reaction normally requires heat (usually >100°C), is promoted by low moisture content, and is accelerated in an alkaline environment as the amino groups are deprotonated and hence have an increased nucleophilicity. Various reducing sugars have differing rates of reaction in the Maillard reaction; pentoses such as ribose, xylose and arabinose are more reactive than hexoses such as glucose, fructose and galactose. Different sugars give different breakdown products and hence unique flavour and colour.

2.2.4.3 Toxic sugar derivatives

The Maillard reaction, while desirable in many respects, does have certain implications for the loss of essential amino acids (cysteine and methionine), the formation of mutagenic compounds and the formation of compounds that can cause protein cross-linking, which is implicated in diabetes. The most concerning aspect is the potential for toxic sugar derivatives with mutagenic properties, primarily the group of compounds called *heterocyclic amines*. These are particularly associated with cooked meat, especially that which has been grilled at high temperature for long cooking times. In recent times the formation of *acrylamide* has been an issue of concern in potato-based snack foods.

2.3 Proteins

Proteins are polymers of amino acids linked together by peptide bonds. They can also be referred to as *polypeptides*. Proteins are key constituents of food, contributing towards organoleptic properties (particularly texture) and nutritive value. Proteins participate in tissue building and are therefore abundant in muscle and plant tissues.

2.3.1 Amino acids – the building blocks of proteins

2.3.1.1 Amino acid structure

The general structure of an *amino acid* is depicted in Fig. 2.10, and consists of an amino group (NH_2), a carboxyl group (COOH), a hydrogen atom and a distinctive R group all bonded to a single carbon atom, called the α-*carbon*. The R group is called the *side chain* and determines the identity of the amino acid.

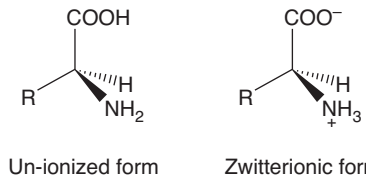


Figure 2.10 The general structure of an amino acid.

Amino acids in solution at neutral pH are predominantly *zwitterions*. The ionization state varies with pH: at acidic pH, the carboxyl group is un-ionized and the amino group is ionized; at alkaline pH, the carboxyl group is ionized and the amino group is un-ionized.

There are 20 different amino acids that are commonly found in proteins. The R group is different in each case and can be classified according to several criteria into four main types: *basic*, *non-polar* (hydrophobic), *polar* (uncharged) and *acidic*. Tables 2.1, 2.2 and 2.3 categorize the amino acids according to these types. The four different functional groups of amino acids are arranged in a tetrahedral array around the α-carbon atom; therefore, all amino acids are optically active apart from glycine. Of the possible L- or D-isomers, proteins contain only L-isomers of amino acids.

Some proteins contain *non-standard amino acids* in addition to the 20 standard amino acids (Fig. 2.11). These are formed by modification of a standard amino acid following its incorporation into the polypeptide chain (*post-translational modification*). Two examples that are encountered often in food proteins are *hydroxyproline* and *O-phosphoserine*. Hydroxyproline occurs in collagen and O-phosphoserine occurs in caseins.

2.3.1.2 Peptide bonds

The *peptide bond* is the covalent bond between amino acids that links them to form peptides and polypeptides (Fig. 2.12). A peptide bond is formed between the α-carboxyl group and the α-amino group of two amino acids by a condensation (or dehydration synthesis) reaction with the loss of water. Peptides are compounds formed by linking small numbers of amino acids (up to 50). A polypeptide is a chain of 50–100 amino acid residues. A protein is a polypeptide chain of 100+ amino acid residues and has a positively charged nitrogen-containing amino group at one end (*N-terminus*) and a negatively charged carboxyl group at its other end (*C-terminus*).

Table 2.1 Basic and acidic amino acids.

