# Chapter 22
# Nutritionally Relevant Proteins

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## 22.1 Introduction

Amino acids, peptides, and proteins are essential constituents of food. They are indispensable food components and directly contribute to the flavor of food and are precursors for aroma compounds and colors formed during thermal or enzymatic reactions in production, processing, and storage of food. Proteins contribute to the physical properties of food; they have ability to build or stabilize gels, foams, emulsions, and fibrillar structures.

The most important sources of animal proteins are eggs, meat, and milk. These proteins contain the essential amino acids leucine, isoleucine, lysine, valine, threonine, tryptophan, phenylalanine, and methionine. Most proteins of plant origin do not contain some of these amino acids. Consequently, these proteins do not have full nutritional value. However, essential amino acids are present in the proteins from legumes, oilseeds, and grains. Legumes are added, sometimes after modification to traditional

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**Table 22.1** Digestibility of various proteins in humans (FAO/WHO/UNU 1985)

| Protein source | Digestibility (%) | Protein source | Digestibility (%) |
|---|---|---|---|
| Egg | 97 | Millet | 79 |
| Milk, cheese | 95 | Peas | 88 |
| Meat, fish | 94 | Peanut | 94 |
| Maize | 85 | Soy flour | 86 |
| Rice (polished) | 88 | Soy protein isolate | 95 |
| Wheat (whole) | 86 | Beans | 78 |
| Wheat flour, white | 96 | Corn, cereal | 70 |
| Wheat gluten | 99 | Wheat, cereal | 77 |
| Oatmeal | 86 | Rice cereal | 75 |

foods, such as meat and cereal products, and also used in the production of novel food items such as meat, fish, and milk substitutes. Plant proteins, mostly originating from soybean, are frequently added to processed meat products either for economic reasons or to improve their functional properties (Gaso-Sokac et al. 2011).

Proteins are formed from amino acids through amide linkages. Most proteins are post-translationally modified. The most frequent post-translational modifications are phoshorylation and glycosylation, but other less frequent modifications such as alkylation and sulfatation are also very important for protein function and their nutritive value (Blom et al. 2004).

Proteins differ in their nutritive value. Several factors, such as content of essential amino acids and digestibility, contribute to these differences and to "quality" of a protein. The daily protein requirement therefore depends on the type and composition of proteins in a diet. High-quality proteins are those that contain all the essential amino acids at levels greater than the FAO/WHO/UNU (1985) reference levels, and the digestibility comparable to or better than those of egg white or milk proteins. As a rule, animal proteins have a better nutritive value than proteins of plant origin.

For example, proteins of major cereals and other foods of plant origin are often deficient in at least one of the essential amino acids. The essential amino acids whose concentrations in a protein are below the levels of a reference protein are termed *limiting amino acids*. The nutritional quality of a protein or protein mixture is ideal when it contains all of the essential amino acids in proportions that produce optimum rates of growth and/or optimum maintenance capability.

Digestibility is defined as the proportion of food nitrogen that is absorbed after ingestion. Although the content of essential amino acids is the primary indicator of protein quality, true protein quality also depends on the extent to which these amino acids are utilized in the body. Digestibility of various proteins in humans is listed in Table 22.1 (FAO/WHO/UNU 1985).

Antinutritional factors:

Most plant protein isolates and concentrates contain trypsin and chymotrypsin inhibitors (Kunitz type and Bowman–Birk type) and lectins. These inhibitors impair complete hydrolysis of legume and oilseed proteins by pancreatic proteases. Lectins,

which are glycoproteins, bind to intestinal mucosa cells and interfere with absorption of amino acids. Lectins and Kunitz type protease inhibitors are thermolabile, whereas the Bowman–Birk-type inhibitor is stable under normal thermal processing conditions. Thus, heat-treated legume and oilseed proteins are generally more digestible than native protein isolates (despite some residual Bowman–Birk-type inhibitor). Plant proteins also contain other antinutritional factors, such as tannins and phytate. Tannins, which are condensed products of polyphenols, covalently react with ε-amino groups of lysyl residues. This inhibits trypsin-catalyzed cleavage of the lysyl peptide bond.

In last few years proteomics technology has been frequently used in food technology for process validation, optimization, and quality control (Gaso-Sokac et al. 2011). The use of proteomics for characterization of nutritionally important proteins, detection of trace components of protein and peptide origin that are important for human nutrition, and detection of potentially harmful components in human food of both animal and plant origin (Gaso-Sokac et al. 2010) are also very important. The analysis scheme of the food proteome is shown in Fig. 22.1

## 22.2 Animal Proteins

### *22.2.1 Egg Proteins*

Eggs are a valuable source of protein and are important ingredients in many food products. In food processing whole eggs or egg ingredients (egg white and egg yolk) are often used as coagulating, foaming, and emulsifying agents, while also contributing nutrients and flavor to different foods (Campbell et al. 2003; Kiosseoglou 2003). Individual components of eggs also have potentially useful biological functions, such as antimicrobial activity, protease inhibitory function, and antigenic or immunogenic characteristics (Raikos et al. 2006). Potential allergens in eggs, such as some genetic variants of the ovomucoid from egg white, are also important from a food safety point of view (Rupa and Mine 2008).

Egg white proteins differ markedly from the proteins of egg yolk in biological function and in composition. The technological functions usually assigned to these two distinct portions of the egg are also different. Although the most important functional property of egg white in food products is its ability to form stable foams, the functional significance of egg yolk is largely connected with its capacity to stabilize fat–water emulsions.

