Chemical Composition of Eggs and Egg Products

12

Hoon H. Sunwoo and Naiyana Gujral

Contents

H.H. Sunwoo (✉) • N. Gujral
3-142H KATZ Group for Health Research, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada
e-mail: hsunwoo@ualberta.ca; gujral@ualberta.ca

P.C.K. Cheung, B.M. Mehta (eds.), *Handbook of Food Chemistry*,
DOI 10.1007/978-3-642-36605-5_28

Abstract

For many years the egg has been subjected to negative publicity generally related to the cholesterol content which also resulted in decreased consumption. This was a negative attribute even though eggs offered many positive effects in the consumer's diet. Comprehensive research has now shown that dietary cholesterol does not significantly influence serum cholesterol. Eggs are now being recognized as a highly nutritious food with unique components which offer potential nutraceuticals with specific health benefits. With these recognized benefits, egg consumption has increased substantially in recent years. Much of this higher consumption has resulted from the increased use of eggs as an ingredient in a variety of further processed egg products. The polyfunctional property of eggs continues to make them the preferred ingredient in many food formulations. Also, eggs are considered a healthy food that does not increase serum cholesterol which fits well into high-protein low-carbohydrate diets. The egg is considered as nature's most perfect food containing excellent source of protein of high biological value, high ratio of unsaturated fatty acids to saturated fatty acids, and excellent source of minerals and all the vitamins. Vitamin C and lower concentration of calcium are the only nutrients lacking in eggs. The yolk provides all of the fat and contains half of the protein, most of the calcium, phosphorus, iron, zinc, and vitamins B_6, B_{12}, A, and folic acid, and half of the riboflavin and thiamine. Egg white contains about half of the protein and riboflavin.

Introduction

Unlike mammals, the embryos of birds are not fed by the mother during their development and therefore lack the ability to eliminate metabolic waste. Consequently, the egg yolk provides vital nutrients (protein, lipids, vitamins, and minerals) that are extremely well metabolized by the chicken embryo. Egg yolk is also a very attractive source of nutrients for humans. Its coefficient of digestive use is comparable to that of milk, and the biological value of proteins in the egg is even superior to that of milk proteins (Bourgeois-Adragna 1994). Because of its versatility, hen egg yolk is a multifunctional ingredient widely used in many food products. It possesses emulsifying, gelling, coloring, aromatic, and antioxidant properties. Each constituent of the yolk possesses unique physical and chemical characteristics responsible for its own functional properties. Environmental conditions and preservative treatment can influence and modulate these functional properties. Due to its

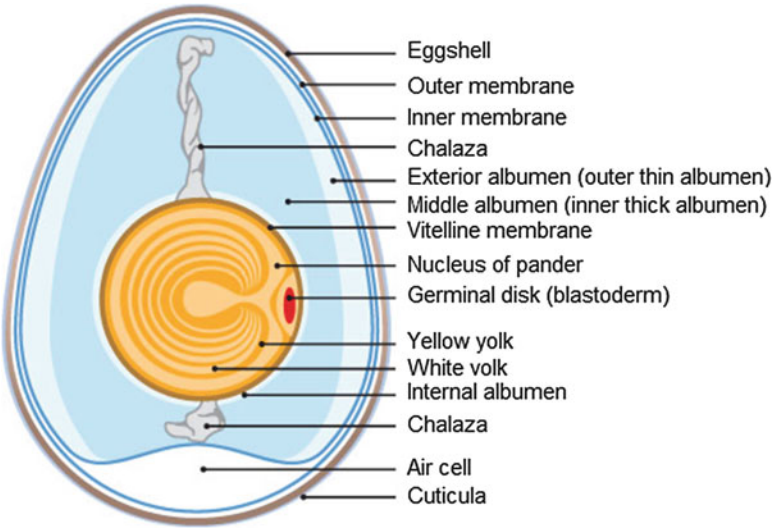

Fig. 1 Schematic drawing of egg

original role as an embryonic chamber, the yolk contains many constituents essential for life. Thus, the yolk is a major source of active factors usable in medical, pharmaceutical, cosmetic, nutraceutical, and biotechnological industries.