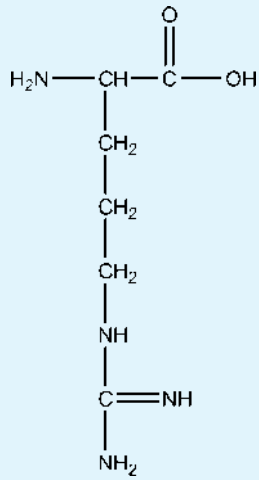
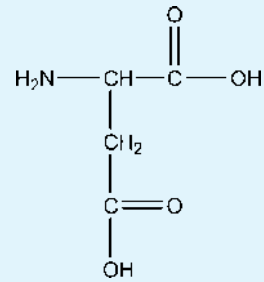
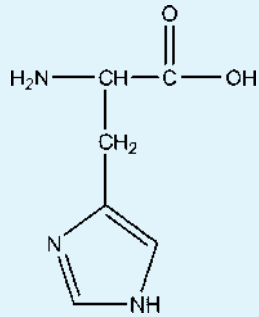
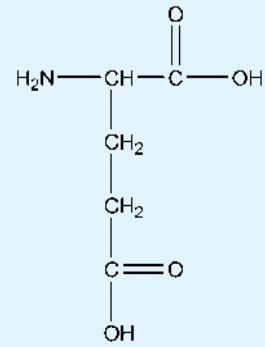
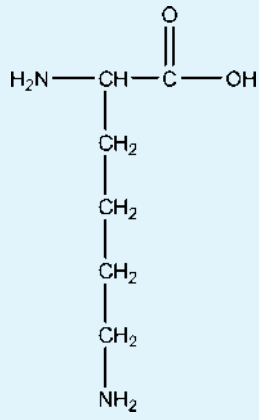
Amino acid	Single letter code	Structural formula	Amino acid	Single letter code	Structural formula
Basic:			**Acidic:**		
Arginine (Arg)	R		Aspartic acid (Asp)	D	
Histidine (His)	H		Glutamic acid (Glu)	E	
Lysine (Lys)	K				

Table 2.2 Non-polar amino acids.

