Chapter 3
Proteins

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Abstract In this chapter, proteins and amino acids will be introduced according to their chemical structures, classification as well as those important physicochemical and biochemical properties. It has been elucidated in detail about the mechanisms and the influencing factors involved in protein denaturation and in governing the functional properties of proteins. This chapter also introduces the functionality evaluations of proteins and their applications in the food industry, and the main methods used in protein modification. After this, readers should acquire the physicochemical and nutritional changes of proteins during food processing and storage and the controlling techniques for these changes.

Keywords Proteins · Amino · Physicochemical properties · Biochemical properties · Functional properties · Protein modification

Proteins are highly complex biopolymers and are made up of carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, and certain metal irons (such as Zn and Fe) at the elemental level. Natural proteins have molecular weights in the range from 10^4 to 10^5 Da, whereas some proteins have molecular weights up to 10^6 Da. Proteins are major components of a cell/organism, accounting for more than 50% of the dry weight of cells. Proteins provide essential nutrients for the growth and sustainability of life. Several proteins, such as enzymes and hormones, are regarded as biocatalysts to regulate the growth, digestion, metabolic activities, and secretion, whereas several proteins take part in energy transfer in the body, such as insulin, hemoglobin, and growth hormone. Certain proteins are also recognized as essential substances in the biological system. Immunoglobulin could take an important part in the immune

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system of the body. However, several proteins like trypsin inhibitors are regarded as anti-nutritional factors. Overall, proteins contribute to food processing for the textural and flavor attributes of foods.

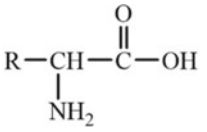
Amino acids are the basic structural units of proteins. There are 20 or 18 kinds of amino acids in food proteins. The amino acids link together via the amide bonds to build native proteins. Generally, proteins can be classified into three categories according to their chemical compositions, namely simple, conjugated, and derived proteins. Among the three types of proteins, simple proteins refer to these proteins only consist of amino acids, whereas conjugated proteins are these proteins that also contain the non-protein constituents or prosthetic groups. The so-called derived proteins are these proteins derived from simple or conjugated proteins by enzymatic or chemical methods. Proteins can also be classified according to their functional properties, including structural proteins, bioactive proteins, and food proteins. In most textbooks, the first protein classification is generally adopted and further subdivided according to their solubility.

To fulfill protein requirements for the human, it is necessary to develop food proteins via new technologies and make full use of the existing protein resources. Therefore, it is essential for the food scientists to understand the physical, chemical, and biological properties of proteins as well as the potential effects of food processing and storage on protein properties.

3.1 Amino Acids

3.1.1 Chemical Structures and Classification of Amino Acids

Native amino acids (except for praline) consist of at least a carboxyl and amino groups, together with a side chain (i.e. residual) R group. The amino group is bonded directly to the α-carbon atom of amino acids. Therefore, amino acids are generally referred to as α-amino acids. Amino acids may have both L- and D-configuration (certain bacteria), but only the amino acids in L-configuration can be used by humans.



The R represents different residual groups of amino acids

Amino acids are usually classified into four groups, based on their different residual groups.

(1) Non-polar amino acids having hydrophobic R group. The hydrophobicity of R group increases with an increasing number of C atoms in the hydrocarbon chain. Examples of non-polar amino acids include alanine, glycine,

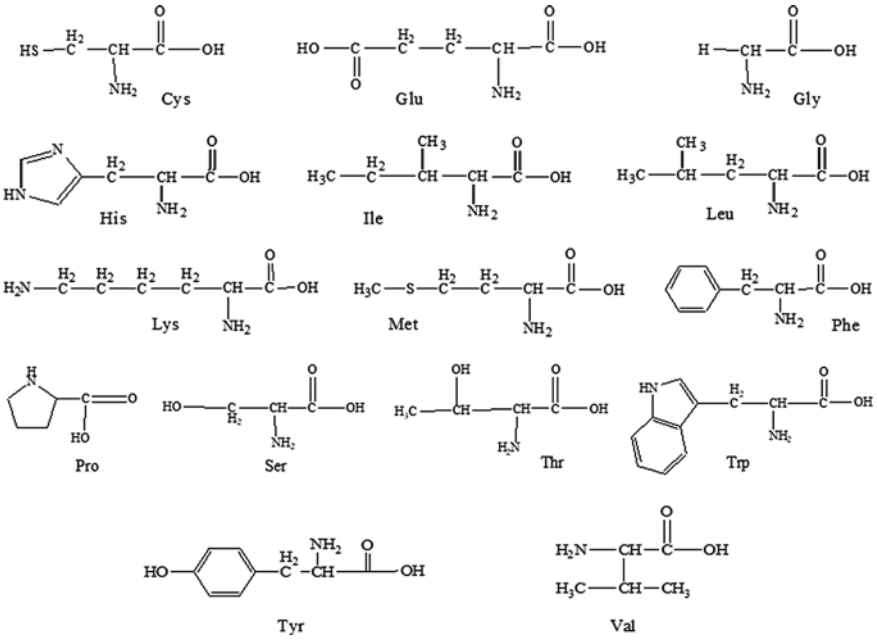


Fig. 3.1 Molecular structure of amino acids at neutral pH

leucine, isoleucine, valine, proline, tryptophan, phenylalanine, and methionine. It should be noted here that proline is an α-subamino acid.

(2) Polar but uncharged amino acids. The R group is polar but ionizable and can form hydrogen bonds with other polar groups. Typical polar but uncharged amino acids are serine, threonine, tyrosine, cysteine, asparagine, glutamine, and glycine. However, the residual groups of tyrosine and cysteine are ionizable under strongly alkaline conditions, so tyrosine and cysteine have polarity greater than the other five polar amino acids. Cysteine is usually present in the form of cystine. Both asparagine and glutamine can be hydrolyzed to remove the amide group and thus converted to respective aspartic and glutamic acids.

(3) Basic amino acids include lysine, arginine, and histidine. The residual groups of those amino acids are amino or imino groups, so they are polar and positively charged at the pH values below their pKa values.

(4) Acidic amino acids include glutamic and aspartic acids containing a carboxyl group in their side chain. The carboxyl group is dissociated to lose protons, and thus negatively charged.

There are also other derivatives of common amino acids with special structure, such as hydroxyproline and 5-hydroxylysine found in collagen, methyl histidine and, α-N-methyllysine in animal muscle protein, while we are not introduced to other amino acids here.

The chemical structures of these amino acids are shown in Fig. 3.1. Several physicochemical constants of these amino acids are given in Tables 3.1, 3.2, and 3.3.

Table 3.1 Primary α-amino acids that occur in proteins and their solubility and melting point

Name	Three letters abbreviation	One letter abbreviation	Molecular weight	Solubility in water at 25 °C (g/L)	Melting point (°C)
Alanine	Ala	A	89.1	167.2	279
Arginine	Arg	R	174.2	855.6	238
Asparagine	Asn	N	132.2	28.5	236
Aspartic acid	Asp	D	133.1	5.0	269–271
Cysteine	Cys	C	121.1	0.05	175–178
Glutamine	Gln	Q	146.1	7.2	185-–186
Glutamic acid	Glu	E	147.1	8.5	247
Glycine	Gly	G	75.1	249.9	290
Histidine	His	H	155.2	41.9	277
Isoleucine	Ile	I	132.2	34.5	283–284
Leucine	Leu	L	131.2	21.7	337
Lysine	Lys	K	146.2	739.0	224
Methionine	Met	M	149.2	56.2	283
Phenylalanine	Phe	F	165.2	27.6	283
Proline	Pro	P	115.1	1620.0	220–222
Serine	Ser	S	105.1	422.0	228
Threonine	Thr	T	119.1	13.2	253
Trytophan	Trp	W	204.2	13.6	282
Tyrosine	Tyr	Y	181.2	0.4	344
Valine	Val	V	117.1	58.1	293

Table 3.2 Specific optical rotation (°) of amino acids (the medium and temperature are not shown here)

Name	$[\alpha]_D(H_2O)$	Name	$[\alpha]_D(H_2O)$
Alanine	+14.7	Lysine	+25.9
Arginine	+26.9	Methionine	+21.2
Aspartic acid	+34.3	Phenylalanine	−35.1
Cystine	−214.4	Proline	−52.6
Glutamic acid	+31.2	Serine	+14.5
Glycine	0	Threonine	−28.4
Histidine	−39.0	Trytophan	−31.5
Isoleucine	+40.6	Tyrosine	−8.6
Leucine	+15.1	Valine	+28.8

Table 3.3 pKa and pI values of ionizable groups in free amino acids and proteins at 25 °C

Name	pK_{a1} (α-COOH)	pK_{a2} (α-NH_3^+)	pK_{aR} (Side chain)	pI
Alanine	2.35	9.69		6.02
Arginine	2.17	9.04	12.48	10.76
Asparagine	2.02	8.80		5.41
Aspartic acid	1.88	9.60	3.65	2.77
Cysteine	1.96	10.28	8.18	5.07
Glutamine	2.17	9.13		5.65
Glutamic acid	2.19	9.67	4.25	3.22
Glycine	2.34	9.60		5.98
Histidine	1.82	9.17	6.00	7.59
Isoleucine	2.36	9.68		6.02
Leucine	2.30	9.60		5.98
Lysine	2.18	8.95	10.53	9.74
Methionine	2.28	9.21		5.74
Phenylalanine	1.83	9.13		5.48
Proline	1.94	10.6		6.30
Serine	2.20	9.15		5.68
Threonine	2.21	9.15		5.68
Trytophan	2.38	9.39		5.89
Tyrosine	2.20	9.11	10.07	5.66
Valine	2.32	9.62		5.96

3.1.2 Physicochemical Properties of Amino Acids

(1) Optical activity

Amino acids (except for glycine) have at least one chiral center at the α-carbon atom (as shown in Fig. 3.1) and hence exhibit optical activity. The data in Table 3.2 list the optical properties of amino acids. The direction and magnitude of the optical rotation depend not only on the nature of these residual R groups but also on the pH, temperature, and other factors of the aqueous solution. The optical properties of amino acids can be used in their quantitative measurement and qualitative identification.

(2) UV absorption and fluorescence

Amino acids have no absorption in the visible region but have absorption near 210 nm in the ultraviolet region (Fig. 3.2). Tyr, Trp, and Phe contain aromatic rings thereby have strong ultraviolet absorption (Fig. 3.2). The three amino acids show maximum wavelengths of 278, 279, and 259 nm, with molar extinction coefficients of 1,340, 5,590, and 190 mol/cm, respectively. These coefficients are often used as reflectors

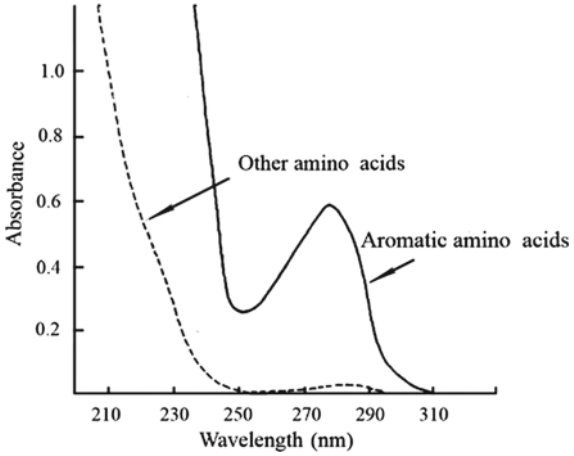


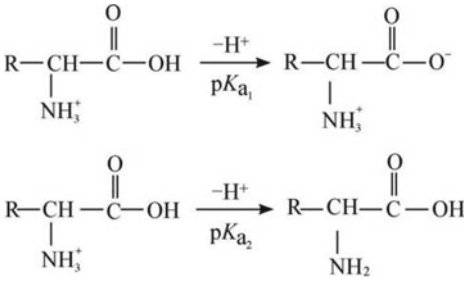
Fig. 3.2 Ultraviolet absorbance spectra of some amino acids

to monitor these three aromatic amino acids. The bound Tyr and Trp have maximum absorption near 280 nm and are responsible for quantitative analysis of proteins using ultraviolet absorption.

Tyr, Trp, and Phe can also be excited to produce fluorescence in the ultraviolet region with emission wavelengths of 304 and 348 nm at the excitation wavelength of 280 nm, respectively. The three aromatic acids also exhibit fluorescence with emission wavelengths of 282 nm at the excitation wavelength of 260 nm. Other amino acids do not exhibit fluorescence.

(3) Dissociation

All amino acids contain at least an amino and one carboxyl groups and are mainly in a form of dipolar ions or zwitterions in neutral aqueous solutions. In other words, an amino acid can either accept a proton as alkaline or dissociate a proton as an acid. In this way, a mono-amino- and mono-carboxyl amino acid after being fully protonated can be regarded as a dibasic acid with two dissociation constants corresponding to the carboxyl group (pKa_1) and protonated amino group (pKa_2). A third dissociation constant (pKa_R) occurs when the side chain of amino acid has dissociable groups such as the α-amino or α-carboxyl groups of basic or acidic amino acids. The dissociation constants and isoelectric points (pI) of these amino acids are shown in Table 3.3.



When the molecules of an amino acid are electrically neutral in solution (i.e. the net charge is zero), they do not move in the electric field. The corresponding pH environment is called the isoelectric point (pI) of the amino acid. The solubility hereof the amino acid is the lowest. The pI values of amino acids can be estimated from their pKa_1, pKa_2, and pKa_3 values using the following expressions.

For a mono-amino and mono-carboxyl amino acid, $2pI = pKa_1 + pKa_2$.

For a basic amino acid, $2pI = pKa_2 + pKa_3$.

For an acidic amino acid, $2pI = pKa_1 + pKa_3$.

The subscript numbers 1, 2, and 3 refer to the α-carboxyl, α-amino, and side-chain ionizable groups, respectively.

The isoelectric point property can be used to selectively separate an amino acid from a mixture of amino acids. In addition, when amino acids are combined to form proteins, the isoelectric properties of proteins are also affected by the dissociation of amino acids.

(4) Hydrophobicity

Hydrophobicity of an amino acid can be defined as the free energy change arising from transferring 1 mol amino acid from ethanol to water phases. In the case of neglecting the change in activity coefficients, the free energy change can be expressed as below.

$$\Delta G^o = -RT \ln(\frac{S_{ethanol}}{S_{water}})$$

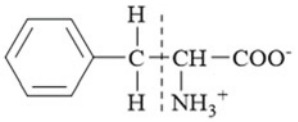
where, $S_{ethanol}$ and S_{water} represent the solubility of the amino acid in ethanol and water (mol/L), respectively.

As is true of all other thermodynamic parameters, ΔG° also has an additive function. If an amino acid molecular has multiple groups, its ΔG° can be the sum of the free energy changes when transferring multiple groups in the amino acid from ethanol to water phases. That is, $\Delta G^\circ = \Sigma \Delta G^\circ$.

An amino acid molecule can be divided into two parts, one is a glycine group and the other is a residual group (R group). For example, Phe can be considered as below, and the free energy change of transferring Phe from ethanol to water phases can then be considered as below. Thus, the hydrophobicity of the side chains can be determined by subtracting $\Delta G^\circ_{glycine}$ from ΔG°.

Table 3.4 Hydrophobicity of amino acid side chain at 25 °C determined using Tanford method

Amino acid	ΔG° (ethanol→water) (kJ/mol)	Amino acid	ΔG° (ethanol→water) (kJ/mol)
Ala	2.09	Leu	9.61
Arg	3.1	Lys	6.25
Asn	0	Met	5.43
Asp	2.09	Phe	10.45
Cys	4.18	Pro	10.87
Gln	−0.42	Ser	−1.25
Glu	2.09	Thr	1.67
Gly	0	Trp	14.21
His	2.09	Tyr	9.61
Ile	12.54	Val	6.27



Benzyl group Glycyl group

$$\Delta G^{\circ} = \Delta G^{\circ}_{(\text{side chain})} + \Delta G^{\circ}_{(\text{glycine})}, \text{ or } \Delta G^{\circ}_{(\text{side chain})} = \Delta G^{\circ} - \Delta G^{\circ}_{(\text{glycine})}$$

The hydrophobicity of these residual groups can be determined by measuring the solubility of each amino acid in two different media (Tanford method, see Table 3.4). The more positive the value of an amino acid is, the more hydrophobic it behaves, where presumably the R group tends to be located inside of proteins. Otherwise, the more negative the value is, the more hydrophilic the amino acid possesses. Thereby, this hydrophilic R group tends to be distributed on the surface of protein molecules. However, an exception is Lys. Lys has a positive hydrophobicity value but is a hydrophilic amino acid due to its four methylene groups. Adsorption coefficients of amino acids are proportional to their hydrophobicity values, and hydrophobicity is commonly used to predict the adsorption behavior of amino acids on a carrier of hydrophobic compounds.

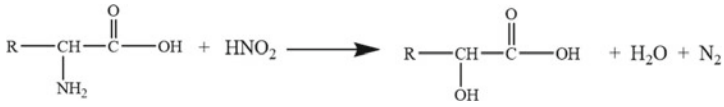
3.1.3 Chemical Reactions of Amino Acids

These functional groups such as amino, carboxyl, and residual groups in amino acid molecules can undergo various chemical reactions.

3.1.3.1 The Reactions of Amino Groups

(1) Reaction with nitrite

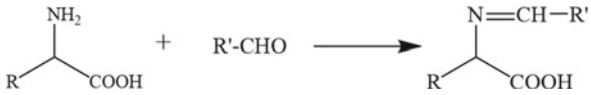
α-NH_2 of amino acids would react quantitatively with HNO_2 to yield nitrogen and hydroxyl acids. The generated N_2 is measured by the volume while its level is directly proportional to the amount of amino acids.



Different from α-NH_2, the ε-NH_2 has a weak reaction with HNO_2. The α-NH_2 of Pro does not react with HNO_2. Other amino acids such as Arg, His, and Trp do not interact with HNO_2 as their side chains form a cyclic structure with the nitrogen atom when being treated with HNO_2.

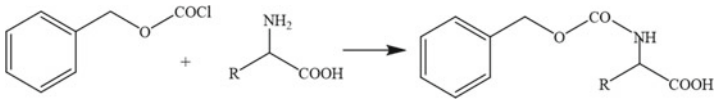
(2) Reaction with aldehydes

The α-NH_2 of amino acids reacts with an aldehyde compound to yield a product known as Schiff-base compound, which is an intermediate product of a non-enzymatic browning reaction (i.e. the Maillard reaction).



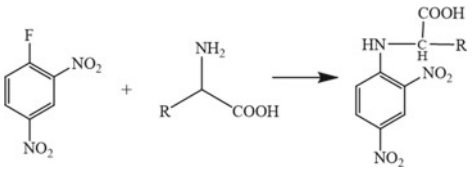
(3) Acylation

Acylation of amino acids (α-NH_2 group) by benzyloxyformyl chloride under alkaline conditions is utilized in the synthesis of peptides.



(4) Alkylation

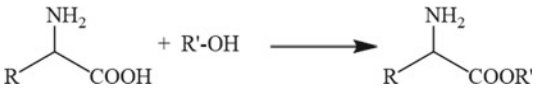
Derivatization of amino acids (ε-NH_2 group) by dinitrofluorobenzene yields stable yellow compounds, which can be used to label N-terminal amino acid residues and free ε-NH_2 groups present in peptides and proteins.



3.1.3.2 The Reactions of Carboxyl Groups

(1) Esterification

Methyl or ethyl esters of amino acid can be obtained in anhydrous methanol or ethanol in the presence of dry HCl.



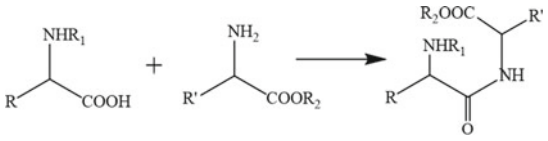
(2) Decarboxylation

Escherichia coli over-expressing the glutamate decarboxylase can catalyze decarboxylation of glutamic acid, which can be used for the analysis of glutamic acid.