The Structure of the Egg

The parts of the egg are shown schematically in Fig. 1. It is generally accepted that a hen can produce an egg in about 2 weeks. This is true, except for the very small core of the yolk. At the time of hatching, the ovary of a female chick possesses many small ova – the number has been estimated to be over 3,000. The yolk of the egg is formed in three stages: (1) the part formed during embryonic development of the female chick, (2) the normal slow development of the ovum from the time of hatching of the chick to a point in sexual maturity some 10 days before ovulation, and (3) the accelerated growth period during the last 10 days before ovulation, after which it is released into the oviduct, a part of the female reproductive system. In the oviduct, the egg white is formed from the layers of secretions of the anterior section of the hen's oviduct to surround the yolk; finally, the shell membrane and shell are deposited to complete the egg formation process.

The yolk is formed during the final 10–12 days prior to the laying of the egg. Its structure consists of the latebra, germinal disk, and concentric layers of light and dark surrounded by the vitelline membrane. The yolk comprises 30–33 % of the total egg weight. At the time of ovulation, the yolk sac, or follicular membrane, releases the fully developed yolk into the open upper end of the oviduct.

The white, or albumen, of the egg is formed in a matter of a few hours and represents approximately 60 % of the total egg weight. The white occurs in four layers (see Fig. 1) in most chicken eggs. These are the chalaziferous or inner continuous with the chalaza. The next outer layer is the inner thin white (layer 3) surrounded by the outer thick white (layer 2). The outer layer of white is the outer thin layer (layer 1). The percentage of the total white found in each of the four layers varies widely, depending on the strain of the layering hen, age of the hen, and age of the egg.

The next layers of the egg are the inner and outer shell membranes. These relatively thin keratin-like membranes act as one of the egg's chief defenses against bacterial invasion. The inner membrane is thinner than the outer membrane, but together they are only 0.01–0.02 mm thick.

The outer shell largely consists of calcium carbonate (94 %), with other components including magnesium carbonate (1 %), calcium phosphate (1 %), and organic matter that are mostly protein (4 %). The shell color of the colored eggs is due to pigments (ooporphins) deposited on the shell surface. The shell is formed in a distinct pattern with pores for gas exchange. Even though the pores are partially sealed by keratin, they allow carbon dioxide and moisture to escape from the egg. Under some conditions, the pores also permit bacterial penetration as far as the shell membranes.

The last structural part of the egg is the air cell. This develops as a separation of the two shell membranes, usually at the large end of the egg, due to the shrinking of egg contents during cooling. The air cell continues to increase in size as moisture and carbon dioxide are lost throughout the existence of the intact egg (Romanoff and Romanoff 1949).

The process of egg formation is a complex series of hormone-controlled reactions, and the study of these reactions requires a thorough knowledge of reproductive physiology. The brief description given here is sufficient for a person interested in eggs as a human food or for commerce.

Physical Properties of the Egg

The viscosity of egg albumen is dependent on the age, mixing treatment, temperature, and rate of shear (Romanoff and Romanoff 1949; Tung et al. 1970). Albumen is pseudoplastic in nature at 32 °C between shear rates of 8.1 and 147/s. At a constant shear rate, the albumen viscosity decreases with time and approaches equilibrium within a few minutes. The egg yolk is a pseudoplastic non-Newtonian fluid. The shear stress (dynes/cm^2)–shear rate (/s) relationship is linear. The particulate matter, or granules, in the egg yolk must be responsible for this nonlinearity, since the plasma (yolk without the granules) is essentially a Newtonian fluid.

The pH of albumen from a newly laid egg is between 7.6 and 8.5. During the storage of shell eggs, the pH of albumen increases at a temperature-dependent rate to a maximum value of about 9.7. After 3 days of storage at 3 °C, the pH of albumen

was 9.18. After 21 days of storage, the albumen had a pH close to 9.4, regardless of storage temperatures between 3 °C and 35 °C. The increase in pH is the result of the loss of carbon dioxide from the eggshell through the pores in the shell. The pH level is dependent on the equilibrium between dissolved carbon dioxide, bicarbonate ions, carbonate ions, and protein. The pH of freshly laid yolk is generally about 6, but during storage the pH gradually increases to between 6.4 and 6.9. At storage temperatures of 2 °C and 37 °C, the yolk reached a pH value of 6.4, in about 50 days and 18 days, respectively.