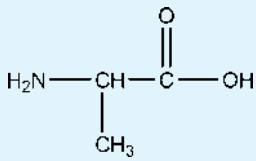
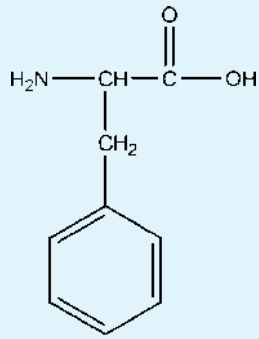
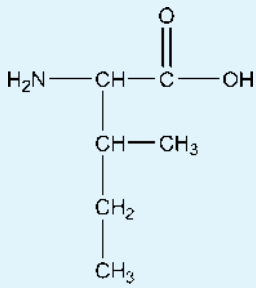
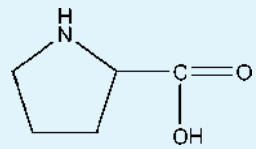
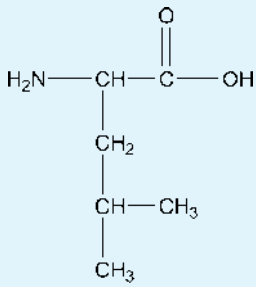
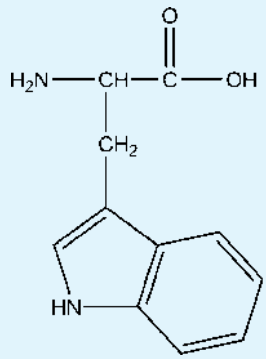
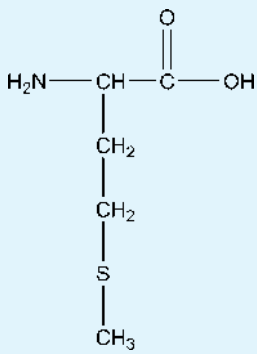
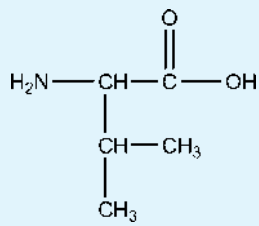
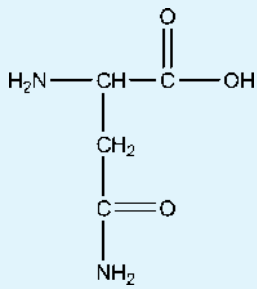
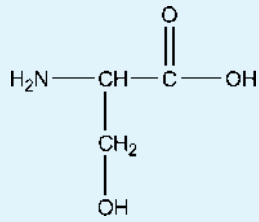
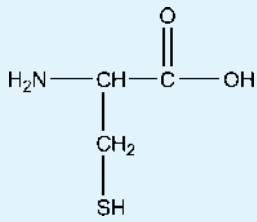
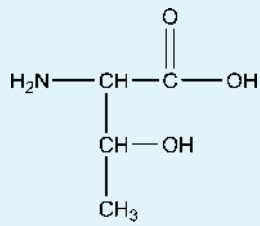
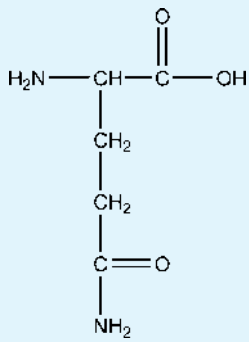
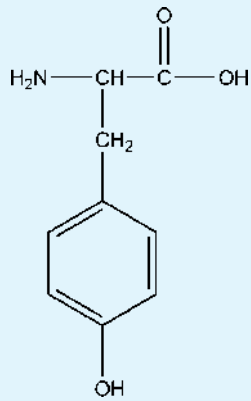
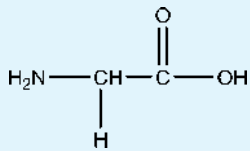
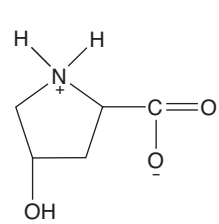
Amino acid	Single letter code	Structural formula	Amino acid	Single letter code	Structural formula
Alanine (Ala)	A		Phenylalanine (Phe)	F	
Isoleucine (Ile)	I		Proline (Pro)	P	
Leucine (Leu)	L		Tryptophan (Trp)	W	
Methionine (Met)	M		Valine (Val)	V	

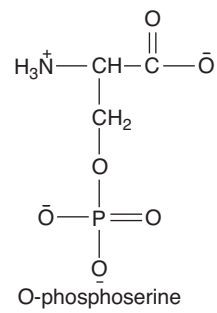
Table 2.3 Polar amino acids.

Amino acid	Single letter code	Structural formula	Amino acid	Single letter code	Structural formula
Asparagine (Asn)	N		Serine (Ser)	S	
Cysteine (Cys)	C		Threonine (Thr)	T	
Glutamine (Gln)	Q		Tyrosine (Tyr)	Y	
Glycine (Gly)	G				

A special feature of the peptide bond is its *partial double bond character*. This arises because the peptide bond is stabilized by *resonance hybridization* between two structures, one single bonded between the carbon and nitrogen atoms, the other double bonded. As a consequence, the peptide bond is planar and stable. This has implications for the possible conformations adopted by a polypeptide chain, since no rotation is possible around the peptide bond. However, rotation is possible around bonds between the α-carbons and the amino nitrogen and carbonyl carbon of their residue.



Hydroxyproline



O-phosphoserine

Figure 2.11 Chemical structures of some non-standard amino acids common in food proteins.

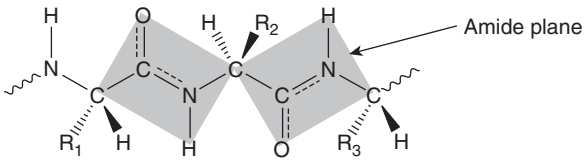


Figure 2.12 Peptide bonds in a polypeptide. The partial double bond character is represented by the dashed double bonds. The shaded boxes highlight atoms that exist within the same plane.

