Southgate D.A.T. 1985. McCance and Widdowson's the composition of foods. Elsevier. Amsterdam.
- Picard B., Berri C., Lafaucheur L., Molette C., Sayd T., Terlouw C. 2010. Skeletal muscle proteomics in livestock production. *Briefings Funct Genom Proteom* 9, 259-278.

- Rerat A., Calmes R., Vaissade P., Finot P.-A. 2002. Nutritional and metabolic consequences of the early Maillard reaction of heat treated milk in the pig. Significance for man. *Eur J Nutr* 41, 1-11.
- Ricroch A.E., Bergé J.B., Kuntz M. 2011. Evaluation of genetically engineered crops using transcriptomic, proteomic, and metabolomic profiling techniques. *Plant Physiol* 155, 1752-1761.
- Sayd T., Morzel M., Chambon C., Franck M., Figwer P., Larzul C., Le Roy P., Monin G., Cherel P., Laville E. 2006. Proteome analysis of the sarcoplasmic fraction of pig Semimembranosus muscle: Implications on meat color development. *J Agric Food Chem* 54, 2732-2737.
- Scaloni A. 2006. Mass spectrometry approaches for the molecular characterisation of oxidatively/nitrosatively modified proteins. In: Dalle-Donne I., Scaloni A., Desiderio D.M., Nibbering N.M., eds). *Redox Proteomics*, John Wiley and Sons, Hoboken, pp 59-122.
- Schäfer K., Goddinger D., Höcker H. 1997. Photodegradation of tryptophan in wool. *J Soc Dyers & Colourists* 133, 350-355.
- Seiquer I., Díaz-Alguacil J., Delgado-Andrade C., López-Frías M., Muñoz Hoyos A., Galdó G., Navarro M.P. 2006. Diets rich in Maillard reaction products affect protein digestibility in adolescent males aged 11-14 y. *The American Journal of Clinical Nutrition* 83, 1082-1088.
- Sentandreu M.A., Armenteros M.N., Calvete J.J., Ouali A., Aristoy M.-C.N., Toldra F. 2007. Proteomic identification of actin-derived oligopeptides in dry-cured ham. *J Agric Food Chem* 55, 3613-3619.
- Sentandreu M.A., Fraser P.D., Halket J., Patel R., Bramley P.M. 2010. A proteomic-based approach for detection of chicken in meat mixes. *J Proteome Res* 9, 3374-3383.
- Shibata M., Matsumoto K., Oe M., Ohnishi-Kameyama M., Ojima K., Nakajima I., Muroya S., Chikuni K. 2009. Differential expression of the skeletal muscle proteome in grazed cattle. *J Anim Sci* 87, 2700-2708.
- Shin S.H., Park H., Park D. 2003. Influence of different oligosaccharides and inulin on heterocyclic aromatic amine formation and overall mutagenicity in fried ground beef patties. *J Agric Food Chem* 51, 6726-6730.
- Silvestre D., Ferrer E., Gaya J., Jareno E., Miranda M., Muriach M., Romero F.J. 2006. Available lysine content in human milk: stability during manipulation prior to ingestion. *Biofactors* 26, 71-79.
- Simat T.J., Steinhart H. 1998. Oxidation of free tryptophan and tryptophan residues in peptides and proteins. *J Agric Food Chem* 46, 490-498.
- Sionkowska A., Kaminska A. 1999. Thermal helix-coil transition in UV irradiated collagen from rat tail tendon. *Int J Biol Macromol* 24, 337-340.
- Smolenski G., Haines S., Kwan F.Y.S., Bond J., Farr V., Davis S.R., Stelwagen K., Wheeler T.T. 2007. Characterisation of host defence proteins in milk using a proteomic approach. *J Proteome Res* 6, 207-215.
- Spanier A.M., Flores M., Toldra F., Aristoy M.C., Bett K.L., P. B., Bland J.M. 2004. Meat flavor: contribution of proteins and peptides to the flavor of beef. In: Shahidi F., Spanier A.M., Ho C.-T., Braggins T., eds). *Quality of fresh and processed foods*. Kluwer Academic/Plenum Publishers. New York. pp 33-49.
- Stadtman E.R. 2006. Protein oxidation and aging. *Free Rad Res* 40, 1250-1258.

