Chapter 5

Egg Components in Food Systems

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I. INTRODUCTION

The avian egg has a long history of being recognized as an important food ingredient and nutrient source for humans. With advances in technology during the twentieth century, the functional properties and chemical composition of avian eggs have been intensively studied, and most of these studies have focused on the egg produced by the domestic chicken. It is vital and basic for food science and biotechnology to comprehensively understand the chemical composition of the egg, and several reviews have been written which describe in detail the chemical composition of eggs (Li-Chan and Nakai, 1989; Huopalahti *et al.*, 2007; Li-Chan and Kim, 2008). Likewise, the functionalities of eggs on a molecular basis have been studied by innovative research which is summarized by multiple scientific reviews (Li-Chan and Nakai, 1989; Mine, 1995, 2002; Campbell *et al.*, 2003; Lomakina and Mikova, 2006). Understanding the functional properties of eggs and the chemistry behind these properties is important for the application of hen's eggs in the food industry to develop novel food products and improve the quality of existing products. The food industry also endeavors to prolong the shelf-life of eggs, protect the nutritional value of eggs

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during the storage period, and incorporate egg products into other marketable products such as cake and ice cream via food processing (Burley and Vadehra, 1989c). However, chemical changes in egg components and their functional properties are the consequences of food processing. Some of these changes are desirable, but some have to be prevented. Thus, it is helpful to improve the quality of egg products by preventing the chemical changes in eggs caused by food processing.

The objective of this chapter is to provide detailed information on egg composition and functionality in order to improve the understanding of the changes that occur in eggs during food processing. Details of composition, biosynthesis, and processing-induced changes in eggshell, albumen, and yolk are also summarized.

II. STRUCTURE AND CHEMICAL COMPONENTS OF EGGS

A. Structure of Eggs

The three main constituents of eggs are the eggshell (9–11%), the albumen, also referred to as egg white (60–63%), and the egg yolk (28–29%), as indicated in Table 5.1 and Figure 5.1. The egg yolk is located in the center of the egg surrounded by albumen, and enclosed by the eggshell. There is also a layer of eggshell membrane in the interval between the albumen and eggshell. The structures of each of these egg constituents are described in this section.

1. Structure of Eggshell

The eggshell has a polycrystalline structure which includes a porous layer of cuticle, a calcite layer, and two shell membranes. The cuticle layer contains 7000–17,000 unevenly distributed pore canals used to exchange gases. The structure of the eggshell layer is presented in Figure 5.2. There are four main layers in the eggshell: (1) the cuticle, a 10–30 μm thin layer which contains mineralized and organic layers as well as most of the pigment; (2) the palisade region, which is a dense vertical crystal layer about 200 μm thick and is composed of a calcified spongy matrix with a crystalline structure; (3) the mammillary layer, also referred to as the inner calcified layer, which is located in the basal part of calcified columns and includes the calcium reserve assembly and crown region; and (4) the shell membranes including the inner (20 μm) and outer membranes (50 μm) which are located between the albumen and the mammillary layer and are formed by organic fibers and used to protect against penetration by microorganisms. The complex eggshell structure is formed through a sophisticated process. The organic cores in the mammillary layer are formed as a seeding site used to grow calcium carbonate crystals, which is eventually built up to form the cuticle. Owing to the growth of calcite crystallites being inhibited by the fiber component of shell membranes, the orientation of the crystal in the palisade layer is outward (Nys *et al.*, 2004; Li-Chan and Kim, 2008).

2. Structure of Egg White

The egg white, or albumen, is comprised of four separate layers. About 23.3% of albumen is made up of a thin layer attached to the shell inner membrane, while the majority of albumen (57.3%) is composed of a viscous or thick white layer. Around 16.8% of albumen is composed of an inner thin white layer, and 2.7% is composed of a chalaziferous layer (Burley and Vadehra, 1989a; Li-Chan and Kim, 2008). The viscosity varies between thick and thin layers of egg white, because of the different contents of ovomucin. The proportions of egg white layers are affected by hen breed, environmental conditions, size of the egg, and rate of production (Li-Chan *et al.*, 1995). In fresh eggs, thick albumen covers the inner thin albumen and the chalaziferous layer, holding the yolk in the center of the egg.

TABLE 5.1 Composition of Albumen, Yolk, and Whole Egg–Wet Basis

Egg Component	% Protein	% Lipid	% Carbohydrate	% Ash
Albumen	9.7–10.6	0.03	0.4–0.9	0.5–0.6
Yolk	15.7–16.6	32.0–35.0	0.2–1.0	1.1
Whole egg	12.8–13.4	10.5–11.8	0.3–1.0	0.8–1.0

Adapted from Li-Chan, 1995.

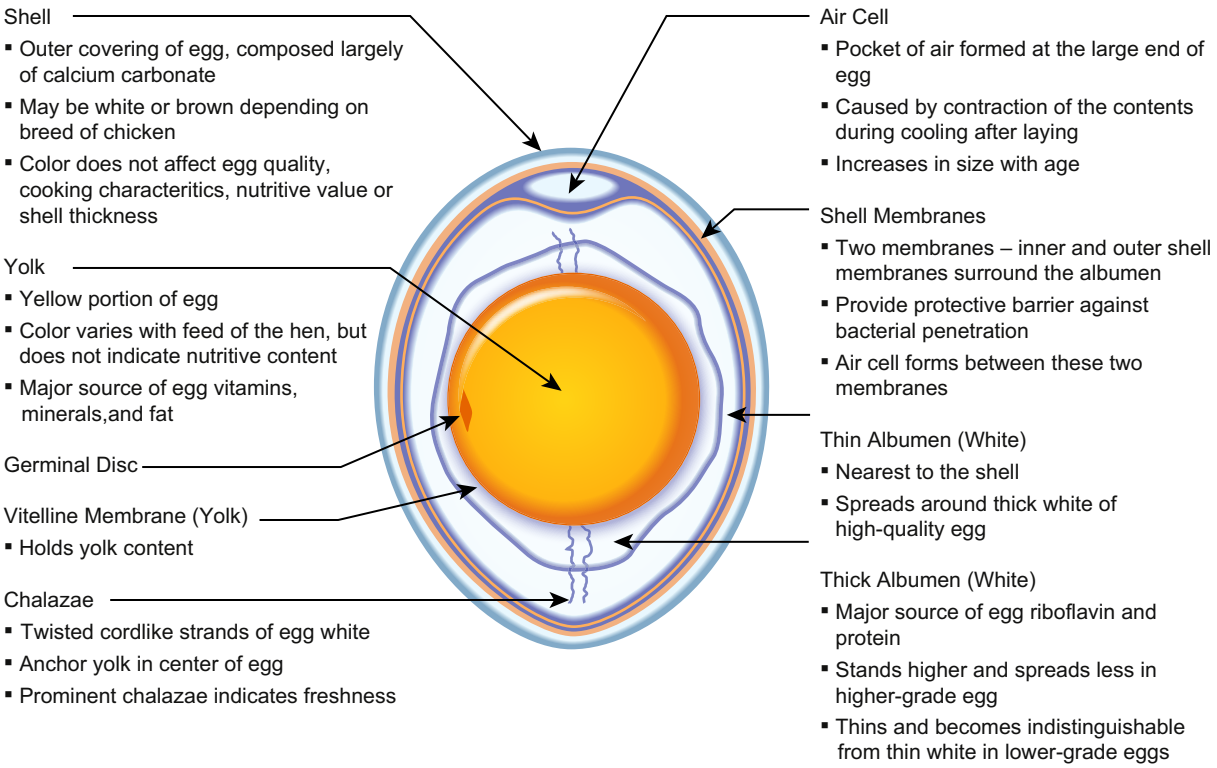

FIGURE 5.1 Structure of the hen's egg. The egg is composed of a shell, shell membranes, an air cell, the chalazae, the albumen (egg white), and the yolk. The yolk is centered in the albumen and surrounded by the vitelline membrane, which is colorless. The germinal disk, where fertilization takes place, is attached to the yolk. On opposite sides of the yolk are two twisted cord-like strands known as chalazae. Their function is to support the yolk in the center of the albumen. Surrounding the albumen are two shell membranes and the shell itself. *(Adapted from American Egg Board, 1981, http://www.aeb.org).*

3. Structure of Egg Yolk

The macrostructure of egg yolk consists of vitelline membrane, yellow and white yolk, as shown in Figure 5.3. The vitelline membrane is a thin (about 10 μm) protein fiber membrane which contains three multiple layers (Mineki and Kobayashi, 1998). The yellow yolk is composed of a light yellow layer (0.25−0.45 mm thick) and a deep yellow layer (2 mm thick) of plasma which are mainly formed by lipid−protein particles. These particles have been classified as

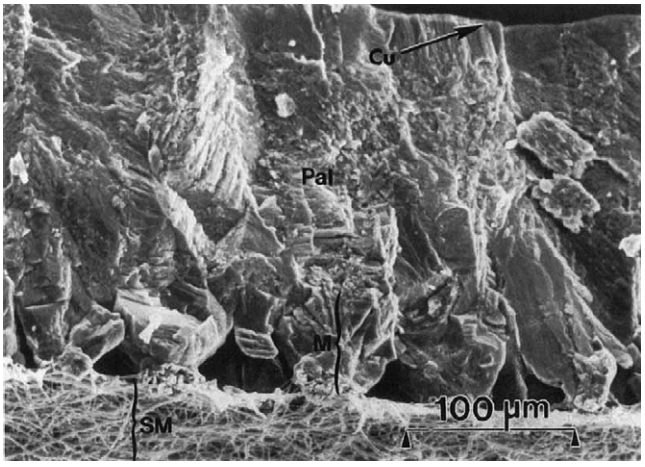

FIGURE 5.2 Scanning electron micrograph of a fractured eggshell showing a cross-section of the mineralized and non-mineralized zones. The shell membranes (SM) are a non-mineralized, collagen-based matrix interposed between the egg white and the mineralized shell. The mammillary zone (M) or cone region is a mineralized zone on the outer surface of the outer shell membrane and forms the base for the palisade region (Pal), which extends to the outermost portion of the eggshell, the cuticle (Cu). *(From Dennis* et al.*, 1996.)*