The most abundant three proteins in egg white are *ovalbumin, conalbumin* (ovotransferrin), and *ovomucoid* (about 77% of total protein) as listed in Table 22.2. Together with *ovomucin, lysosyme*, and *ovoglobulins* $G_2$ and $G_3$, the content of these highly abundant proteins in egg white is over 92% (Belitz et al. 2004). Most egg white proteins are glycosylated, and some of them contain a high amount of carbohydrates. There are some thorough proteomic investigations of both high and low

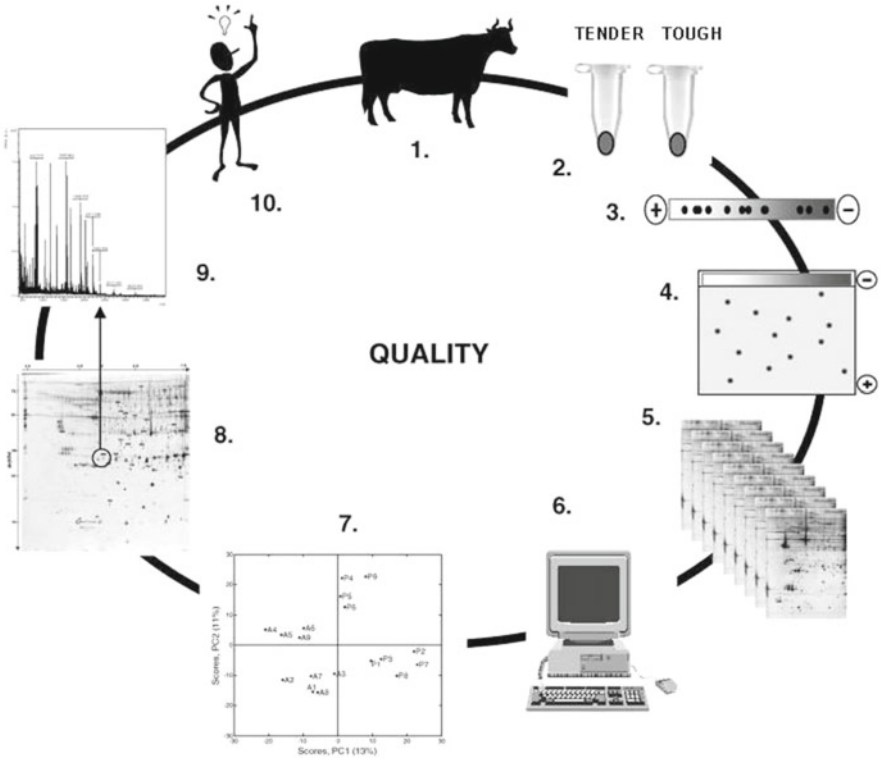


**Fig. 22.1** Workflow in the proteome analysis using 2-D electrophoresis or chromatographic separation methods and mass spectrometry. *1*. animal or sample chosen for the analysis; *2*. sample extraction; *3*. isoelectric focusing or first chromatographic step; *4*. SDS-PAGE or next chromatographic step as a second dimension; *5*. alignments and comparisons of the images; *6*. data analysis; *7*. data interpretation and selection of significantly changed proteins; *8*. extraction of significantly changed protein spots; *9*. protein identification by mass spectrometry; *10*. interpretation of the results (Reproduced from Hollung *et al.* (*5*) with permission from Elsevier Ltd, copyright 2007)

abundant egg proteins. Because of their post-translational modifications, some high-abundance proteins, such as ovotransferrin and ovomucoid also show a very high level of polymorphism (Raikos et al. 2006; Guérin-Dubiard et al. 2006). Egg white is a very complex mixture and further proteomic analyses resulted in identification of 148 distinct gene products (D'Ambrosio et al. 2008; Mann and Mann 2008; Farinazzo et al. 2009; D'Alessandro et al. 2010).

Egg yolk is a fat-in-water emulsion with about 50% dry matter; it contains one-third of proteins and two-thirds of lipids. Consequently, the egg yolk contains a high amount of lipoproteins, such as *lipovitellins, lipovitellenins,* and *phosvitin*. Again, most egg yolk proteins are also post-translationally modified. By the use of combinatorial ligand libraries and other high-resolution techniques, more than 250 distinct gene products were identified in egg yolk (Mann and Mann 2008; Farinazzo

**Table 22.2** Proteins of egg white (Belitz et al. 2004)

| Protein | Percent of the total protein[a] | Denaturation temperature (°C) | Molecular weight (kdal) | Isoelectric point (pH) |
|---|---|---|---|---|
| Ovalbumin | 54 | 84.5 | 44.5 | 4.5 |
| Conalbumin (Ovotransferin) | 12 | 61.5 | 76 | 6.1 |
| Ovomucoid | 11 | 70.0 | 28 | 4.1 |
| Ovomucin | 3.5 | | 5.5–8.3×10$^6$ | 4.5–5.0 |
| Lysozyme (Ovoglobulin $G_1$) | 3.4 | 75.0 | 14.3 | 10.7 |
| Ovoglobulin $G_2$ | 4 | 92.5 | 30–45 | 5.5 |
| Ovoglobulin $G_3$ | 4 | | | 5.8 |
| Flavoprotein | 0.8 | | 32 | 4.0 |
| Ovoglycoprotein | 1.0 | | 24 | 3.9 |
| Ovomacroglobulin | 0.5 | | 760–900 | 4.5 |
| Ovoinhibitor | 0.1 | | 49 | 5.1 |
| Avidin | 0.05 | 68.3[b] | 9.5 | |
| Cystatin (ficin inhibitor) | 0.05 | | 12.7 | 5.1 |

[a]Average values are presented
[b]Four times 15.6.kdal + approx. 10% carbohydrate

et al. 2009; D'Alessandro et al. 2010). Some of these proteins play an important role in antimicrobial response and vitamin binding.

The chicken egg proteome is still not complete, but some newly identified proteins are of biopharmaceutical interest as potentially physiologically active substances.