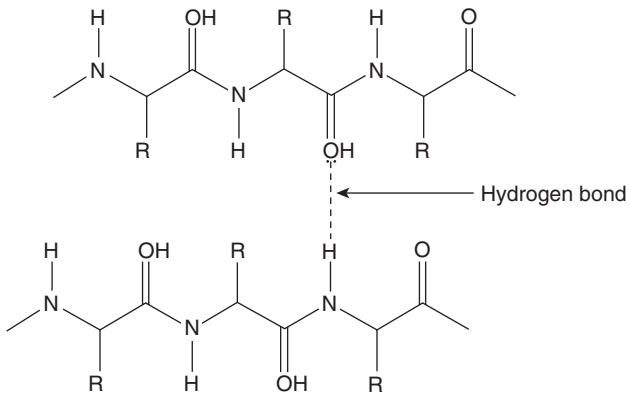


Figure 2.13 Hydrogen bonding between two polypeptides.

2.3.2 Molecular structure of proteins

2.3.2.1 Primary structure

The *primary structure* of a protein is simply the sequence of amino acids listed from the N-terminal amino acid. There are more than a billion possible sequences of the 20 amino acids and every protein will have a unique primary structure which determines how the protein folds into a three-dimensional conformation. If we compare the primary sequence Leu-Val-Phe-Gly-Arg-Cys-Glu-Leu-Ala-Ala with Gly-Leu-Arg-Phe-Cys-Val-Ala-Glu-Ala-Leu, these two peptides have the same number of amino acids, the same kinds of amino acids, but have different primary structures.

2.3.2.2 Secondary structure

The secondary structure of a protein describes the arrangement of the protein backbone (polypeptide chain) due to *hydrogen bonding* between its amino acid residues. Hydrogen bonding can occur between an amide hydrogen atom and a lone pair of electrons on a carbonyl oxygen atom, as shown in Fig. 2.13.

The peptide bond is planar, offering no rotation around its axis. This leaves only two bonds within each amino acid residue that have free rotation, namely the α-carbon to amino nitrogen and α-carbon to carboxyl carbon bonds. The rotations around these bonds are represented by the *dihedral angles* ϕ (phi) and ψ (psi), as shown for a tripeptide of alanine in Fig. 2.14. Ramachandran plotted ϕ and ψ combinations from known protein structures and found that there are certain sterically favourable combinations that form the basis for the preferred secondary structures. He also found that unfavourable orbital overlap precludes some combinations: $\phi = 0^\circ$ and $\psi = 180^\circ$; $\phi = 180^\circ$ and $\psi = 0^\circ$; $\phi = 0^\circ$ and $\psi = 0^\circ$.

Two kinds of hydrogen bonded secondary structures occur frequently with features that repeat at regular intervals. These *periodic structures* are the α-*helix* and the β-*pleated sheet*. The β-*pleated sheet* can give a two-dimensional array and can involve more than one polypeptide chain.

α-*Helix*

The α-helix is a coiled rod-like structure and involves a single polypeptide chain. The 'α' denotes that if you were to view the helix down its axis then you would note that it spirals clockwise away from you. The α-helix is stabilized by hydrogen bonding parallel to the helix axis and the carbonyl group of each residue is hydrogen bonded to the amide group of the residue that is four residues away if counting from the N-terminus. There are 3.6 residues for each turn of the helix and the dihedral angles are $\phi = -57^\circ$ and $\psi = -48^\circ$. The R group of each residue protrudes from the helix and plays no role in the formation of

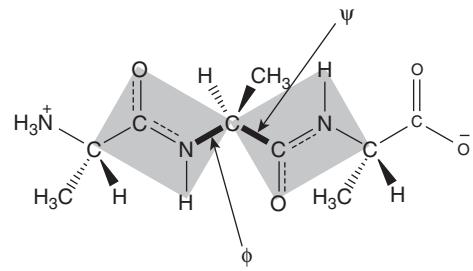


Figure 2.14 Bonds adjacent to peptide bonds with free rotation are depicted in bold with their respective dihedral angles ϕ and ψ.