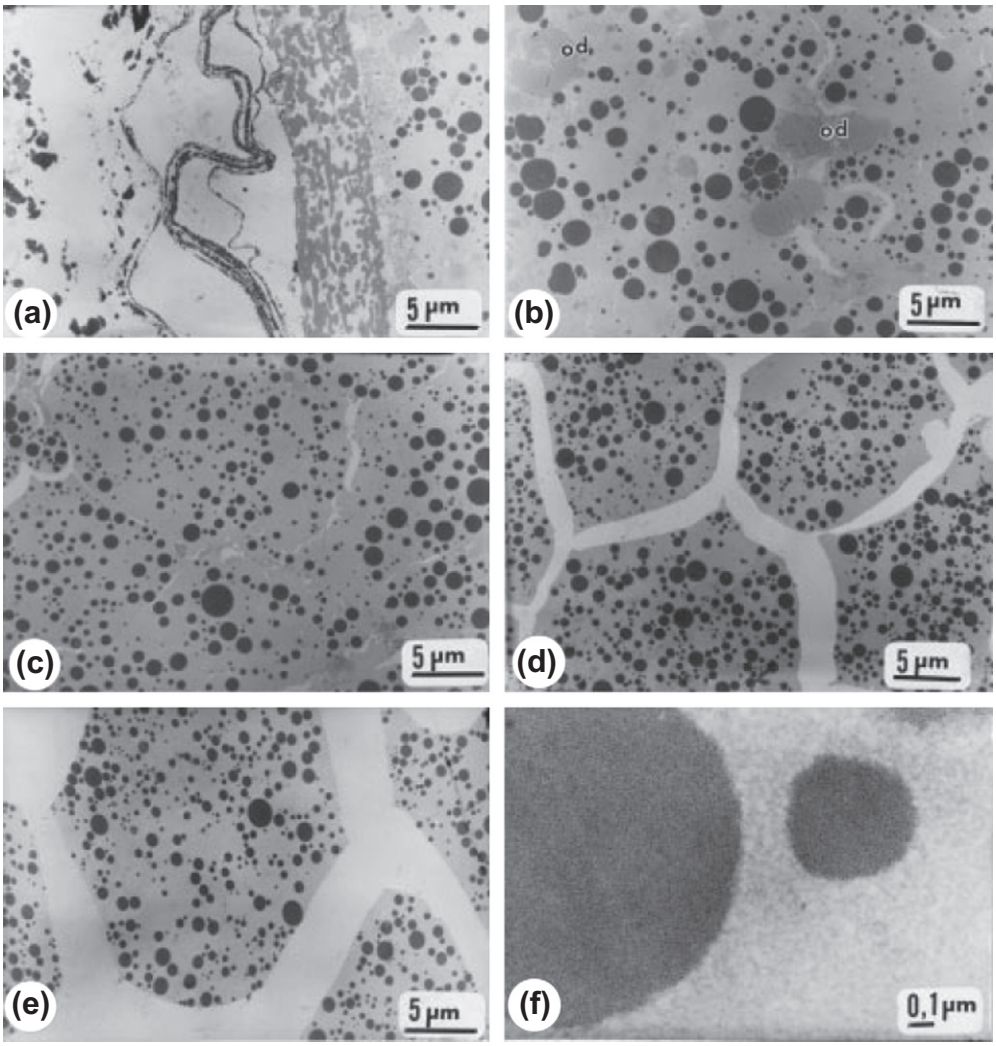

FIGURE 5.3 Transmission electron micrographs of fresh egg yolk. (a) Vitelline membrane; (b) cortical layer of yolk (od: oil droplet); (c) yolk sphere bordering cortical layer; (d) yolk sphere in outer layer; (e) yolk sphere in inner layer; (f) protein granules in yolk sphere. *(From Mineki and Kobayashi, 1997.)*

spheres (4−150 μm diameter), profiles (12−48 μm), or granules (0.3−2 μm) depending on their size. Egg yolk can be separated into two distinct fractions by dilution and centrifugation, resulting in a dark orange supernatant (plasma) and a pale pellet (granule) (Figure 5.4) (Anton, 2007). The white yolk accounts for only 2% of the total egg yolk by weight and contains several structures including the latebra, neck of latebra, nucleus of pander, and embryonic disk. The embryonic disk is 2−3 mm in diameter, is located in the nucleus of the pander, and is used by the developing embryo (Mineki and Kobayashi, 1998).

The microstructure of egg yolk has been analyzed using microscopy techniques including light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM), and the detailed structure of the yolk sphere and vitelline membrane has been shown (Mineki and Kobayashi, 1998). Mineki and Kobayashi used the frozen-section method as a novel approach to analyze egg yolk by fixing the egg yolk specimen at extremely low temperatures, followed by a second fixation step using chemicals. The result of their study is shown in Figure 5.3. The cortical layer of egg yolk (Figure 5.3b, c) was described as a distinct structure characterized by undeveloped yolk spheres with a shapeless membrane structure, small granules, possibly proteins, and oil spheres seen as larger granules. Furthermore, the yolk spheres observed in the outer layer (Figure 5.3d) were described as a round shape and smaller than the polyhedral spheres observed in the inner layer (Figure 5.3e). In the yolk spheres, protein granules with high electron density were shown to be highly dispersed (Figure 5.3f).

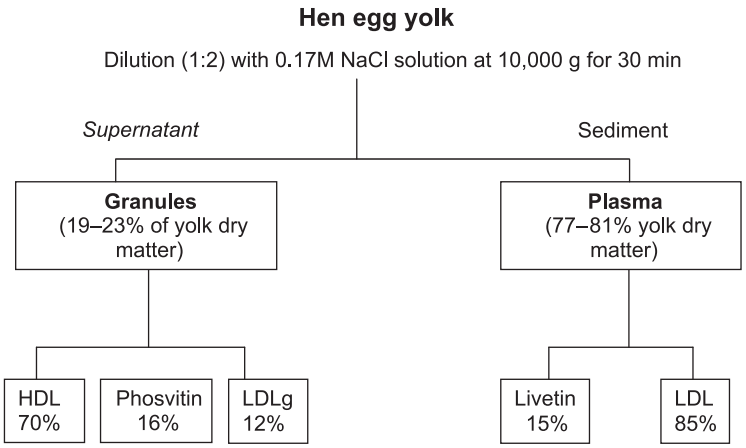

FIGURE 5.4 Fractionation of egg yolk into granules and plasma. *(From Anton, 2007.)*

B. Chemical Composition of Eggs

Eggs are made up of a variety of chemical components, including water, protein, fatty acids, minerals, vitamins, and pigments. Accordingly, the egg is recognized as a valuable food with high nutrient value. Eggs comprise 75% water, after which they are mainly made up of protein and lipid. Eggs also contain a smaller amount of carbohydrate, which includes glucose, sucrose, fructose, lactose, maltose, and galactose (Li-Chan and Kim, 2008). Sugino *et al.* (1997) indicated that the chemical composition of eggs is affected by the feed as well as by other factors including the species and age of the hen.

1. Chemical Composition of Eggshell

The eggshell consists of 95% minerals, primarily calcium and others including phosphorus and magnesium (Sugino *et al.*, 1997). About 3.5% of the organic composition of eggshells is protein, fatty acids, and polysaccharides rich in sulfated molecules (Nys *et al.*, 1999; Li-Chan and Kim, 2008). Li-Chan and Kim (2008) reviewed some organic acid components in the eggshell which are present in trace amounts. The cuticle layer, a thin organic layer covering the mineral crystal layer of the eggshell that provides protection from microbial infections, is composed of 90% insoluble protein, 5% carbohydrate, and 3% ash. This layer also contains a large proportion of the pigments which contribute to the different colors of eggs.

Following the cuticle layer, the vertical crystal layer is a thin monolayer on top of the palisade layer and is composed of calcium carbonate. As a foundation to the crystallization of calcium carbonate, there is a group of proteins called eggshell matrix proteins that belong to the basal part of the palisade layer and mammillary layer. Hunton (2005) reviewed the distribution of eggshell matrix proteins. Some eggshell matrix proteins are also widely expressed in egg white or in various chicken tissues, including ovalbumin, lysozyme, ovotransferrin, osteopontin, and clusterin. Osteopontin, a phosphorylated glycoprotein, is distributed in the basal part of the eggshell including membrane fibers, and the mammillary and palisade layers. The main function of osteopontin is to prevent calcium oxalate crystal precipitation extending over the basal part of the eggshell. Ovalbumin, ovotransferrin, and lysozyme are major egg white proteins distributed in both the mammillary layer and the shell membrane. The main role of these proteins in eggs is antimicrobial. Furthermore, ovotransferrin and lysozyme both have a dual role in the eggshell. These two proteins in eggshell membranes and the basal calcified layer influence the nucleation and crystallization of calcite and inhibit microorganism growth in egg albumen (Hincke *et al.*, 2000; Gautron *et al.*, 2001b).

Another group of eggshell matrix proteins is specific to the eggshell and identified only in domestic hens. These proteins include ovocleidin (OC)-17, -23, and -116, and ovocalyxin (OCX)-21, -25, -32, and -36. These specific eggshell matrix proteins have been successfully purified and identified as having particular functional properties which regulate the eggshell mineralization process (Dominguez-Vera *et al.*, 2000; Li-Chan and Kim, 2008). The mineralization develops in an acellular uterine fluid which contains the ionic and matrix precursors of the eggshell. These proteins, produced from uterine tubular gland cells, are widely distributed throughout the mammillary and

palisade layers of the eggshell (Gautron *et al.*, 1997). OC-17 is a 142 amino acid phosphorylated protein with C-type lectin-like domains, which was the first matrix protein identified which regulates the precipitation of calcium carbonate in eggshell (Mann and Siedler, 1999; Reyes-Grajeda *et al.*, 2004). The glycosylated form of OC-17 protein is OC-23, which has a molecular mass of 23 kDa (Li-Chan and Kim, 2008). OC-116 is a 742 amino acid glycosylated protein with two disulfide bonds, which plays an important role in controlling calcite growth during eggshell calcification (Hincke *et al.*, 1999). OCX-32, identified by Gautron *et al.*, is secreted by the surface epithelial cells of the uterus during the phase of termination of eggshell formation (Gautron *et al.*, 2001a). Xing *et al.* (2007) recently determined that OCX-32 has the capacity to reinforce the antimicrobial activity of the eggshell. Gautron and Nys (2007) have also successfully cloned OCX-21, -25, and -36. OCX-36 is expressed abundantly during shell calcification and plays a role in natural defense mechanisms in eggs. However, the functional properties of OCX-21 and -25 have not been determined.

The inner and outer shell membranes consist of mainly protein and a smaller amount of ash and glucose based on the dry weight (Sugino *et al.*, 1997). The eggshell membranes are primarily constituted by collagens, keratin sulfate, and matrix proteins. The matrix proteins, including ovotransferrin, lysozyme, and ovalbumin, are distributed in the eggshell membranes to enhance the antimicrobial defense system of the egg. Dermatan, a proteoglycan substance, and keratin sulfate, are covered by these fibers to form a core (Dennis *et al.*, 1996). The mammillae are the vesicles of calcium reserve body containing the calcium binding molecule, which is made up of keratin sulfate (Li-Chan and Kim, 2008). The cross-linked collagen fiber system plays an important role in protecting the egg from contamination by microorganisms.

2. Chemical Composition of Egg White

About 60% of the total egg weight is composed of egg white. The major constituent of egg white is water, which makes up more than 80% of egg white (Li-Chan and Kim, 2008). The proteins in the egg white were first purified and fractionated by Rhodes *et al.* (1958). On a dry weight basis, proteins are the main component of egg white and predominantly include 54% ovalbumin, 12−13% ovotransferrin, 11% ovomucoid, 3.5% lysozyme, 2% G2 and G3 ovoglobulins, and 1.5−3% ovomucin. Some trace amounts of other proteins are also found in egg white, including ovostatin, ovoflavoproteins, avidin, and enzymes such as α-mannosidase, β-galactosidase, and catalase (Li-Chan and Nakai, 1989). The physicochemical and biological functions of the main egg white proteins are summarized in Table 5.2.