### *22.2.2 Proteins of Meat and Meat Products*

In human nutrition, meat is the most valuable source of protein and essential amino acids. The proteins/enzymes of muscle can be categorized based on biological function or chemical properties. Proteins involved in the physical process of contraction are those contained in the sarcomere. These can be divided based on location, such as thick or thin filaments, or on function, such as force generating or regulating proteins (Hollung et al. 2007; Bendixen 2005). An additional, frequently neglected factor that influences meat quality and digestibility is the content and composition of the intramuscular connective tissue (Purslow 2005)

*Myosin* is the major protein of the thick filaments, which comprises 45% of the myofibrillar proteins. It is an elongated protein molecule about 160 nm in length with a molecular mass of approximately 480,000 D. Myosin contains a total of six polypeptide chains, two heavy chains and four light chains. Myosin heavy chains have "head" and "tail" regions, reflecting the respective globular and rod portions of the molecules. The biological functions of myosin reside in heavy chains. Myosin can be cleaved in the middle region by proteolytic enzymes, such as trypsin,

producing two fractions of the protein. One of these is called light meromyosin and the other, which contains the globular head structures of the myosin molecule is called heavy meromyosin. Separated heavy meromyosin retains its ability to interact with actin and its ATPase activity.

*Actin* is the major protein of the thin filaments and comprises 20% of myofibrilar protein of muscle. Actin is bound to the structure of the muscle much more firmly than is myosin. Its shape can be described as two peanut-shaped domains of equal size lying side by side. Actin monomers, called globular actin or G-actin, are assembled in a double-helical structure called fibrous actin, or F-actin. This constitutes the main portion of the thin filament. G-actin is composed of 374–375 amino acids and has a molecular mass of 42,000–48,000 D.

*Tropomyosin*, representing 5% of myofibrilar protein, is composed of two alpha-helical polypeptides wound together into a two-strained, coiled-coil supersecondary structure. It resembles the tail or rod portion of the myosin molecule. In skeletal muscle two polypeptides, alpha- and beta-tropomyosin, can combine to form a tropomyosin dimmer. The alpha- and beta-tropomyosin polypeptides have molecular masses of 37,000 and 33,000 D, respectively. They are found in muscle as the alpha-alpha or beta-beta homodimers and the alpha-beta heterodimer. Tropomyosin aggregates end to end and binds to actin filaments along each groove of the actin double helix such that each molecule interacts with seven G-actin monomers.

The content and the relative concentration of different types of *collagen* vary and are dependent on the type of the meat. In conclusion, collagens, as well as different types of proteoglycans are responsible for the so-called "background" feature of the meat and indirectly also for its digestibility. Furthermore, the turnover of the connective tissue, especially the turnover of different types of collagen, and further changes of other main proteins of the meat is controlled by matrix proteases and their specific inhibitors (Belcerzak et al. 2001; Purslow 2002).

In summary, in meat science and for the further exploration of meat proteins, proteomics can be used for: (1) proteome mapping and meat identification, (2) determination of proteome changes due to genetic variations, (3) determination of changes due to the pre-slaughter conditions, (4) determination of post-mortem changes, and (5) study and detection of changes in peptide composition during meat storage and processing (Hollung et al. 2007; Bendixen 2005; Bauchart et al. 2006).

Proteolytic degradation of muscle that occurs post mortem and degradation of proteins during meat processing and aging results in the production of protein fragments (Geesink and Koohmaraie 1999). These polypeptides can be further digested into smaller peptides or even single amino acids (Geesink and Koohmaraie 1999; Mullen et al. 2000). Unfortunately, there are only a few studies dealing with polypeptides and small peptides in aged and cooked meat, but they play a key role for aroma and taste of cooked or dry-cured products (Purslow 2005; Bauchart et al. 2006).

Plant proteins, mostly originating from soybean or other leguminosae, are frequently added to processed meat products either for economic reasons or to improve their functional properties. Leitner et al. (2006) used liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) in order to detect soybean proteins in meat products. In all soybean

protein-containing meat samples, the plant protein glycinin G4 subunit A4 was identified, and this protein can be used as a target for a simpler analytical method in order to identify the addition of soybean proteins to meat products, and possible adulteration of meat and meat products.

### *22.2.3 Seafood and Seafood Proteins*

Proteins are also the essential components of seafood which are gaining increasing importance in nutrition, especially in developed countries. The wide variability of proteins that are present in seafood and their highly variable composition offer a large potential to originate a broad variety of different products (Piñeiro et al. 2003). The complexity of seafood also implies the extreme variability of the seafood proteomes, and only some basic questions can be addressed in this short overview. There are some literature data about the use of proteomics for the identification of allergens in seafood (Gaso-Sokac et al. 2010, 2011) and this important point is briefly discussed in this review.

In their review about the use of proteomics as a tool for the investigation of seafood and other marine products, Piñeiro et al. (2003) recommend the use of proteomics for detection of allergens in food of this origin. However, there are still only few studies in this field. Taka et al. (2000) characterized an allergenic parvalbumin from frog by the use of LC-ESI-MS. The main crustacean allergens are proteins tropomyosin and arginine kinase (Lehrer et al. 2003; Ishikawa et al. 2001). Tropomyosin is a myofibrillar protein of 35–38 kDa, and proteins from six species of crustaceans have also been cloned (Motoyama et al. 2007). Arginine kinase from some commercially relevant shrimp species was characterized by the use of proteomic methods (Ortea et al. 2009). Some additional shrimp allergens such as sarcoplasmatic calcium binding protein (SCP) have also been detected (Yu et al. 2003; Shiomi et al. 2008). Interestingly, this protein was previously detected as an allergen in crayfish *Procambarus clarkii* (Gao et al. 2006). The problem of allergens in the seafood was recently discussed (Gaso-Sokac et al. 2010).