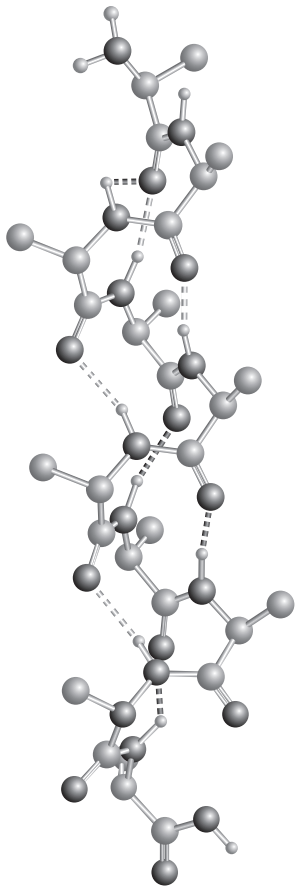


Figure 2.15 Ball and stick representation of an α-helix structure showing the position of hydrogen bonds between amide hydrogens (small white spheres) and carbonyl oxygens (larger dark spheres).

hydrogen bonding as part of the α-helix structure. In the illustration of an α-helix structure shown in Fig. 2.15, the hydrogen bonds are shown as dotted lines between backbone amide hydrogens and backbone carbonyl oxygens.

Proteins contain varying amounts of α-helix structure. Properties of α-helices include strength and low solubility in water. These properties arise because all amide hydrogen and carbonyl oxygen is involved in hydrogen bonds. Multiple strands of α-helix may entwine to make a protofibril such as in the muscle protein myosin.

All amino acids can be found in α-helix structure apart from *proline*, which disrupts α-helix. This is because its cyclic structure causes a bend in the backbone as a result of the restricted C–N bond rotation. This prevents the α-amino group from participating in intrachain hydrogen bonding.

β-*Pleated sheet*

In β-pleated sheets the peptide backbone is almost completely extended (termed a β-*strand*) and hydrogen bonding is perpendicular to the direction of the polypeptide chain. Hydrogen bonds form between different parts of a single chain that is doubled back on itself (*intrachain bonds*) or between different chains (*interchain bond*) giving rise to a repeated zigzag structure (see Fig. 2.16). β-Sheets can be either *parallel* (where the β-strands run in the same direction) or *antiparallel* (where the β-strands run in opposite directions). The dihedral angles are $\phi = -119^\circ$ and $\psi = +113^\circ$ for pure parallel, and $\phi = -139^\circ$ and $\psi = +135^\circ$ for pure antiparallel β-sheets.

β-*Turns*

β-*Turns* are essentially hairpin turns in the polypeptide chain that allow it to reverse direction. In a β-turn the carbonyl oxygen of one residue is hydrogen bonded to the amide proton of a residue three