Ovalbumin is the most abundant constituent of egg white proteins. It is a phosphoglycoprotein with a molecular mass of 45 kDa and is composed of 386 amino acid residues (McReynolds *et al.*, 1978; Huntington and Stein, 2001; Lechevalier *et al.*, 2007). As a secretory protein, ovalbumin has a hydrophobic sequence between residues 21 and 47, used as an internal signal sequence involved in transmembrane location instead of a classical N-terminal leader sequence (Huntington and Stein, 2001). Half of the amino acids in ovalbumin are hydrophobic and one-third are negatively charged; consequently, the protein has an isoelectric point of 4.5 (Li-Chan and Nakai, 1989). Fothergill and Fothergill (1970) determined that ovalbumin contained six cysteine residues, but only two of them were involved in a disulfide bond and the other four were composed in a sulfhydryl (SH) group. Three of these SH groups are masked in the native state whereas the fourth is reactive only in the denatured protein (Lechevalier *et al.*, 2007). Apart from cysteine residues, ovalbumin also contains one carbohydrate unit and zero, one, or two residues of phosphoserine. Furthermore, the ovalbumin structure has four crystallographically independent molecule parts and the distance between the position of the helical reactive center loop and the protein core is 2−3 Å (Huntington and Stein, 2001). It also contains three β-sheets and nine α-helices (Stein *et al.*, 1991). As result of denaturation, ovalbumin can be transformed into a more heat-stable *S*-ovalbumin, which is an intermediate species (Lechevalier *et al.*, 2007). High pH and temperature both increase the rate of conversion. The appearance of *S*-ovalbumin can be enhanced by storage time of eggs. The *S*-ovalbumin level may reach 81% in an egg after 6 months' storage at low temperature (Vaderhra and Nath, 1973). About 2−5% loss of α-helices and increases in antiparallel β-sheets are attributed to the conformational changes in *S*-ovalbumin (Huntington and Stein, 2001). Ovalbumin belongs to the serpin (serine protease inhibitor) superfamily but lacks inhibitory activity; its biological function remains largely unknown (Huntington and Stein, 2001). For the application of egg ovalbumin in the food industry, the functionality of ovalbumin is mainly responsible for the gelling properties of egg white (Mine, 1995). Ovalbumin is also the major allergen found in egg white and can activate immunoglobulin E (IgE)-mediated allergic reactions in mammals. The structural and physicochemical properties of ovalbumin are reviewed in detail by Huntington and Stein (2001) and Lechevalier *et al.* (2007).

TABLE 5.2 Physicochemical Properties of Proteins Found in Egg White

Protein	% (w/w)	p*I*	M_w (kDa)	T_d(°C)	Cysteines	−SH	S−S
Ovalbumin	54	4.5−4.9	45	75−84	6	4	1
Ovotransferrin (conalbumin)	12−13	6.0−6.1	77.7	61−65 (76.5, Al^{3+})	30	−	15
Ovomucoid	11	4.1	28	77	18	−	9
Ovomucin	1.5−3.5	4.5−5.0	110, 5500−8300, 220−270,000		(2)	−	
Lysozyme	3.4−3.5	10.7	14.3−14.6	69−77	6		4
G2 ovoglobulin	1.0	4.9−5.5	47−49				
G3 ovoglobulin	1.0	4.8, 5.8	49−50				
Ovoflavoprotein	0.8	4.0	32−35, 80		5		2
Ovostatin	0.5	4.5−4.7	760−900				
Cystatin	0.05	5.1	12				
Avidin	0.05	10.0	55−68.3		2		1

From Mine (1995).

Egg white lysozyme, which is the *N*-acetylmuramic hydrolase used to hydrolyze peptidoglycan at the β1−4 glycosidic bond, has a molecular weight of 14.4 kDa and consists of 129 amino acid residues (Lechevalier *et al.*, 2007; Li-Chan and Kim, 2008). It is a basic protein with an isoelectric point of 10.7. Lysozyme is made up of two domains linked by a long α-helix, while also separated by a helix-loop-helix (HLH) (Young *et al.*, 1994). HLH has recently been shown to possess membrane-permeabilizing and antibacterial activity (Ibrahim *et al.*, 2001). As an enzyme, lysozyme has its polar groups outside and hydrophobic groups buried inside the molecule. The conformational transition in lysozyme, called 'hinge-bending', results in a relative movement of its two lobes and provides a site for catalysis to access the substrates. Ibrahim *et al.* (1997) demonstrated that the calcium cation was able to induce conformational changes in lysozyme to activate its antibacterial activity. Some of the antibacterial activity of lysozyme has been shown to be independent of its catalytic function via analysis of the activity of antibacterial peptides prepared from the enzymatic hydrolysis of lysozyme (Mine and Kovacs-Nolan, 2006).

Ovotransferrin, also referred to as conalbumin, is a glycoprotein found in egg white. It contains 686 amino acid residues and has a molecular mass of 78−80 kDa and an isoelectric point of 6.0. It has the capacity to bind with various ions, especially ferric Fe^{3+} ions, thereby transferring iron into host cells by membrane receptors (Mason *et al.*, 1996). Ovotransferrin has two lobes with four domains, including an N- and a C-terminal lobe. Each lobe consists of two distinct alpha/beta domains of similar size and with a single binding site as well as 15 disulfide bridges (Superti *et al.*, 2007; Li-Chan and Kim, 2008). Ovotransferrin has antimicrobial activity against various Gram-negative and Gram-positive bacteria, fungi, and viruses.

Another glycoprotein distributed in the egg white is ovomucoid, which is a thermally stable protein and makes up 11% of the egg white protein (Li-Chan and Nakai, 1989). Ovomucoid contains about a quarter of asparaginyl-linked carbohydrate moieties, and is made up of 186 amino acids with a molecular mass of 28 kDa (Kato *et al.*, 1987b; Li-Chan and Nakai, 1989). It is a trypsin inhibitor with three distinct domains cross-linked by intradomain disulfide bonds. In total, nine disulfide bonds are identified in ovomucoid, while no free sulfhydryl groups are found. The active site is found only in domain II, which is attributed to inhibiting trypsin activity in the chicken egg white (Li-Chan and Nakai, 1989). Ovomucoid purified from chicken egg white has allergenic potential to trigger IgE-mediated reactions in humans (Mine and Zhang, 2001; Mine and Rupa, 2004).

Ovomucin is a sulfated glycoprotein found in egg white with insoluble and soluble subtypes, which contains proteins and a smaller amount of carbohydrates. The carbohydrates found in ovomucin are in the form of oligosaccharides (Hiidenhovi, 2007). Insoluble ovomucin plays a key role in forming the gel-like insoluble fraction of the thick albumen in egg white, while soluble ovomucin is mainly distributed in the outer and inner albumen (Burley and

Vadehra, 1989d; Li-Chan and Kim, 2008). Ovomucin consists of two subunits, α- and β-ovomucin; these are found in both insoluble and soluble subtypes of ovomucin in different proportions (Li-Chan and Kim, 2008). α-Ovomucin contains 91% protein and 9% carbohydrate with 2087 amino acid residues and a molecular mass of 230−250 kDa (Watanabe *et al.*, 2004). β-Ovomucin consists of 872 amino acids and has a molecular mass of about 400−720 kDa (Itoh *et al.*, 1987; Hiidenhovi, 2007; Hammershøj *et al.*, 2008).

The other proteins in albumen include ovoglycoprotein, flavoprotein, avidin, protease, and minor proteins including lipocalins, clusterin, and Ch21 proteins. These are described in detail by Li-Chan and Kim (2008) and Huopalahti *et al.* (2007).

In addition to proteins, other types of chemical components are found in the egg white (Li-Chan and Kim, 2008). Carbohydrates are present in egg white in the form of both conjugated oligosaccharides and free glucose. A low content of lipid, 0.03% of egg albumen by weight, is also found in egg white, as are trace amounts of various minerals and water-soluble vitamins. Li-Chan and Kim (2008) provide a detailed review of the chemical composition of egg white.

3. Chemical Composition of Egg Yolk

Egg yolk contains 50% solids with a lipid:protein ratio of 2:1 (Li-Chan and Kim, 2008). The distribution of protein and lipid in the vitelline is different from the yolk itself. It contains 87% protein, 10% carbohydrate, and 3% lipid on a dry weight basis (Li-Chan and Kim, 2008). Egg yolk can be separated into two phases: plasma and granule. Plasma is composed of up to 80% liquid yolk with a higher content of lipid, while granules contain approximately three times more proteins than plasma (Li-Chan *et al.*, 1995). The components of egg yolk are shown in Figure 5.4 and the compositional analysis of fresh yolk is summarized in Table 5.3.

The protein content of liquid yolk is approximately 16%. Egg yolk protein constitutes mainly 16% high-density lipoproteins (HDL), 68% low-density lipoproteins (LDL), 10% livetins, 4% phosvitin and very low-density lipoproteins (VLDL) (McCully *et al.*, 1962). Approximately two-thirds of yolk solids are LDLs, which are spherical particles with a lipid core surrounded by a layer of phospholipid and protein (Anton *et al.*, 2003). The LDLs are composed of about 14% protein and 86% lipid, which includes 74% neutral lipid and 26% phospholipid (Martin *et al.*, 1964). Because LDLs consist of apoproteins and phospholipids, they have amphiphilic properties and can be dispersed at the oil−water interface. Thus, LDLs are the essential component responsible for the emulsifying properties of egg yolk. There are six types of apoprotein in the LDLs of egg yolk, with molecular weights between 15 and 130 kDa and a p*I* range from 6.3 to 7.5. Apoprotein I constitutes about 70% of total apoproteins, and is less soluble in water. Apoprotein II contains a high proportion of amphipathic α-helix chains, which contribute to dispersion at the oil−water interface (Anton *et al.*, 2003). VLDLs are precursors of egg yolk LDL and transferred from the hen's blood into the ovary.

HDLs in hen's egg yolk, also referred to as lipovitellins, are distributed in granules. They have a molecular mass of 400 kDa and contain 75−80% proteins and 20−25% lipid, comprising 65% phospholipids, 30% triglycerides, and 5% cholesterol (Cook and Martin, 1969; Anton *et al.*, 2003). Two subtypes of lipovitellin, α and β, are found in egg yolk in a ratio of 1:1.5 and contain different amino acid sequences as well as phosphorus and carbohydrate residues (Li-Chan and Kim, 2008). There are about five types of apoprotein identified in HDLs with molecular masses ranging from 35 to 110 kDa; these apoproteins are glycosylated by mannose, galactose, glucosamine, and sialic acid

TABLE 5.3 Compositional Analysis of Egg Yolk

	Fresh Yolk (%)	Dry Yolk (%)
Water	51.1	–
Lipids	3.6	62.5
Proteins	16.0	33.0
Carbohydrates	0.6	1.2
Minerals	1.7	3.5

From Li-Chan *et al.* (1995).

(Anton *et al.*, 2007). Furthermore, HDLs and phosvitins have the same precursor of vitellogenin which is synthesized in the hen's liver. A granular complex in egg yolk is formed by HDLs and phosvitin via phosphocalcic bridges (Wang *et al.*, 1983). Li-Chan and Kim (2008) indicated that egg yolk HDLs may have therapeutic potential including antioxidant and antimicrobial properties.

Phosvitin is a phosphoglycoprotein and makes up 4% of egg yolk dry matter (Anton *et al.*, 2007). Almost half of the amino acids in phosvitin are serine residues, with 90% phosphorylation, and form a central hydrophilic area surrounded by two hydrophobic areas at the N- and C-termini. Two subtypes of phosvitin, α- and β-phosvitin, are found in chicken egg yolk and account for 80% of the phosphorus binding proteins in yolk (Li-Chan and Kim, 2008). The higher content of phosphoserine residues and special conformational structure of phosvitin contribute to its resistance to heat denaturation and proteolytic cleavage (Juneja and Kim, 1997; Anton *et al.*, 2000). Phosvitin has strong chelating properties, and is a natural metal binding biomolecule.

Livetin accounts for 30% of the plasma proteins in egg yolk. Livetin contains 20% α-livetin, a serum albumin, 50% β-livetin, an α_2-glycoprotein, and 30% γ-livetin, immunoglobulin Y (IgY), which is similar to mammalian IgG (Schade and Chacana, 2007). The α-livetin has a molecular weight of 70 kDa and a p*I* value between 4.3 and 5.7, and has allergenic activity to induce type I hypersensitivity (Schade and Chacana, 2007; Williams, 1962). The β-livetin has a molecular weight of 45 kDa and contains 7% hexose. The molecular weight of γ-livetin (IgY) is approximately 167 kDa. The IgY antibody is made up of two light chains and two heavy chains, and is transferred to the yolk to passively protect the developing embryo.