### *22.2.4 Milk Proteins*

Because of the immense importance for human nutrition, milk proteins have been studied continuously for more than 100 years. In 1877 Hammarsten distinguished three main proteins in milk: casein, lactalbumin, and lactoglobulin (Belitz et al. 2004). Later it was revealed that the milk protein system is much more complex. Using ultracentrifugation and electrophoresis it was proven that casein consists of tree fractions, namely α-, β- and γ-casein. The most important proteins of milk are listed in Table 22.3. Other protein constituents such as enzymes are present in much lower quantities (they are not listed in Table 22.3). The two important groups of

**Table 22.3** Belitz et al. 2004

| Fraction | Genetic variants | Portion[a] | Isoionic point | Molecular weight[b](kdal) |
|---|---|---|---|---|
| *Caseins* | | 80 | | |
| $\alpha_{s1}$-Casein | A,B,C,D,E | 34 | 4.92–5.35 | 23.6[c] |
| $\alpha_{s2}$-Casein | A,B,C,D | 8 | | 25.2[d] |
| χ-Casein | A,B,C,E | 9 | 5.77–6.07 | 19[e] |
| β-Casein | $A^1$,$A^2$,$A^3$,B,C,D | 25 | 5.20–5.85 | 24 |
| γ-Casein | | 4 | 5.8–6.0 | 12–21 |
| $\gamma_1$-Casein | $A^1$,$A^2$,$A^3$,B | | | 20.5 |
| $\gamma_2$-Casein | $A^1$/$A^2$,$A^3$,B | | | 11.8 |
| $\gamma_3$-Casein | $A^1$/$A^2$/$A^3$,B | | | 11.6 |
| *Whey proteins* | | 20 | | |
| β-Lactoglobulin | A,B,C,D,E,F,G | 9 | 5.35–5.41 | 18.3 |
| α- Lactalbumin | A,B,C | 4 | 4.2–4.5[f] | 14.2 |
| Serum albumin | A | 1 | 5.13 | 66.3 |
| Immunoglobulin | | 2 | | |
| IgG1 | | | 5.5–6.8 | 162 |
| IgG2 | | | 7.5–8.3 | 152 |
| IgA | | | – | 400[g] |
| IgM | | | – | 950[h] |
| FSC(s)[i] | | | | 80 |
| Proteose-peptone | | 4 | 3.3–3.7 | |

[a]As% of skim milk total protein
[b]Monomers
[c]Variant B
[d]Variant A
[e]Variant $A^2$
[f]Isoelectric point
[g]Dimer
[h]Pentamer
[i]Free secretory component

proteins, proteins and glycoproteins, are related to the milk fat globule membrane (MFGM, see Fig. 22.2) and whey proteins have recently been the topic of intensive investigation (Gaso-Sokac et al. 2011).

In milk separation by centrifugation, three fractions can be obtained by the following steps: (1) whole milk is centrifuged to obtain the milk fat globule (MFG) and the low fat milk fractions; (2) protein-rich, skimmed milk is ultracentrifuged and separated in two fractions, the whey protein and pellet fraction; and (3) in the last step the casein protein fraction is obtained by washing the pellet from the previous step (Pogacic et al. 2010). The milk proteome and glycoproteome have been topics of extensive investigations since the creation of the proteomics methodology (Gagnaire et al. 2009; Johnson and Lucey 2006). This technology is currently used for: (1) the analysis of high-abundance proteins, (2) the analysis of

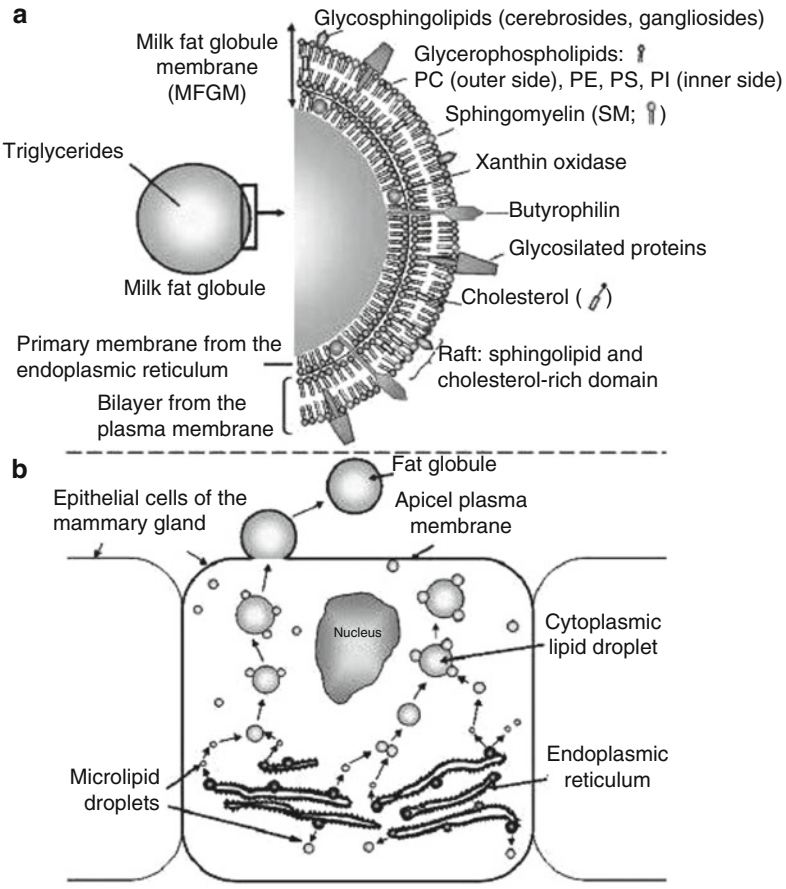


**Fig. 22.2** Milk fat globule membrane. Schematic presentation of: (**a**) the structure of the milk fat globule membrane; and (**b**) the pathways for the synthesis and secretion of milk fat globules (Reproduced from C. Lopez *et al.* (*151*) with permission of the American Chemical Society, copyright 2008)

low-abundance proteins, (3) the analysis of proteins in whey, (4) the characterization of milk fat globule and milk fat globule membrane, and (5) the characterization of products containing complex dairy matrices such as cheese and yogurt (Gaso-Sokac et al. 2011).