3.1.3.3 The Reactions Involving Both Amino and Carboxyl Groups

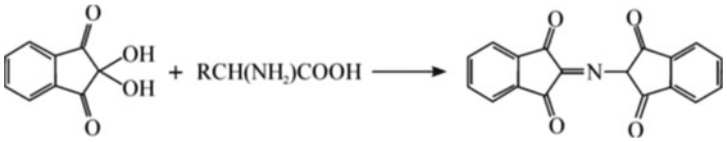
(1) Forming peptide bonds

The connecting reaction between the carboxyl group of amino acid and the amino group of another amino acid leads to covalently linking of the two amino acids via an amide bond (also known as a peptide bond), which is the basis of protein synthesis.



(2) Reaction with ninhydrin

Under weak alkaline conditions, ninhydrin can react with amino acids, forming a final purple product known as Ruhemann's purple, which has a maximum absorbance at 570 nm. This reaction can be used to quantify amino acids as well as proteins. Pro is an exception as it has no α-amino group, and its reaction product has a maximum absorbance at 440 nm and thereby gives yellow color.



3.1.3.4 Reaction of the Side Chain Groups

The side chain group (R group) influences the chemical properties of an α-amino acid. A functional group in the side chain will add many reactions that the α-amino acid can take part in. Phenolic reaction is one of the most popular assays for side chain group of amino acids. The principle of the Folin-phenol assay is the reduction of the Folin-phenol reagent in the presence of phenolic compounds, which results in the production of molybdenum–tungsten blue. Besides the phenolic reaction, those having chemical groups that form bonds with sulfhydryls (–SH) are the most common reactions for proteins. Sulfhydryls, also called thiols, exist in proteins in the side chain of cysteine amino acids. Sulfhydryl groups are often transformed into disulfide bonds (–S–S–) within or between polypeptide chains in the presence of an oxidizing agent. Typically, –S–S– group is reversible to reduced sulfhydryl groups (–SH) in the presence of a reducing agent. This conversion has an important influence on the functional properties of proteins.

$$—SH + —SH \rightarrow —S—S—$$

In the identification of amino acids or proteins, some side chain groups show important chemical reactions, which are listed in Table 3.5.

3.1.4 Preparation of Amino Acids

(1) Protein hydrolysis

Amino acids can be produced by acidic, basic, or a series of enzymatic hydrolysis of native proteins. Separation of single amino acid from a mixture can be performed by

Table 3.5 Several important color reactions of amino acids and proteins

Reaction	Reagent	Amino Acid/Group/Bond	Color
Millons's reaction	Mercuric nitrate, mercurous nitrate	Phenol group (Tyr)	Red
Xanthoproteic reaction	Concentrated nitric acid	Benzene ring/Tyr, Trp	Yellow but orange with alkaline addition
Hopkins–Cole reaction	Glyoxylic acid	Trp/Indole ring	Purple
Ninhydrin reaction	Hydrated ninhydrin	α-, ε-amino groups	Blue or purple
Ehrlich reaction	p-dimethylaminobenzaldehyde	Indole ring (Trp)	Blue
Sakaguchi reaction	α-naphthol, sodium hypochlorite	Arg	Intense red
Sullivan reaction	1,2-naphthoquinone 4-sulfonate, sodium sulfite, sodium thiosulfate, sodium cyanide	Cystine, Cys	Red

isoelectric precipitation and crystallization. A combination of two or more of these methods is often needed to get pure compounds. However, strong acid or alkaline catalyzed destroys some amino acids in proteins such as Trp, Asn, and Glu.

(2) Chemical synthesis

Chemical synthesis leads to the production of both L- and D-amino acids. However, pharmaceutical and medical applications often need chiral pure L- or D-amino acids. Several enzymatic processes thus are used to convert the D/L-racemate into the pure isomer. The best known and industrial operated process is the enzymatic conversion of D/L-Met and Trp.

(3) Microbial fermentation

Amino acids can be produced via microorganism fermentation by utilizing inexpensive by-products as hydrocarbon sources. In fact, microbiological method has been successively employed to prepare Glu and Lys. This method is a promising way in the future.

3.2 Proteins and Peptides

3.2.1 Protein Structures

Proteins are macromolecules composing amino acids as structural units. The rotation of the peptide bonds in protein molecules leads to different protein configuration or conformation. Therefore, the spatial structures of proteins are very complicated and usually are described at the three structural levels.

(1) Primary structure

The primary structure of a protein denotes the linear sequence in which the constituent amino acids are linked via peptide bonds, listing the amino acids starting at the amino-terminal end through to the carboxyl-terminal end. The primary structure of several proteins has already been determined, such as insulin, hemoglobin, cytochrome C. Milk proteins like α-a_{S1}-, α-a_{S2}-, and β-$_{A2}$-caseins are also known to us. The total number of amino acid residues in the sequences varies; for example, a few proteins are illustrated by several dozens of amino acid residues, whereas a majority of proteins contain as many as several hundred (100–500) residues. Some uncommon proteins may have amino acid residues of up to thousands. However, the primary structures of some proteins are not yet fully determined.

In general, primary structures of proteins to a large extent determine their basic properties. Interactions between atoms of the molecular backbone (primary structure) have an influence on the next local folded structures such as secondary and tertiary structures. It is theoretically possible to create vast amino acid sequences as the proteins are built from a set of amino acids. For example, when a protein is constructed from 100 amino acid residues by all 20 amino acids, the possible sequences of the protein might statistically be up to 20^{100} (equally to 10^{130}). However, only about, 10^4–10^5 proteins are synthesized in nature, while only thousand proteins among these proteins have been isolated and characterized.

(2) Secondary structure

Secondary structure refers to the periodic spatial arrangement of amino acid residues of protein molecules at certain segments of the polypeptide chain. In proteins, three types of helical structures, namely α-helix, π-helix, and γ-helix are found, while the α-helix is the major form. Another common structural feature found in proteins is extended sheet-like structure, which mainly includes β-strand and β-bend. In addition, random coil structure is also a dominant structure for some proteins, which is characterized by the absence of symmetrical axis or symmetrical surfaces. Hydrogen bonds play an important role in conformational stability in the secondary structure of proteins.

The α-helical structure (right-handed α-helix) is an ordered and the most stable conformation in proteins. Each helical rotation involves 3.6 amino acid residues, with each residue extending the axial length by 0.15 nm. The axial length occupied per

rotation is 0.54 nm. The apparent diameter of the helix is 0.6 nm. In this structure, each backbone N–H group in the polypeptide chain is hydrogen-bonded to the C=O group of its neighboring loop. Therefore, the hydrogen orientation of the α-helix is the same as the dipole orientation. Pro is an exception due to its two attributes that hinder α-helix formation. On one hand, ring structure formed by propyl side chain to the amino group restricts the rotation of N–C_α. On the other hand, there is no hydrogen available at the nitrogen atom. Thus, the peptide chains rich in Pro cannot form α-helix; alternatively, they tend to assume a random coil structure. One example is β-casein, in which about 17% of total amino acids are Pro. Therefore, β-casein shows a disordered or random structure.

The β-sheet structure is a structural feature in a cylindrical or nearly cylindrical shape and is a more extended form than the α-helical structure. Conversion from α-helix to β-sheet is usually observed when α-helix type proteins are heated. In this β-sheet configuration, the polypeptide chains interact with each other through hydrogen bonding, involving the backbone carbonyl group and amide proton on neighboring strands. In general, depending on N → C directional orientations of the strands, two forms of β-sheet structure are found in proteins, namely parallel β-sheet and anti-parallel β-sheet. Neighboring strands run in the same orientation from the N- to C- terminal ends in parallel β-sheet structures, whereas an anti-parallel β-sheet may alternate the orientation of N-terminal end in the peptide chain. In the two forms, the side chains of successive amino acid residues in a strand alternately locate themselves above and below the plane sheet.

Another common secondary structure found in proteins is the β-bend or β-turn, which can be regarded as a special helix form with “zero distance”. This structure confers polypeptide chain folding back on itself, and the stability of the bend is maintained via hydrogen bonds.

(3) Tertiary structure

The tertiary structure of a protein refers to the spatial arrangement attained when a polypeptide chain with a secondary structure is further folded by various forces to form a compact three-dimensional form. From an energetics viewpoint, the formation of tertiary structure involves the optimization of various interactions such as hydrogen bonds, electrostatic interactions, disulfide bonds, and van der Waals forces between various groups in protein. In most globular proteins, the R groups of the polar amino acids are generally located on the water accessible surface, while the R groups of the non-polar amino acids are inevitably buried in the interior of proteins. However, the non-polar amino acids of certain lipoproteins mainly attach to the molecular surface of the protein particles.

(4) Quaternary structure

Quaternary structure refers to the spatial arrangement of proteins with two or more polypeptide chains in a special way. This arrangement may lead to the formation of biological properties. The peptide chains of these quaternary complexes are generally referred to as subunits, which are the same or different and have their own primary,

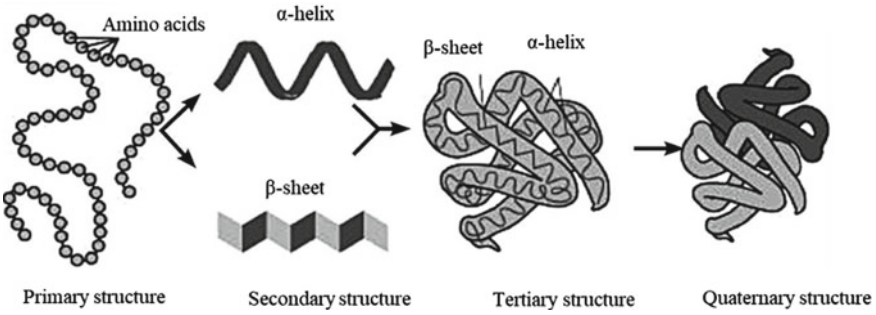


Fig. 3.3 Schematic diagram of structural levels of proteins

secondary, and tertiary structures. The interaction between peptide chains is primarily dominated by non-covalent interactions such as hydrogen bonding and hydrophobic interaction. When the hydrophobic amino acid content of a protein is more than 30%, it exhibits a greater tendency to form an oligomeric structure than those contain fewer hydrophobic amino acid residues.

The levels of protein structure are represented in Fig. 3.3.

3.2.2 The Forces Involved in the Structural Stability of Proteins

As mentioned earlier, the secondary structure of the proteins is primarily maintained via hydrogen bonds between various amino acid residues. Hydrogen bond, electrostatic interactions, hydrophobic interaction, and van der Waals interaction make contribution to protein folding of the tertiary and quaternary structures. The characteristics of the forces involved in the structural stability of proteins are given in Table 3.6. The strong driving force (bond energy) involved in the structural stability of observed proteins is only the covalent bonds, mainly disulfide bonds, whereas other forces have lower bond energy than covalent bonds. Any change in the environment of the proteins certainly would influence their folding pattern. When major structural changes appear in the secondary, tertiary, and quaternary structures, protein denaturation occurs.

The forces involved in structural stability of proteins can also be depicted in Fig. 3.4.

Table 3.6 Forces involved in structural stability of proteins

Type	Bond energy (kJ/mol)	Interaction distance (nm)	Functional groups from side chains	Reagents/conditions for weakening forces	Force enhancement
Covalent bonds	330–380	0.1–0.2	Disulfide moiety	Cysteine, Na_2SO_3, CH_3CH_2SH, etc.	–
Hydrogen bonds	8–40	0.2–0.3	Amide, carboxyl, and phenolic groups	Guanidine, urea, detergent, acid, heating	Cooling
Hydrophobic interaction	4–12	0.3–0.5	Aliphatic and aromatic side chains	Organic solvents, surfactants	Heating
Electrostatic interaction	42–84	0.2–0.3	Carboxyl and amino groups	High or low pH, salt solution	–
van der Waals interaction	1–9	–	Permanent, induced, and instantaneous dipples	–	–

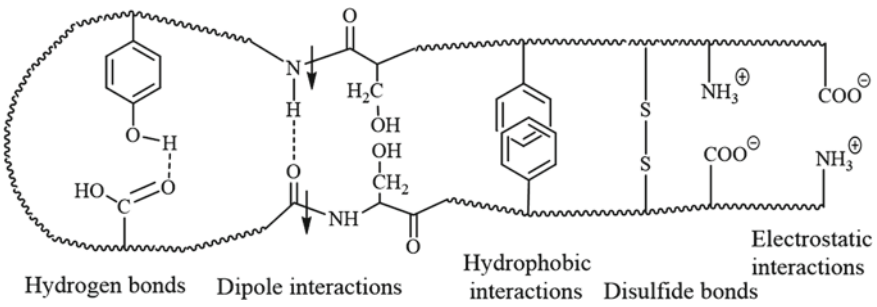


Fig. 3.4 Schematic diagram of the forces involved in structural stability of proteins

3.2.3 Protein Classification

There are a number of ways of classifying proteins. Generally, proteins can be classified into three categories based on their chemical compositions, namely simple, conjugated, and derived proteins. Proteins also can be classified according to their solubility as water-soluble, salt-soluble, alkaline (acid)-soluble, and alcohol-soluble proteins. The solubility characteristics and major sources of various common proteins are shown in Table 3.7, and the related factors affecting protein solubility can be referred to in the relevant textbooks.

Table 3.7 Protein classification and typical proteins

Proteins		Characteristics	Presence	Typical examples
Simple proteins	Albumins	Soluble in water, diluted salt, acid/alkaline solutions, and precipitated by saturated ammonium sulfate as well as heat coagulation	Animal cells and body fluids	Albumin, α-lactalbumin, ovalbumin
	Globulins	Soluble in dilute salt, acid/alkaline solutions but insoluble in pure water, and precipitated in half-saturated ammonium sulfate, also almost coagulation when heated	Animal cells and body fluids	Serum globulin, β-lactoglobulin, glycinin, myosin, lysozyme
	Glutenins	Soluble in dilute acid/alkaline solutions but not in pure water, ethanol, and neutral salt solution	Plant seeds	Gluten, glutenin
	Prolamines	Soluble in dilute acid/alkaline and 66–80% ethanol–water solution, but insoluble in pure water and salt solution. Contain large amounts of Pro and Glu but only small amounts of Lys	Plant seeds	Gliadin, zein
	Scleroproteins	Usually insoluble in water, salt, diluted acid/alkaline solution, resistance to protease	Animal tissues	Collagen, elastin, keratin

(continued)

Table 3.7 (continued)

Proteins		Characteristics	Presence	Typical examples
	Histones	Soluble in pure water but insoluble in ammonia, and precipitated in aqueous phosphotungstic acid solution (acid or neutral pH)	Animal cells	Thymus histone, erythrocyte histone, nuclear protein
	Protamines	Soluble in dilute acid and pure water but insoluble in ammonia, and precipitated in aqueous phosphotungstic acid solution (acid or neutral pH). Contain large amounts of the Arg	Mature germ cells	Fish protamine
Conjugated proteins	Nucleoproteins	Nucleic acid such as ribonucleic acid and deoxyribonucleic acid	Animal and plant cells	Thymus histone, viral protein
	Phosphoproteins	Containing phosphate group and can be attacked by phosphatase	Animal cells and body fluids	Casein, egg yolk phosphoprotein
	Metalloproteins	Containing metals like iron and copper, also having some pigments	Plant/animal cells and their body	Hemoglobin, myoglobin, cytochrome c, catalase
	Glycoprotein	Containing saccharide groups	Animal cells	Serum glycoprotein, ovomucoid
Derived proteins	Primary derivatives	Initially denatured protein; acid- or base-denatured protein		Rennet-coagulated casein
	Secondary derivatives	Decomposition products of proteins with significant property changes		Peptides

3.2.4 Physical and Chemical Properties of Proteins

3.2.4.1 Acid–Base Properties of Proteins

As we know, proteins contain ionizable amino and carboxyl groups in N- and C-terminal ends, respectively, that is, proteins can be considered as ampholytes. In addition, the side chains of proteins also may have ionizable groups. Proteins should exist as polyvalent ions. The dissociation and the resulted total charge of a protein are depending on these ionizable groups as well as the pH value of the solution. Typically, a protein carries a charge of zero at a certain pH known as isoelectric point (pI). At its isoelectric pH (pI), a protein does not possess any charge and thus will not move in an applied electric field and exhibits the lowest solubility. When the pH > pI, a protein holding a net negative charge moves toward the anode. Similarly, when the pH < pI, a protein has a net positive charge and thereby moves toward the cathode.

3.2.4.2 Protein Hydrolysis

Under the action of acid/alkaline/enzyme, proteins receive a cleavage in peptide bonds, resulting in the formation of a serial of intermediate products with various chain lengths such as poly-peptides, oligo-peptides, and di-peptides. Surely, the final products are amino acids.

Alkaline hydrolysis can damage Cys and Arg, while acid hydrolysis can destroy Trp. Alkaline hydrolysis also induces racemization of amino acids. However, enzymatic hydrolysis can avoid amino acid loss, due to its mild reaction conditions. One protease is unable to convert proteins into free amino acids. In general, several proteases are used. In addition, the enzymatic hydrolysis rate is slower than the chemical hydrolysis.

3.2.4.3 Color Reaction

Biuret reaction is an important reaction used to detect the presence of proteins; however, this reaction is not specific for proteins. In an alkaline environment, the nitrogen of the peptide bonds can donate its lone pair of electrons to Cu^{2+}, forming a stable and violet-colored complex (Fig. 3.5). At least two peptide linkages must be present due to the coordination of Cu^{2+} with –CONH– group. This means that di-peptides and free amino acids are unable to give the test. In a neutral environment, protein or polypeptides and ninhydrin reagent give blue or purple color. Of course, the ninhydrin reagent can also react with ammonium salt and amino acids.

In addition, some color reactions are based on specific reactions of certain amino acids. An example of these reactions is the presence of phenolic groups of Tyr or thiol

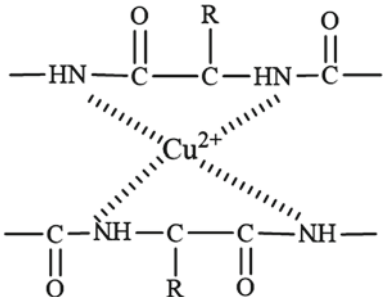


Fig. 3.5 Formation of protein–Cu^{2+}complex by the so-called biuret reaction

groups of His. These reactions can also be used in the qualitative and quantitative analyses of proteins.

3.2.4.4 Hydrophobicity

Proteins also have their hydrophobicity. Hydrophobicity of a protein can be represented by averaging the hydrophobicity of each amino acid, if its amino acid composition is known. That is, the hydrophobicity values of proteins can be determined by dividing the sum of the hydrophobicity values of amino acids by the number of amino acid residues.

$$\Delta \bar{G}^o = \frac{\Sigma \Delta G^o}{n}$$

Hydrophobicity of the proteins is one of the major factors governing physicochemical properties of proteins such as structure, interfacial, and fat-binding properties, and others. The hydrophobicity can be used to reflect the interaction between protein and water or other chemicals. The relationship between hydrophobicity, surface tension, and emulsifying activity index of several proteins is shown in Fig. 3.6.

3.2.5 Peptides

Peptides are short chains of amino acid monomers linked via amide (peptide) bonds. Peptides have lower molecular weights than proteins. Peptides are distinguished depending on the length of peptide chain that formed. The shortest peptide, consisting of two amino acids, is called di-peptides, followed by tri-peptides, tetra-peptides, and so on. On the other hand, a polypeptide chain consists of less than 10 amino acid residues usually called oligo-peptide. Likewise, poly-peptide is the general term for the peptides containing 10 or more amino acid residues. Therefore, the properties of peptides are different from amino acids or proteins. The amount of peptides in foods is very lower than proteins, and their functional properties are usually ignored.