The main constituents in dry egg yolk are lipids, which make up 62% of egg yolk powder (Juneja, 1997). Among those lipids, triglycerides, phospholipids, and cholesterol occupy 65%, 31%, and 4%, respectively. The fatty acid compositional analysis of these lipids is summarized in Table 5.4. Triglycerides and phospholipids are both glycerolipids with a glycerol backbone. Phospholipids have a glycerol−phosphate backbone. In addition, as amphiphilic molecules, phospholipids have both polar and non-polar groups with emulsification properties. The two major components of egg yolk phospholipids are phosphatidylcholine and phosphatidylethanolamine, with the remainder

TABLE 5.4 Proteins and Lipids in Egg Yolk

Constituent	Major Components	Relative %
Proteins[a]	Apovitellenin I–VI	37.3
	Lipovitellin apoproteins	
	α-Lipovitellin	26.7
	β-Lipovitellin	13.3
	Livetins	
	α-Livetin (serum albumin)	2.7
	β-Livetin (α_2-glycoprotein)	4.0
	γ-Livetin (γ-globulin)	2.7
	Phosvitin	13.3
	Biotin binding protein	Trace
Lipids[b]	Triglyceride	65
	Phosphatidylcholine	26
	Phosphatidylethanolamine	3.8
	Lysophosphatidylcholine	0.6
	Cholesterol	4
	Sphingomyelin	0.6

[a]*Modified from Burley and Vahedra (1989a).*
[b]*Adapted from Juneja (1997).*
From Li-Chan and Kim (2008).

consisting of lysophosphatidylcholine, lysophosphatidylethanolamine, and sphingomyelins (Li-Chan and Kim, 2008). The cholesterol content is about 1.6% in raw egg yolk. About 80% of total egg yolk cholesterol is free cholesterol and the remainder exists in the form of cholesterol esters.

There are smaller amounts of carbohydrate present, around 1% of the dry mass of egg yolk (Li-Chan and Kim, 2008). Glucose is a free carbohydrate and a major constituent found in egg yolk. The rest of the carbohydrates found in egg yolk are in a conjugated form, such as sialic acid bound to glycoproteins and glycolipids (Li-Chan and Kim, 2008). Egg yolk also contains a trace amount of vitamins, minerals, and pigments, which are listed in Table 5.5. The mineral content in egg yolk is about 1%. Phosphorus is the most abundant mineral owing to the high content of phospholipid (Li-Chan and Kim, 2008). Egg yolk contains various vitamins and is a good source of vitamins A, D, E, and B_{12}. Water-soluble vitamins including folic acid, riboflavin, and niacin are present in both egg white and yolk. As the precursor of vitamin A, carotenoids are also present in egg yolk and are its main

TABLE 5.5 Minerals and Vitamins Present in Whole Egg, Egg Albumen, and Egg Yolk

Constituent (units)	Whole Egg	Egg Albumen	Egg Yolk
Minerals (mg)			
Calcium	29.2	3.8	25.2
Chlorine	96.0	66.1	29.9
Cobalt	0.033	0.009	0.024
Iodine	0.026	0.001	0.024
Iron	1.08	0.053	1.02
Magnesium	6.33	4.15	2.15
Manganese	0.021	0.002	0.019
Phosphorus	111	8	102
Potassium	74	57	17
Sodium	71	63	9
Sulfur	90	62	28
Zinc	0.72	0.05	0.66
Vitamins			
Vitamin A (IU)	264	–	260
Vitamin D (IU)	27	–	27
Vitamin E (mg)	0.88	–	0.87
Vitamin B_{12} (μg)	0.48	–	0.48
Choline (mg)	11.0	2.58	8.35
Folic acid (mg)	237	0.46	238
Inositol (mg)	0.023	0.006	0.026
Niacin (mg)	5.94	1.52	4.35
Pantothenic acid (mg)	0.045	0.035	0.010
Pyroxidine (mg)	0.83	0.09	0.73
Riboflavin (mg)	0.065	0.008	0.057
Thiamine (mg)	0.18	0.11	0.07
	0.05	0.004	0.048

Adapted from Watkins (1995); Li-Chan and Kim (2008).

pigment. It should be noted that carotenoids cannot be synthesized by the hen; the carotenoids in egg yolk are obtained from the feed of laying hens.

III. BIOSYNTHESIS OF EGGS

A. Introduction to the Egg Formation Process

The chicken egg as a developed ovum is formed in the ovary, where it grows into a follicle, and is finally delivered to the uterus, where it can be further assembled (Burley and Vadehra, 1989c; Okubo *et al.*, 1997). The constituents used for egg yolk development are supplied by the liver and delivered in the blood. First, the oocyte in the ovary begins to grow into a white follicle which is surrounded by the hen's veins. Later, a yellow follicle starts to develop at the base of a white follicle in the ovary approximately 7−10 days before ovulation, resulting in the deposition of yellow egg yolk (Okubo *et al.*, 1997). Eventually, the encapsulated yellow follicle, known as the mature ovum, is ovulated into the oviduct, a process which takes about 24−27 hours. The oviduct in laying hens is about 60−80 cm long and is composed of five regions: the infundibulum, magnum, isthmus, uterus, and vagina (Burley and Vadehra, 1989a). The length and ovum-holding time of these five portions are distinguished by the particular functionality of each region (Okubo *et al.*, 1997). The longest part of the oviduct is the magnum, which is also recognized as the albumen-secreting region. The eggs stay in this region for approximately 2−3 hours to accumulate albumen. The uterus has thick walls, and it is here that the eggshell is assembled. The eggs are held in the uterus for about 21 hours to complete the process of eggshell mineralization before the egg is laid.

B. Biosynthesis of Eggshell and Related Biochemical Changes

1. Regulation of Eggshell Biosynthesis

The eggshell is an essential part of all avian eggs and has a highly organized and porous structure to sustain water and gas exchanges, provide calcium to the embryo during development, and protect the egg from microbial infections. Eggshells are mainly made up of inorganic mineral and organic matrix components, the latter being composed of proteins, glycoproteins, and proteoglycans provided by uterine fluid (Hincke and Wellman-Labadie, 2008). The main inorganic component of eggshell is calcium ions, which are transported through the uterine mucosa by binding with calbindin. Subsequently, bicarbonate ions are produced from calcium and hydration of carbon dioxide by carbonic anhydrase catalysis (Gautron *et al.*, 1997). The synthesis of the eggshell is regulated by the endocrine system of the laying hen, which includes estrogen, calcitonin, parathyroid hormone, and 1,25-dihydroxyvitamin D_3 (Burley and Vadehra, 1989b). The concentration of blood calcium and rhythms of laying eggs are controlled by hormones of the endocrine system. Formation of a whole eggshell is initiated by deposition of the shell membranes on the outside surface of the albumen in the isthmus region of the oviduct. The organic matrix of the eggshell membrane is mainly made up of a fibrillar protein formed by a disulfide cross-linking network, and collagens including types I, V, and X (Nys *et al.*, 2004; Hincke and Wellman-Labadie, 2008). These components are synthesized and released by numerous tubular gland cells and other types of cell located in the isthmus. A lysine-derived cross-linked structure of eggshell membrane is produced with the assistance of a copper-containing enzyme (Burley and Vadehra, 1989c). This cross-linked fiberic structure plays a dual role in the control of eggshell mineralization by preventing calcification toward the inner membranes and forming nucleation sites on the outer surface of membranes.

2. Biomineralization of Eggshell

The biomineralization of eggshell is a distinct process compared with the mineralized tissues in other species, both vertebrate and invertebrate. In other species, the matrix usually contains collagenous or non-collagenous elements that directly interact with the mineral phase to form a biphasic calcified matrix and control the deposition of minerals (Addadi and Weiner, 1992; Belcher *et al.*, 1996; Robey, 1996). For the avian shell, there is also a spatial separation in the eggshell between its organic framework and mineralized components, by which the eggshell membrane interacts with organic aggregates known as mammillary knobs (Hincke and Wellman-Labadie, 2008). The mammillary knobs are distributed on the outer surface of eggshell membrane and used as a nucleus for calcium carbonate aggregating into a polycrystalline structure. The calcification is a sequential process with a specific timeline related to ovulation. A variety of biochemical changes are associated with the biomineralization of eggshell, including changes in the

protein matrix composition and formation of calcite in the uterine fluid. The acellular uterine fluid contains both organic and inorganic precursors of the shell matrix, thereby influencing the precipitation kinetics, morphology, and orientation of crystals. The protein profile collected from uterine fluid is different during the three stages of eggshell formation, i.e. the initial, growth, and terminal phases (Dominguez-Vera *et al.*, 2000). Recent studies have shown that the uterine fluid can enhance calcium carbonate precipitation in both the initial and growth phases, whereas it has inhibitory effects on mineralization (Gautron *et al.*, 1997; Dominguez-Vera *et al.*, 2000).

In the initial phase of mineralization of the eggshell, numerous proteins have been identified in uterine fluid that are involved in calcium carbonate precipitation and calcite crystalline formation, which mainly include ovocleidins, ovalbumin, albumen, osteopontin, and ovotransferrin. Ovotransferrin and ovalbumin are predominant in the uterine fluid in this phase and have calcium-binding properties, which may play a role in regulating the nucleation of calcite (Gautron *et al.*, 2001b). OC-17 and -116 are also prevalent in the uterine fluid during the initial phase. OC-17 may be involved in regulating calcite morphology as crystal growth of the eggshell (Nys *et al.*, 2004). OC-116, known as ovoglycan, which is a dermatan sulfate proteoglycan, is a unique eggshell matrix protein belonging to the secretory calcium-binding phosphoprotein (SCPP) family and produced from the granular cells of the uterine epithelium of the isthmus (Arias and Fernandez, 2001; Hincke and Wellman-Labadie, 2008). OC-116 may have the same functionality as a proteoglycan which plays a key role in promoting cartilage calcification and collagen mineralization (Hunter, 2001; Hincke and Wellman-Labadie, 2008).

In the growth phase, the uterine fluid still actively promotes precipitation kinetics of calcium carbonate. However, some protein constituents collected from uterine fluid in this phase have inhibitory effects on the precipitation of calcite and delay calcite growth by directly binding to the crystal (Gautron *et al.*, 1997; Nys *et al.*, 2004). OCX-32 and -36 are present in uterine fluid during the growth phase. Owing to its involvement in natural defense mechanisms, OCX-36 may enhance the development of the eggshell during this phase, but its activity in the mineralization of calcite is unknown (Hincke and Wellman-Labadie, 2008). OCX-32 is capable of inhibiting mineralization and therefore plays a role in controlling the deposition of calcium carbonate during the eggshell growth phase (Nys *et al.*, 2004; Hincke and Wellman-Labadie, 2008). Some proteins, including lysozyme, were identified with biphasic effects on regulating calcium carbonate precipitation relevant to their concentration in the uterine fluid (Hernandez-Hernandez *et al.*, 2003). At high concentration, these proteins can block the growth of calcite crystallization by binding to the crystal surface. The biphasic roles of these proteins may inhibit the growth of calcite crystals at the end of the eggshell formation process.

In the terminal phase, the uterine fluid mainly contains an organic matrix which is assumed to have inhibitory effects on the precipitation of calcite. The organic matrix contained in this phase delays the pH drop associated with calcium carbonate precipitation, thereby inhibiting the growth of calcite crystals (Gautron *et al.*, 1997). The proteins identified in the organic matrix collected from this stage are primarily OCX-32 and -36 (Nys *et al.*, 2004; Hincke and Wellman-Labadie, 2008). It takes about 1.5 hours to terminate mineralization and compose the organic cuticle layer surrounding the eggshell before egg ovulation. In the whole process of mineralization and formation of eggshells, the matrix components in the uterine fluid play an active role in the control of the calcite growth kinetics and crystal morphology.