The Chemical Composition of the Egg

Egg Protein

The protein value of the whole egg protein is considered to be 100 and is used as standard for measuring nutritional quality of other food proteins. The addition of two eggs in the diet provides 12 g of protein, which will meet 30 % of the recommended dietary allowance in the United States. Protein is the major component of egg white; there are more than 40 different kinds of proteins that make up 11 % of its entire composition. Due to their functional and pharmacological properties, egg proteins are desirable ingredients in many baked foods. It follows that egg proteins are desirable in the drug industry. Egg white consists of a solution of proteins, containing the major proteins such as ovalbumin, ovotransferrin, ovomucoid, ovomucin, and lysozyme which account for >83 % of the total egg white proteins. Other minor proteins are also found at low concentration and account for <17 % of the egg white proteins. Egg white proteins are predominantly globular proteins having an acidic pI, the exception being lysozyme and avidin. Ovalbumin is the major protein and constitutes up to 54 % of the total egg white proteins. It typically serves as the major source of amino acids for the developing embryo. Ovotransferrin and ovomucoid constitute about 12 % and 11 % proteins, respectively (Sugino et al. 1997). Ovotransferrin is implicated in the transfer of iron to target cells and could therefore be used as a nutritional ingredient in iron-enriched foods. Other proteins include ovomucin, ovoglobulin, ovomacroglobulin, ovoglycoprotein, flavoprotein, ovoinhibitor, avidin, and cystatin. Egg white also contains enzymes, such as lysozyme, phosphatase, and catalase. Among these enzymes, lysozymes constitute about 3.5 %. They are widely used in the food industry due to their antibacterial properties.

The egg proteins distributed in the yolk exist as lipoproteins, of which there are low density and high density. Low-density lipoprotein (LDL) is the major protein and accounts for up to 65 % of the total yolk proteins. The high-density lipoprotein exists as a complex with phosphoprotein known as "phosvitin." About 80 % of phosphorus in eggs is contained in phosvitin, which is derived from vitellogenin formed in the liver (Sugino et al. 1997). Other yolk proteins include livetin, a water-soluble, non-lipid glycoprotein, and riboflavin-binding protein, a hydrophilic phosphoglycoprotein.

Egg Lipids

The lipids in eggs have attracted attention both at scientific and consumer levels due to the link between high dietary fat consumption and coronary heart diseases. The fat in the egg is exclusively in the yolk and comprises 5.5–6 g in an average 60 g egg. Almost all lipids are present in lipoprotein complexes within the yolk. Trace levels of lipids have been observed in the whites. Yolk lipids are made mainly of triacylglycerol, phospholipid, and free cholesterol. Triacylglycerol and phospholipids are the major components of yolk lipids, comprising up to 65 % and 32 %, respectively. Fatty acids may be of different chain lengths, degrees of saturation, and configurations. Because of the varying degrees of unsaturation in fatty acid and differing effects on health and well-being, these fatty acids are of interest to researchers. Consequently, the most significant characteristic of dietary lipids is the content of different types of fatty acids. Fatty acids are classified into three families, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (PUFA).

Egg Minerals

Minerals are contained within the egg yolk. The egg yolk contains 1 % minerals, with phosphorus as the most abundant mineral component. More than 60 % of the total phosphorus in egg yolks is contained in phospholipids. The major inorganic components of egg white are sulfur, potassium, sodium, and chloride. Phosphorus, calcium, and magnesium are next in importance. Table shows the content of major minerals in eggs (Table 1).

Table 1 Mineral content of edible egg portion and their approximate proportion in egg white and yolk

Nutrient	Whole egg (mg)	White (%)	Yolk (%)
Phosphorus	89	5	95
Chlorine	87.1	70	30
Sulfur	82	70	30
Sodium	63	90	10
Potassium	60	80	20
Calcium	25	8	92
Magnesium	5	80.0	20.0
Iron	0.72	5	95
Zinc	0.55	n/a	95
Iodine	0.024	5	95
Manganese	0.012	10	90
Copper	0.007	35	65

Based on 59 g shell weight, with 50 g total liquid whole egg, 33.4 g white, and 16.6 g yolk
Adapted from Cherian (2006), Table 153.4, p. 153–5
Abbreviation: *n/a* not applicable

Table 2 Vitamin content of egg edible portion and their approximate proportion in egg white and yolk