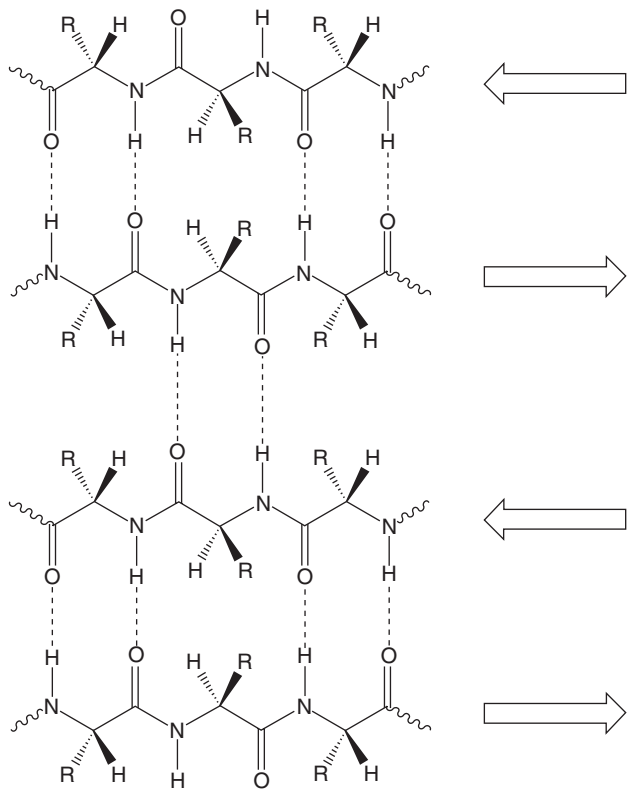


Figure 2.16 Hydrogen bonding within an antiparallel β-sheet structure. The arrow shows the direction of the polypeptide chain.

residues away. Proline and glycine are prevalent in β-turns. β-Turns are estimated to comprise between a quarter and a third of all residues in proteins and they are commonly found to link two strands of anti-parallel β-sheet.

Collagen triple helix

Collagen is a component of bone and connective tissue and is organized as strong water-insoluble fibres. It has a unique periodic structure comprising of three polypeptide chains wrapped around each other in a repeat sequence of X-Pro-Gly or X-Hyp-Gly (where X can be any amino acid). Proline and hydroxyproline make up to 30% of the residues in collagen, and hydroxylysine is also present. Every third position in the *collagen triple helix* is Gly because every third residue must sit inside the helix and only Gly is small enough.

The individual collagen chains are also helices and the three strands are held together by hydrogen bonds involving hydroxyproline and hydroxylysine residues. The molecular weight of the triple-stranded array is approximately 300,000 Daltons, involving approximately 800 amino acid residues. Intra- and intermolecular cross-linking stabilizes the collagen triple helix structure, especially covalent bonds between lysine and histidine. The amount of cross-linking increases with age. A major role for vitamin C (L-ascorbic acid) in vivo is in making collagen: proline and lysine in collagen are converted to 4-hydroxyproline and 5-hydroxylysine using this vitamin. Scurvy is a disease arising from a deficiency of vitamin C, and results in skin lesions, bleeding gums and fragile blood vessels.

2.3.2.3 Tertiary structure

The *tertiary structure* of a protein is the three-dimensional arrangement of all atoms within the molecule, and takes into account the conformations of side chains and the arrangement of helical and pleated sheet sections with respect to each other. Proteins *fold* to make the most stable structure and this structure will generally minimize solvent contact with residues of opposing polarity and hence minimize overall free energy. Therefore, in aqueous solution, proteins generally exist with their hydrophobic residues to the inside and their hydrophilic residues to the outside of their three-dimensional conformation.

There are two categories of tertiary structures:

- *Fibrous proteins* – overall shape is a long rod; mechanically strong; usually play a structural role in nature; relatively insoluble in water and unaffected by moderate changes in temperature and pH.
- *Globular proteins* – helical and pleated sheet sections fold back on each other; interactions between side chains important for protein folding; polar residues face surface and interact with solvent; non-polar residues face interior and interact with each other; structure is not static; generally more sensitive to temperature and pH change than their fibrous counterparts.

The tertiary structure of a protein is held together by interactions between the side chains. These can be through *non-covalent* interactions or *covalent* bonds. The most common non-covalent interactions are *electrostatic* (ionic bonds, salt bridges, ion pairing), *hydrogen bonds*, *hydrophobic interactions* and *van der Waals dispersion forces*. Covalent bonds in protein structure are primarily *disulphide bonds* (sulphur bridges) between cysteine residues, although other types of covalent bond can form between residues.

Electrostatic interactions

Some amino acids contain an extra carboxyl group (aspartic acid and glutamic acid) or an extra amino group (lysine, arginine, histidine). These groups can be ionized and therefore an ionic bond could be formed between the negative and the positive group if the chains folded in such a way that they were close to each other.