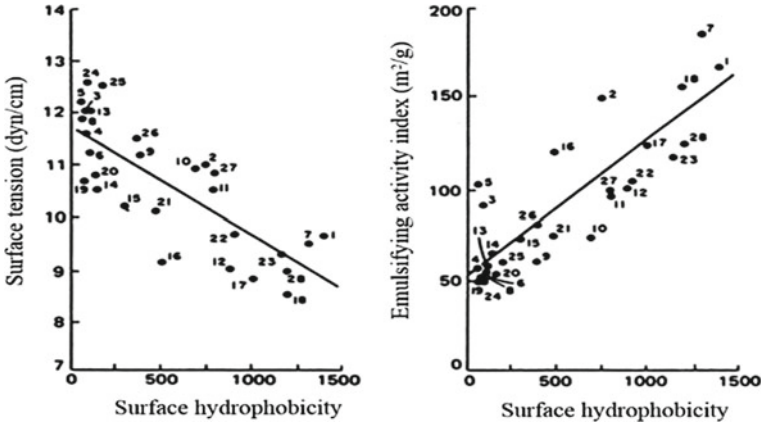


Fig. 3.6 Relationship between surface hydrophobicity. of proteins and the interfacial property of the emulsions prepared by corn oil and proteins (0.2%, w/w)

3.2.5.1 Physiochemical Properties of Peptides

(1) Dissociation

Peptides like amino acids and proteins have their dissociable functional groups. pK values and isoelectric points of peptides can be determined. The dissociation of peptides is also influenced by peptide composition and the aqueous environment they are exposed such as pH and ionic strengths. However, the dissociation of peptides is not as intensive explored as that of proteins or amino acids.

(2) Solubility, viscosity, and osmotic pressure of peptides

Peptides with small molecular weights generally have high solubility, which also shows small changes at various pH values. In comparison with proteins, peptides remain soluble at higher concentrations and over a wide pH range, making them ideal for the processing of some acidic foods. In addition, small peptides have similar behavior than amino acids in trichloroacetic acid (commonly used in protein precipitation); that is, small peptides are soluble in 3% or more of trichloroacetic acid solution, while large peptides are precipitated by 3% trichloroacetic acid.

The viscosity of peptide solution is significantly lower than that of protein solution. Peptides do not facilitate to form gels. The osmotic pressure of the peptide solution is lower than that of the free amino acid and thereby is beneficial for gastrointestinal absorption. Some small peptides have even better absorption than free amino acids, which is important to food nutrition.

(3) Chemical properties of peptides

The chemical properties of peptides are generally like those of proteins and amino acids, and thus can undergo those reactions that most amino acids carry out. The

biuret reaction can also be used for quantitative determinations of peptides. It should be noted that this reaction cannot be used to distinguish polypeptide and proteins.

3.2.5.2 Bioactive Peptides

Bioactive peptides play vital roles in human health and nutrition. The tetra- and tri-peptide gastrins are the most favorite bioactive peptides to promote gastric acid secretion. Glutathione takes part in redox reaction and can scavenge free radicals and hydrogen peroxide to exert protective functions in the body.

$$\mathrm{ROOH} + 2\,\mathrm{GSH} \xrightarrow{\text{Enzyme}} \mathrm{ROH} + \mathrm{GSSG} + \mathrm{H_2O}$$

$$\mathrm{H_2O_2} + 2\,\mathrm{GSH} \xrightarrow{\text{Enzyme}} \mathrm{GSSG} + 2\,\mathrm{H_2O}$$

Some bioactive peptides can be obtained by selective degradation of native proteins, e.g. enzymatic hydrolysis. The explored physiological functions of the polypeptides are summarized below.

(1) Mineral binding peptides enhance mineral absorption in human. Caseinphosphopeptide is derived during casein hydrolysis with trypsin. Caseinphosphopeptide contains a plurality of phosphoserines, which enable its calcium-binding activities. Calcium and caseinphosphopeptide form soluble complexes to allow calcium solubility or prevent the formation of insoluble calcium phosphate. The enhancement of the intestinal calcium paracellular absorption is thus observed.

(2) Certain hydrolysates from the native proteins show anti-hypertensive effect. These peptides from soybean and casein have been found to have significant anti-hypertensive effect and thus are referred to anti-hypertensive peptides. Inhibition on angiotensin-converting enzyme by these peptides leads to anti-hypertension.

(3) Several peptides also show immuno-modulatory effects and can stimulate the proliferation of human immune lymphocytes and enhance the phagocytic ability of macrophages. These peptides can promote pathogen resistance in both humans and animals.

(4) Several peptides also have anti-microbial action. Nisin is an anti-microbial peptide produced by the subsp. *Lactis* in *Lactococcus lactis*. Nisin consists of 34 amino acids and has strong inhibition on many of the Gram-positive bacteria. Hence, Nisin is not as resistant as common antibiotics due to its rapid degradation by chymotrypsin in the digestive tract.

(5) Other bioactive peptides. The ratio of the branched-chain amino acids to the aromatic amino acids in mixtures of the amino acids or oligo-peptides is expressed as Fischer ratio. Peptides with high Fischer ratio, useful for patients with liver diseases, are also explored for nutritional and therapeutic applications.

3.3 Protein Denaturation

Protein molecules are formed by amino acids that are linked together in specific orders and balanced by intra-molecular and inter-molecular forces. Finally, they form spatial structures, namely primary, secondary, tertiary, and quaternary structures. Hence, protein conformation is the result of many actions. However, this conformation is unstable and there will be some changes to varying extents in the secondary, tertiary, and quaternary structures of proteins under acidic, alkaline, heat, organic solvent, or radiation treatments, in which the process is called protein denaturation. Therefore, protein denaturation does not involve changes in the sequences of amino acid connection, namely the primary structures of proteins.

Protein denaturation can affect the structures, physiochemical, and biological properties of proteins, generally including:

(1) Exposure of the hydrophobic groups inside protein molecules; protein solubility thus decreases in water;
(2) Loss of certain bioactivity of proteins such as the loss of enzyme or immune activity;
(3) More peptide bonds of proteins are exposed, which are, therefore, more susceptible to hydrolysis by proteases;
(4) Changes in the water-binding capacity of proteins;
(5) Changes in the viscosity of protein dispersion system;
(6) Loss of crystallization capacity of proteins.

The extent of protein denaturation can be evaluated by measuring the changes in some properties of proteins such as optical, sedimentation, viscosity, electrophoretic, thermodynamic properties, etc. In addition, protein denaturation can also be studied by immunological methods, e.g. via the well-known enzyme-linked immunosorbent assay (ELISA).

The denaturation of native proteins is sometimes reversible. When denaturation factors are removed, proteins will return to their native structures, namely protein renaturation. In general, proteins are more likely to undergo reversible denaturation under mild conditions, while irreversible denaturation will occur under those severe conditions. When disulfide bonds that stabilize protein conformation are destroyed, it is difficult for denatured proteins to renaturate.

The factors that cause protein denaturation include physical (e.g. temperature, pH), chemical, chemical reagent, mechanical processing, etc. No matter what factor causes protein denaturation, from the viewpoint of proteins themselves, protein degeneration is analogous to a physical change process, which does not involve any chemical reaction.

3.3.1 Physical Denaturation

(1) Heating treatment

Heating treatment is a commonly used process during food processing, which is the most common factor responsible for protein degeneration. Proteins undergo drastic changes at a certain temperature known as denaturation temperature. Protein denaturation with thermal treatment leads to a considerable extension of protein deformation. For instance, native serum protein is ellipsoidal (length: width ratio of 3:1). After thermal denaturation, this ratio is increased to 5.5:1. The molecular shape of the protein is significantly extended.

For the chemical reactions, temperature coefficients are usually in a range of 3–4. However, for protein denaturation with thermal treatment, the temperature coefficients may be up to 600. This property is important for food processing. High-temperature instantaneous sterilization and ultra-high temperature sterilization technology thus employ high temperature to greatly enhance the speed of protein denaturation and inactivation of bioactive proteins or microorganisms in a short time, whereas there is a slight increase in chemical reaction speed in terms of other nutrients to ensure less loss of nutrients.

Thermal denaturation of proteins is related to protein compositions, concentrations, water activity, pH, and ionic strengths. When proteins have more hydrophobic amino acids, they are more stable than those with more hydrophilic amino acids. Thus, bioactive proteins are stable in dry state and show strong resistance to temperature change; however, they are liable to denaturation in the moist and thermal state.

(2) Freezing treatment

Low-temperature treatment can also cause denaturation of some proteins. L-Threonine cystinase is stable at room temperature, but unstable at 0 °C. Soybean 11S proteins and milk protein will aggregate and precipitate when they are cooled or frozen. Some enzymes (e.g. oxidases) can be activated at relatively low temperatures.

Low-temperature protein denaturation may be caused by changes in hydration environment of proteins. The force balance to maintain protein structures is destroyed, and hydration layer of some chemical groups is also destroyed. The interaction between these groups may cause protein aggregation or subunit rearrangement. It is also possible that the salt effect of the frozen system induces protein denaturation. In addition, concentration increase caused by freezing may lead to the increased exchange reactions of intra-molecular and inter-molecular disulfide bonds in proteins, thus leading to protein denaturation.

(3) Mechanical treatment

Due to the shearing force, both kneading and whipping can extend protein molecules and destroy α-spiral structure, which then leads to protein denaturation.

The greater the shearing rate, the greater the degree of protein denaturation. For example, when a 10–20% whey protein solution at pH 3.5–4.5 and 80–120 °C is

subjected to a shearing rate of 8,000–10,000 s^{-1}, it forms a protein-based fat substitute. Mechanical denaturation of proteins is also used in the production of salad dressing and ice cream.

(4) Static high-pressure treatment

High static pressure treatment can also lead to protein denaturation. Although native proteins have relatively stable conformation, spherical protein molecules are not rigid. Some void spaces show certain flexibility and compressibility, invariably inside the protein molecules. The protein molecules, therefore, will be deformed (i.e. denaturation) under high pressure. At normal temperatures, denaturation of proteins occurs at pressures ranging from 100 to 1,000 MPa. Sometimes, high pressure can lead to protein denaturation or enzyme inactivation, which is restored when the high pressure is removed.

High static pressure treatment neither causes the destruction of nutrients, color, and flavor of foods nor form harmful compounds. High-pressure treatment of meat products can lead to lysis of muscle fibers in muscle tissues, thereby resulting in improved quality for meat products.

(5) Electromagnetic radiation

The effect of electromagnetic waves on protein structure is related to the used wavelength and energy of electromagnetic wave. Visible light has little effect on protein conformation due to its long wavelength and low energy. Using high-energy electromagnetic wave such as UV, X-ray, and γ-ray can affect protein conformation. High-energy rays absorbed by aromatic amino acids can induce conformational changes of proteins and even chemical changes of amino acid residues such as destruction of covalent bonds, ionization, and free radicalization. Therefore, high-energy rays can not only lead to protein denaturation but also may affect the nutritional values of proteins.

Radiation preservation of foods has little effect on proteins as the used radiation dosage is relatively low. On the other hand, the dissociation of water can reduce the dissociation of other substances in foods.

(6) Interfacial effect

Irreversible protein degeneration occurs when proteins are adsorbed at gas–liquid, liquid–solid, or liquid–liquid interfaces. The water molecules at the gas–liquid interface have higher energy than those molecules in bulk solution and can interact with protein molecules, which gives rise to the increase in molecular energy of proteins. When some chemical effects (bonds) in protein molecules are broken, the structure stretches a little. Finally, water molecules enter internal protein molecules, which further leads to the extension of protein molecules. The hydrophobic and hydrophilic residues of proteins, respectively, thus arrange to the different polarity of two phases (air/oil and water), which eventually lead to protein denaturation.

Protein molecules have porous structures, which can be easily absorbed in the interface. If protein structure is tight, stabilized by disulfide bonds, or absence of

obvious hydrophobic and hydrophilic regions, proteins cannot be absorbed in the interface easily, so interfacial protein denaturation is difficult.

3.3.2 Chemical Denaturation

(1) Acidic and alkaline factors (pH)

Most proteins are stable within a specific pH range. However, if they are in extreme pH conditions, dissociation of these dissociable groups within protein molecules such as amino and carboxyl groups will occur and thereby generating strong intra-molecular electrostatic interactions, which then lead to stretching and denaturation of protein. If accompanied by heating treatment, the denaturation rate of proteins will be even higher. In some cases, however, proteins (e.g. enzymes) can be restored to their native structures, when pH is adjusted to its original range after acid–base treatment.

Proteins are more stable at their isoelectric points than at any other pH value. At neutral pH, proteins do not have too much net charge and intra-molecular electrostatic repulsive force is relatively small, most proteins are, therefore, relatively stable in the neutral condition.

(2) Salts

Metal ions such as $Ca^{2+}$and Mg^{2+} may be the constituent parts of proteins and play an important role in protein conformation. Therefore, the removal of Ca^{2+} and Mg^{2+} will reduce the stability of protein molecules toward heat and enzyme treatments. It is easy for Cu^{2+}, Fe^{2+}, Hg^{2+}, Pb^{2+}, and Ag^{2+} to interact with the -SH groups of protein molecules or to convert disulfide bonds into -SH groups, which changes the force that stabilizes protein structure and thereby leads to protein denaturation. Hg^{2+} and Pb^{2+} can react with the His and Trp residues of proteins and bring protein denaturation.

For various anions, their influences on the structural stability of proteins follow the series: $F^- < SO_4{}^{2-} < Cl^- < Br^- < I^- < ClO_4{}^- < SCN^- < Cl_3CCOO^-$. At high concentration, anions have stronger impact on protein structure than cations. Generally, chloride ions, fluorine ions, and sulfate ions are structure stabilizers of proteins, whereas thiocyanate and trichloroacetic acid are structure destabilizers of proteins.

(3) Organic solvents

Most organic solvents can bring about protein denaturation as they reduce the dielectric constant of solution and increase intra-molecular electrostatic force of protein molecules. Or else, they may destroy or increase hydrogen bonds within protein molecules and change the original force that stabilizes protein conformation. In addition, they can enter into the hydrophobic regions of proteins and destroy the

hydrophobic interaction of protein molecules. They may give rise to the changed protein structure and denaturation.

At low concentrations, organic solvents have little effect on protein structure, and some of them even have stabilizing effects. However, at high concentrations, all organic solvents can cause denaturation of proteins.

(4) Organic compounds

High concentrations of urea and guanidine salts (4–8 mol/L) will break down the hydrogen bonds in protein molecules, leading to protein denaturation. Surfactants such as sodium dodecyl sulfonate can destroy the hydrophobic regions of proteins and promote the stretching of protein molecules and therefore are powerful denaturing agents.

(5) Reducing agents

With -SH groups, mercaptoethanol ($HSCH_2CH_2OH$), cysteine, and dithiothreonol can reduce disulfide bonds existing in protein molecules and thus change the original conformation of proteins, resulting in irreversible degeneration of proteins.

$$HSCH_2CH_2OH + —S—S—Pr \rightarrow —S—SCH_2CH_2OH + HS—Pr$$

For food processing, protein denaturation is generally favorable, but in some cases such as enzyme separation and milk concentration, it should be avoided. At this time, excessive denaturation of proteins will lead to enzyme inactivation or precipitation formation, which is an undesirable change.

3.4 Functional Properties of Proteins

The functional properties of proteins are these physiochemical properties beneficial to the characteristics of foods, apart from their nutritional values, such as gelation, solubility, foamability, emulsification, viscosity, etc. The functional properties of proteins affect the sensory quality of foods, especially in terms of texture, and they also play an important role in the physical characteristics of food products, food processing, or storage, which can generally be divided into three categories.

(1) Hydration properties, which depend on the interaction between proteins and water, including water adsorption and retention, wettability, swelling, adhesion, dispersibility, and solubility.
(2) Structural properties (interaction between protein molecules) such as precipitation, gelation, texturization, dough formation, etc.
(3) Surface properties, involving the role of proteins in two phases with different polarity, mainly including foaming and emulsification.

According to some functions of proteins in food sensory quality, the fourth property, sensory property, can be classified, which involves the turbidity, color, flavor binding, chewiness, and smoothness of proteins in foods.

Functional properties of proteins are not only independent and completely different from each other but also related to each other. For example, gelation involves both the interaction between protein molecules (formation of three-dimensional spatial network structure) and the interaction between protein molecules and water molecules (water retention). Viscosity and solubility of proteins are also related to the interaction between proteins and water.

The functional properties of proteins are the results of synergistic action of many related factors. The physiochemical properties (molecular sizes, shapes, chemical compositions, and structures) of proteins as well as many external factors have an impact on the functional properties of proteins. Overall, the factors affecting the functional properties of proteins can be divided into three aspects: (1) the inherent properties of proteins, (2) environmental conditions, and (3) food processing.

In general, certain functional property of protein is not the result of certain physicochemical property, so it is difficult to explain how the physicochemical property of proteins plays a role in the functional properties. However, some physicochemical constants of proteins are well correlated with other functional properties (Table 3.8).

Proteins are not only important nutritional components but also their functional properties are incomparable and indispensable, compared with some other food components, and are crucial for the quality of some foods. The functional properties of proteins required in common foods are shown in Table 3.9.

Table 3.8 Contribution of hydrophobicity, charge density, and structure of proteins to functional properties

Functional properties	Hydrophobicity	Charge density	Structure
Solubility	No contribution	With a contribution	No contribution
Emulsification	Contribution by surface hydrophobicity	Generally, no contribution	With a contribution
Foaming	Contribution by total hydrophobicity	No contribution	With a contribution
Fat binding	Contribution by surface hydrophobicity	Generally, no contribution	No contribution
Water binding	No contribution	With a contribution	In doubt
Thermal coagulation	Contribution by total hydrophobicity	No contribution	With a contribution
Dough formation	Slight contribution	No contribution	With a contribution

Table 3.9 Functional properties of proteins required in various foods

Foods	Functional properties
Beverages	Solubility, thermal stability, viscosity at different pH values
Soup, sauce	Viscosity, emulsification, water binding
Dough baking products (bread, cake, etc.)	Molding and forming viscoelastic film, cohesion, thermal denaturation and gelation, emulsification, water absorption, foaming, and browning
Dairy products (cheese, ice cream, dessert, etc.)	Emulsification, fat retention, viscosity, foaming, gelation, coagulation
Egg	Foaming, gelation
Meat products (sausages, etc.)	Emulsification, gelation, cohesion, absorption, and retention of water and fat
Meat substitutes (histochemical plant proteins)	Absorption and retention of water and fat, insolubility, hardness, chewiness, cohesion, thermal denaturation
Food coating	Cohesion, adhesion
Confectionery (milk chocolate, etc.)	Dispersion, emulsification

3.4.1 Protein Hydration

Most foods are hydrated systems. Physiochemical and rheological properties of each component are not only affected by water but also by water activity. Protein conformation is largely related to the interaction between proteins and water. In addition, hydration process of proteins is also involved in the application of protein concentrate or isolate produced from different raw materials in foods. The ability of proteins to absorb and retain water affects not only the viscosity and other properties of proteins but also the quality and the quantity (directly related to the product cost) of food products. Therefore, it is very useful to study the hydration and rehydration of proteins.

Protein hydration has arisen from the interaction of various polar groups on the surface of protein molecules with water molecules. In general, about 0.3 g/g of water can bind firmly to proteins, while about 0.3 g/g of water may bind loosely to protein. Due to the different contents of amino acids, different proteins have various water-binding capacities. Polar groups of amino acids have the stronger binding capacities to water, thus ionized amino acids and protein salts have relatively higher binding capacities. The binding capacities of different amino acid residues to water are shown in Table 3.10.

Protein concentration, medium pH, temperature, ionic strength, and the presence of other components can affect protein–protein and protein–water interactions. The total water-binding amount of proteins increases with an increase in protein concentration, but proteins usually show minimal hydration at their isoelectric points.