C. Biosynthesis of Egg Albumen

The egg albumen proteins are primarily synthesized by tubular gland cells which are distributed along the oviduct wall. The egg albumen is assembled in the magnum after a developed ovum reaches this region. The biosynthesis of egg albumen proteins begins only in response to hormone stimulation (Schutz *et al.*, 1978; Burley and Vadehra, 1989d). The proportions of the three major egg albumen proteins synthesized from the tubular gland cells are 50−60% ovalbumin, 8% ovomucoid, and 2−3% lysozyme (Palmiter, 1972; Schutz *et al.*, 1978). Synthesis of ovalbumin results from primary stimulation by estrogen followed by withdrawal and secondary stimulation. The estrogen stimulation leads to an increase in ovalbumin messenger RNA expression and subsequently enhances the glycosylation of albumen in the chicken oviduct (Schutz *et al.*, 1978; Burley and Vadehra, 1989d). Other mechanisms, such as the signal transduction, are also involved in regulating the synthesis of egg albumen proteins. Cooney *et al.* (1993) identified that the upstream promoter transcriptional factor (COUP-TF) of chicken ovalbumin regulated the hormone expression via interaction with other transcriptional factors. The expression of hormones involved in regulating egg albumen protein secretions from the oviduct is under the control of the particular signals which, in turn, are stimulated by those secreted proteins; therefore, an integrated control system is established. Other egg albumen proteins produced by hens are mainly antimicrobial proteins, such as lysozyme and ovotransferrin, which are used to protect the embryo from microbial infections. The lysozyme in egg albumen is primarily produced by

tubular gland cells in the oviduct, rather than being delivered from the hen's blood (Shawkey *et al.*, 2008). The biosynthesis of ovotransferrin is regulated by hormones in the oviduct including estrogen and progesterone (Lee *et al.*, 1978; Shawkey *et al.*, 2008). The activities of the antimicrobial proteins are enhanced during the hatching process owing to an increase in temperature. The production and deposition of antimicrobial proteins in eggs are a typical protective system for reproduction and genetics established during the evolutionary process.

The gel structure of egg albumen is primarily attributed to the interactions of glycoproteins. The main constituents of egg albumen are glycoproteins, including ovalbumin, lysozyme, and ovomucin. Glycoproteins are a group of macromolecules with carbohydrate moieties attached to the polypeptide chains through covalent bonds, such as *N*-acylglycosylamine linkages (Robinson, 1972). The variable degrees of modification in amino acid residues result in the generation of different protein moieties of the egg white glycoproteins; these modifications include N-acetylation of N-terminal residues, phosphorylation of serine and threonine residues, and methylation of lysine and arginine residues (Robinson, 1972). The particular physical properties of egg white are attributed to the interactions of chemical bonds in the egg glycoproteins. Moreover, both ovomucin and lysozyme contain substantial amounts of cysteine residues which can form disulfide bonds and subsequently form a lysozyme−ovomucin complex. This complex contributes to a rigid gel network being established in the egg white, whereas the disulfide bond of the lysozyme−ovomucin complex can be inhibited by existing divalent cations, such as magnesium and calcium (Robinson, 1972). An integrated regulation of the gel structure in the egg white depends on interaction among the glycoproteins and other constituents of the egg white.

D. Biosynthesis of Egg Yolk

The early formation of egg yolk is initiated after differentiation of the embryo. The egg yolk cells are assembled in the epithelium of the ovaries. Subsequently, the yolk cells divide rapidly and form oocytes, surrounded by continuous follicle cells (Burley and Vadehra, 1989b). The egg yolk develops inside the wall of the hen's ovarian follicle which is supplied with blood capillaries by which the egg yolk protein precursor, known as vitellogenin, is transferred and deposited into the yolk. Egg yolk proteins are primarily synthesized in the liver under hormonal control, and then transported into the developing oocyte via the blood (Burley and Vadehra, 1989b; Stevens, 2004). The activation of transcription and translation of genes encoding egg proteins is also involved in this process (Stevens, 2004). The whole process of egg yolk formation involves an increase in protein and lipid synthesis in the liver and is influenced by the hen's habitat and dietary nutrients (Stevens, 2004).

1. Formation of Vitellogenin

About 95% of the egg yolk protein is made up of LDL, lipovitellin, phosvitin, and livetin. After entering the yolk, vitellogenin is enzymatically cleaved into phosvitin and lipovitellin, which are continually incorporated into granules (Burley and Vadehra, 1989b). Vitellogenin is synthesized in liver cells in response to estrogen stimulation. Estrogen triggers signals that induce vitellogenin translation on the rough endoplasmic reticulum of the hepatocytes by binding with membrane receptors (Stevens, 2004). The completed vitellogenin polypeptide is formed by glycosylation and subsequent phosphorylation in the hepatocytes prior to secretion. The vitellogenin circulating in the blood is eventually picked up by the oocyte (Stevens, 2004).

2. Synthesis of Yolk Low-Density Lipoprotein

Egg yolk lipids are synthesized in the liver and transported to peripheral tissues in the form of VLDL, which is composed of protein and triglyceride, phospholipid, cholesterol, and cholesteryl esters. Apolipoprotein B (apoB), composed of apoVLDL I and II, is the primary protein constituent of VLDL and is produced in the liver following stimulation by estrogen (Burley and Vadehra, 1989b). The proteins apoB and apoVLDL are synthesized on the rough endoplasmic reticulum, whereas VLDL is assembled in the Golgi apparatus (Burley and Vadehra, 1989b). Estrogen and other transacting proteins, known as liver-enriched transcription factors, help to induce the transcriptional production of apoB (Beekman *et al.*, 1991; Stevens, 2004). Evans *et al.* (1987) suggested that five genes of yolk proteins are potentially regulated by estrogen in chicken liver, including *vtgI*, *vtgII*, *vtgIII*, *apoVLDL-II*, and *apoB*. These genes play a key role in controlling the biosynthesis of egg yolk lipoproteins.

3. Biosynthesis of Yolk Livetins

The livetins consist of soluble proteins in egg yolk and contain three subtypes, α, β, and γ. Unlike other egg yolk proteins, the biosynthesis of livetins is not controlled by estrogen (Burley and Vadehra, 1989b). The α- and β-livetins from egg yolk have similar characteristics to serum albumen and α_2-glycoprotein, respectively (Schade and Chacana, 2007). Furthermore, γ-livetin (IgY) is produced in the bone marrow, while the other two types of livetin are synthesized in the liver. γ-Livetin belongs to a group of chicken immunoglobulins, known as antibodies, which are transferred from blood into egg yolk, and IgY is the only type of antibody found in egg yolk (Burley and Vadehra, 1989b; Schade and Chacana, 2007). The IgY antibody is produced by hens and transferred into the egg yolk to protect their offspring from microbial infections (Schade and Chacana, 2007). Owing to its potential involvement in passive immunity, research on the biological functions and activities of egg yolk γ-livetin is increasing.

IV. CHANGES IN EGG COMPONENTS INDUCED BY FOOD PROCESSING

Eggs are an excellent source of nutrients and play a critical role in influencing the food consumer market. The nutrient components in egg include protein, lipid, various vitamins, and minerals. Because they are rich in protein and lipid, eggs have been associated with a variety of functionalities and are widely applied in the food processing industry. Moreover, egg processing technology has been improved to develop higher quality and more stable egg products to meet the increased demand for processed egg products (Froning, 2008). The main objectives of improving egg processing technology are to extend the shelf-life of egg products and to incorporate egg ingredients into other marketable products based on the chemical and physical properties of eggs. However, regardless of processing techniques, the processing itself can lead to chemical and physical changes in the egg components. In this section, processing-induced chemical changes in eggs will be discussed, as well as the chemical modifications of egg constituents that are generally applied in the food industry to improve their functionality.

A. Denaturation of Egg Proteins

The processing of egg products usually results in changes to the egg proteins. These changes are caused by the modification of protein structure, called denaturation. Under normal conditions, with constant pH and temperature, the protein molecule assumes one specific conformation, referred to as the native state (Boye *et al.*, 1997). In the native state, the protein molecule has a minimal free energy and is considered thermodynamically stable. Any changes to normal conditions cause changes in the thermodynamic homeostasis of the protein molecule, thereby altering the native structure of the molecule. The denaturation of proteins is defined as a process in which protein molecules lose their native structure and change into a more disordered arrangement through the spatial rearrangement of polypeptide chains within the molecule (Kauzmann, 1959). The conformational changes in protein molecules that occur during denaturation are more specific in their secondary, tertiary, and quaternary structural levels. The primary structure of protein molecules, composed of polypeptide sequences, is not affected by denaturation. Denaturation cannot affect the hydrolysis of peptide bonds in the protein.

To produce egg products or incorporate egg ingredients into other food products, denatured or partly denatured egg proteins are required, in order to improve their functional properties. The denaturation or partial denaturation of egg proteins is beneficial for egg foaming and emulsifying abilities and to enhance digestibility and palatability (Burley and Vadehra, 1989c). However, the denaturation of egg proteins needs to be avoided during the process of egg preservation as egg albumen aggregation increases with aging. Denaturation can be induced by a variety of physicochemical agents including heat, pH, salt, and surface effects. The process of heat-induced denaturation and formation of aggregates of ovalbumin is represented in Figures 5.5 and 5.6. In egg proteins, especially ovalbumin, there is an intermediate state known as the molten globule state, in which proteins are partly denatured and maintain their native compactness (Mine, 1995). The molten globule state is defined as a stable partially folded conformation that can be distinguished from both the native and completely denatured states.

B. Changes in Egg Proteins during Preservation

The composition of eggs undergoes a variety of chemical changes with increasing storage time. The major changes in the different parts of the egg as a result of aging have been identified and are as follows: (1) the pH of the albumen is increased by the loss of carbon dioxide; (2) the vitelline membrane becomes weak and eventually disappears;

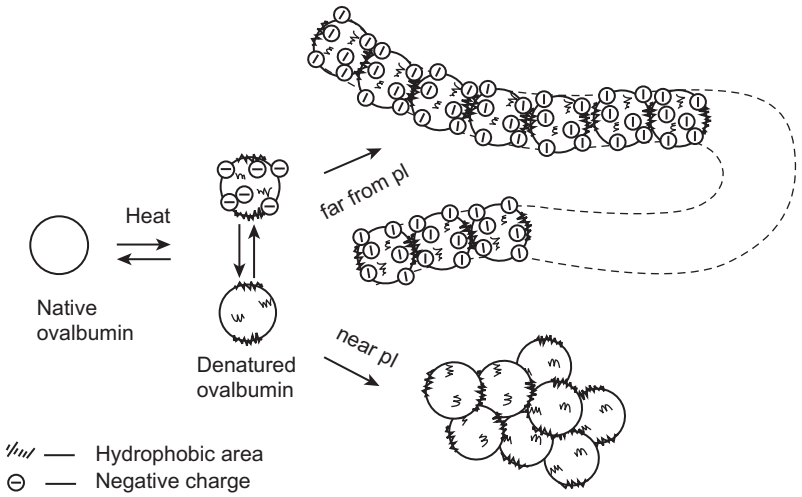

FIGURE 5.5 Model for heat denaturation and formation of aggregates of ovalbumin. *(From Doi and Kitabatake, 1989.)*