#### 22.2.4.1 Caseins and Other High-Abundance Proteins in Milk and Milk Products

There are only a few highly abundant proteins that are present in milk from all species. In bovine milk, these proteins are casein (CN), β-lactoglobin (β-LG),

α-lactalbumin (α-LA), and bovine serum albumin (BSA) with relative abundances of approximately 80:10:4:1 (Conti et al. 2007; O'Donnell et al. 2004). Similar to other biological fluids, such as blood plasma, despite the fact that it contains a small number of primary proteins, the milk proteome is still extremely complex. A great deal of this complexity is the consequence of post-translational modifications and the presence of numerous genetic variants of this limited list of proteins (Casado et al. 2009).

Caseins are a group of unique milk-specific proteins. These proteins represent about 80% of the total protein in bovine milk. Caseins are a mixture of proteins and subclasses of proteins. The most abundant caseins are $\alpha_{S1}$-, $\alpha_{S2}$-, β-, and κ-casein (Fox and Brodkorb 2008). Isolation of κ-casein revolutionized the ideas on the structure of the so-called casein micelle in milk, and a realistic model of its structure became possible only after the characterization of the soluble form of this most abundant milk protein (Waugh et al. 1970). Many technologically and nutritionally important properties of milk, such as its white color, stability to heat or ethanol, coagulation by cheese-making enzymes, and gelation characteristics, are due to the properties of casein micelles. It is for these reasons that properties of casein micelles are still a topic of extensive studies (Johnson and Lucey 2006; Fox and Brodkorb 2008; Waugh et al. 1970; Glantz et al. 2010). It has been known for more than 100 years that the colloidal caseinate particles contain calcium. Therefore, in nutrition, milk is one of the most important sources of calcium. The phosphorylation of caseins and other post-translational modifications of this protein have a vital role in the interaction with calcium phosphate and the organization of the casein micelle (Sørensen et al. 2003). Determination of post-translational modification of major protein components provides the scientific basis for coagulation and cheese-making processes used in dairy production. Further characterization of these proteins is also of fundamental importance for identification of the origin and quality assessment of milk and milk products (Holt 1998; Sørensen et al. 2003; Di Luccia et al. 2009; Matéos et al. 2009; Roncada et al. 2002).

The other high-abundance milk proteins, β-LG, α-LA, and BSA, are major components of the whey (Farrell et al. 2004). The concentration of β-LG in skimmed milk is about 2–4 mg/mL. This protein occurs with high frequency in cows as two genetic variants, variant A and variant B. Because of the different physicochemical characteristics of the two β-LG molecules, the presence of one or the other of these variants significantly affects the properties of the milk. The A variant is expressed at a higher level than the B variant, or the less frequently occurring C variant (Farrell et al. 2004; Ng-Kwai-Hang and Grosclaude 2003). β-lactoglobulin may also be glycosylated, but lactosylation of this protein is a more important chemical modification, which is caused by heating of the milk or whey (Morgan et al. 1998).

Bovine skimmed milk contains α-LA at a concentration of 1.2–1.5 mg/mL. In bovine milk, the mature α-LA is also present in two genetic variants, variants A and B. This protein binds bivalent metals such as zinc and calcium. α-LA is important

for normal function of the mammary gland, such as milk secretion and lactose content in milk (Farrell et al. 2004).

#### 22.2.4.2 Low-Abundance Proteins

The low-abundance proteins in milk can be identified by mass spectrometry after proper sample preparation and removal of high-abundance proteins (Manso et al. 2005; Pampel et al. 2007; Al-Ghobashy et al. 2009). Blood plasma proteins, such as BSA, serotransferrin, and lactoferrin, are only observed in colostrum, which may have special physiological importance for children in the early nursing period. Among more than 400 spots in 2-D electrophoresis that were separated from bovine milk, identified proteins include $\beta_2$-microglobulin, complement components, $\alpha_1$-antitrypsin, prealbumin, fructose-biphosphate aldolase A, and casein fragments (Yamada et al. 2002).

The immunoglobulin fraction accounts for about 1% (w/w) of total milk protein, and therefore these proteins can be classified as a kind of "medium-abundance proteins." In milk, IgG, IgA, and IgM have been isolated and characterized. Immunoglobulins in colostrum and milk have a protecting function, especially for the newborn (Farrell et al. 2004).

Lactoferrin is a specific, iron-binding protein that also occurs in milk of most mammalian species. The lactoferrin concentration in milk is relatively low, and varies between 20 and 200 mg/L. This protein increases noticeably in response to inflammation or infection. Consequently, lactoferrin plays an important role in the host defense against infection and inflammation (Ward et al. 2002). Antibacterial and antiviral activity of this protein against both DNA and RNA viruses have been detected (Vogel et al. 2002; van der Strate et al. 2001), and lactoferrin is now being isolated and purified from cheese whey and commercially utilized in the pharmaceutical and food industries (Marshall 2004).

#### 22.2.4.3 Whey Proteins

Whey is considered as a functional milk fraction with a content of proteins and bioactive polypeptides that have a positive effect on the health (Madureira et al. 2007), and whey protein fractions are increasingly incorporated as functional ingredients in food, not only in infant formulas, but also for adults (Panchaud et al. 2005). As ingredients in food, whey proteins can provide antimicrobial activity, immune modulation, improve muscle strength, and may delay and/or ameliorate conditions in different diseases, such as osteoporosis and cardiovascular diseases (Madureira et al. 2007; Marshall 2004).

The identification of low-abundance proteins in whey is challenging due to their wide dynamic concentration range. Namely, in comparison with highly abundant proteins, the concentrations of minor protein components vary by at least a factor of

$10^6$ (Panchaud et al. 2005). These proteins may play important physiological and eventually therapeutic roles in nutrition and as additives to cosmetic products.