Hydrogen bonds

Hydrogen bonds can form between side chains since many amino acids contain groups in their side chains which have a hydrogen atom attached to either an oxygen or a nitrogen atom. This is a classic situation where hydrogen bonding can occur. For example, the amino acid serine contains a hydroxyl group in its side chain; therefore, hydrogen bonding could occur between two serine residues in different parts of a folded chain.

Hydrophobic interactions

Non-polar molecules or groups tend to cluster together in water; these associations are called hydrophobic interactions. The driving force for hydrophobic interactions is not the attraction of the

non-polar molecules for one another, but is due to entropic factors relating to the strength of hydrogen bonding between water molecules.

Van der Waals dispersion forces

Several amino acids have quite large hydrocarbon groups in their side chains (e.g. leucine, isoleucine and phenylalanine). Temporary fluctuating dipoles in one of these groups could induce opposite dipoles in another group on a nearby folded chain. The dispersion forces set up would be enough to hold the folded structure together, although van der Waals forces are weaker and less specific than electrostatic and hydrogen bonds.

Disulphide bonding

If two cysteine side chains are oriented next to each other because of folding in the peptide chain, they can react to form a covalent bond called a *disulphide bond* or a *sulphur bridge*.

2.3.2.4 Quaternary structure

Not all proteins possess *quaternary structure*; it is only a property of proteins that consist of more than one polypeptide chain. Each chain is a subunit of the *oligomer* (protein), which is commonly a dimer, trimer or tetramer. Haemoglobin has quaternary structure. It is a tetramer consisting of two α- and two β-chains. The chains are similar to myoglobin and haemoglobin is able to bind four oxygen atoms through positive cooperativity.

2.3.3 Denaturation of proteins

The forces that stabilize the secondary, tertiary and quaternary structures of proteins can be disrupted through various chemical or physical treatments. This disruption of the native protein structure is defined as protein *denaturation*, which is an important process that may occur during the processing of foods. Denaturation is a change in a protein which causes an alteration in its physical and/or biological properties without rupture of its peptide bonds. It is generally observed as unfolding of the protein molecule from its uniquely ordered structure to a randomly ordered peptide chain. In the case of globular proteins, the denaturing process is often followed by *aggregation*, since previously buried hydrophobic residues are exposed to solution.

Denaturation is accompanied by a loss of native biological activity, but also affects physical properties. Some important consequences of protein denaturation are:

- Loss of biological activity (e.g. enzyme activity).
- Loss of solubility and changes to water-binding capacity.
- Increased intrinsic viscosity.
- Increased susceptibility to proteolysis.

Denaturation can be reversible, but if disulphide bonds are broken the denaturation process is often considered irreversible. Different proteins have different susceptibilities to denaturation since their individual structures are different. There are various denaturing agents that can destabilize protein structures that are categorized as physical agents or chemical agents.

Physical agents include heat, mechanical treatment, hydrostatic pressure, irradiation, and adsorption at interfaces. *Heat* is the most commonly encountered physical agent and is able to destabilize many bonds within proteins, including electrostatic bonds, hydrogen bonds and van der Waals interactions. Heat denaturation is useful in food processing since it tends to lead to improvement of sensory properties and protein digestibility, and can be used to manipulate foaming and emulsifying properties. Heating also promotes the participation of proteins in the Maillard reaction, which leads to the loss of nutritionally available lysine residues.

Chemical agents to denature proteins include acids, alkalis, metals, organic solvents and various organic solutes. Exposure to *acids* or *alkalis* (i.e. pH changes) affects the overall net charge on a protein, which will change the extent of electrostatic interactions, both attractive and repulsive. Most proteins are stable within a pH range around their isoelectric point (zero net charge) and the effects of acids or alkalis are normally reversible.