Nutrient	Whole egg (mg or μg)	White (%)	Yolk (%)
Vitamin A (IU)	317	n/a	100
Vitamin D (IU)	24.5	n/a	100
Vitamin E (mg)	0.70	n/a	100
Vitamin B_{12} (μg)	0.50	15.0	85
Biotin (μg)	9.98	25	75
Choline (mg)	215.1	0.2	99.8
Folic acid (μg)	23	4	96
Inositol (mg)	5.39	25	75
Niacin (mg)	0.037	80	20
Pantothenic acid (mg)	0.063	5	95
Pyridoxine (mg)	0.07	5	95
Riboflavin (mg)	0.25	60	40
Thiamine (mg)	0.03	7	93

Based on 59 g shell weight, with 50 g total liquid whole egg, 33.4 g white, and 16.6 g yolk
Adapted from Cherian (2006), Table 153.3, p. 153–4
Abbreviation: *n/a* not applicable

Egg Vitamins

The chicken egg is considered a good source of most vitamins, except vitamin C. As shown in the table, vitamins A, D, and E are located exclusively in the yolk. Choline, folic acid, and pantothenic acid are located mainly in the yolk. Niacin appears to be located mainly in the white. Comparing the two, it seems that of the components of the edible egg, the egg yolk contains the more significant percentage of vitamins of an egg. Eggs contain both fat-soluble and water-soluble vitamins (Table 2). Most fat-soluble vitamins are concentrated in the yolk. Although several factors, such as age, strain of bird, and age of bird, are involved, diet is the most important factor for regulating egg vitamin content. Transfer efficiency of a vitamin depends on the vitamin level in the diet, feed intake, rate of egg production, and egg weight. The transfer efficiency may vary between vitamins. For example, vitamin A has a transfer efficiency between 60 % and 80 %; riboflavin, pantothenic acid, and biotin have transfer efficiencies between 15 % and 25 %; and vitamin K, thiamine, and folacin have 5–10 % transfer efficiencies (Naber and Squires 1993).

Egg Shell

The egg shell is a complex compound composed of 95 % minerals, of which calcium is more than 98 %. Other inorganic components include phosphorus, magnesium, and trace amounts of iron and sulfur comprising less than 0.05 %. Egg shell powder is considered to be a good source of highly bioactive calcium and could be used as an ingredient for human consumption. Carbohydrates in egg shell

are composed of glycosaminoglycans that are anionic polysaccharides consisting of hyaluronic acid (48 %) and galactosminoglycan (52 %) (Nakano et al. 2001). These carbohydrates have wide application in the cosmetics, pharmaceutical, and food industry.

Egg Shell Membrane

The egg shell membrane is composed of collagen-like proteins (collagen type I and V), in a ratio of 100 of type I to 1 of type V. Coarse fibers (2.5 μm in diameter) contain more type I collagen, while type V collagen predominates in the fine fibers (0.6 μm in diameter) and is largely located in the inner membrane.

The egg shell membrane contains several bacteriolytic enzymes, such a lysozyme and *N*-acetylglucosaminidase as well as other membrane proteins that have been through to have beneficial effects in treating injuries. The peptides derived from the membrane were shown to stimulate skin fibroblasts in vitro (Suguro et al. 2000). The egg shell membrane proteins are currently utilized as a cosmetic ingredient for their emollient properties.

Vitelline Membrane

The vitelline membrane, or yolk membrane, surrounds the yolk and prevents the egg yolk from mixing with the albumen. The membrane is the final barrier to microorganisms invading into the yolk. Along with being a physical barrier, it has the essential role in embryogenesis of allowing small molecules to cross the membrane. The dry weight of the membrane is 5–10 mg per egg depending on the size of eggs.

The vitelline membrane consists of the outer layer, continuous membrane, and inner layer. The outer layer contains ovomucin and lysozyme that are also found in egg white. Other compositions of the outer membrane are lectin, vitelline membrane outer protein I and II. The inner layer contains membrane glycoprotein I (27 kDa) and II (240 kDa), both of which play a structural role in the inner layer.