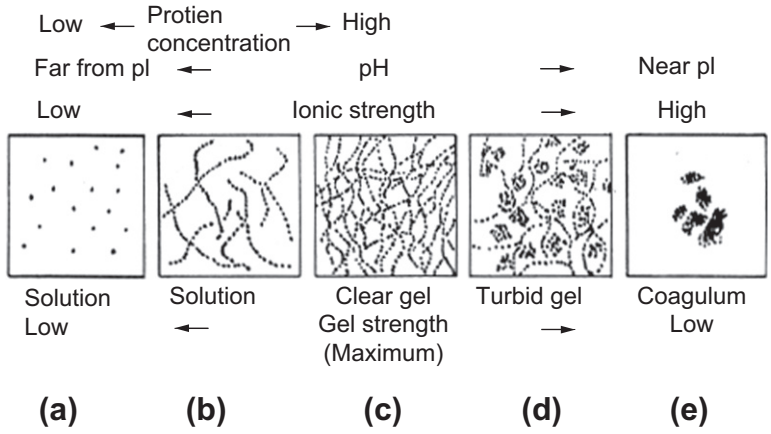

FIGURE 5.6 Factors affecting the texture of heat-induced ovalbumin gels. (a) At pH values far from the p*I* and at low ionic strength, linear aggregates are formed. (b) With decreasing electrostatic repulsion at low ionic strength or at 7.0 > pH > p*I*, three-dimensional networks form a transparent gel. (c) At intermediate ionic strength of pH, both linear aggregates and random aggregates are formed. In this case, the linear aggregates form a cross-linked primary gel network and the random aggregates are interdispersed within this network. This mixed gel of linear and random aggregates has either a translucent or an opaque appearance depending on the relative amounts of the linear and random aggregates. (d) At high ionic strength or at pH values near the p*I*, proteins aggregate to form a turbid gel composed of random aggregates. Among these gel types, the transparent and the opaque/translucent gels exhibit higher gel strength and water-holding capacity than the others. *(From Doi and Kitabatake,* et al.*, 1989.)*

(3) sulfhydryl (SH) groups in the egg yolk are cross-linked and lipids are oxidized; and (4) water is lost by evaporation from the eggshell (Burley and Vadehra, 1989c). The changes caused by the aging process can induce irreversible alterations to egg products which are attributed to the denaturation of egg proteins. The denaturation of egg proteins involves two parts: endothermic denaturation and exothermic aggregation. In the denaturation process, the hydrogen bonds and non-covalent bonds of egg proteins are extensively rearranged; therefore, the egg proteins aggregate and form a cross-linked network (Burley and Vadehra, 1989c).

1. Effects of Aging on Eggs

As eggs age, the gelling characteristics of albumen deteriorate and consequently albumen becomes watery. Meanwhile, the pH of the albumen progressively rises from an initial value of 7.6 to a final value of 9.5 (Fromm, 1967). The modification of ovalbumin among egg white proteins is significantly increased with the aging time. *S*-ovalbumin was detected in egg albumen after 34 days of aging (Rossi and Schiraldi, 1992). The formation of a relatively stable intermediate state in egg albumen during aging is attributed to the conversion of *n*-ovalbumin into *S*-ovalbumin. This transformation results in a cohesive film forming on the interface between air and water, thereby reducing foam stability (Lomakina and Mikova, 2006). The gelation and precipitation of egg albumen are initiated after denaturation, and subsequently increase the viscosity of egg albumen.

Mineki and Kobayashi (1998) determined that the storage duration and conditions can influence the microstructure of egg yolk. Longer storage time or increased temperature results in expansion of the interstitial spaces

between yolk spheres and fusion of granules. The vitelline membrane of the egg yolk becomes more elastic and weaker, and eventually disintegrates. Kirunda and McKee (2000) indicated that the original integrity of the vitelline membrane dissipates as a result of aging. The deterioration of the vitelline membrane as a result of aging is influenced by the same factors causing degeneration of the gel structure in the egg albumen (Feeney *et al.*, 1952). The rate of deterioration of the vitelline membrane is enhanced by increasing storage temperature (Kirunda and McKee, 2000). Fromm (1967) identified that the weight of the vitelline membrane was decreased by a half after five days at 35°C, meanwhile the protein and hexosamine concentration of the vitelline membrane also decreased. The deterioration of the vitelline membrane is due to disruption of the cross-linked fibers which make up the structure of the vitelline membrane (Fromm, 1967). The degradation of the glycoprotein II structure and the disulfide bonds of the ovomucin is mainly attributed to the disruption of the fibrous structure of the vitelline membrane during aging (Kido *et al.*, 1976; Kato *et al.*, 1979). In addition, excess water penetrating into the yolk from the egg albumen results in a decrease in elasticity of the vitelline membrane during prolonged storage (Kirunda and McKee, 2000).

2. Effects of Heat on Eggs

Heat is the most common factor causing denaturation of proteins. Heat treatment of globular proteins leads to an increase in free energy and thermal motion of the whole protein molecule. Therefore, the thermodynamic homeostasis of the native proteins is disrupted, which is sustained by intermolecular and intramolecular bonds (Boye *et al.*, 1997). The thermal denaturation leads to disruption of the original bonds in the native proteins and the formation of a new three-dimensional network which results from two types of protein aggregations, thermal coagulation and thermal gelation (Boye *et al.*, 1997). Heat treatment is generally applied in commercial egg production to eliminate bacterial contamination or facilitate the production of desirable products. New technology is needed to minimize the effects of heat-induced denaturation on egg proteins. A flash heating method has been used to preserve eggs by placing eggs in boiling water for 5 seconds to form a thin layer next to the shell (Romanoff and Romanoff, 1944). The undesired effect of heat treatment on whole eggs is the development of a dark layer on the surface of the egg yolk, caused by the deposition of ferrous sulfide residues, which result from the reaction between hydrogen sulfide from the albumen and iron in the yolk granules (Tinkler and Soar, 1920; Burley and Vadehra, 1989c). The following subsections illustrate the effects of heating on individual egg components as well as mixed yolk and albumen.

a. Effects of Heat on Yolk

Heat treatment of egg yolk results in changes to yolk lipoproteins. A sharp increase in the viscosity of egg yolks was observed after heating above 65°C, and coagulation of the yolk occurred around 70°C (Denmat *et al.*, 1999). This is caused by heat-induced denaturation of LDL and lipovitellins in the egg yolk (Burley and Vadehra, 1989c). However, some egg yolk proteins, such as phosvitin and some livetins, are less sensitive to heat treatment. The denatured egg yolk proteins are able to form a cross-linked network which results in thermal gelation. Nguyen and Burley (1984) showed that sulfhydryl−disulfide interactions of denatured LDL are mostly responsible for the gelation of heated egg yolk, owing to LDL in egg yolk containing SH groups, which are used to attach to apoprotein. According to Denmat *et al.* (1999), egg yolk granules are less sensitive than egg yolk plasma to heat treatment. The different constituents of egg yolk have been shown to have different sensitivity to heat treatment. This may be caused by the distinct distribution of egg yolk proteins in the different constituents of egg yolk.

b. Effects of Heat on Albumen

Owing to the substantial role of egg white proteins in the food industry, the effects of heat on egg albumen have been extensively studied (Seideman *et al.*, 1963; Shimada and Matsushita, 1980; Egelandsdal, 1980; Mine *et al.*, 1990; Rossi and Schiraldi, 1992). The egg albumen undergoes coagulation and gelation upon heating. Therefore, heat treatment of egg albumen can facilitate the improvement of water solubility, foaming, and emulsification capacities of food products. The major protein constituents of egg albumen, including ovalbumin and conalbumin, have a high heat coagulability (Shimada and Matsushita, 1980). Payawal *et al.* (1946) identified a series of discontinuous changes in egg albumen induced by heating. Based on their results, the egg albumen liquid changes appearance from turbid to clear after heating from 63°C to 70°C. The egg albumen forms a clear gel on the surface at 63°C. Precipitation of the egg albumen mixture is initiated between 63°C and 66°C, but a clear gel appearance is detected at 66°C. Finally, egg albumen forms a white coagulum above 66°C and takes on a solid white appearance at even higher temperatures.

Using differential scanning calorimetry, Donovan *et al.* (1975) observed that heat-induced denaturation of the three main protein constituents of egg albumen was represented by endothermic peaks within 60−100°C. The contributions of these three proteins to the whole denaturation of egg albumen were 64.5% from ovalbumin, 16.8% from conalbumin, and 3.7% from lysozyme. Ovotransferrin is the least heat-stable protein, with a denaturation temperature of about 57°C. Globulins, ovalbumin, and lysozyme have denaturation temperatures around 72°C, 71.5°C, and 81.5°C, respectively (Cunningham, 1994). Ovomucoid and ovotransferrin contribute less to the denaturation of egg albumen owing to their lower heat coagulability.

The intermolecular interactions produce a continuous three-dimensional network exhibiting structural rigidity, which plays a control role in the thermocoagulation of proteins. Different chemical interactions, including hydrophobic, ionic, and intermolecular sulfhydryl−disulfide interactions, are involved in the heat-induced aggregation among the heterogeneous proteins in egg albumen (Shimada and Matsushita, 1980). The formation of a cross-linked gel network is mainly attributed to sulfhydryl−disulfide exchanges in egg albumen after heat treatment, while hydrophobic interactions are primarily responsible for the coagulation of egg albumen, as hydrophobic interactions are enhanced by increasing temperature (Shimada and Matsushita, 1980). The heat denaturation of egg white proteins is significantly increased by the exposure of hydrophobic residues on the molecular surface (Mine *et al.*, 1990). Mine *et al.* (1990) described the changes in secondary structure content in egg white proteins during heat denaturation. The β-sheet content was increased more than three-fold, while the helical content was decreased with increasing temperature. According to their results, the heat-denatured egg albumen contained a considerable amount of protein secondary structure which formed a cross-linked network by disulfide bonding, and this network of β-sheet structures is strengthened by the exposed hydrophobic residues. The heat-induced denaturation of egg albumen is represented by the aggregation of egg white proteins, resulting from the transformation of the protein from a native to a denatured state.

c. Pasteurization of Eggs by Heat

Pasteurization has been widely used in the egg production industry to destroy microbial contamination and has been generally recognized as a valid method since the twentieth century. Among pathogenic bacteria, salmonellae are of primary concern in the egg processing industry because of the high prevalence detected in eggs and egg products. The compositional differences in egg constituents account for the wide range of pasteurization conditions (Cunningham, 1994). Since salmonellae are most heat resistant at mild acidic pH or near-neutral pH, they are more viable in egg yolk (pH 6.0) than in egg albumen (pH 9.1). Owing to the differences in pH values, solids, and the nature of constituents of the whole egg, the heat resistance of salmonellae is different in egg albumen and yolk. Therefore, the pasteurization conditions applied to egg yolk are more severe than for egg albumen (Cunningham, 1994). The heat-induced denaturation of egg products is also affected by the mechanical conditions of the pasteurization process, including equipment design, flow rate, and temperature differential between the heating medium and egg products (Cunningham, 1994).

The physical and chemical changes induced by the heating of egg proteins can adversely affect the quality of resulting products. Pasteurization of egg albumen at 60°C results in a rise in viscosity and a decrease in foaming activity (Burley and Vadehra, 1989c). The reduction in the foaming capacity of egg albumen is caused by the denaturation of ovotransferrin during the pasteurization process and the irreversible denaturation of the ovomucin−lysozyme network (Lomakina and Mikova, 2006). Metal ions can be added to restore the foaming properties of egg albumen after pasteurization. During pasteurization of liquid egg albumen, chemical reagents are commonly added to stabilize conalbumin, which forms a heat-stable complex by binding with metal ions such as aluminum sulfate. Hydrogen peroxide, a well-known bactericidal agent, is also added to liquid egg albumen to eliminate microbial contamination so as to allow the pasteurization of egg albumen at relatively low temperatures. Pasteurization standards for egg processing have been established in different countries. The United States Department of Agriculture (USDA) requires the liquid whole egg to be heated for 3.5 minutes at 60°C (Burley and Vadehra, 1989c; Cunningham, 1994). Under these conditions, the pasteurized egg products are considered safe to be consumed.