The presence of *organic solvents* weakens hydrophobic interactions since non-polar side chains become more soluble. *Organic solutes* can have a variety of effects. Urea alters the structure of water in such a way as to weaken hydrophobic interactions, leading to protein unfolding. Sodium dodecyl sulphate (SDS) is an anionic detergent that binds irreversibly to charged groups within a protein, inducing a large net negative charge that increases electrostatic repulsion, leading to unfolding. Reducing

agents, such as mercaptoethanol and dithiothreitol, break disulphide bonds in proteins.

2.3.4 Post-translational modification

Post-translational modification of a protein is a chemical change that has occurred after the protein was synthesized by the body. Post-translational modification can create new functional families of proteins by attachment of biochemical functional groups to reactive groups on amino acid side chains, such as phosphate (*phosphoproteins*), various lipids (*lipoproteins*) and carbohydrates (*glycoproteins*). More simple modifications of amino acid side chains are also possible, such as the hydroxylation of lysine and proline encountered in collagen due to the action of vitamin C.

Enzymes also cause post-translational modification, such as peptide bond cleavage by specific proteases. One example of particular relevance in food production is the action of chymosin on casein. Chymosin cleaves the peptide bond between phenylalanine and methionine in κ-casein, which is used to bring about extensive precipitation and curd formation during cheese making.

2.3.5 Nutritional properties of proteins

Food proteins have an important nutritional role and are primarily used by the body to supply nitrogen and amino acids from which the body synthesizes its own proteins. Within the gastrointestinal tract, hydrolytic enzymes break down food proteins into their component amino acids, which are then used by the body to synthesize other substances. The liver balances the pattern of amino acid supply against the needs of synthesis.

In terms of nutritive value, proteins are classified according to their content of non-essential and essential amino acids. *Non-essential amino acids* are synthesized by the body and require only an adequate supply of amino nitrogen and carbohydrate. However, humans cannot synthesize some *essential amino acids* and these must be supplied by our diet. The essential amino acids are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

Protein efficiency ratio (PER) is used as a measure of how well food protein sources supply essential amino acids. Human breast milk is treated as a standard and is given a PER score of 100%. In general, animal protein foods (eggs, milk and meat) are very efficient sources, whereas plant protein foods are less efficient since they are usually deficient in either lysine or methionine. For this reason, vegetarians need to consume a balanced diet of plant products to ensure sufficient supply of these two amino acids.

2.4 Lipids

Lipids are a group of molecules that contribute to the structure of living cells and are also used in the body for the purpose of energy storage. Dietary lipids have important roles for provision of energy and as carriers of fat-soluble vitamins. Generally speaking, all lipids are soluble in non-polar organic solvents and have low solubility in water. Dietary lipids are commonly referred to as *oils* and *fats*. Edible oils are liquid at room temperature, whereas fats are solid or semi-solid at room temperature. The lipids found in oils and fats are chemically very diverse, but are predominantly long-chain *fatty acid esters*. Other lipid types encountered in foods are also either fatty acids or derivatives of fatty acids, and include *triglycerides*, *phospholipids*, *sterols* and *tocopherols*.

Lipids can be broadly classified into three main groups:

- *Simple lipids* yield two classes of product when hydrolyzed, e.g. glycerides (acylglycerols) which are hydrolyzed to give glycerol and a fatty acid.
- *Complex lipids* yield three or more classes of product when hydrolyzed, e.g. phospholipids, which are hydrolyzed to give alcohols, fatty acids and phosphoric acid.
- *Derived lipids* are non-hydrolyzable and do not fit into either of the above classes, e.g. sterol, tocopherol and vitamin A.

2.4.1 Lipid structure and nomenclature

2.4.1.1 Fatty acids

A *fatty acid* is a carboxylic acid having a long unbranched aliphatic tail or chain, and can be described chemically as an *aliphatic monocarboxylic acid*. The aliphatic chain can be either *saturated* (no double bonds between carbons) or *unsaturated* (one or more double bonds between carbons). Saturated