- Stadtman E.R., Berlett B.S. 1991. Fenton chemistry. Amino acid oxidation. *J Biol Chem* 266, 17201-17211.
- Stadtman E.R., Levine R.L. 2006a. Chemical modification of proteins by reactive oxygen species. In: Dalle-Donne I., Scaloni A., Butterfield D.A., (eds). *Redox Proteomics - From Protein Modifications to Cellular Dysfunctions and Diseases*. John Wiley and Sons, Inc. Hoboken. pp 3-23.
- Stadtman E.R., Levine R.L. 2006b. Chemical modification of proteins by reactive oxygen species. In: Dalle-Donne I., Scaloni A., Butterfield D.A., (eds). *Redox proteomics - from protein modifications to cellular disfunctions and diseases*. John Wiley and Sons. Hoboken. pp 3-23.
- Stelwagen K., Carpenter E., Haigh B., Hodgkinson A., Wheeler T.T. 2009. Immune components of bovine colostrum and milk. *J Anim Sci* 87, 3-9.
- Sun Q., Faustman C., Senecal A., Wilkinson A.L., Furr H. 2001. Aldehyde reactivity with 2-thiobarbituric acid and TBARS in freeze-dried beef during accelerated storage. *Meat Sci* 57, 55-60.
- Taylor C.M., Wang W. 2007. Histidinoalanine: a crosslinking amino acid. *Tetrahedron* 63, 9033-9047.
- Taylor J.L.S., Demyttenaere J.C.R., Abbaspour Tehrani K., Olave C.A., Regniers L., Verschaeve L., Maes A., Elgorashi E.E., van Staden J., De Kimpe N. 2003. Genotoxicity of melanoidin fractions derived from a standard glucose/glycine model. *J Agric Food Chem* 52, 318-323.
- te Pas M.F.W., Jansen J., Broekman K.C.J.A., Reimert H., Heuven H.C.M. 2009. Postmortem proteome degradation profiles of longissimus muscle in Yorkshire and Duroc pigs and their relationship with pork quality traits. *Meat Sci* 83, 744-751.
- Thomas S.N., Lu B.-W., Nikolskaya T., Nikolsky Y., Yang A.J. 2006. MudPIT (multidimensional protein identification technology) for identification of post-translational protein modifications in complex biological mixtures. In: Dalle-Donne I., Scaloni A., Butterfield D.A., (eds). *Redox Proteomics - From Protein Modifications to Cellular Dysfunctions and Diseases*. John Wiley and Sons, Inc. Hoboken. pp 233-252.
- Trautinger F. 2007. Damaged proteins: Repair or removal? In: Giacomoni P.U., (ed). *Biophysical and physiological effects of solar radiation on human skin*. RSC Publishing. Cambridge, UK. pp 311-319.
- Uribarri J., Tuttle K.R. 2006. Advanced glycation end products and nephrotoxicity of high-protein diets. *Clinical Journal of the American Society of Nephrology* 1, 1293-1299.
- Van de Wiel D.F.M., Zhang W. 2008. Identification of pork quality parameters by proteomics. *Meat Sci* 77, 46-53.
- Vanderghem C., Blecker C., Danthine S., Deroanne C., Haubruge E., Guillonnet F., De Pauw E., Francis F. 2008. Proteome analysis of the bovine milk fat globule: Enhancement of membrane purification. *Int Dairy J* 18, 885-893.
- Veiseth-Kent E., Grove H., Færgestad E.M., Fjæra S.O. 2010. Changes in muscle and blood plasma proteomes of Atlantic salmon (*Salmo salar*) induced by crowding. *Aquaculture* 309, 272-279.
- Wedholm A. 2008. Variation in milk protein composition and its importance for the quality of cheese milk. Uppsala: Swedish University of Agricultural Sciences.

- Wiese S., Reidegeld K.A., Meyer H.E., Warscheid B. 2007. Protein labeling by iTRAQ: A new tool for quantitative mass spectrometry in proteome research. *Proteomics* 7, 340-350.
- Wood J.D., Enser M., Fisher A.V., Nute G.R., Richardson R.I., Sheard P.R. 1999. Manipulating meat quality and composition. *Proc Nutr Sci* 58, 363-370.
- Wu W.W., Wang G., Baek S.J., Shen R.-F. 2006. Comparative study of three proteomic quantitative methods, DIGE, cICAT, and iTRAQ, using 2D gel- or LC-MALDI TOF/TOF. *J Proteome Res* 5, 651-658.
- Xiong Y.L. 2000. Protein oxidation and implications for muscle food quality. In: Decker E., Faustman C., Lopez-Bote C.J., eds). *Antioxidants in muscle foods - nutritional strategies to improve quality*. Wiley Interscience, Hoboken, pp 85-112.
- Xue K., Liu B., Yang J., Xue D. 2010. The integrated risk assessment of transgenic rice: A comparative proteomics approach. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 9, 1693-1700.
- Zapata I., Zerby H.N., Wick M. 2009. Functional proteomic analysis predicts beef tenderness and the tenderness differential. *J Agric Food Chem* 57, 4956-4963.
- Żegota H., Kołodziejczyk K., Król M., Król B. 2005. o-Tyrosine hydroxylation by OH-radicals. 2,3-DOPA and 2,5-DOPA formation in  $\gamma$ -irradiated aqueous solution. *Radiat Phys Chem* 72, 25-33.



## **Proteomic Applications in Biology**

Edited by Dr. Joshua Heazlewood

ISBN 978-953-307-613-3

Hard cover, 264 pages

**Publisher** InTech

**Published online** 18, January, 2012

**Published in print edition** January, 2012

The past decade has seen the field of proteomics expand from a highly technical endeavor to a widely utilized technique. The objective of this book is to highlight the ways in which proteomics is currently being employed to address issues in the biological sciences. Although there have been significant advances in techniques involving the utilization of proteomics in biology, fundamental approaches involving basic sample visualization and protein identification still represent the principle techniques used by the vast majority of researchers to solve problems in biology. The work presented in this book extends from overviews of proteomics in specific biological subject areas to novel studies that have employed a proteomics-based approach. Collectively they demonstrate the power of established and developing proteomic techniques to characterize complex biological systems.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Stefan Clerens, Jeffrey E. Plowman and Jolon M. Dyer (2012). Food Proteomics: Mapping Modifications, Proteomic Applications in Biology, Dr. Joshua Heazlewood (Ed.), ISBN: 978-953-307-613-3, InTech, Available from: <http://www.intechopen.com/books/proteomic-applications-in-biology/food-proteomics-mapping-modifications>

# **INTECH**

open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.