3. Effects of Freezing on Eggs

The freezing process facilitates the preservation and production of egg products. Frozen egg products are normally produced from the liquid form of egg white, yolk, or other egg ingredient-containing food products. After freezing

and thawing, the mixture of yolk−albumen becomes non-homogeneous and aggregated, and possibly undergoes gelation.

a. Effects of Freezing on Whole Egg Mixture

Torten and Eisenberg (1982) studied freezing-induced alterations in colloidal properties of the whole egg. Increases in viscosity and surface tension were detected in whole egg samples after freezing. Based on these results, it was concluded that the freezing process disrupts the three-dimensional system in eggs; thus, complexes are formed by random intermolecular contacts, which results in a lumpy appearance in the yolk−white mixture after thawing. Therefore, the freezing process can lead to major textural changes in egg products. The viscosity of the supernatant fraction from the thawed liquid of mixed whole egg is consistent with the unfrozen whole egg mixture. The precipitate fraction from the whole egg mixture, which contains the yolk granules, does not undergo gelation upon freezing (Cotterill, 1994). Accordingly, the gelation of the whole egg mixture depends on the interaction of components from both egg yolk and albumen. Freezing has no significant effects on the functional properties of whole egg mixture (Miller and Winter, 1950).

b. Effects of Freezing on Egg-Yolk Gelation

During freezing and storage of egg yolks below −6°C, viscosity is increased and gelation occurs. Below this temperature, the gelation of egg yolk is irreversible. However, the gelation of egg yolk does not occur when it is frozen at a supercooling or high freezing rate (Lopez *et al.*, 1954). The freezing rate, temperature, and thawing conditions play an integral role in the control of egg yolk gelation. It is preferable to minimize the gelation of egg yolk when it is used as an ingredient in food products. The irreversible gelation of egg yolk can be controlled by adding solutes such as salt and sucrose (Burley and Vadehra, 1989c; Cotterill, 1994). One possible mechanism is that the solutes depress the freezing point of the products, thereby inhibiting protein denaturation caused by freezing (Burley and Vadehra, 1989c). LDL, as the major lipoprotein in egg yolk, is the primary component altered by freezing. Kamat *et al.* (1976) determined that solute addition inhibits the gelation of egg yolk lipoproteins by increasing solvation at interfacial regions to form stabilized layers. Wakamatu *et al.* (1983) studied the freezing-induced gelation of egg yolk LDL and the effects of salt on this process. The pH changes in the unfrozen phase and transformation of water into ice also affect LDL aggregation. The formation of ice crystals results in the denaturation of proteins and subsequent aggregation of yolk lipoproteins (Cotterill, 1994). A high concentration of salt in the unfrozen phase disrupts the egg yolk granules. In addition, pretreating yolk with proteolytic and lipolytic enzymes can help to inhibit freezing-induced gelation (Lopez *et al.*, 1955).

Several intramolecular and intermolecular interactions are involved in the aggregation of egg yolk lipoproteins, and these are summarized by Cotterill (1994). The calcium and phosvitin in egg yolk may contribute towards the formation of intracellular bridges to combine proteins, as identified in the gelled fraction of yolk (Cotterill, 1994). Hydrophobic bonding, hydrogen bonding, and electrostatic forces all contribute to interactions between protein molecules in the gelled yolk after freezing-induced disruption in the egg yolk components (Palmer *et al.*, 1970). Burley and Vadehra (1989c) observed that the electrophoretic behavior, chromatographic properties, and electron microscopic appearance are altered in egg yolk LDL as a result of freezing-induced gelation.

c. Effects of Cooling and Freezing on the Vitelline Membrane

Deformation of the vitelline membrane and loss of its elasticity occur during the cold storage progress. However, changes in the strength of the vitelline membrane are not noticeable under cold storage conditions. Jones and Musgrove (2005) showed that the vitelline membrane strength was consistent, with no significant decrease, when stored for up to 10 weeks at 4°C in a cool room at 80% relative humidity. In contrast, freezing causes changes in the strength of the vitelline membrane; it is reduced during extended cold storage (Jones *et al.*, 2002). The water-holding capacity of the isolated membrane is increased by freezing at −18°C (Cotterill, 1994). Thus, the properties of the vitelline membrane may be altered by freezing. During the slow freezing process, the formation of ice crystals can also cause physical damage by puncturing the vitelline membrane.

d. Effects of Freezing on Egg Albumen

The functional properties of egg albumen are affected by freezing. Frozen storage results in a decrease in the foaming ability of egg albumen. The amount of thick white of the total albumen is dramatically decreased when frozen at

−16°C and stored for 3 months at −3°C (Moran, 1925). One possible reason is that denaturation of proteins occurs, leading to coagulation of the thick white under frozen storage conditions (Cotterill, 1994). Wootton *et al.* (1981) observed that ovotransferrin was the most susceptible and, along with ovalbumin, was responsible for the freezing-induced changes in egg albumen. Davis *et al.* (1952) determined that the formation of ice crystals was a major factor causing damage to cooked egg albumen when it was frozen.

4. Effects of Irradiation on Eggs

Electromagnetic and ionizing radiation, at a dosage up to 3 kGy, has been approved for use in shell eggs by the US Food and Drug Administration (USDA, 2000). Irradiation has been regarded as an alternative method to heat pasteurization for shell eggs. The irradiation of the whole shell egg influences the physical properties of the egg by weakening the vitelline membrane and decreasing the viscosity of the albumen two-fold (Burley and Vadehra, 1989c). Loss of ovomucin, ovotransferrin, and ovalbumin was observed using gel electrophoresis in egg albumen following exposure to irradiation (Burley and Vadehra, 1989c). The viscosity of egg albumen is dramatically reduced after irradiation, because irradiation-induced denaturation of proteins results in the transformation of carbohydrate and protein moieties, and subsequently the disruption of ovomucin complexes (Ma *et al.*, 1990). Irradiation-induced changes in ovomucin may be attributed to cleavage of *O*-glycosides from the protein moiety (Wong and Kitts, 2002). Wong and Kitts (2002) reported that egg albumen irradiated with dosages between 2 and 4 kGy sustained a loss of thick albumen accompanied by an increase in free SH groups, which led to a reduction in the foam volume and gel hardness of albumen. In this study, the number of SH groups released from albumen did not increase with the elevated dosage of electron beam.

Since denaturation enhances the formation of a rigid film at the interface, the functional properties of fresh eggs, including foaming, emulsifying activity, gel rigidity, and angel cake volume, are improved by irradiation (Ma, 1996). However, Min *et al.* (2005) demonstrated that the foaming capacity and foam stability of egg albumen were reduced by increasing the dosage of irradiation. The deterioration in foaming properties of egg albumen is possibly caused by irradiation-induced oxidative changes in globulins, ovomucin, and lysozyme.

Irradiation-induced changes in egg yolk are less drastic than those in egg albumen. The major effect of irradiation on egg yolk is oxidation of polyunsaturated fatty acids, which results in the formation of hydroperoxides (Thakur and Singh, 1994). The emulsifying capacity and gelling ability of egg yolk are not affected by exposure to irradiation. No significant effects of irradiation on frozen and spray-dried egg have been detected. Ma *et al.* (1993) showed that no noticeable changes were induced in irradiation-treated frozen liquid egg white, by analyzing the scanning calorimetric profiles and electrophoretic patterns of egg protein components, as well as the functional properties. In a study of the effects of irradiation on the functionality of frozen liquid egg yolk, Huang *et al.* (1997) demonstrated that the emulsifying capacity of irradiated samples was significantly higher than that of non-irradiated samples during the frozen storage progress.

C. Changes in Egg Protein Functionality Induced by Processing

The unique functional properties of eggs have numerous benefits for their application as ingredients in a variety of food products. Egg products incorporated into other food products can improve the profits of the food industry owing to the high nutritional value of eggs. When used as food ingredients, egg products can contribute various functionalities including foaming, gelation, and emulsification properties (Table 5.6). The two main components of eggs directly responsible for their functional properties are proteins and lipids. In the food industry, there is increasing concern about the chemical and structural changes induced in egg ingredients by the physicochemical procedures used in food processing. These changes are associated with modifications in the functional properties of egg components. The major focus of this section is to improve understanding of the food processing-induced modifications in egg functional properties.

1. Effects of Dry-Heating on Gelation

A gel can be described as a continuous solid cross-linked system, which consists of a three-dimensional network embedded in an aqueous solvent (Smith, 1994). Gels have water-holding capacity in food products with rheological properties. Heat-induced gelation in food proteins generally happens during heat processing. Gel formation involves two main processes: (1) heat leads to the denaturation of proteins, which depends on the time,

TABLE 5.6 Functional Properties Attributed to Egg Proteins in Food Systems

Function	Underlying Mechanisms	Examples
Water binding	Hydrogen bonding and ionic hydration	Cakes and bread
Gelation	Water entrapment and immobilization, network formation	Gels, cakes, baked goods
Cohesion, adhesion	Hydrophobic, ionic, and hydrogen bonds	Pasta, baked goods
Emulsification	Adsorption and film formation at interface	Cakes, dressings
Foaming	Adsorption and film formation at interface	Whipped toppings, ice cream, cakes, desserts
Aroma − flavor binding	Hydrophobic bonds, entrapment	Low-fat bakery products, doughnuts

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temperature, and nature of the proteins; and (2) the unfolded proteins aggregate and form intermolecular interactions, which leads to the development of a coagulum or gel, depending on the conditions (Raikos *et al.*, 2007). The difference between a protein coagulum and a gel is that a gel has an ordered network system formed by the polymerization of protein molecules, whereas a coagulum consists of a disorganized aggregated structure (Hayakawa and Nakai, 1985).

Egg white proteins play an important role in improving the consistency of food products by forming heat-induced gels which provide a medium for holding flavor and a homogeneous texture (Sun and Hayakawa, 2002). The egg white proteins are mainly responsible for the gelation properties of eggs owing to their higher content, flexibility, thiol content, and ability to denature and form a cross-linked network. As the major constituent of egg albumen, ovalbumin plays a key role in gel formation. The factors affecting the textural changes in heat-induced ovalbumin gels are illustrated in Figure 5.6. The rigidity and turbidity of heat-induced ovalbumin gels are influenced by factors such as pH, ionic strength, and protein concentration (Hatta *et al.*, 1986). The heat-induced gelation of ovalbumin and other egg white proteins is attributed to intermolecular interactions of the denatured proteins. The aggregation of ovalbumin after heat treatment results from hydrophobic interaction and disulfide bonding, due to sulfyhydryl−disulfide exchange reactions and sulfyhydryl oxidation (Sun and Hayakawa, 2002).

Kato *et al.* (1989) first observed that heating in a dry state was an effective approach by which to improve the gelation properties of egg albumen. The dry-heating process can result in the formation of transparent and firm ovalbumin gels within a wide range of pH and ionic strengths (Matsudomi *et al.*, 1991). Matsudomi *et al.* (1991) suggested that the possible mechanism for this is that dry-heating provides a balance between attractive and repulsive forces, which facilitates the formation of soluble linear aggregates by partially unfolded ovalbumin. Mild alkaline treatment contributes to the development of firm and elastic ovalbumin gels during the dry-heating process (Mine, 1996, 1997). The gelation and polymerization of proteins are improved by the increasing hydrophobicity with the heating time and sulfyhydryl−disulfide interchange. Watanabe *et al.* (2000) demonstrated that dry-heated ovalbumin has inhibitory effects on the coagulation of ovotransferrin, because ovalbumin interferes with the interaction of ovotransferrin molecules via the formation of disulfide bonds with ovotransferrin.