Table 3.10 Hydration capacities of amino acid residues (mol water/mol residues)

Amino acid residue		Hydration capacity	Amino acid residue		Hydration capacity
Polar residue	Asn	2	Ionic residue	Asp	6
	Gln	2		Glu	7
	Pro	3		Tyr	7
	Ser, The	2		Arg	3
	Trp	2		His	4
	Asp (unionized)	2		Lys	4
	Glu (unionized)	2	Nonpoloar	Ala	1
	Tyr	3		Gly	1
	Arg (unionized)	4		Phe	0
	Lys (unionized)	4		Val, Ile, Leu, Met	1

After animal slaughter, the water-binding capacities of muscle tissues are the worst during rigor mortis, which is caused by the decrease of muscle pH from 6.5 to 5.0 (close to isoelectric point), resulting in the decreased tenderness and poor quality of meat products. In general, the water-binding capacities of proteins decrease with the increase of temperature, which is due to the destruction of hydrogen bonds formed between proteins and water by heating, the reduction of the interaction between proteins and water, the denaturation and aggregation of proteins during heating, the reduced surface area of proteins, as well as the reduced water-binding effectiveness of polar amino acids (see Fig. 3.7). However, the heating treatment also sometimes improves the water-binding capacities of proteins. Proteins with very compact structures can undergo subunit dissociation and molecular extension during heating treatment, which exposes some previously covered peptide bonds and polar groups to protein surface and thus improves water-binding capacity. Or else, protein gelation can occur when proteins are heated. The yielded three-dimensional network can hold a large amount of water and thereby improves the water-binding capacities of proteins. Ions present in the protein system also have an impact on the water-binding capacities of proteins, which is the result of competitive effects of water–salt–protein. In usual, low salt concentration can increase the water-binding capacities of proteins (salting-in), while high salt concentration decreases the water-binding capacities of proteins (salting-out) and may even cause protein dehydration.

For some monomer proteins, their water-binding capacities can be estimated by an empirical formula and amino acid compositions. The calculated results are in good agreement with the experimental results. For some proteins composed of multiple subunits, the calculated values are generally greater than the experimental values.

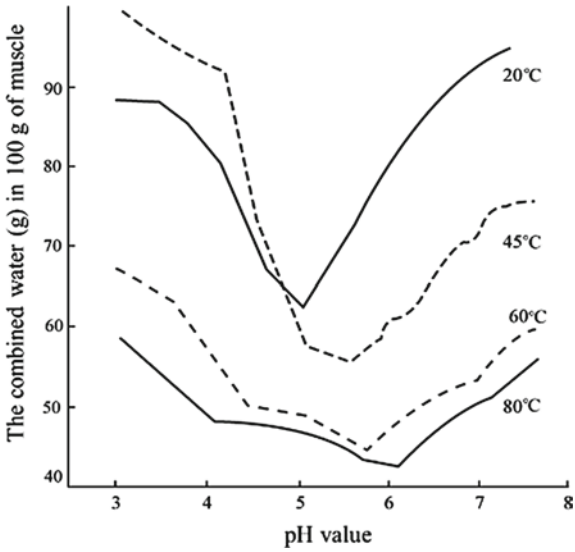


Fig. 3.7 Water-binding capacities of muscle protein at different temperatures and pH values

$$\text{Water-binding capacity (g water/g protein)} = f_C + 0.4f_P + 0.2\,f_N.$$

where, f_C, f_P, and f_N represent the percentage of charged, polar, and non-polar (i.e. hydrophobic) amino acid residues in protein molecules, respectively. It can be seen from the coefficients that the charged amino acids contribute most to water-binding capacity but the non-polar amino acids have the least influence on water-binding capacity.

Water-binding capacity plays an important role in the texture of various foods, especially meat products and dough. Other functional properties of proteins, such as the gelation and emulsification, are also related to protein hydration. During food processing, protein hydration is usually measured or reflected by water-holding capacity or water retention. Water-holding capacity refers to the ability of proteins to retain (or bind) water in their tissues. The retained water includes adsorbed water, physical retained water, and hydrodynamic water. Water-holding capacity can affect tenderness, juiciness, and softness of foods. Therefore, water-holding capacity is of great significance to food quality.

3.4.2 Protein Solubility

As organic macro-molecules, proteins exist in the dispersed (colloidal) state in water. Accordingly, proteins do not have true solubility in water. The protein amount dispersed in water is usually measured using an empirical index called protein solubility. Protein solubility is very important to protein ingredients, which determines their extraction, separation, and purification. Denaturation degree of proteins can

also be reflected by assessing their solubility changes. In addition, the application of proteins in acidic beverages is directly governed by their solubility.

The commonly used indices for protein solubility include protein dispersibility index (PDI), nitrogen solubility index (NSI), and water soluble nitrogen (WSN).

$$PDI\ (\%) = \frac{The\ weight\ of\ water\ dispersed\ protein}{The\ weight\ of\ total\ protein} \times 100$$

$$NSI\ (\%) = \frac{The\ weight\ of\ water\ soluble\ nitrogen}{The\ weight\ of\ total\ nitrogen} \times 100$$

$$WSN\ (\%) = \frac{The\ weight\ of\ water\ soluble\ nitrogen}{The\ sample\ weight} \times 100$$

Some conditions such as pH, ionic strength, temperature, and solvent type can affect protein solubility. Protein solubility is usually the lowest at a pH value near isoelectric point. When pH values are higher or lower than the isoelectric points, the net charges of proteins are negatively or positively charged, and their solubility levels thus increase (Fig. 3.8). Protein solubility is the lowest at isoelectric point, but this behavior is different between proteins. Casein and soy protein isolate are almost insoluble at their isoelectric points, while whey protein concentrate is still very soluble at isoelectric point. The proteins with solubility varying greatly with pH values can be conveniently extracted and separated by changing pH values. Proteins with solubility varying slightly with pH value need to be separated and extracted by other methods.

Salts have different impacts on protein solubility. When the concentration of neutral salt is 0.1–1 mol/L, protein solubility in water can be increased (salting-in).

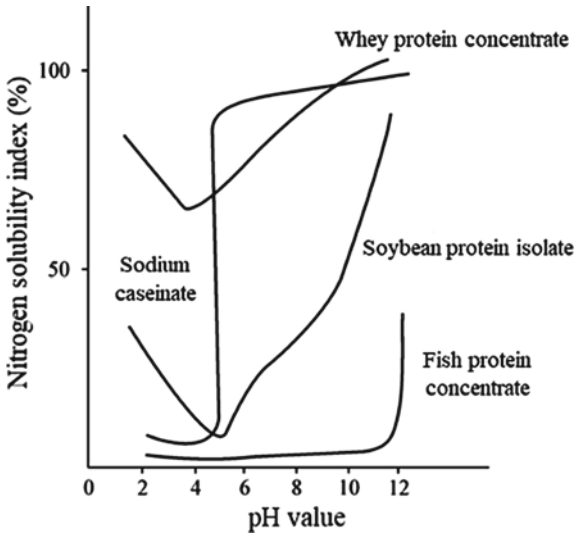


Fig. 3.8 NSI of several proteins at various pH values

When the concentration of neutral salt is greater than 1 mol/L, protein solubility in water can be reduced and even precipitation can occur (salting-out). When salting-in or salting-out happens, the mathematical relationship between solubility and salt ionic strength follows the two formulas below.

$$Salt-in: S = S_0 + k\mu^{0.5}$$
$$Salt-out: \log S = \log S_0 - k\mu$$

Due to their reduction of dielectric constant of organic solvents, electrostatic repulsion between protein molecules is weakened by organic solvents such as acetone, ethanol, etc. The attraction between protein molecules is relatively increased, so protein aggregation and even precipitation can occur. In other words, organic solvents reduce protein solubility.

Protein solubility is irreversibly decreased after heating treatment of proteins. Protein extraction and purification process may lead to a certain degree of protein insolubility, such as defatted soybean powder. Protein concentrate and isolate thus have nitrogen solubility index varying from 10 to 90%. Generally, when other conditions are fixed, protein solubility increases with temperature at the range of 0–40 °C; as the temperature is further increased, stretching and especially denaturation of protein molecules occur, and protein solubility is eventually decreased (Table 3.11).

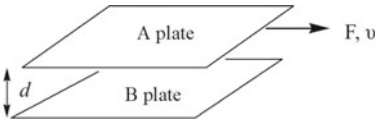
It is generally believed that proteins with higher initial solubility can rapidly dispersed in large quantities in the system, so a good dispersion system is obtained, which is conducive to the diffusion of protein molecules to the air or water–oil interface and improvement of other functional properties of proteins.

Table 3.11 The relative changes of solubility of some proteins after processing

Protein	Treatment	Solubility	Protein	Treatment	Solubility
Serum protein	Native	100	Albumin	Native	100
	Heating	27		80 °C, 15 s	91
β-lactoglobulins	Native	100		80 °C, 30 s	76
	Heating	6		80 °C, 60 s	71
Soybean protein isolate	Native	100		80 °C, 120 s	49
	100 °C, 15 s	100	Rapeseed protein isolate	Native	100
	100 °C, 30 s	92		100 °C, 15 s	57
	100 °C, 60 s	54		100 °C, 30 s	39
	100 °C, 120 s	15		100 °C, 60 s	14
				100 °C, 120 s	11

3.4.3 Viscosity

The viscosity of a fluid can be illustrated by two plates with relative movement. The plates are filled with fluid, and the relative movement of plates occurs under external force (F). If the fluid has a high viscosity, the plates will move very slowly, whereas the plates will move very fast. Therefore, viscosity is a measure of the resistance of fluid against motion. Generally, viscosity coefficient μ is used to represent the viscosity level of a kind of liquid. It is the numerical ratio of shear force (τ) and shear rate (γ), while shear rate is the ratio of movement velocity (υ) and the distance (d) of two plates with relative movement.



$$\tau = \mu \times \gamma = \mu \times \frac{v}{d}$$

Newtonian fluid (ideal fluid) has a fixed μ value. That is, μ value does not change along with the change of shear stress or rate. However, the dispersion systems composed of macro-molecules (including solution, emulsion, suspension liquid, gels, etc.) do not have the property of Newtonian fluid. The μ values of the dispersion systems will change as fluid shear rate or the stress changes, and their relationship in numerical value varies.

$$\tau = \mathrm{m} \times \gamma^{n} \text{ (m is consistency coefficient, n is flow index, } n < 1)$$

There are many factors influencing the viscosity of proteins, including sterilization, pH shift, proteolysis, and the presence of inorganic ions. The main factor influencing viscosity characteristics of protein fluid is the apparent diameters of dispersed protein molecules or particles, which varies with the following parameters: (1) the inherent characteristics of protein molecules, such as sizes, volumes, structures, charges, and protein concentrations; (2) the interaction between proteins and solvents (water) molecules; and (3) the interactions between protein molecules.

The μ value of a protein solution will be decreased with the increased velocity. This phenomenon is called “shear thinning”. The reasons are as follows: (1) protein molecules gradually move in the same direction so that protein molecules are orderly arranged, and thereby the friction resistance generated by liquid flow is reduced; (2) protein hydration environment deforms in the direction of motion; and (3) hydrogen bonds and other weak bonds are broken, resulting in the dissociation of protein aggregates and network structures as well as the reduction of protein volume. In summary, shear thinning can be explained by a reduction in the apparent diameters of protein molecules or particles in the direction of motion.

The breakdown of weak forces in protein molecule usually slowly occurs as protein solution flows. Therefore, the apparent viscosity of protein solution decreases with the increase of time before it reaches equilibrium. When the shear stops, the original aggregates may or may not reform. If protein aggregates can be reformed, the viscosity coefficient will not be decreased. The system is thixotropic. Both whey protein concentrate and soybean protein isolate can produce a thixotropic system.

There is no simple relationship between protein viscosity and solubility. The insoluble proteins obtained by thermal denaturation do not produce high viscosity after dispersion in water. However, for whey proteins with good solubility but poor water absorption and swelling ability, they also cannot form high viscosity dispersion system in water. For those proteins (such as soy protein, sodium caseinate) with great initial water absorption capacities, their dispersions have high viscosity, so there is a positive correlation between water adsorption capacity of proteins and dispersion viscosity.

The viscosity and consistency of protein system are main functional properties of liquid foods, such as beverage, broth, and soup, which affect food quality and processing like transportation, blending, heating, and cooling.

3.4.4 Protein Gelation

The scientific terms gelation, association, aggregation, polymerization, precipitation, flocculation as well as coagulation of proteins are used to reflect the changes of protein molecules at different levels of aggregation. These terms have certain differences. Protein association refers to the changes at the subunit or molecular level. Protein polymerization or aggregation generally refers to the generation of larger protein polymers. Protein precipitation refers to all precipitation reactions because of partial or total loss of protein solubility. Protein flocculation refers to the disordered aggregation reaction without protein denaturation. Protein coagulation is the disordered aggregation of the denatured proteins, and more, protein gelation is the orderly aggregation reaction of the denatured proteins.

Gel is the product formed after protein gelation. Protein gels have ordered three-dimensional network structure, contain other components, and play important role in food texture (e.g. meat). Proteins can not only form a semi-solid viscoelastic texture but also have the functions of water holding, fat stabilization, and adhesion. For some protein foods such as tofu and yogurt, protein gelation is the basis of quality formation.

The network structure of protein gels is the result of balanced protein–protein interaction, protein–water interaction as well as attraction and repulsive force between adjacent peptide chains. Electrostatic attraction and protein–protein interaction (including hydrogen bonds and hydrophobic interaction, etc.) are conducive to the proximity of protein peptide chains, while electrostatic repulsion and protein–water interaction are reasons for the separation of protein peptide chains. In most cases, heat treatment is necessary for protein gelation (protein denaturation and extension of

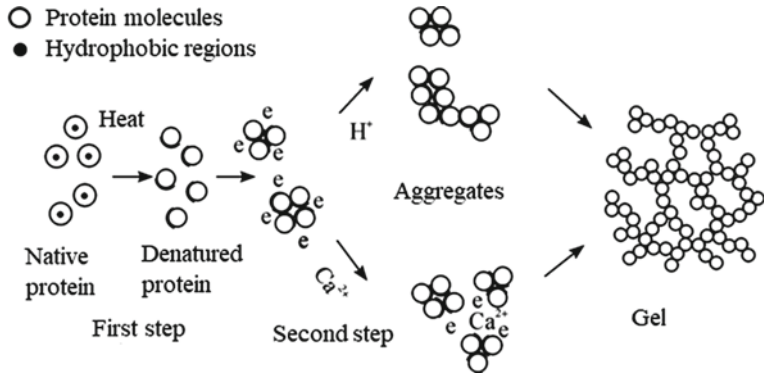


Fig. 3.9 Schematic diagram of gelation process of soybean protein

peptide chains), followed by cooling (formation of hydrogen bonds between peptide chains).

When protein gels are formed, the addition of a small amount of acid or Ca^{2+} can improve the speed and strength of protein gels. Sometimes, proteins can form gels without heating. Some proteins via addition of Ca^{2+} salt, appropriate enzymatic hydrolysis, and addition of alkaline solution, followed by adjustment of pH value to isoelectric point may also yield protein gels. The role of calcium ions is to form the so-called salt bridges.

Generally, the process of protein gelation can be divided into two steps: (1) conformation change or partial extension of protein molecules, and the occurrence of denaturation and (2) the denatured protein molecules gradually aggregate to form a network structure that can hold water and other substances in an orderly manner (Fig. 3.9).

According to the used pathway of gel formation, gels are generally divided into heat-induced gels (such as gels formed by heating egg albumin) and non-heat-induced gels (gel formed by adjustment of pH, addition of bivalent metal ions, or partial protein hydrolysis). According to the thermal stability of protein gels, they can be classified into thermal reversible gel (such as gelatin that can form solution when heated and restore to gel after cooling) and thermal irreversible gel (such as egg white protein and soy protein; gel state is formed by heat treatment never change). The thermally reversible gels are stable mainly through the formation of hydrogen bonds between protein molecules. The thermally irreversible gels mostly involve the formation of disulfide bonds between protein molecules. Once disulfide bonds are formed, they are not easy to break again and cannot be damaged by heating.

When proteins form gels, protein molecules may arrange in two different ways (Fig. 3.10). The gel formed by orderly cluster arrangement is transparent or translucent, such as the gels yield from serum protein, lysozyme, egg albumin, soybean globulin, etc. The gel formed by free aggregation is opaque, such as myosin gel formed under high ionic strength and gels formed by whey protein and β-lactoglobulin. Among common protein gels, these two different ways may simultaneously exist

Fig. 3.10 Schematic diagram of network structure of protein gels. **a** Orderly aggregation; **b** Free aggregation

and are affected by gelling conditions (such as protein concentration, pH, ion type, ion strength, heating temperature, heating time, etc.).

Protein gelation is usually produced by protein solutions, but insoluble proteins or protein dispersions in brine can also form gels. Therefore, protein solubility is not a necessary but only conducive factor for protein gelation.

Gelation is a very important functional property of proteins and plays an important role in preparation of many foods such as dairy products, gels, and various heated minced meat and fish products. Protein gelation can be used to form solid elastic gels, improves water/fat holding capacity, and also contributes to emulsification and foaming stability of food ingredients. Gelation is one of the most important functions of proteins and one of the most frequently considered indices in food processing.

3.4.5 Protein Texturization

Proteins are the basis of texture or structure of many foods such as animal muscle. However, some proteins in nature do not have the corresponding texture and chewing properties such as the isolated soluble plant proteins or milk proteins. Therefore, there are certain limitations in application of these protein ingredients in food processing. However, these proteins can currently be processed via the so-called protein texturization to form films or fibrous products with chewing properties and good water-holding properties and maintain good properties after hydration or heating treatment in the future. The texturized proteins can be used as meat substitutes or substitutes in foods. In addition, protein texturization can also be used to reorganize some by-products of animal proteins.

There are three common methods for protein texturization.

(1) Thermal protein coagulation and film formation

When the water of concentrated soybean protein solution is evaporated on a smooth hot metal surface, proteins show thermal coagulation and form hydrated protein

film. Soybean protein solution kept at 95 °C for a few hours thus yields a thin layer film of proteins. These protein films are textured proteins with stable structure against heating treatment and normal chewing properties. Yuba, a traditional soybean product, is processed by the above method.

If protein solution (such as the ethanol solution of zein) is uniformly coated on the surface of a smooth object, proteins can also form a uniform film (protein film) by interaction with each other after solvent evaporates. Protein films have various mechanical strengths and barrier functions against water, oxygen, and other gases, which can be used as edible packaging materials.

(2) Thermoplastic protein extrusion

Thermoplastic extrusion method is to let protein mixture pass through a cylinder with the function of rotating screw. Under the action of high pressure, high temperature, and strong shear, solid materials can be transformed into a viscous state and then quickly pass through the cylinder and enter atmospheric environment. The moisture evaporates rapidly, resulting in the formation of a highly swelling and dried porous structure. The final product is called textured protein (commonly known as puffed protein). After water adsorption, the product has a fibrous elastic structure with chewing properties and is stable under sterilization conditions. It can be used as meat substitute and filler for meatballs, hamburgers, etc. This method is the most commonly used method for protein texturization at present.

Despite the absence of muscle fiber structure, thermoplastic protein extrusion results in the texturized protein with similar textural feature. The texturized soybean flour shows uniform texture and fibrous laminar structure characteristics (Fig. 3.11).

(3) Protein fiber formation

This is another way to form texturized proteins, sharing same production principles of synthetic fibers. Protein solution with high concentration is prepared at pH > 10. Due to the increase of electrostatic repulsion, protein molecules are dissociated and fully extended. After degasification and clarification, protein solution passes through a nozzle with many holes under high pressure. At this time, the

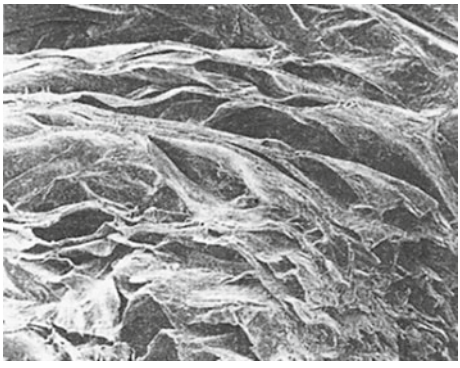


Fig. 3.11 Microstructure of texturized soybean protein

stretched protein molecules are oriented along the outflow direction and extended in a parallel way, and thereby orderly arranged. When the liquid from the nozzle enters an acidic solution containing NaCl, due to both isoelectric point and salt-out effect, the occurred protein coagulation induces the formation of hydrated protein fiber through hydrogen, ionic, and disulfide bonds, and other forces. By rotating the roller, protein fibers are stretched to increase their mechanical resistance and chewiness and reduce their water holding capacity. Then, through the heating of roller to remove part of the water, both fiber adhesion and protein toughness are improved. Finally, through a series of processing, such as seasoning, adhesion, cutting, and molding, artificial meat, or protein product analogous to meats can be formed.