#### 22.2.4.4 Milk Fat Globule and Milk Fat Globule Membrane

Lipid molecules are provided in milk via a unique delivery system, milk fat globules (Argov et al. 2008). In previous research, the lipid fraction in milk was oversimplified as a relatively pure mixture of triacylglycerols (Timmen and Patton 1988), and the unique structure and composition of MFG was overlooked. In MFG, the milk fat globule membrane is the protective coat that surrounds lipid globules. The MFGM prevents flocculation and coalescence of lipid droplets in milk and protects the milk fat against lipolysis (Argov et al. 2008). In order to secrete MFG, portions of the mammary epithelial cell membranes are sacrificed. This process results in a unique structure of MFG (Timmen and Patton 1988). Recent investigations of the MFG and MFGM proteome have provided new insights into mammary function and the mechanism of milk secretion (Reinhardt and Lipolis 2008). Mather (2000) gave an early overview of the proteins found in the MFGM. The eight most abundant MFGM proteins are: mucin1, xantine dehydrogenase/oxidase, periodic acid Schiff III and Schiff 6/7 proteins, CD36, butyrophilin, adipophilin, and fatty-acid binding protein. The MFGM is organized as a trilayer, and its structure is shown in Fig. 22.2 (Lopez et al. 2008).

MFGM proteins also have other important functions, such as defense against pathogens (Sando et al. 2009; Smolenski et al. 2007). One of the major proteins in MFGM, xantine dehydogenase/oxidase, has a direct antibacterial activity, and inhibits bacterial growth through the formation of hydrogen peroxide or the stimulation of lactoperoxidase in milk (Martin et al. 2004). Additional factors with beneficial health properties, such as cholesterol lowering and inhibition of cancer cell growth, were also documented for MFGM (Dewettinck et al. 2008).

#### 22.2.4.5 Peptides

Peptides originating from milk proteins have various health-promoting effects such as: regulation of digestive enzymes and modulation of nutrient absorption (Yamauchi et al. 2003); regulation of the cardiovascular system, for example, anti-hypertensive effects (Pins and Keenan 2006); regulation of the immune system, for example, enhancing immune cell functions and stimulation of the phagocytic activities of macrophages (Meisel and FitzGerald 2003); regulation of the nervous system, for example, by their opoid activity (Yamauchi et al. 2003); and antioxidative and other health-promoting activities (Madureira et al. 2007).

The physiological activity of milk-derived peptides has been the topic of numerous studies during the last 10 years (Madureira et al. 2007). Experimental evidence exists that bioactive peptides can be released from caseins (see above), α-lactalbumin (α-LA), β-lactoglobin (β-LG), lactoferrin, and serum albumin. Some

of these bioactive peptides have received special names, such as α- and β-lactorphin, β-lactotensin, serophin, albutensin A, lactoferricin B, lactoferrampin, osteopontin, and many others. Their production and biological properties have been the subject of two comprehensive reviews (Korhonen and Pihlanto 2006; Madureira et al. 2010).

#### 22.2.4.6 Milk Proteins as Allergens

Milk products can also cause allergies. However, proteomics tools have only been sparingly applied in the investigation of allergens in these products. It is well known that changes in the main milk protein casein such as carbonylation (Scaloni et al. 2002) or formation of covalent complexes between casein micelles and β-lactoglobulin (Henry et al. 2002) and modification of other proteins (Gagnaire et al. 2009; Gupta and Lee 2007) during the production process, mainly heating, can cause induction of allergies to milk products, but a thorough proteomic and "allergenomic" investigation has still to be performed.

## 22.3 Plant Proteins

### *22.3.1 Seed Proteins*

Seeds, mostly cereals, have always played a key role in human nutrition. Cereal product consumption meets close to 50% of the daily requirement for carbohydrates. Additionally, cereals are an important source of vitamin B, minerals, and trace elements. Frequently neglected is the fact that the consumption of cereals also provides about one-third of the requirement for proteins (Belitz et al. 2004).

Wheat and rice are still the most important cereals and the longitudinal section of the wheat grain is shown in Fig. 22.3.

In comparison to other plant tissues, seeds are relatively rich in protein. The biological role of proteins in the seed is still obscure. The process of germination involves intense and many-sided biochemical activity, requiring the rapid biosynthesis of many enzymes. One can assume that seed proteins provide both the machinery and the raw materials for such biosynthesis.

It is also logical to suppose that, as most other constituents of the cotyledons, seed proteins serve as a food reserve for the seedling, providing the young plant with amino acids and nitrogen until the root system and the photosynthetic apparatus are sufficiently developed. Within the cells of the seed cotyledons these "storage proteins" occur in granules with diameters in the range of 2–20 μ, known as *aleurons* or "*protein bodies*".

Interest in seed proteins arose early in the history of protein chemistry. The role of seeds as an important source of proteins is also the topic of many proteomic

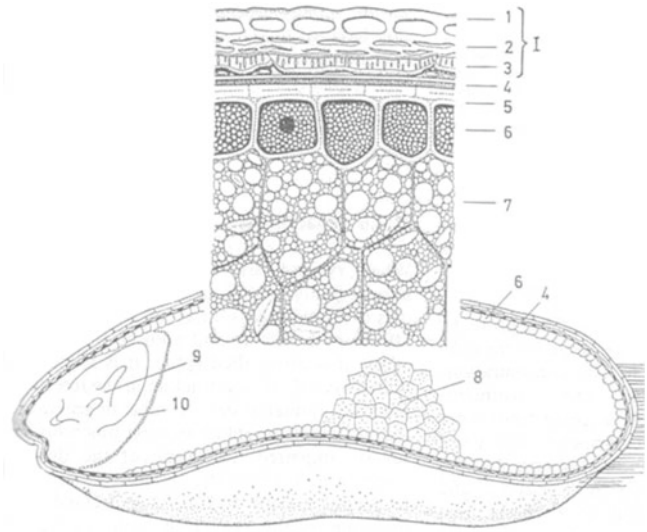


**Fig. 22.3** Longitudinal section of a wheat grain. *I* Pericarp, *1* epidermis (epicarp), *2* hypodermis, *3* tube cells, *4* seed coat (testa), *5* nucellar tissue, *6* aleurone layer, *7* outer starchy endosperm cells, *8* inner starchy endosperm cells, *9* germ and *10* scutellum

analyses (Marsolais et al. 2010; Gong et al. 2012). In a broad review, Miernyk and Hajduch (2011) give an excellent overview of seed proteomics.