2. Effects of Heat on Foaming

Foams are a complex system in which dispersed air bubbles are entrapped by interfacial films between air and liquid or solid continuous phases (Davis and Foegeding, 2007). The denaturation of egg proteins at the air−water interface occurs during blending or whipping processes, which lead to the introduction of air into the protein solution. As a consequence of the application of mechanical forces into a protein solution, which results in an increase in free energy, the proteins unfold and form biphasic films by exposing their hydrophobic groups to the air phase, while their hydrophilic groups remain in the liquid phase. Egg white proteins are optimal foaming agents in the food industry because of their ability to establish a complex of interactions between the various protein components. The hierarchy of the foaming capacity of egg white proteins, from low to high, is as follows: globulins; ovalbumin; ovotransferrin; lysozyme; ovomucoid; and ovomucin (Mine, 1995). Glycoproteins contain hydrophilic carbohydrate moieties, which

increase the viscosity and foam stability by binding with water. The foaming properties of egg white proteins contribute to the spongy and foamy structure in various food products including angel food cakes, meringues, soufflés, and mousses. In these products, egg white proteins are the main surface-active agents that facilitate the stabilization of the dispersed gas phase. Foaming stability can be attributed to a variety of forces, including viscosity of the liquid phase and electrostatic and steric forces between proteins. Among the egg white proteins, globulins and ovalbumin contribute to the formation of foams, whereas ovomucin and lysozyme contribute to foam stability, and ovoglobulins to increasing viscosity (Yang and Baldwin, 1995).

Parameters affecting the foaming properties of egg white proteins include protein concentration, film thickness, ionic strength, pH, temperature, other constituents in the food product, and physicochemical properties of the proteins. Kim and Setser (1982) demonstrated that egg yolk lipids have detrimental effects on the foaming capacity of egg albumen. Recently, Wang and Wang (2009) also showed that egg yolk contamination has the most significant impact on heat-induced foaming properties of fresh egg albumen. Furthermore, intrinsic factors also affect the foaming quality of egg white proteins, including the ratio of thin albumen, storage conditions, aging, and the hen's genetic conditions. As mentioned previously, the increase in *S*-ovalbumin content in egg albumen during the prolonged storage process results in the reduction of foam stability (Lomakina and Mikova, 2006). Numerous studies have been carried out to improve the foaming properties of egg albumen. Kato *et al.* (1994) revealed that the dry-heating of egg albumen could enhance foaming capability and stability four-fold without loss of solubility. An increase in molecular flexibility and surface hydrophobicity after dry-heating facilitates the intermolecular interactions and formation of a cohesive interface film (Kato *et al.*, 1994). Relkin *et al.* (1999) identified that the mild heat treatment of ovalbumin led to the formation of a molten globule state which was an intermediate stable unfolded state. The partial denaturation of ovalbumin in the molten globule state, which is reversible, improves foaming properties by increasing flexibility and surface hydrophobicity (Campbell *et al.*, 2003).

3. Effects of Heat on Emulsification

Fluid emulsions are thermodynamically unstable mixtures of immiscible liquids such as lipid and water (Mangino, 1994). The surface-active molecules are dispersed in an immiscible liquid system and cover the oil−water interface to form emulsions. This process is facilitated by mechanical homogenization. Amphiphilic compounds which contain both polar and non-polar moieties normally have emulsifying activity. Egg yolk itself is an emulsion system in which lipid is dispersed into a continuous aqueous phase. Egg yolk contains substantial amounts of lipoprotein−cholesterol complexes and phospholipids that are efficient emulsifying agents. Accordingly, egg yolk is an essential ingredient in various foods, including mayonnaise and salad dressings, to stabilize emulsions. To study the roles of different components in egg yolk emulsion, the emulsifying abilities of yolk have been divided into two parts: plasma (LDL and livetins), and granules (HDL and phosvitin) (Denmat *et al.*, 1999). The phospholipid−protein interaction and absorption behavior of egg yolk components, including HDL, LDL, phosvitin, and livetin, contributes to the emulsifying properties. The conditions for the emulsifying capacity of egg components are similar to their gelation properties, and mainly affect the formation of interfacial films. These conditions include pH, ionic concentration, and protein concentration. Mine (1998) studied the effects of pH on the emulsifying properties of egg yolk and showed that the size of the emulsion particles formed by egg yolk proteins decreased as pH levels increased. Excellent solubility of egg yolk plasma has been found in common pH ranges and salt concentrations (Sirvente *et al.*, 2007). In addition, heat treatment has different effects on the emulsifying properties of egg yolk plasma and granules. Denmat *et al.* (1999) demonstrated that egg yolk granules were less sensitive than plasma to heat, because the HDL and phosvitin components were bound to form an insoluble complex by phosphocalcic bridges, which protected the protein molecules from thermal denaturation. Based on their results, egg yolk granules have better emulsifying activity and stability than plasma, especially during heat treatment over 72°C. The emulsifying property of plasma was not affected by heating up to 69°C (Denmat *et al.*, 1999). However, after continuously increasing the heating temperature, the solubility of plasma was dramatically decreased, which led to a rise in viscosity due to aggregation of LDL induced by thermal denaturation.

Mine *et al.* (1991) showed that egg white proteins had effective emulsifying capacity to be used as surface-active agents. The emulsifying properties of egg white proteins are more heat resistant than those of the egg yolk components. Kato *et al.* (1989) demonstrated that dry-heating of egg albumen improved the emulsifying capacity and emulsion stability owing to the increased flexibility and surface hydrophobicity of egg white proteins. The thermal denaturation of egg white proteins results in an increase in absorbing ability at the oil−water interface caused by the exposure of hydrophobic residues at the oil phase (Campbell *et al.*, 2003). The emulsifying capacity of egg white ovalbumin is

particularly influenced by factors such as the oil-phase volume, presence of salts, and protein concentrations. Dry-heating of egg albumen is one application of dehydration in food production. Dehydration is a standard method used in food processing for the preservation of food components. The dry-heating process is indeed a promising approach to improve egg albumen functionality. The beneficial effects of dry-heating on the gelling, foaming, and emulsifying properties of egg albumen have been discussed and summarized in this section. The optimal conditions for dry-heating egg albumen to improve the functionalities of egg white proteins are heating at 80°C in a dry state with 7.5% moisture content (Kato *et al.*, 1989). In a recent study, the conformational changes in egg white proteins induced by dry-heating treatment were shown to be dependent on the moisture content (Plancken *et al.*, 2007). Based on the results of this study, the optimal moisture content was below 6.8% during dry-heating of egg albumen at 80°C.

D. Modification of Egg Protein Functionality

Research is being carried out to develop new approaches to modify the denaturation and aggregation of egg proteins in order to improve egg functionality with respect to the preparation of food products with stable qualities. Developments mainly focus on the application of chemical reagents and new processing techniques. Currently, the most successful approach is the use of the Maillard reaction to improve the functional properties of egg proteins.

1. Chemical and Physical Modifications of Eggs

Chemical modification using carboxylation and succinylation of spray-dried egg white solids has been developed to improve the foaming properties. However, Ma *et al.* (1986) demonstrated that carboxyl modification had moderate effects on improving foaming properties in spray-dried egg white solids, and succinylation caused a decrease in foaming ability and low heat coagulation. The enzymatic hydrolysis of egg albumen has been studied to improve egg white protein foaming ability and a variety of enzymes has been analyzed (Lomakina and Mikova, 2006). The application of pepsin in food proteins has been shown to have effects on increasing foaming stability owing to the hydrolysis of hydrophobic regions on the protein surface (Horiuchi and Fukushima, 1978). Hydrolysis of egg albumen with papain has also been found to have a promising effect on foaming ability (Lee and Chen, 2002). Phillips *et al.* (1987) indicated that adding copper ions to fresh egg albumen improved foaming stability through the formation of a copper−ovotransferrin complex, thereby preventing protein denaturation. Knorr *et al.* (2004) reported that a combination of ultrasound and high pressure contributed to an even distribution of protein and lipid in the whole egg liquid and increased its foaming ability.

The emulsifying properties of LDL have also been shown to be improved by high-pressure treatment combined with an alkaline pH. A significant decrease in droplet flocculation of LDL dispersions was detected under the above treatment conditions (Speroni *et al.*, 2005). Consequently, the aggregation and denaturation of proteins were shown to be enhanced without altering the capacity of LDL adsorption at the oil−water interface (Speroni *et al.*, 2005). Kato *et al.* (1987a) studied the modification of phosvitin emulsifying properties by chemical and enzymatic neutralization or the removal of phosphate anionic residues. These modifications resulted in a significant impact on emulsion stability and a decrease in the emulsifying properties of phosvitin. Kitabatake *et al.* (1989) reported that the application of freeze-drying and spray-drying improved the emulsifying properties of egg ovalbumin. Li *et al.* (2004) studied the effects of a combination of phosphorylation and dry-heating on the functional properties of egg white proteins. They showed that dry-heating of egg white proteins in the presence of pyrophosphate improved the emulsifying properties of egg white proteins by increasing the exposure of hydrophobic residues at the oil−water interface. The combination of phosphorylation and dry-heating also enhanced the gelling ability of egg white proteins and led to the formation of a transparent gel, which resulted from hydrophobic interaction and electrostatic-repulsive forces between phosphate residues added to proteins in the process.

2. Improvement of Functional Properties of Eggs using Maillard Reactions

The Maillard reaction is a chemical reaction by which the amino groups of proteins interact with the carbonyl groups of reducing polysaccharides to form covalent cross-links (Danehy, 1986), and it has been recognized as a safe and promising approach to modify egg functionality. The Maillard reaction, a non-enzymatic browning reaction widely applied in the food industry, plays a key role in the development of flavor and color in food products. Previously, the Maillard reaction was prevented during the preservation of fresh eggs by desugarization prior to pasteurization, because it results in browning reactions during the storage of eggs. However, it has been shown that the addition of

carbohydrate moieties to egg protein molecules improves their functional properties, including emulsification and gelation. The carbohydrate moieties added to the protein molecules enhance the amphiphilic nature of the complex of conjugated molecules.

Kato *et al.* (1990) applied this approach to the preparation of ovalbumin−dextran conjugates to improve the functional properties of ovalbumin. The Maillard reaction in egg white proteins can facilitate solubility and improve heat stability owing to the decreased hydrophobicity of protein residues by covalent binding with hydrophilic sugar molecules (Campbell *et al.*, 2003). Handa and Kuroda (1999) reported that application of the Maillard reaction in dried egg albumen improved the gelling properties when carried out at 55°C and 35% relative humidity. In their study, the degree of polymerization was increased with increased heating time. The increase in polymerization may be attributed to disulfide bonding and non-disulfide covalent interactions. Furthermore, Matsudomi *et al.* (2002) found that galactomannan modification of dried egg albumen, using the Maillard reaction, could improve its gelling ability. Several studies have demonstrated that application of the Maillard reaction has a positive impact on the emulsifying properties of egg albumen (Kato *et al.*, 1993; Aoki *et al.*, 1999; Begum *et al.*, 2003). Aoki *et al.* (1999) showed that the emulsifying activity of ovalbumin was improved by conjugation with glucuronic acid through the Maillard reaction under certain conditions. Begum *et al.* (2003) identified that conjugating ovoinhibitor with galactomannan under controlled dry-heating conditions resulted in an improvement in emulsifying properties, with potential benefits for industrial applications. One possible explanation is that the amphiphilic conjugates formed by covalent interaction between sugars and proteins are absorbed better at the oil−water interface, with the hydrophobic residues being exposed to the oil phase, while the hydrophilic side-chains interact with water.

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