Among three methods of protein texturization, thermoplastic protein extrusion is quite economical, simple, and without rigorous requirement of raw materials. Thermoplastic extrusion is not only suitable for raw materials with low protein contents (such as defatted soybean powder) but also for the raw materials with high protein contents. Protein fiber formation can only be used for the processing of protein isolates as they have higher protein contents.

3.4.6 Dough Formation

Wheat, barley, and rye have the same characteristics. In the presence of water, gluten protein in endosperm can form a strong cohesive and viscoelastic paste (dough) through mixing, kneading, and other treatments. Wheat flour has the strongest ability to form paste. After dough formation, gluten in wheat flour and other components such as starch, sugar and polar lipids, non-polar lipids, and soluble proteins are conducive to the formation of gluten three-dimensional network structure as well as the final texture of bread, which are entrapped in this three-dimensional structure.

Gluten protein is mainly composed of glutenin and gliadin, which account for more than 80% of the total protein in flour. The characteristics of dough are directly related to glutenin and gliadin. First, the contents of these dissociable amino acids in glutenin and gliadin are low, so they are insoluble in neutral water. Second, they contain many glutamine and hydroxyl-containing amino acids, so it is easy for them to form intermolecular hydrogen bonds. Gluten thus has a strong ability to absorb water and adhesion property, which is also related to hydrophobic interaction. Finally, glutenin and gliadin contain -SH groups, which can form disulfide bonds, so they are tightly bound together in dough to make it tough. When flour is kneaded, protein molecules are stretched, while disulfide bonds are formed and hydrophobic interaction is enhanced. Gluten protein is then transformed into three-dimensional and viscoelastic network structure. Starch particles and other components are entrapped in this structure. If the reducing agent is added to destroy these disulfide bonds, the cohesive structure of dough is destroyed. When oxidant like $KBrO_3$ is added to promote the formation of disulfide bonds, it is conducive to the elasticity and toughness of dough.

The proper balance between gluten and gliadin is important to bread making. The molecular weight of glutenin is as high as 1×10^6 Da, and glutenin contains many disulfide bonds (intra-chain and inter-chain). The molecular weight of gliadin is only 1×10^4 Da, with only intra-chain disulfide bonds found in gliadin molecules. Gluten determines the elasticity, adhesion, and strength of dough, while gliadin determines the fluidity, extensibility, and expansibility of dough. The strength of bread is related to glutenin. High content of glutenin will inhibit the expansion of residual CO_2 during the fermentation process and bulging of dough.

If the content of gliadin is too high, it will lead to excessive expansion. As a result, the resulting gluten will be easily broken and permeable, and dough will collapse. The addition of polar lipids to dough was beneficial to the interaction between glutenin and gliadin, and the network structure of gluten was improved. However, addition of neutral lipids was unfavorable. Addition of globulin to dough is generally not conducive to dough structure, while addition of denatured globulin can eliminate adverse effects.

When dough is kneaded, the three-dimensional network structure of gluten protein will not be well formed, if kneading strength is insufficient. As a result, the dough strength is insufficient. Excessive kneading can also cause breakage of some disulfide bonds in gluten, resulting in a decrease of dough strength. When dough is baked, water released by gluten is absorbed by the gelatinized starch molecules, but gluten retains nearly half amount of water. Gluten protein is fully stretched during kneading, which cannot be further extended during baking.

3.4.7 Emulsifying Property

Many daily foods are regarded as protein-stable emulsions. The formed dispersions include water-in-oil (W/O) and oil-in-water (O/W) types. Milk, ice cream, margarine, cheese, mayonnaise, and minced meat are the most common water–oil dispersion system, where proteins stabilize this emulsion system. Proteins are adsorbed at the interface between oil droplet and water phase to produce anti-coagulable physical and rheological properties (such as electrostatic repulsion and viscosity). The most important role of soluble proteins is that they could spread to the oil–water interface and adsorb at the interface. Part of proteins will contact the interface and the hydrophobic (or hydrophilic) amino acid residues are oriented to oil (or water) phase, which reduces free energy of system. The rest of the proteins partially will be unfolded and spontaneous adsorbed on the interface to exhibit corresponding interface properties. It is generally believed that the greater the hydrophobicity of proteins, the higher protein concentration adsorbed on the interface. Therefore, it will lead to smaller interfacial tension and stable emulsion system.

Globulins such as serum protein and whey protein have a relatively stable structure and higher surface hydrophilicity, so are not good emulsifiers. Casein is a good emulsifier due to its structural characteristics (random coil) and the relative separation

of hydrophilic and hydrophobic regions in the peptide chain. Soy protein isolate, meat, and fish proteins also have good emulsifying properties.

The emulsion system is thermodynamically unstable, and the interaction between fat globules will inevitably lead to emulsion instability. The result is the complete separation of oil and water phases. In addition, the instability O/W system can be coalescence, flocculation, and stratification. Coalescence refers to the rupture of membrane between fat globules, which leads to the formation of large fat globules. Flocculation refers to the process of aggregation between fat globules without rupture of membranes. Stratification refers to the floating of fat globules due to their density less than the continuous (i.e. water) phase. All kinds of emulsion instability can occur independently or simultaneously.

Protein solubility is positively correlated with emulsifying properties. Generally, insoluble proteins have no influence on emulsion formation. Therefore, the improvement of protein solubility will be conducive to improved emulsification performance. For example, when NaCl exists in minced meat (0.5–1 mol/L), emulsifying capacity of proteins can be improved due to salt-in effect of NaCl. However, once emulsion is formed, adsorption of insoluble proteins on the membrane will promote the stability of fat globules. The pH of solution also affects emulsification. Gelatin and ovalbumin at pI have good emulsification performance. Most of the other proteins like soybean protein, peanut protein, casein, myofibril, and whey protein have better emulsification performance when they are not at pI. At this time, the dissociation of side chains in amino acids will generate greater electrostatic repulsion, which contributes to stability and avoidance of droplet aggregation. At the same time, it is helpful for protein dissolution and water binding, which also improve the stability of protein membrane.

Heating can reduce the viscosity of protein membrane adsorbed in the interface, and thus decrease the stability of emulsion. However, if protein gelation is generated by heating, viscosity and hardness of the membrane can be improved; emulsion stability is thus improved. For example, gelation of myofibrillar protein is beneficial to the stability of emulsion systems, e.g. sausages, which can improve water holding capacity and fat retention of meat products, and enhance adhesion between components at the same time.

Low molecular weight surfactants are generally adverse to emulsion stability of proteins, as they will be completely absorbed at the interface, leading to weakened protein adsorption at the interface, reduction in viscosity of protein membrane, and consequently reduced emulsion stability.

The emulsifying properties of proteins are generally determined by emulsifying activity index (EAI), emulsifying capacity (EC), and emulsifying stability index (ESI), which reflect the ability of proteins to help form emulsion systems and stabilize them. These indices reflect that (1) proteins can reduce the interface tension to help emulsion formation and (2) the emulsion system is stabilized by increasing viscosity of the adsorption membrane, steric resistance, and other factors. There is no correlation between the ability of proteins to form emulsified dispersion system and the ability to stabilize the emulsified dispersion system. The emulsifying properties of some proteins are shown in Tables 3.12 and 3.13.

Table 3.12 Emulsifying activity index of some proteins (ionic strength of solution = 0.1 mol/L)

Protein	Emulsifying activity index		Protein	Emulsifying activity index	
	pH 6.5	pH 8.0		pH 6.5	pH 8.0
Egg albumin	–	49	Whey protein	119	142
Lysozyme	–	50	β-lactoglobulin	–	153
Yeast protein	8	59	Sodium caseinate	149	166
Hemoglobin	–	75	Bovine serum albumin	–	197
Soy protein	41	92	Yeast protein (88% acylation)	322	341

Table 3.13 Emulsifying capacity **and emulsifying stability of some proteins**

Protein source	Type	EC/ (g/g)	ES (24 h)/%	ES (14 d)/%
Soybean	Protein isolate	277	94	88.6
	Soybean flour	184	100	100
Egg	Protein powder	226	11.8	3.3
	Liquid protein	215	0	1.1
Milk	Casein	336	5.2	41.0
	Whey protein	190	100	100

The interaction between proteins and lipids is helpful to the formation and stability of emulsion but it may also have adverse effects. When proteins are extracted from lipid-rich raw materials, the formation of emulsion may decrease extraction efficacy and protein purity.

3.4.8 Foaming Property

Foam refers to the dispersion system formed by gas dispersed in the continuous liquid phase or semi-solid phase. Typical food examples include ice cream, beer, and so on. In a stable foam system, continuous phase of elastic thin layer separates each bubble, and the diameter of bubble ranges from 1 μm to several cm.

Food foam has several characters: (1) the incorporation of a large amount of gas; (2) there is a large surface area between gas and continuous phases; (3) relatively high solute concentration at the interface; (4) rigid or semi-rigid and elastic films that can expand; (5) opaque foam that can reflect light. The main difference between a foam and an emulsion is whether the dispersed phase is gas or fat and that gas takes up a larger percentage of the volume in the foam system. Foam has a large interfacial area, while the interfacial tension is much higher than the emulsified dispersion system. Foam thus is more unstable and prone to rupture. At this point, the roles of proteins are to adsorb at the gas–liquid interface to reduce the interfacial tension, and at the same

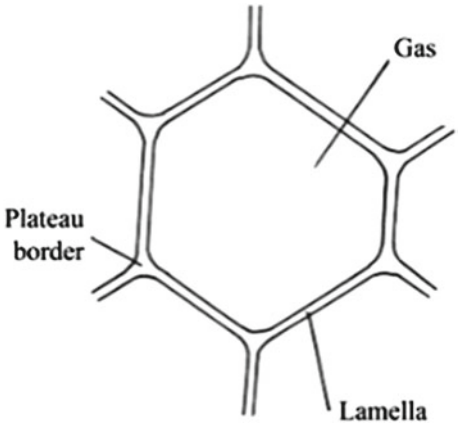


Fig. 3.12 Structure diagram of foam

time to render rheological characteristics and stability of the formed membrane to increase the strength, viscosity, and elasticity of membrane against adverse external effects. The typical structure of foam is shown in Fig. 3.12, in which the properties of lamella have a very important influence on foam stability.

The method for foam formation includes: (1) gas goes through the porous disperser and then passes into protein solution to generate corresponding gas bubbles; (2) in the presence of abundant gas, gas bubbles can be generated by mechanically stirring or oscillating protein solution; (3) under high pressure, gas is dissolved in solution, followed by sudden release of the pressure. The gas expands to form foam. In the process of foam formation, proteins are rapidly diffused and adsorbed at the gas–liquid interface in the first place, and then molecular structure of proteins is rearranged after entering the interface layer. Among them, diffusion process is a decisive factor.

The reasons for foam instability are listed here. (1) The thin layer of foam is drained due to gravity, pressure difference, evaporation, etc., which reduces the thickness of thin layer and eventually leads to foam rupture. (2) Due to the different sizes of bubbles, the pressure of gas in the small bubbles is higher, while the pressure in the large bubbles is lower. Therefore, gas transfers from the small bubbles to the large bubbles through continuous phase, resulting in the decrease of the total foam area, which is a spontaneous process to reduce the free energy of surface. At this point, interfacial expansion will lead to an increase in interfacial tension. To reduce the increased tension, protein molecules (carrying water molecules together) will migrate from the low-tension region to high-tension region, reducing the thickness of the thin layer in original region (Marangoni effect) and thus reducing foam stability. (3) The thin layer that separates bubbles ruptures. The thickness and strength of thin layer are reduced due to drainage and other factors, while the diameter of foam increases through agglomeration, eventually leading to rupture of bubble. Foam drainage correlates with thin layer rupture. The rupture of thin layer increases foam drainage, and enhanced drainage promotes thickness and strength decreases of thin layer, which is equivalent to a vicious cycle.

Table 3.14 Intrinsic properties that affect the foaming properties of proteins

Solubility	Rapid diffusion to the gas–liquid interface
Hydrophobicity	The polar zone and the hydrophobic zone are independently distributed, which can reduce the interfacial tension
Flexibility of the peptide chain	It is beneficial to extension and deformation of protein molecules at the interface
Interactions between peptide chains	It is beneficial to the interaction of protein molecules to form a good viscoelastic and stable adsorption membrane
Dissociation of groups	It is beneficial to the repulsion between bubbles, but high charge density is also adverse to protein adsorption on the membrane
Polar group	The combination of water and interaction between protein molecules are beneficial to the stability of the adsorbed membrane

Factors influencing the foaming properties of proteins include the following nine points:

(1) Intrinsic properties of proteins. A protein with good foaming property should be the protein that can quickly diffuse to the gas–liquid interface, and easy to be adsorbed, expanded, and rearranged at the interface to form a viscoelastic adsorption membrane through the interaction between molecules. β-Casein with a loose, free and curly structure is such a protein. Lysozyme, by contrast, is a tightly wound globulin that has disulfide bonds in multiple molecular sites and thus has a poor foaming property. The relationship between physical and chemical properties of proteins and the foaming properties are shown in Table 3.14.

Proteins with good foaming ability are generally very poor in foaming stability, while proteins with poor foaming ability are relatively good in foaming stability, for foaming ability and foam stability of proteins are two different molecular properties. Foaming ability depends on rapid diffusion of protein molecules, while the decrease of interfacial tension and distribution of hydrophobic groups are mainly determined by solubility and hydrophobicity of protein, and softness of peptide chains. Foaming stability is mainly determined by rheological properties of protein solutions, such as protein hydration in adsorption membranes, protein concentration, membrane thickness, and appropriate molecular interactions of proteins. Usually, egg albumin is the best protein foaming agent. Other proteins such as serum protein, gelatin, casein, gluten, soy protein, etc., also have good foaming properties.

(2) Salts. Salts affect the solubility, viscosity, stretching, and depolymerization of proteins as well as foaming properties. For example, NaCl increases expansion but decreases foaming stability. Ca^{2+} can improve foaming stability by forming salt bridges with the carboxyl groups of proteins.

(3) Sugars. Sugars usually inhibit foam expansion of proteins, but it can improve the viscosity of protein solutions. Hence, foaming stability is improved.

(4) Lipids. When low concentration lipids contaminate protein solution, the foaming ability of proteins will be seriously impaired. Polar lipids can be also adsorbed at the gas–water interface, interfere with protein adsorption, affect the interaction between proteins adsorbed, and thus decrease the foam stability.

(5) Protein concentration. Proteins at 2–8% concentration can reach the maximum degree of expansion, where the liquid phase has the best viscosity and the film has the appropriate thickness and stability. When protein concentration exceeds 10%, the viscosity of protein solution is too high, which affects the foaming ability, resulting in smaller bubbles and stiffened bubbles.

(6) Mechanical treatment. The formation of foam by mechanical treatment requires appropriate agitation, which leads to protein extension. However, stirring strength and time must be moderate. Excessive agitation will cause protein flocculation and reduce the degree of expansion and foam stability of proteins, for the flocculated proteins cannot be properly adsorbed at the interface.

(7) Heating treatment. Heating treatment is generally adverse to foam formation, as it causes gas expansion, viscosity reduction, and bubble rupture. However, proper heat treatment of some compact proteins before foaming is beneficial, since it can cause the unfolding of protein molecules and facilitate their adsorption at the gas–liquid interface. If heating treatment can lead to protein gelation, foaming stability will be greatly improved.

(8) pH. When pH is close to pI, the foam system stabilized by protein is very stable. This is due to that, the repulsive force between protein molecules is very low, which promotes protein–protein interaction and protein adsorption on the membrane. A sticky adsorption membrane thus is formed with enhanced foaming power and stability. The foaming ability of proteins at pH other than pI is generally better, but their stability is generally poor.

To evaluate the foaming property of proteins, one method is used to evaluate their ability to encapsulate gas (i.e., foaming power), while another method is to evaluate the foam life (i.e. foam stability). Foaming power increases with protein concentration, so data comparison at a single concentration is inaccurate. The values of foaming power under three different conditions are usually compared (Table 3.15).

Foam stability is generally a reflector of rupture extent of the foam sample placed for a period, or the drainage speed of the foam sample at different times. Foam stability can be measured by the time required to drain 1/2 liquid volume from the

Table 3.15 Comparison of foaming power values of the three proteins

Proteins	Maximum foaming power (concentration 20–30 g/L)	The concentration (g/L) with 1/2 maximum foaming force	Foaming power at 10 g/L
Gelatin	228	0.4	221
Caseinate	213	1	198
Soy protein isolate	203	2.9	154

foam sample after foam rupture or by the changes of foam volume at different times. Despite any methods, foam stability depends on protein concentration.

3.4.9 Binding of Flavor Compounds

Aldehydes, ketones, acids, phenols, and the fatty decomposition products present in foods may produce off-flavor. These substances can also bind to proteins or other food ingredients. They can be released in food processing and finally perceived by consumers, thereby affecting the sensory quality of foods. However, the interaction between proteins and flavor compounds also has desirable aspects. For example, they can confer texturized plant proteins with meat flavor.

The interaction between proteins and flavor compounds includes physical and chemical binding. The force involved in the physical binding is mainly van der Waals force, which is reversible binding with energy change near 20 kJ/mol. The force involved in chemical binding (usually irreversible binding) includes hydrogen bonds, covalent bonds, electrostatic force, etc. The energy change is more than 40 kJ/mol. It is generally believed that there are some identical but independent binding sites in the structure of proteins, which result in their binding by interacting with flavor compounds (F) as below.

$$\text{Proteins} + \text{nF} \leftrightarrow \text{Proteins-F}_\text{n}$$

The Scatchard model describes the binding of proteins to flavor compounds. Where, V is the amount of flavor compounds (mol/molprotein) when flavor compounds are to proteins to achieve equilibrium, L is the amount of free flavor compounds (mol/L), K is the equilibrium constant of binding (L/mol), and n is the total number of binding sites for flavor compounds in 1 mol of proteins.

$$\frac{V}{[L]} = K(n - V)$$

For proteins composed of single peptide chain, a good result can be obtained by using this model. However, for proteins composed of polypeptide chains, the binding amount of flavor compounds per mole of proteins will decrease with the increase of protein concentration. This is due to that, the interaction of proteins reduces the effectiveness of proteins to bind with flavor compounds, e.g. some sites are hidden. The binding constants of flavor compounds to some proteins are shown in Table 3.16.

The binding of flavor compounds to proteins is affected by environmental factors. Water can improve the binding of polar volatile compounds to proteins but it does not affect the binding of non-polar substances, as water can increase the diffusion rate of polar substances. High salt concentration weakens the hydrophobic interaction of proteins, causing protein unfolding and increasing their binding to carbonyl compounds. Casein binds more carbonyl compounds at neutral or alkaline pH values

Table 3.16 Binding constants of flavor compounds of some proteins

Protein		The flavor compounds bound	N (mol/mol)	K (L/mol)	ΔG° (kJ/mol)
Serum albumin		2-heptanone	6	270	−13.8
		2-nonanone	6	1800	−18.4
β-lactoglobulin		2-heptanone	2	150	−12.4
		2-nonanone	2	480	−15.3
Soy protein	Native	2-heptanone	4	110	−11.6
		2-octanone	4	310	−14.2
		2-nonanone	4	930	−16.9
		Nonanal	4	1094	−17.3
	Partial denaturation	2-nonanone	4	1240	−17.6

than it does under acidic conditions, which is associated with the non-ionization of amino groups. Hydrolysis of proteins generally reduces their ability to bind flavor compounds (especially excessive hydrolyzed proteins), which is related to the destruction of the primary structure or binding sites of proteins. Thermal protein denaturation causes expanding of protein molecules, leading to an increase in the binding capacity of flavor compounds. The presence of lipids promotes the binding and retention of various carbonyl volatiles. When proteins are vacuum freeze-dried, 50% of volatiles initially bound to proteins can be released due to vacuum.