#### 22.3.1.1 Cereal Proteins

The protein content of cereal grains is in the vicinity of 10% (wheat and barley 13%, rice and maize 9%).

The proteins of different cereal flours vary in their amino acid composition (Table 22.4). Lysine content is low in all cereals. Methionine is also low, particularly in wheat, rye, barley, oats, and corn. Both amino acids are significantly lower in flour than in muscle, egg, or milk proteins. By breeding, attempts are being made to improve the content of all essential amino acids. This approach has been successful in the case of high-lysine barley and several corn cultivars.

Wheat

Wheat is the most important cereal in the western world and wheat seed proteins have been investigated for more than 100 years. In 1907 Osborne separated wheat proteins, on the basis of their solubility, into four fractions. Sequential extraction of a flour sample yielded: water-soluble albumins, salt-soluble (e.g., 0.4 mol/L NaCl)

**Table 22.4** Amino acid composition of the total proteins (mole-%) of flours from various cereals (Belitz et al. 2004)

| Amino acid | Wheat | Rye | Barley | Oats | Rice | Millet | Corn |
|---|---|---|---|---|---|---|---|
| Asx | 4.2 | 6.9 | 4.9 | 8.1 | 8.8 | 7.7 | 5.9 |
| Thr | 3.2 | 4.0 | 3.8 | 3.9 | 4.1 | 4.5 | 3.7 |
| Ser | 6.6 | 6.4 | 6.0 | 6.6 | 6.8 | 6.6 | 6.4 |
| Glx | 31.1 | 23.6 | 24.8 | 19.5 | 15.4 | 17.1 | 17.7 |
| Pro | 12.6 | 12.2 | 14.3 | 6.2 | 5.2 | 7.5 | 10.8 |
| Gly | 6.1 | 7.0 | 6.0 | 8.2 | 7.8 | 5.7 | 4.9 |
| Ala | 4.3 | 6.0 | 5.1 | 6.7 | 8.1 | 11.2 | 11.2 |
| Cys | 1.8 | 1.6 | 1.5 | 2.6 | 1.6 | 1.2 | 1.6 |
| Val | 4.9 | 5.5 | 6.1 | 6.2 | 6.7 | 6.7 | 5.0 |
| Met | 1.4 | 1.3 | 1.6 | 1.7 | 2.6 | 2.9 | 1.8 |
| Ile | 3.8 | 3.6 | 3.7 | 4.0 | 4.2 | 3.9 | 3.6 |
| Leu | 6.8 | 6.6 | 6.8 | 7.6 | 8.1 | 9.6 | 14.1 |
| Tyr | 2.3 | 2.2 | 2.7 | 2.8 | 3.8 | 2.7 | 3.1 |
| Phe | 3.8 | 3.9 | 4.3 | 4.4 | 4.1 | 4.0 | 4.0 |
| His | 1.8 | 1.9 | 1.8 | 2.0 | 2.2 | 2.1 | 2.2 |
| Lys | 1.8 | 3.1 | 2.6 | 3.3 | 3.3 | 2.5 | 1.4 |
| Arg | 2.8 | 3.7 | 3.3 | 5.4 | 6.4 | 3.1 | 2.4 |
| Trp | 0.7 | 0.5 | 0.7 | 0.8 | 0.8 | 1.0 | 0.2 |
| Amide group | 31.0 | 24.4 | 26.1 | 19.2 | 15.7 | 22.8 | 19.8 |

globulins, and 70% aqueous ethanol-soluble prolamins. The glutelins remained in the flour residue. They can be separated into two subfractions. For this purpose, all the proteins remaining in the residue are first dissolved in 50% aqueous 1-propanol at 60°C with reduction of the disulfide bonds, for example, with dithioerythritol. The high-molecular (HMW) subunits precipitate out on increasing the propanol concentration to 60%, whereas the low-molecular (LMW) subunits remain in solution. This separation scheme can still be used for fractionation of seed proteins for further proteomic analysis (Miernyk and Hajduch 2011).

Albumins and globulins are derived mostly from cytoplasmic residues and other subcellular fractions that are part of the kernel. Prolamins and glutelins are storage proteins. The most important wheat proteins are glutens. When the flour is mixed with water, gluten proteins form an elastic matrix. This matrix holds carbon dioxide and gives volume to bread during rising. The gluten extracts are also used as additives for many food products. Gluten proteins are composed of monomeric subunits gliadins and polymeric glutenins. Gliadins are subdivided into alpha/beta-, gamma, and omega gliadins. Furthermore, gliadins are subdivided into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) that are linked by intermolecular disulfide bonds. Gliadins and glutenin subunits together are called prolamins (see above). Glutens contain large amounts of proline and glutamine. The content of essential amino acids such as arginine, lysine, and histidine is low, and in a complete nutrition, an additional source for these amino acids is necessary (Belitz et al. 2004). Van den Broeck et al. (2008) give

an overview of the proteomic analysis of gluten proteins. A negative aspect of gluten proteins is that they can trigger an immune response called celiac disease in those genetically susceptible (Sollid 2002). On the other hand, a lot of effort has been put into analyzing the allelic codes for gluten proteins in order to come to the high yield and highly resistant wheat sorts giving a flour with good baking quality (Yahata et al. 2005; van den Broeck et al. 2008).