3.4.10 Binding of Other Compounds

In addition to binding to water, lipids, and volatile compounds, proteins can still bind to metal ions, pigments, dyes, and others. Proteins are also able to bind to a few mutagenic and other active compounds. This binding can produce a detoxification effect or toxic enhancement effect. Sometimes, this binding can still lead to nutritive loss of proteins. The binding of proteins to metal ions facilitates absorption of some essential minerals such as iron and calcium, and the binding of protein to pigments can be used for quantitative analysis of proteins. Isoflavones, which bind to soybean proteins, ensure their beneficial effect.

3.5 Protein Changes During Food Processing and Storage

Food processing may induce several beneficial effects. Enzyme inactivation of the oxidases can prevent potential oxidation, while microorganism inactivation can enhance food preservability. Food processing also can convert some substances

(flavor precursors) into desired flavor compounds. However, nutritional and functional properties of proteins during food processing and storage may undergo some changes. These changes even bring safety issues to the processed foods.

3.5.1 Heat Treatment

Sterilization is widely employed for most foods and has some impacts on protein functionalities. Suitable sterilization conditions are thus recommended. Pasteurization at 72 °C for bovine milk induces inactivation for most enzymes, with a slight effect on whey proteins, flavor, and nutritional value. However, higher pasteurization temperature results in protein aggregation, casein dephosphorylation, and whey protein denaturation, and thus shows significant effect on milk quality. During meat processing, myofibril proteins and myosinogen will be aggregated at 80 °C, while the –SH groups in myofibril proteins are converted into –S-S- groups. At 90 °C, myofibril proteins will yield H_2S, and proteins may react with reducing sugars to undergo the Maillard reaction.

Heat treatment (especially using mild conditions) is beneficial to food proteins. Blanching or stemming treatments lead to enzyme inactivation and thus inhibit the production of off-color or off-flavor by the enzymatic oxidation. The anti-nutritional factors and toxins in plant foods are mostly damaged or inactivated during heat treatment. What is more, suitable heat treatment ensures partly unfolding of proteins and exposure of buried amino acids and thus increases hydrolysis and digestion of proteins. Suitable heat treatment also induces the production of some flavor substances to improve the sensory quality of foods.

However, excessive heat treatment is adverse to proteins, as amino acids have some reactions like deamination, desulfuration, and decarboxylation, which result in amino acid loss and decreased nutritional values. When the processed foods have both proteins and reducing sugars, the lysine residues in proteins can react with reducing sugars to form one product of the Maillard reaction, the Schiff bases. This product is not digestible in the body. The carbonyl compounds, generated from non-reducing sugars at high temperature or from lipid oxidation, also can react with proteins via the Maillard reaction. More importantly, under higher temperature together with long treatment time, proteins in the absence of reducing agents can undergo undesired changes in peptide bonds, resulting in decreased enzymatic digestion and bio-availability. Besides, several carcinogenic/mutagenic products can be formed in meats at high temperature (190–200 °C), due to amino acid pyrolysis.

3.5.2 Freezing Treatment

Foods stored at lower temperature (e.g. freezing treatment) have delayed microorganism growth, inhibited enzyme activity, and decreased rates for chemical reactions. Freezing treatment has no influence on nutritional values of proteins, but usually exerts clear effect on their functional properties. Meat foods after freezing and thawing treatments obtain a damage effect on tissues and cell membranes, and the water–protein interaction is irreversibly replaced by the adverse protein–protein interaction. Meat foods thus show poor quality and lower water retention. Freezing treatment of bovine milk induces casein precipitation after thawing treatment, which brings a worse sensory quality.

Protein denaturation under freezing conditions is controlled by the employed freezing rate. In general, a quick-freezing rate ensures the formation of smaller ice crystals, which results in weaker mechanical action on the cells and less protein denaturation. It is widely recommended using quick freezing treatment for foods to ensure minimum flavor and texture loss.

3.5.3 Dehydration Treatment

Food dehydration reduces food mass and especially water activity and thus has beneficial effect on food storage. However, it also brings some adverse impacts on proteins.

Hot-air drying now is less used in the food industry, as this technology results in poor rehydration, rigid texture, and lower flavor quality for the dehydrated meats and fishes. Vacuum drying has less impact on meat quality than hot-air drying. In this case, the applied partial pressure of oxygen as well as temperature is kept at lower levels and thereby leads to slow rates in oxidation and the Maillard reaction, respectively. Drum drying has been used in the production of milk powder; however, this technology is also mostly replaced by spray drying technology, as it usually results in decreased protein solubility and burnt flavor in the milk powder. Vacuum freeze-drying is widely used in the present, as this technology ensures proteins with slight denaturation but good rehydration. Vacuum freeze-drying has no effect on nutritional values and bioavailability of proteins and is especially suitable for bioactive proteins like enzymes and probiotics.

The most used drying technology is spray drying. Liquid foods are atomized into small droplets and heated by the hot air to evaporate water very quickly. This technology has little impact on proteins and thus is used in the production of protein ingredients or food rich in proteins.

3.5.4 Radiation Treatment

Low-dose radiation cannot damage protein structure and therefore has no impact on nutritional values of amino acids or proteins. However, high-dose radiation induces water dissociation to form the most active free radicals, OH. These radicals thus can react with proteins, resulting in many reactions such as deamination and cross-linking. Consequently, proteins have altered functional properties.

3.5.5 Alkaline Treatment

When alkaline treatment is used in food processing, especially in the condition of strong alkaline combined with temperature, some adverse reactions related to proteins will occur. Nutritional proteins may be seriously damaged. For example, proteins may undergo dephosphorization (phosphatized Ser) or desulfurization (Cys) to produce the active dehydroalanine residues (Fig. 3.13).

After that, dehydroalanine residues can react with lysine and cysteine residues to the respective lysinoalanine and lanthionine residues (Fig. 3.14). Proteins thus have decreased nutritional values and digestibility.

Alkaline treatment at a temperature higher than 200 °C may lead to racemization of amino acids (Fig. 3.15). Native L-amino acids are thus partly converted into D-amino acids, which results in decreased bioavailability as these D-amino acids have no biological values in the body. Alkaline treatment of proteins also destroys several amino acids like Arg, Ser, Thr, and Lys.

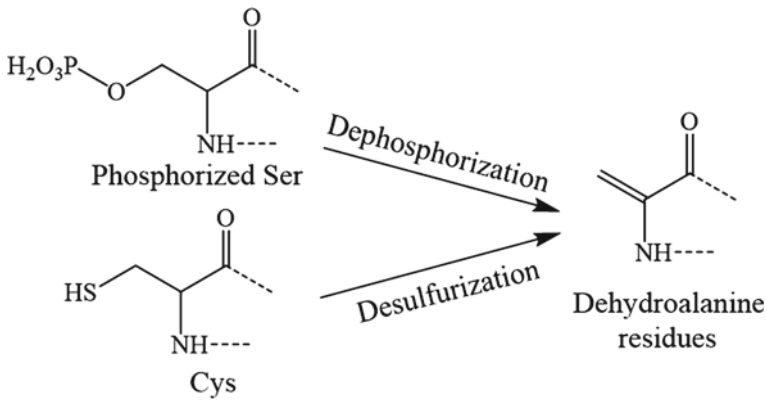


Fig. 3.13 Two pathways for the formation dehydroalanine residues

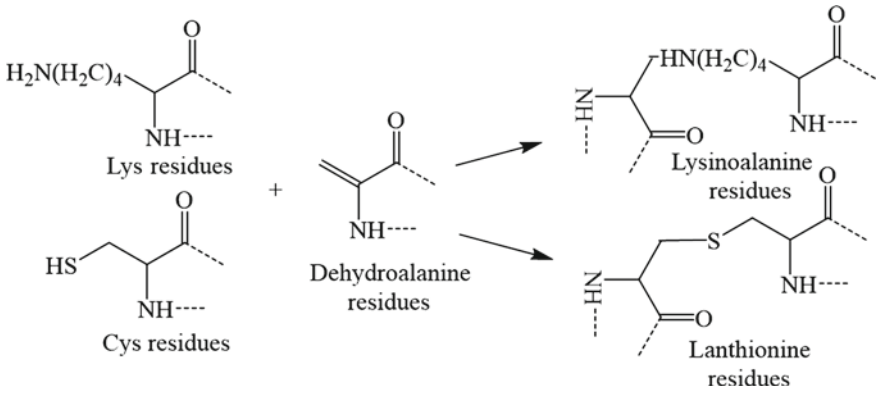


Fig. 3.14 The cross-linking of Lys and Cys residues of proteins with the formed dehydroalanine residues

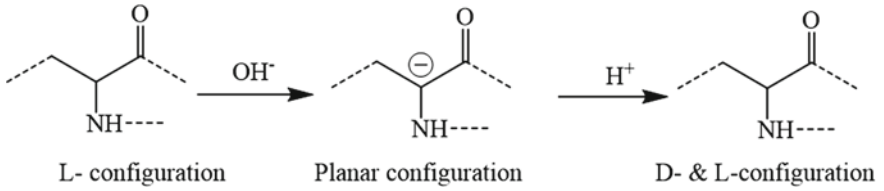


Fig. 3.15 Racemization of amino acids under alkaline condition

3.5.6 Other Protein Reactions

3.5.6.1 The Reaction of Proteins with the Free Radicals from Lipid Oxidation

Lipid (unsaturated fatty acids) oxidation leads to the formation of alkoxy and peroxyl (LOO·) radicals. These radicals in turn can react with proteins (Pr), resulting in protein cross-linking.

$$\mathrm{LOO\cdot + Pr \rightarrow LOOPr\cdot \text{ and } LOOPr\cdot + O_2 \rightarrow LOOPrOO\cdot \rightarrow \cdots\cdots\cdots}$$

$$\mathrm{Or\ LOO\cdot + Pr \rightarrow LOOH + Pr\cdot \text{ and } Pr\cdot + Pr \rightarrow Pr\text{-}Pr\cdot \rightarrow Pr\text{-}Pr\text{-}Pr\cdot}$$

Protein cross-linking brings decreased nutritional values. Malondialdehyde, one product from lipid oxidation, is regarded as an important factor inducing adverse changes in protein functionalities.

Oxidized lipids have been revealed to destroy amino acids, especially when water content in the reaction system is low. The damaging extents of amino acids are given in Table 3.17.

Table 3.17 Amino acid loss in the five proteins arisen from oxidized lipids

Reactants		Reaction conditions		Amino acid loss (%)
Proteins	Lipids	Times	Temperatures (°C)	
Cytochrome c	linolenic acid	5 h	37	His, 59; Ser, 55; Pro, 53; Val, 49; Arg, 42; Met, 38; Cys, 35
Trypsin	linoleic acid	40 min	37	Met, 83; His, 12
Lysozyme	linoleic acid	8 d	37	Trp, 56; His, 42; Lys, 17; Met, 14; Arg, 9
Casein	Ethyl linoleate	4 d	60	Lys, 50; Met, 47; Ile, 30; Phe, 30; Arg, 19; His, 28; Thr, 27; Ala, 27
Albumen	Ethyl linoleate	24 h	55	Met, 17; Ser, 10; Lys, 9; Leu, 8; Ala, 8

3.5.6.2 The Reaction Between Proteins and Nitrites

Nitrates exist in soil, water as well as plant and animal foods. Nitrates can be inverted into nitrites under reducing condition (e.g. by microorganisms). Nitrates in vegetables may be converted to nitrite during normal storage. Vegetable pickling or rotting process also leads to nitrate conversion.

Nitrites set nonnegligible safety risk to the human, as they can react with other substances to form nitroso compounds. The well-known N-nitroso amines are formed by nitrites and secondary amines and are considered as the most carcinogenic compounds in foods. Nitrites react with free or bound amino acids like Pro and Trp in foods especially for processed meat products (Fig. 3.16). Other secondary amines, such as those generated from the Maillard reaction, also can react with nitrites. Other amino acids like Cys, Arg, and Tyr also have potential to react with nitrites.

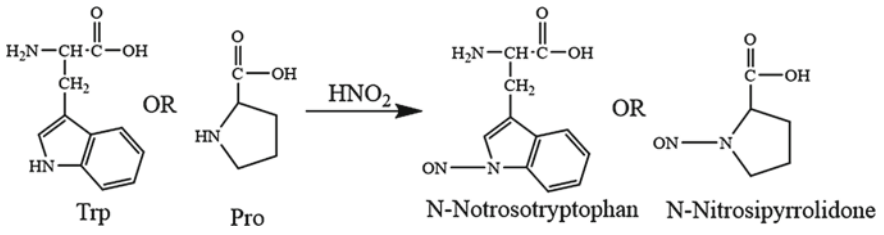
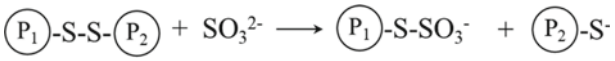


Fig. 3.16 The two nitrosamines generated from two amino acids

3.5.6.3 The Reaction of Proteins with Sulfites and Other Reducing Agents

Sulfites have a reducing property and are able to break the –S-S- bonds in the proteins, yielding S-sulfonate derivatives.



If other reducing agents such as Cys or β-mercaptoethanol are used, the S-sulfonate derivatives will be converted into Cys residues. Under acidic or alkaline pH, the S-sulfonate derivatives will decompose to disulfides. Clearly, the S-sulfonation does not decrease the bioavailability of Cys; however, this treatment induces protein unfolding and finally property changes, due to increased electrostatic repulsion and disulfide breakage.

3.5.6.4 Oxidation of Amino Acid Residues

Several oxidants such as H_2O_2 and hypochlorites, and those oxidative substances formed during food processing and storage all are potential to induce oxidation of sensitive amino acids (Met, Cys, Trp, His, and Tyr). The S-containing amino acids in acidic conditions can be oxidized to several products like sulfinic acids, sulfoxides, and sulphones (Fig. 3.17). Both sulfoxides and sulphones are biological unavailable. In the presence of peroxidases (e.g. horseradish peroxidase) and H_2O_2, Tyr residues in proteins can be converted into the dityrosine residues (Fig. 3.18), leading to protein cross-linking. In general, oxidized amino acids have lower bioavailability and even are harmful to the body.

Under mild, acidic, and oxidative conditions, for example, using performic acid (HCO_3H), dimethylsulfoxide (DMSO) or N-bromosuccinimide (NBS), Trp can be

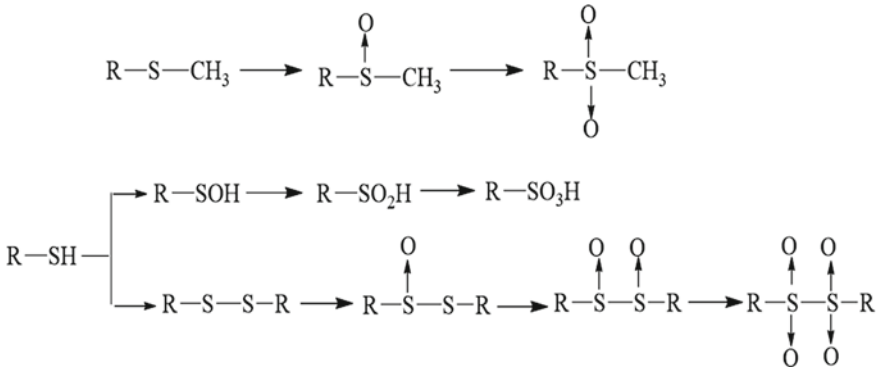


Fig. 3.17 The oxidative products of S-containing amino acids

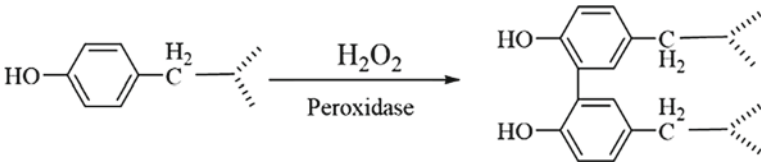


Fig. 3.18 Enzymatic cross-linking of the Tyr residues

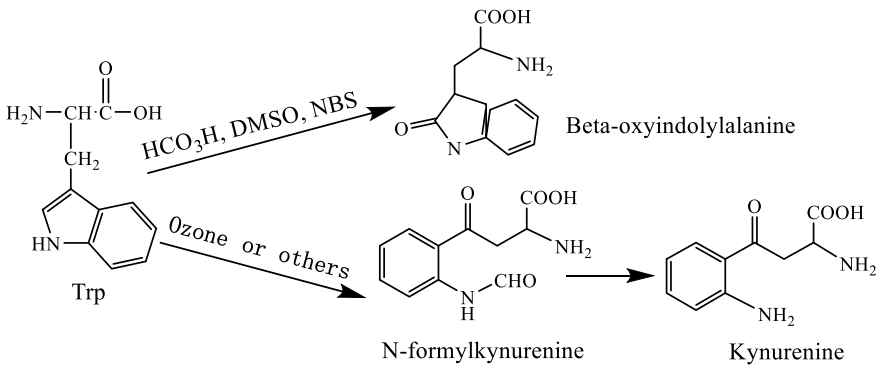


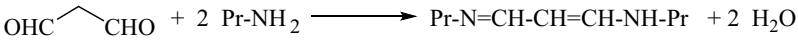
Fig. 3.19 Trp oxidation and resultant products

oxidized to β-oxyindolylalanine; however, using stronger oxidants such as ozone and H_2O_2 lead to the formation of other decomposition products (Fig. 3.19). Kynurenine has high toxicity and is carcinogenic in animals.

The products from phyto-oxidation of riboflavin also can induce oxidation of amino acids. Sensitive amino acids include Tyr, Cys, Trp, ad His. Oxidation for the S-containing amino acids and Trp usually occurs following decreased reaction rate consistently as Met > Cys > Trp.

3.5.6.5 Protein Cross-Linking

Free $–NH_2$ (mainly from Lys residues) can react with aldehydes to form condensation products, the Schiff bases. Malonaldehyde is one product of lipid oxidation. One malonaldehyde molecule can react with two $–NH_2$ or protein molecules as below, which leads to protein cross-linking. In addition, glutaraldehyde has similar ability to induce protein cross-linking.



Protein cross-linking alters protein properties significantly, such as solubility and water holding capacity. The cross-linked proteins even show resistance to protease-induced hydrolysis. Malonaldehyde is also one of the oxidative products from lipids

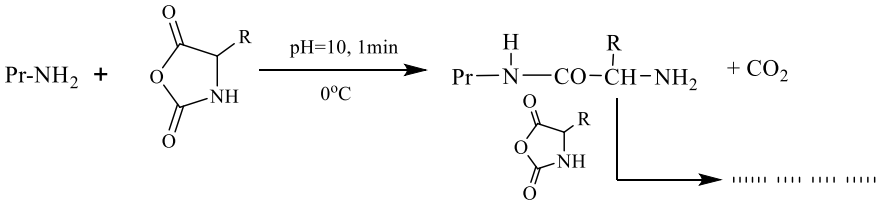


Fig. 3.20 Elongation of the side chain of proteins by carbonylated dehydrated anhydrides

in the body. Malonaldehyde thus reacts with proteins, while the resultant products are accumulated in the body. As the age increases, lipofuscin is yielded and regarded as a biomarker of aging.

3.5.6.6 Elongation Reaction of the Side Chain

When carbonylated dehydrated anhydrides react with proteins, $–NH_2$ in the side chain (e.g. Lys) will be elongated as shown in Fig. 3.20. The generated iso-peptide bonds are also bioavailable. Thus, this reaction can be used to change amino acid composition or fortify the essential amino acids.

3.5.6.7 Acrylamide Formation

Several high-temperature processed foods such as fried potato chips show lower contents of acrylamide, whilst those low-temperature processed foods only contain a few acrylamides. In general, acrylamide is regarded with two formation approaches in high-temperature foods, via lipid decomposition and the reaction between amino acid Asp and reducing sugar glucose (Fig. 3.21). These approaches have been verified using model reaction systems. Acrylamide is thus regarded as a potential toxic substance generated in food processing.

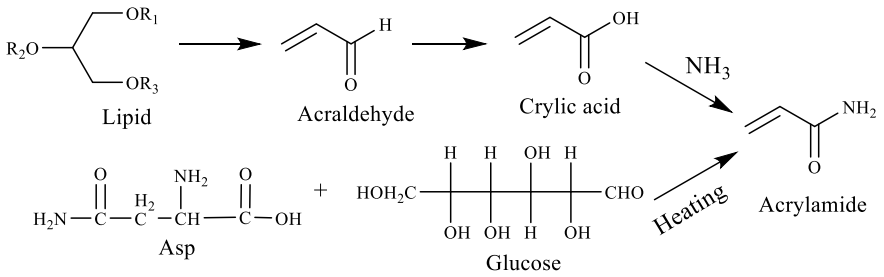


Fig. 3.21 Potential formation approaches of acrylamide in the high-temperature processed foods

Table 3.18 The aldehydes and flavor characteristics formed during the reaction of various amino acids and glucose

Amino acids	Aldehydes	Flavor characters	
		100–150 °C	180 °C
Gly	Formaldehyde	Caramel	Burnt
Ala	Acetaldehyde	Caramel	Burnt
Val	2-Methyl propanal	Cake	Chocolate
Leu	3-Methyl butanal	Bread, chocolate	Burnt cheese
Ser	Glycolaldehyde	Maple sugar	Burnt
Phe	Hyacinthine	Rosebush	Caramel
Met	Thioformaldehyde	Potato, cabbage	Potato
Pro		Corn	Bread
Ser		Bread, butter	
Arg		Bread, popcorn	Burnt
Lys		Potato	Fried potato
Asp			Caramel
Glu		Caramel	Burnt
Ile	2-Methyl butyraldehyde	Cake, moldy	Burnt cheese
Thr	2-Hydroxyl propanol	Maple sugar, chocolate	Burnt

3.5.7 Effect of Protein Reactions During Food Processing and Storage on Sensory Quality of Foods

3.5.7.1 The Flavor Substances Generated from the Maillard Reaction

Strecker reaction, one reaction involved in the Maillard reaction, is an important way to generate flavor substances for processed foods. The Maillard reaction is essential to sensory quality of bread. However, excessive reaction extent leads to a negative effect on sensory quality, for example, the burnt flavor. In the model reaction systems containing various amino acids and glucose, different reaction temperatures may result in different flavor characters (Table 3.18).

3.5.7.2 The Flavor Substances from the Rotten Aquatic Products

In rotten aquatic products, proteins are decomposed to produce various substances, yielding adverse impacts on both flavor and safety of aquatic products. When the polluted microorganisms in aquatic products are proliferated into higher levels, the secreted proteases thus hydrolyze proteins into amino acids, followed by deamination and decarboxylation of amino acids. These reactions lead to the formation of amine compounds with lower molecular weights and unacceptable off-flavor. For

example, Glu, Lys, His, and Trp are converted into γ-aminobutyric acid, cadaverine, histamine, and indole, respectively. Both cadaverine and histamine have high toxic effects on the body and may induce allergic food poisoning. Thus, it is recommended to control cadaverine and histamine levels in various aquatic products. Cadaverine and histamine levels also can be used to reflect the freshness of aquatic products.

3.5.7.3 Flavor Substances Formed During Milk Processing

Fresh milk usually contains acetone, acetaldehyde, butyric acid, methyl sulfides, and other volatiles. Heating treatment of milk leads to the formation of desired flavor. In usual, bovine milk and related products contain dimethyl sulfide as the most important flavor substance, which is generated from Met as flavor precursor (Fig. 3.22). Other S-containing flavor substances are generated from the Cys in the whey proteins. Overall, protein decomposition is considered important to milk flavor quality.

During cheese ripening, protein or amino acids are the main resources to form flavor substances. The main reactions involved in the flavor formation are decarboxylation, deamination, desulfurization, Strecker degradation, and others. The formed compounds include amines, alcohols, aldehydes, S-containing compounds, and others.

3.5.8 Chemical Modification of Proteins

The side chains of proteins have several active chemical groups. Thus, some external chemical groups can be introduced into protein molecules via various chemical reactions. Attachment of these external chemical groups in the side chains of proteins brings modified structure and especially property changes. However, some side reactions accompanied with the modifying reactions may be unacceptable or harmful. Several chemical modifications used for the chemical groups in protein side chains are summarized below.

(1) Hydrolysis. This reaction is also regarded as a conversion of chemical groups, for example, using a hydrolysis reaction to convert Gln and Asn to Glu and Asp, respectively.

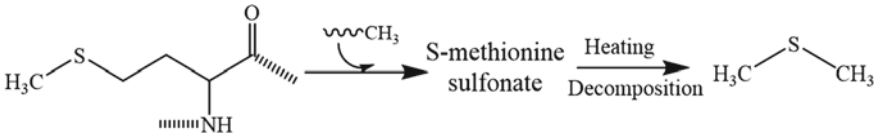


Fig. 3.22 The formation of dimethyl sulfide during milk heating

Table 3.19 The chemical reactions and groups involved in chemical modification of proteins

Chemical groups/bonds	Chemical modification	Chemical groups/bonds	Chemical modification
-NH_2 groups	Acylation, alkylation	Carboxyl groups	Esterification, amidation
-S-S- bonds	Oxidation, reduction	-SH groups	Oxidation, alkylation
Sulfide groups	Oxidation, alkylation	Phenolic groups	Acylation, electrophilic substitution
Imidazolyl groups	Oxidation, alkylation	Indolyl groups	Oxidation, alkylation

(2) Alkylation. This reaction introduces alkyl (e.g. carboxyl methyl) groups into protein side chains. These alkyl groups may be attached to –OH, –NH_2, and –SH groups of the side chains.
(3) Acylation. Various carboxyl groups also can be introduced into side chains. Organic acids of lower molecular weights, dicarboxylic acids, and long-chain fatty acids all can be used to modify proteins. The main reaction sites are –OH and –NH_2 groups of the side chains.
(4) Phosphorylation. Using phosphorus oxychloride or polyphosphates, proteins can be conjugated with phosphate groups. Main reaction sites involve –OH and –NH_2 groups of the side chains.

Table 3.19 shows the main chemical groups and reaction types involved in chemical protein modifications.

When proteins are modified with these reactions, their property changes depend on the introduced chemical groups. The introduction of several ionic groups like carboxyl methyl, dicarboxylic acid, and phosphate groups brings enhanced intramolecular electrostatic repulsion and greater unfolding, and thereby alters protein solubility. The introduction of carboxyl and phosphate groups also increases the Ca-sensitivity of protein. Hydrolysis of amide groups in protein side chains, in general, results in increased protein solubility and improved foaming and emulsifying properties. If proteins are introduced with non-polar groups such as long-chain fatty acids, protein hydrophobicity is enhanced. Modified proteins thus have changed interface properties. Acylation of proteins with long-chain fatty acids improves emulsifying properties.

3.5.9 Enzymatic Modification of Proteins

Enzymatic modification of proteins usually has no safety consideration, as this approach does not alter chemical structures of amino acids. Several enzymatic reactions may be used in protein modification, especially, enzymatic hydrolysis, the plastein reaction, and enzymatic cross-linking.

3.5.9.1 Limited Enzymatic Hydrolysis

Enzymatic hydrolysis of proteins with higher hydrolysis extent yields smaller peptides and free amino acids. Most functional properties of proteins are thus destroyed totally. Modified proteins only have higher solubility at a wide pH range. Using limited hydrolysis and specific proteases, modified proteins can be endowed with improved emulsifying and foaming activity but damaged gelation. Limited hydrolysis leads the exposure of hydrophobic groups, modified proteins have decreased solubility. Limited protein hydrolysis has important application in cheese production, as rennin's effect on caseins ensures the formation of cheese curds.

Enzymatic hydrolysis of proteins may result in the formation of bitter peptides, which have potential impact on sensory quality of foods. Bitter intensity of bitter peptides is highly depending on amino acid compositions of proteins and the used proteases. In general, the proteins with average hydrophobicity larger than 5.85 kJ mol^{-1} are easily to produce bitter peptides, while using the non-specific proteases other than the specific proteases also may generate more bitter peptides.

3.5.9.2 Plastein Reaction

Th plastein reaction of proteins contains several reaction stages. First, proteins are enzymatic hydrolyzed by proteases to yield protein hydrolysates (or peptides). Second, protein hydrolysates are treated with proteases for protein re-synthesis (i.e. plastein reaction), yielding the plasteins. Protein hydrolysis is done with normal conditions, while plastein reaction is done under higher substrate concentration. In general, plastein reaction involves three chemical reaction and interaction; that is, transpeptidation, condensation, and hydrophobic interaction. If extrinsic amino acids are added into reaction system, amino acid compositions of the plasteins are thus changed. Thus, plastein reaction has potential to alter protein nutritional values or bio-functions. Plastein reaction has been used to fortify proteins with essential amino acids, or increase bio-activities such as anti-oxidation, inhibition on angiotensin-converting enzyme, anti-coagulation, and others.

3.5.9.3 Enzymatic Cross-Linking

Transglutaminase (TGase) can catalyze the acyl transfer reactions between the Lys and Gln residues of proteins and induce the formation of new covalent bonds, i.e. ε-(γ-glutamyl) lysine isopeptide bonds. Under mild reaction conditions, TGase results in both intra-molecular and inter-molecular protein cross-linking. Modified proteins are thus protein polymers with different extents of polymerization. TGase modification thus is a widely used enzymatic protein cross-linking. Overall, many proteins including caseins, whey proteins, soybean proteins, cereal proteins, and meat proteins have been modified using TGase to improve the quality attributes of foods like

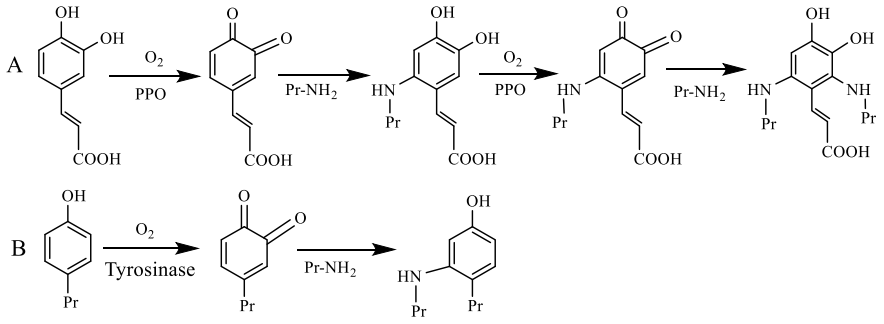


Fig. 3.23 The polyphenoloxidas (PPO)-induced protein cross-linking. Approach A, in the presence of caffeic acid; Approach B, in the absence of phenolic compounds of lower molecular weights; Pr, protein

yoghurt and bread. TGase-induced cross-linking of β-casein leads to decreased emulsifying activity but enhanced stability for the resultant casein-fat emulsion. Suitable addition of TGase to cross-link cereal proteins improves bread quality; however, higher TGase usage brings worse bead quality. TGase usage in milk processing may decrease yogurt syneresis, via the induced protein cross-linking.

Tyr residue in proteins also can undergo cross-linking through the catalysis action of peroxidases. The tyrosinases from mushrooms have potential to cross-link lysozyme, casein, and ribonuclease, in the presence of phenolic compounds (i.e. cross-linkers) of lower molecular weights (cross-linkers). Tyr in proteins also can be used as a cross-linker. The proposed two chemical mechanisms are depicted in Fig. 3.23.

The transglutaminase (TGase, EC 2.3.2.13) from the liver of guinea pig is very expensive and thus mostly used in biological researches. The TGase from *Streptoverticilliummobaraense* is composed of 331 amino acid residues and has molecular weight about 40 kDa and a Cys residue in its active center. Catalysis activity for microbial TGase is independent of calcium ions, while many metal ions (except for Cu^{2+}, Zn^{2+}, Pb^{2+}) have no or slightly effect on TGase activity. Suitable pH values and temperature for TGase catalysis are 5–8 and 37–50 °C.

TGase belongs to one of the acyl transferases and can induce acyl transfer and other reactions (Fig. 3.24). The Gln residues (γ-amide groups) of proteins are acyl donors while the Lys residues (ε-NH_2 groups) of proteins are acyl receptors. Protein cross-linking thus occurs via forming the intra-molecular or inter-molecular ε-(γ-glutamyl) lysine isopeptide bonds (Reaction I, Fig. 3.27). Protein cross-linking can be applied for the reaction system containing homologous protein even heterogeneous proteins, resulting in changed functional properties in rheology, gelation, emulsification, and hydration. TGase-induced protein cross-linking has no adverse impact on nutritional values of proteins. Other amine compounds including the NH_2-containing saccharides can be used as acyl receptors to replace the Lys residues. In these cases, amine compounds or saccharides can be conjugated into protein molecules at the Gln residues (Reaction II, Fig. 3.27). Several studies have used this approach to connect

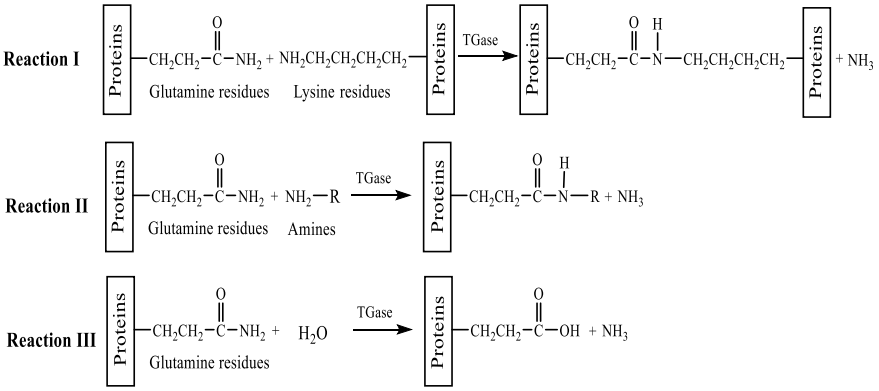


Fig. 3.24 The three reactions catalyzed by transglutaminase

soybean and milk proteins or protein hydrolysates with glucosamine or oligochitosan, resulting in the formation of glycated proteins or peptides. This interesting protein glycation (TGase-type protein glycation) also has protein cross-linking and is clearly different from the Maillard-type protein glycation. Moreover, TGase also has weak ability to catalyze the hydrolysis of the Gln residues to form Glu residues (Reaction III, Fig. 3.27). However, this hydrolysis only occurs in the absence of acyl receptors. Overall, TGase has important application in the food industry and can be used to cross-link and glycate food proteins for property modification.

3.6 The Main Food Proteins

Food proteins mostly come from edible animals and plants. The animal-derived proteins (such as those from meats, milks, eggs, and fishes) and cereal proteins are regarded as traditional edible proteins and are important food components or ingredients (Table 3.20). In Asia countries, traditional edible proteins also include soybean proteins. Meanwhile, the so-called new protein resources are also proposed as potential proteins for the food industry. The new protein resources include single cell proteins (SCPs), leaf proteins, and alga proteins and are the main targets for the research and development of new protein resources in the future.

3.6.1 Soybean Proteins

Soybean proteins are mainly composed of globulins and other minor proteins. These globulins are soluble in the water at the pH value far away from their isoelectric point (near pH 4.5), or soluble in salt solutions. Based on their essential amino acid

Table 3.20 Main food proteins and their applications

Resources	Proteins	Applications
Cereals	Glutes, corn proteins	Breakfast, bakery products, whipped toppings
Eggs	Whole egg, albumen, lipovitellinin	Various applications including emulsification, foaming, cohesion–adhesion, gelation
Fishes	Muscles, collagen/gelatin	Gelation, comminuted meat products
Animals	Muscles, collagen/gelatin, the serum proteins from porcine or bovine blood	Gelation, emulsification, water holding
Milks	Whole milk, skimmed milk, caseinate, whey protein powder	Various applications including emulsification, cohesion–adhesion, thickening
Oil seeds	The protein products from soybean, peanut, sesame, and others, various protein concentrates and isolates	Bakery products, protein beverages, meat analogs, or substitutes

compositions, soybean proteins have nutritional value close to that of the animal proteins and are rich in higher lysine but lack of the S-containing amino acids. Overall, soybean proteins can be regarded as one of these protein resources with both good nutritional and functional properties and have potential application in various processed foods.

Based on their sedimentation coefficients, soybean proteins can be classified into four different parts. The soybean proteins, extracted with water and ultracentrifuged at fixed conditions, are grouped into the so-called 2S, 7S, 11S, and 15S globulin parts. Here, the symbol S represents the Svedburg unit. The 2S globulin part contributes to about 20% of the total soybean proteins and mainly contains protease inhibitors, cytochrome C, allantoicase, and two other proteins. The dominant 7S globulin part contributes to about 37% of the total soybean proteins and mainly is composed of β-amylase, hemagglutinin, lipoxygenases, and 7S globulins. 11S globulins (about 1/3 of the total soybean proteins) are the major components of the 11S globulin part. However, detailed compositions of the 15S globulin part are still unknown in the present. The 15S globulin part contributes to about 10% of the total soybean proteins and might be the polymers of soybean globulins. In brief, the 7S and 11S globulin parts contribute to about 70% of total proteins, and thereby are regarded as the most important fractions in soybean proteins. What is more, composition classification of soybean proteins is strictly dependent on the used conditions, for condition change induces protein disaggregation or aggregation. For example, when the used salt concentration (or ionic strength) is decreased from 0.5 to 0.1 mol/L, 11S globulins will be aggregated into the 9S globulins.

When soybean is extracted with solvents to prepare edible oil, the left by-product is defatted soybean flour. Defatted soybean flour mainly contains proteins and carbohydrates and is now used to prepare various soybean protein products, which can be applied in food processing as protein ingredients (Table 3.21).

(1) The defatted soybean flour (DSF) is yielded from the dehulled soybean by a solvent extraction using the so-called 6# solvent (mainly containing hexane). DSF is also subjected to an important treatment (i.e. flash evaporation), aiming to remove residual solvent and to inactivate the anti-nutritional factors in DSF. In usual, a half of DSF are proteins with less loss in functional properties.

More importantly, flash evaporation of the extracting solvent ensures less protein denaturation and higher nitrogen solubility index for DSF. DSF also has higher

Table 3.21 Soybean protein products and their applications in various foods

Functional properties	Main mechanisms	Foods	Product types
Solubility	Protein hydration, pH effect	Beverages	F, C, I, H
Water adsorption and binding	Water binding via hydrogen bond interaction, water entrapment	Meat products, bread, cake	F, C
Viscosity	Thickening, water binding	Soup	F, C, I
Gelation	Formation protein network via various interactions, water entrapment	Meat products, cheese	C, I
Cohesion–adhesion	Hydrophobic interaction, hydrogen bonding	Meat, bakery products, pasta products	F, C, I
Elasticity	Disulfide bonds, gel deformation	Meat and bakery products	I
Emulsification	Interfacial adsorption and emulsion stability	Sausages, cake, soup	F, C, I
Fat binding	Hydrophobic interaction	Meat products	F, C, I
Flavor binding	Hydrophobic and other interactions, flavor entrapment	Meat analogs or substitutes, bakery products	C, I H
Foaming	Interfacial adsorption, film formation	Desserts, dressings	I, W, H
Color control	Bleaching with lipoxygenase	Bread	F

Notes C, soybean protein concentrate; F, defatted soybean flour; H, hydrolyzed soybean proteins; I, soybean protein isolate; W, soybean whey protein

lipoxygenase activity and thereby can be applied in the bleaching treatment of wheat flour.

(2) The soybean protein concentrate (SPC) is usually prepared as follows. DSF is extracted with pH 4.5 water or ethanol–water, or first wet-heated and then extracted with water. These treatments lead to the removal of the soluble oligosaccharides from DSF and result in higher protein content (about 70%) for SPC. Protease inhibitors and some proteins in DSF are also lost in the soybean whey fraction. SPC thus has lower protease inhibitor levels together with lower protein recovery. In usual, about 2/3 raw proteins are recovered.

(3) The soybean protein isolate (SPI) is usually prepared using the alkali-extraction and acid-precipitation procedures. DSF is extracted with diluted alkaline solution, and the resultant supernatant (protein solution) is acidified into isoelectric point (pH 4.5) to precipitate proteins. After that, protein precipitate is neutralized and spray-dried to obtain SPI. SPI is free of cellulose and anti-nutritional factors and has higher protein content (about 90%) with good properties in dispersion, emulsification, gelation, and thickening.