Other important proteins in wheat are puroindolins a and b, and some enzymes and enzyme inhibitors, such as amylases, proteolytic enzymes, lipases, and enzymes involved in oxido-reductive processes, as well as amylase and protease inhibitors. Puroindolins are the cysteine-rich proteins that also contain segments in their amino acid sequences (Belitz et al. 2004; Branlard et al. 2003). Puroindolins are the lipid-binding proteins that have influence on the proper texture of dough during the baking process (Branlard et al. 2003). In an early paper, Islam et al. (2003) gave an overview of the wheat proteome and the relationship between chromosome deletion and protein expression.

Rice

Rice is the most important cereal in Asia and in developing countries. This crop feeds one-fourth of the world population, and its genome was sequenced relatively early (Komatsu et al. 2003) and it is also the reason for the early start of proteomic investigation of this important crop (Komatsu et al. 2003). Traditional rice milling involves steeping in hot water and steaming in autoclaves, followed by drying and polishing. This treatment causes removal and destruction of some nutritionally important components, such as proteins and vitamins. It may be the reason that most studies of rice proteomics are focused on the whole plant and plant development (Agrawal and Rakwal 2011), as well as discussion about proteomic changes in transgenic rice (Xue et al. 2012). The proteomic papers dealing with the rice proteins important for nutrition are relatively rare. From the nutritional point of view, together with the proteins from maize and millet, rice proteins are important as a protein source for celiac disease patients (Belitz et al. 2004; Moroni et al. 2010).

#### 22.3.1.2 Celiac Disease

In genetically susceptible individuals, some cereals such as wheat, rye, and barley can cause celiac disease. This disease affects infants as well as adolescents. Recent epidemiological studies indicate that about 1% of the world population suffers from this disease (Moroni et al. 2010). It is associated with a loss of villous structure of the intestinal mucosa, and, depending on severity of the disease, the nutrient absorption function can be impaired (Sollid 2002). After consumption of food containing the above-mentioned glutens, specific peptides from prolamins trigger an immune response that causes damage. This leads to a range of symptoms including altered bowel habits, malnutrition, and weight loss (Rodrigo 2006) Individuals with celiac

**Table 22.5** Legumes: protein distribution (%) by *Osborne* fractions

| Fraction | Soy-beans | Peanuts | Peas | Mungo beans | Broad beans |
|---|---|---|---|---|---|
| Albumin | 10 | 15 | 21 | 4 | 20 |
| Globulin | 90 | 70 | 66 | 67 | 60 |
| Glutelin | 0 | 10 | 12 | 29 | 15 |

disease are sensitive to the prolamin fractions of wheat, barley, and rye. A simple change of the diet to rice, millet, and maize can eliminate the cause of the disease. Van den Broeck et al. (2008) give the complete proteomic analysis of the gluten proteins involved in celiac disease in different wheat varieties.

#### 22.3.1.3 Legumes

Soybeans are nutritionally the most important legume as a protein source, because of their high content of essential amino acids (see above). The fractionation of legume proteins developed early in the last century by Osborne (1907) using solubility procedures to yield three fractions: albumins, globulins, and glutelins. As shown in Table 22.5, globulins are the predominant fraction in all legumes.

Globulins seem to have a function in seeds as storage proteins. Further simple fractionation of these proteins by ultracentrifugation or chromatography yields separation into two major components present in all legumes: vicilin and legumin. Legumin from soybeans is called glycinin and from peanuts arachin. The modern proteomic analyses of soybean also show that most of the seed proteins (60–80%), for example, in soybeans (Krishnan et al. 2009) as well as in common bean seeds (Marsolais et al. 2010) belong to the above-mentioned group of storage proteins.

The low-abundance proteins in seeds also have important nutritional value as protease inhibitors (e.g., in soybeans), or allergens (mostly in peanuts, see below), and it is important to know the whole proteome of nutritionally important legumes (Krishnan et al. 2009). Many legume proteins, mostly from peanuts are responsible for many allergic reactions. Most allergies in the United States are caused by peanuts and peanut-containing food products (Stevenson et al. 2009). Several proteins detected in peanut seed such as Ara h1-4 are responsible for these reactions. Proteomic analyses show different contents of these allergens in different peanut varieties. Interestingly, these proteins are absent in genetically engineered peanut seeds (Chassaigne et al. 2009; Stevenson et al. 2009). Other potential nutritional risks are lectins that are present in many legume seeds. If not inactivated or degraded during processing, these proteins can cause outbreaks of gastroenteritis, nausea, diarrhea, and other, even more severe, side reactions (Gaso-Sokac et al. 2010; Noah et al. 1980).

Finally, some legumes, especially soybeans, are genetically modified. Such food and food products are already on the market, especially in the United States. Proteomics are widely used for characterization of genetically engineered food, and

there is a broad discussion about this topic in nutrition and medicine (Sakata et al. 2009; Stevenson et al. 2009; Gaso-Sokac et al. 2010).

### *22.3.2 Fruit and Vegetable Proteomics*

Proteins, such as enzymes and inhibitors, as well as structural proteins play a key role in the molecular physiology of fruit development and ripening, as well as for stability of this food during transport and storage (Palma et al. 2011; Chan et al. 2007). Palma et al. (2011) give a proteomic overview about the proteome change in the ripening process, and the following events where proteins are involved take place during ripening of fruits, such as red pepper: taste alternation, intense metabolism, respiration and emitting volatile components, destruction of chlorophyll and synthesis of new pigments, pectins, and new proteins. Similar changes also occur in other fruits and vegetables during the growth and ripening processes. In this review, proteomic changes in different fruits and vegetables such as tomato, grape, citrus, peach, strawberries, and apple were listed. Chan et al. (2007) demonstrate impressively that proteins play a key role in stability of peach fruits during storage, and describe some enzymatic processes responsible for these changes. Finally, proteins also play an important role for quality of all fruit juice based and other beverages, and there are intensive proteomic studies in this field (Garibaldi and Giuffrida 2010; Colgrave et al. 2012).

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