3.6.2 Milk Proteins

Milk proteins mainly are composed of caseins and whey proteins. Caseins contribute to about 80% of total milk proteins and are characterized as α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein. Whey proteins contribute to about 20% of total milk proteins, including β-lactoglobulin, α-lactoalbumin, and other minor components such as immunoglobulins, lactoferrin, lysozyme, lactoperoxidase, and other peptides or proteins.

In the milk, milk proteins exist mostly in a format of protein aggregates. Caseins can form casein micelles up to a level of 10^{14}/mL milk. Casein micelles usually show particle sizes of 30–300 nm. Some larger casein micelles even have a size larger than 600 nm. Colloidal phosphate calcium plays an important role in the formation and stability of casein micelles. The structure and involved interactions for casein micelles or sub-micelles are proposed as those shown in Fig. 3.25.

Caseins can be separated from the milk using acid precipitation (pH 4.6) or rennin treatment. The resultant products thus show property differences. Casein products (especially sodium caseinate) are important protein ingredients in the food industry. Sodium caseinate has good dispersion and stability at pH > 6, is a commercial protein additive with very good emulsification, water-binding, thickening, foaming, and gelation. Both whey protein concentrates and isolate (WPC and WPI) also are good protein ingredients and have special application in infant foods, and more, whey protein products can be used in acidic foods with good dispersion and solubility, compared with casein products. Whey protein products also can be applied in the preparation of mimic fat. Table 3.22 summarizes the main chemical features of commercial milk protein products.

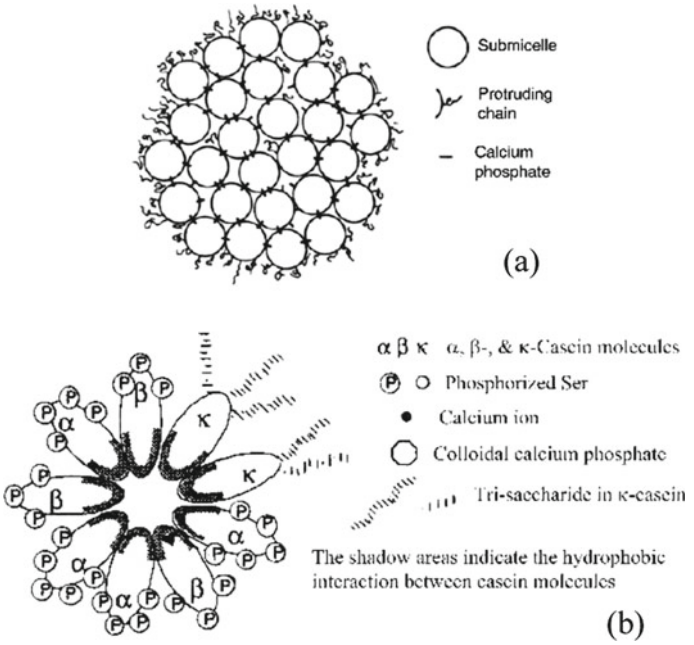


Fig. 3.25 The structures of casein micelle (**a**) and interaction involved in structure formation (**b**)

Table 3.22 Main chemical features of commercial milk protein products

Milk protein products	Preparation methods	Contents(%, dry basis)			
		Protein	Ash	Lactose	Fat
Caseins	Acid precipitation	95	2.2	0.2	1.5
	Rennin treatment	89	7.5	–	1.5
	Co-precipitation	89–94	4.5	1.5	1.5
Whey protein concentrate	Ultrafiltration	59.5	4.2	28.2	5.1
	Ultrafiltration + reverse osmosis	80.1	2.6	5.9	7.1
Whey protein isolate	Spherosil S method	96.4	1.8	0.1	0.9
	Vistec method	92.1	3.6	0.4	1.3

Milk proteins also have several important bio-functions (Table 3.23), which can provide desired health benefits to the body.

Table 3.23 Relationship between surface hydrophobicity

Milk proteins	Important bio-functions
α, β, κ-Caseins	Metal (Cu, Fe, Ca) carrier, precursors of active peptides
α-lactalbumin	Ca carrier, immuno-modulation, anti-cancer effect
β-lactoglobulin	Retinol carrier, fatty acid-binding, potential anti-oxidants
Immunoglobulins	Immune function
Lactoferrin	Anti-bacterial, anti-virus, immuno-modulative, anti-oxidative, anti-cancer, and Fe-binding functions
Lactoperoxidase	Anti-bacterial activity
Lysozyme	Anti-bacterial activity, synergized with immunoglobulins and lactoferrin
Glycopeptides	Anti-bacterial and anti-virus effect

3.6.3 Meat Proteins

Animal meats or muscles are important dietary foods of the human and are also main resources of food proteins. The proteins in animal muscles are usually classified as three groups, that is, myofibrillar, sarcoplasmic, and stromal proteins, comprising about 55, 30, and 15% of the total muscle proteins, respectively. Sarcoplasmic proteins can be extracted by water and diluted salt solutions from muscle tissues, while myofibrillar proteins are only extractable using concentrated salt solutions. Stromal proteins are insoluble proteins.

Myofibrillar proteins comprise myosin, actin, and other proteins. Myosin has a molecular weight of about 500 kDa and an isoelectric point around 5.4, possesses ATPase activity, and will be coagulated at a temperature up to 50–55 °C. Actin monomer has a molecular weight of about 43 kDa and an isoelectric point around 4.7 and can bind with myosin to form actomyosin. In myofibrillar proteins, muscle contraction is the result of the myosin–actin interaction.

Sarcoplasmic proteins are mainly composed of myoglobin, albumin, and other proteins. Myoglobin molecule contains one Fe^{2+}. It has an isoelectric point around 6.8 and makes critical contribution to meat color or quality. However, myoglobin is very sensitive to condition change (especially oxidative condition), as Fe^{2+} is easily converted into Fe^{3+}, which results in an undesirable brown color in meat. Other albumin proteins such as myogen also have instable properties, for example, they may undergo denaturation around a temperature of 50 °C.

Stromal proteins mainly comprise collagen and elastin. Collagen is chemically rich in glycine, proline, and hydroxyl praline and has both intra-molecular and inter-molecular cross-linking. Cross-linking extent of collagen increases as animal age increases. Higher cross-linking extent induces stable properties for collagen but worse tenderness for meat quality. However, long-time heating may convert collagen into gelatin. Gelatin is soluble in hot water can produce heat-reversible gels and thereby has various applications in the food industry. Elastin has no hydroxyl proline

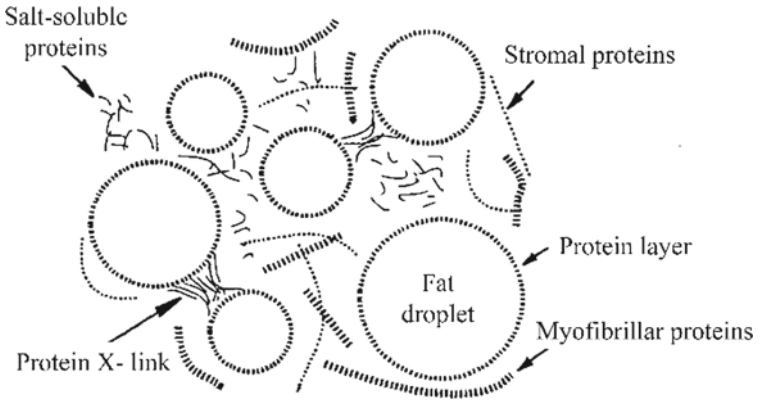


Fig. 3.26 The roles of muscle proteins in meat products for emulsion

and tryptophan but is rich in proline, glycine, and valine. Elastin is resistant to both pepsin and trypsin digestion but can be hydrolyzed by elastase.

In meat, especially comminuted meat products, all proteins make contribution to the formation and stability of the emulsion (Fig. 3.26). Longer chopping time ensures a thick protein film around fat droplets and thus brings emulsion stability. Moreover, myosin has the best emulsifying properties than other meat proteins.

Tenderness is a critical reflector of meat quality, especially for meat texture. Plant-derived proteases such as papain, bromelin, and ficin can be used in meat tenderization, via enzymatic hydrolysis of meat proteins. These proteases are usually injected into animal muscles after animal slaughter. In general, bromelin has better action on collagen but shows slightly weaker action on elastin and myofibrillar proteins. Papain exhibits highly action on myofibrillar proteins but less action on collagen. Ficin, however, has good hydrolysis effect on both myofibrillar and stromal proteins

3.6.4 Egg Proteins

The eggs especially hen eggs have been used as dietary foods for a very long time. Whole egg comprises the edible egg white and yolk and the inedible egg shell. The egg white is mainly composed of globulin, a few carbohydrates, and negligible lipids. The carbohydrates in the egg white exist in two states: free or bound with proteins, while glucose is the most abundant carbohydrate. The main components of the egg yolk are proteins and lipids, while carbohydrate content is very low. What is more, most of the lipids are bound with proteins, which results in a critical influence on the functional properties of the yolk proteins.

The egg yolk can be used as emulsifier in the food industry. Emulsifying properties of the egg yolk mostly depend on its lipoproteins. Among the three lipoproteins (vitellin, phosvitin, and α-/β-lipoproteins) in the egg yolk, lipoproteins have better

emulsification. Low-density lipoprotein in the egg yolk is also better in emulsifying properties than bovine serum albumin at same protein concentration. The addition of lipolipids has no impact on the emulsifying properties of low-density lipoprotein. In general, good emulsification of the low-density lipoprotein is due to its lipids–proteins complexes, which has high binding capacity to fat.

The egg white can be used as foaming agent with better performance than caseinate. Among these proteins in the egg white, they are detected with decreased foaming activities as the order of ovomucin, ovoglobulin, ovotransferrin, ovalbumin, ovomucoid, and lysozyme. Both ovomucoid and lysozyme have stable structures due to inter-molecular disulfide bonds; therefore, they are difficult in molecule deformation and have lower abilities in interface adsorption. The egg white is also important gelling agent in the food industry, as it can produce heat-irreversible gels easily. The gel strength and turbidity of the resultant gels are governed by medium pH and ionic strength. Heat-denatured ovalbumin has similar molecular conformation than the native one. Under a pH value near isoelectric point or higher ionic strength, the denatured ovalbumin performs a random aggregation via hydrophobic interaction; however, under a pH value far away isoelectric point and lower ionic strength, the denatured ovalbumin performs an ordered linear aggregation, as electrostatic repulsion between protein molecules prevents the occurrence of the random aggregation (Fig. 3.27).

Medium pH and ionic strength also have impacts on gelling properties of ovalbumin. Ovalbumin gels have higher gel strength under mild pH and ionic strength but give worse transparency under higher ionic strength and a pH value near isoelectric point (Fig. 3.28).

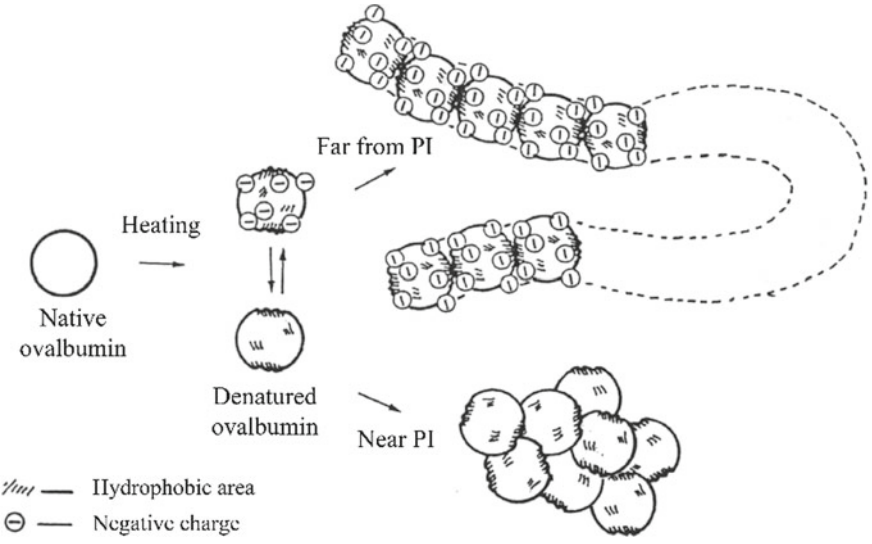


Fig. 3.27 Proposed model for the heat denaturation and formation of aggregates of ovalbumin

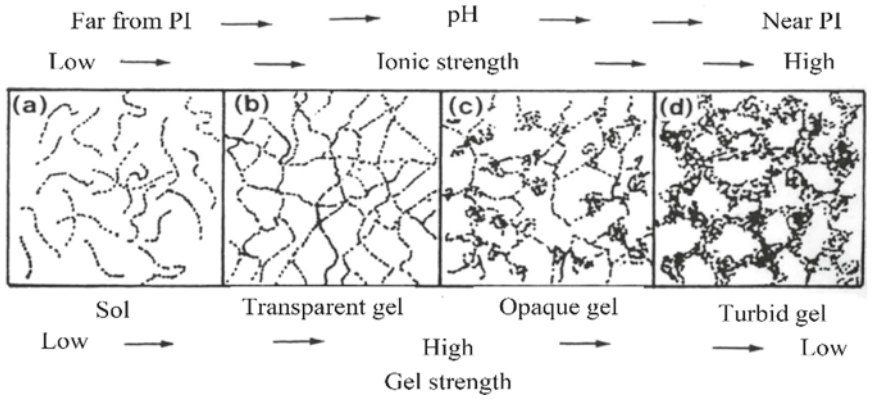


Fig. 3.28 Proposed model for the gel networks produced with heated ovalbumin

3.6.5 Cereal Proteins

Cereals including wheat, rice, corn, barley, and oat are traditional plant foods. Cereal proteins are classified into four kinds: albumins, globulins, gliadins, and glutenins (Table 3.24). Protein contents of these cereals vary with cultivated varieties, plant regions, growth conditions, and others. In general, gliadins and glutenins are major protein in cereals, accounting for 85% of total proteins. Nutritional values of cereals are thus highly controlled by gliadins and glutenins. Gliadins have lower content in lysine. Thus, the first limited amino acid in cereal proteins is lysine.

Gliadins and glutenins have poor solubility in water and, in usual, are regarded as two components of the well-known gluten in the food industry. Gliadins are composed with a single peptide chain with molecular weight of about 30–60 kDa, have intra-molecular disulfide bonds, and are usually divided into α-, β-, γ-, and ω-gliadins. Glutenins are composed of the so-called low and high molecular weight (LMW and HMW) subunits, with molecular weights of about 31–48 and 97–136 kDa, respectively. A glutenin molecule usually has about 3–5 HMW and 15 LMW subunits. Glutenins also have disulfide bonds between these subunits.

The disulfide bonds have a critical effect on gluten properties, for they govern solubility of both gliadins and glutenins. When reducing agents are added to wheat

Table 3.24 Relationship between surface hydrophobicity

Cereals	Albumins	Globulins	Gliadins	Glutenins
Wheat	5	10	69	16
Rice	5	10	5	80
Corn	4	2	55	39
Sorghum	8	8	52	32

flour, they induce an exchange reaction for the disulfide bonds. Thus, protein solubility increases while dough strength decreases. However, oxidative agents such as benzoperoxide can be used to improve flour and dough quality, as these oxidative agents are able to promote the formation of disulfide bonds. Active soybean flour also has similar effect. In addition, the interaction between gluten and starches may result in decreased starch retrogradation.

3.6.6 New Protein Resources

3.6.6.1 Single Cell Proteins (SCPs)

The proteins from several microorganisms and microalgae are regarded as SCPs and have potential application as food proteins. Compared with traditional food proteins, SCPs have some advantages in their production. SCP production is not dependent on climate and region conditions and can be done on industrial scale. The cells grow at higher rate, thus bringing higher protein production. Organic residues are also potential substrates for cell growth. In general, these proteins from algae, yeasts, and bacteria are the most important SCPs. The main chemical compositions of these organisms are given in Table 3.25.

The yeasts contain about 50% proteins (on dry basis), which lack the S-containing amino acids. Moreover, the yeasts have higher nucleic acid contents. Excessive yeast intake might induce higher uric acid level and metabolic disturbance. The bacteria contain about 75% proteins (on dry basis) that are also poor in the S-containing amino acids, and lipids in bacteria are mainly composed of saturated fatty acids. Both yeasts and bacteria thus cannot be used as food proteins directly. In usual, it is recommended to treat the cells with several steps, to remove the undesired cell wall, nucleic acid, and ash. The refined SCPs thus have similar chemical compositions to SPI. Fortification of SCPs with the S-containing amino acids leads to improved nutritional values.

Two microalgae chlorella and spirulina have application potential as new protein resources. They contain proteins, about 50–60% of dry basis. These proteins are rich in essential amino acids except for the S-containing amino acids.

Table 3.25 Relationship between surface hydrophobicity

Components	Algae	Yeasts	Bacteria	Molds
Nitrogen	7.5–10	7.5–8.5	11.5–12.5	5–8
Lipids	7–20	2–6	1.5–30	2–8
Ash	8–10	5.0–9.5	3–7	9–14
Nucleic acid	3–8	6–12	8–16	Very changeable

3.6.6.2 Leaf Proteins

Leaves cereal grasses and legumes contain 2–4% of proteins. After mechanical expression, the leaf juices are subjected to a treatment for the elimination of anti-nutritional factors, followed by a heat treatment to coagulate crude proteins. The dried protein products contain about 60% proteins, 10% lipids, 10% ash, and other substances (such as vitamins and colorants). Decolorized leaf protein products have better sensory quality and can be used in cereals for lysine fortification.

3.6.6.3 Fish Proteins

The proteins from those non-commercial fishes also can be used as potential dietary proteins for the human. The fresh fishes are recommended with water and lipid removal, which leads to decreased off-flavor arisen from the auto-oxidation of unsaturated fatty acids. If fishbone and entrails are also removed, fish protein products will have protein contents larger than 95%.

Fish proteins as one of those animal proteins have similar amino acid compositions to egg and milk proteins. However, fish proteins usually have poor dispersion, solubility, and water binding. With special treatments such as hydrolysis on fish proteins, an improvement in functional properties can be achieved.

3.7 Summary

Amino acids are basic structural elements of proteins. Proteins possess primary, secondary, tertiary, and quaternary structures, via covalent connection of amino acids and various forces. Proteins have stable spatial conformation and show various functional properties in food systems. However, condition changes may induce altered spatial conformation and forces and thereby will lead to protein denaturation and especially property changes. Food processing brings protein denaturation and various reactions for proteins (like cross-linking, decomposition, and hydrolysis) or amino acids (like oxidation and isomerization). These changes or reactions will yield negative or positive effects on protein nutrition, property, and safety as well as food quality.

Proteins are key components in foods and make contribution to nutritional values and quality attributes of foods. Proteins in some cases are critical factors to control food texture. Proteins after their hydration may form protein dispersion in water and thus provide beneficial properties for processed foods. The important foods from animals, plants, and soybean usually have good functional properties in gelation, emulsification, foaming, texturization, viscosity, and others. Proteins also can be endowed with modified properties via various structural modifications.

Questions

1. Please give a scientific definition for these technical terms:
   Hydrophobicity of amino acids, simple proteins, complex proteins, protein structure, denaturation, functional property, shear-thinning, cross-linking, Plastein reaction
2. Based on the chemical classification of amino acids, summarize the main reactions for the $-NH_2$ groups.
3. Please list the main results arisen from protein denaturation, the normal denaturation approaches, and the involved mechanisms.
4. Please explain the potential benefits of using the UHT technology in liquid foods, from a chemical kinetics point of view.
5. Please summarize these functional properties of proteins, the chemical mechanisms involved, and their application in the food industry.
6. Please explain different roles of wheat proteins in dough formation.
7. Please summarize interface properties of food proteins and explain the roles of milk and muscle proteins in dairy and meat products.
8. Please summarize the adverse reactions of proteins subjected to strong alkaline and high-temperature treatments.
9. Please summarize main protein modification and the beneficial effects of using enzymatic protein modification.
10. Please read a paper reporting single cell proteins (SCPs) extraction and separation or describe how bioactive proteins are separated from milk.

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