Proteins of the Albumen

Albumen may be regarded as a protein system consisting of ovomucin fibers in an aqueous solution of numerous globular proteins. The albumin proteins and their characteristics are presented in Table 3. The major proteins listed in the table are regarded as ovalbumin, conalbumin (ovotransferrin), ovomucoid, lysozyme, globulins, and ovomucin. The protein compositions of the thin and thick layers of albumen differ primarily in their ovomucin content. The ovomucin content in thick white is about four times than that of thin white. The principal protein fractions of albumen can be separated and purified by the stepwise addition of

Table 3 Proteins in egg albumen

Protein	% of albumen proteins	Molecular weight (kDa)	Characteristics
Major proteins			
Ovalbumin	54	45	Heat-stable polypeptide containing phosphorus and carbohydrate
Ovotransferrin	12	76	Metal-binding transport protein
Ovomucoid	11	28	Trypsin inhibitor
G2 globulin	4	30–45	–
G3 globulin	4	–	–
Ovomucin	3.5	5,500–8,300	Maintaining structure and viscosity of egg white
Lysozyme	3.4	14.3	Damage cell wall bacteria
Minor proteins			
Ovoinhibitor	1.5	49	Serine protease inhibitor
Ovoglycoprotein	1	24.4	Sialoprotein
Ovoflavoprotein	0.8	32	Riboflavin-binding protein
Ovomacroglobulin	0.5	769	Strongly antigenic protein
Cystatin	0.05	12.7	Thiol protease inhibitor
Avidin	0.05	68.3	Biotin-binding protein

Adapted from Etches (2008), Table 7.1, p. 293

ammonium sulfate and ion-exchange techniques using carboxymethyl cellulose and dimethylamino ethyl cellulose. Several electrophoresis methods have been used to characterize albumen protein fractions, including ovalbumins A1 and A2, globulins G2 and G3, ovoglobulins, conalbumin, and lysozymes.

Major Protein of Albumen

Ovalbumin

Ovalbumin, the predominant protein in albumen, is classified as a phosphoglycoprotein. This is because carbohydrate and phosphate moieties are attached to the polypeptide. A hen's ovalbumin sequence contains 385 amino acids. The N-terminal amino acid is acetylated glycine and the C-terminal amino acid is proline. The molecular weight of the polypeptide is 43,669 Da. Ovalbumin contains two phosphate residues on serines 68 and 344 that can be removed by phosphatases.

Purified ovalbumin is made up of three components. These are A1, A2, and A3, all of which differ in phosphorus content. Ovalbumins A1, A2, and A3 containing two, one, and no phosphate groups per molecule, respectively, are present in albumen fraction in relative portions of about 85:12:3. The molecule contains a carbohydrate chain attached at asparagine 292. Ovalbumin is the only albumen

protein to contain free sulfhydryl groups. Each ovalbumin molecule contains four sulfhydryl groups, three of which are reactive to *p*-chloromercuribenzoate in the native protein and the fourth in the denatured protein.

Ovalbumin in solution is readily denatured and coagulated by exposure to new surface (e.g., shaking) but is resistant to thermal denaturation. Heating of albumen at pH 9 to 62 °C for 3.5 min altered only 3–5 % of the ovalbumin, whereas heating albumen at pH 7 changed negligible amounts of this protein. During the storage of eggs, a proportion of the ovalbumin is transformed into a more heat-stable protein referred to as *S*-ovalbumin. The content of *S*-ovalbumin increases from 5 % in fresh eggs to 81 % in eggs cold stored for 6 months. At a heating rate of 10 °C/min at pH 9.0, the denaturation temperature of ovalbumin is 84.5 °C compared with 92.5 °C for *S*-ovalbumin (Donovan and Mapes 1976). It has been proposed that probably a thiol–disulfide exchange is involved in the conversion of ovalbumin to *S*-ovalbumin on storage (Smith 1964). Also, the greater stability, compactness, and hydrophobicity of the S-form contrast with that of ovalbumin (Nakamura and Ishimaru 1981).

Ovotransferrin

Ovotransferrin is a monomeric glycoprotein consisting of single polypeptide chain of 686 amino acids. The molecular weight of ovotransferrin is about 78 kDa; this constitutes 13 % of total proteins in egg white. This protein consists of two lobes, each containing a specific binding site for iron, although ovotransferrin does not contain iron in the egg. Copper, zinc, or aluminum may also bind to this site. It is the most abundant heat-sensitive egg white protein, and the complexation of iron or aluminum significantly increases its heat stability (Lin et al. 1994). The molecule does not contain iron in the egg, due to the low concentration of free iron, so that ovotransferrin has been known as a bacterial inhibitor. The denaturation temperature of ovotransferrin is the lowest among egg white proteins and forms aggregates by heating at 60 °C, like most heat-labile proteins in egg white (Matsudomi et al. 1991). However, iron-bound ovotransferrin, the holo-form, is relatively stable to chemical and thermal denaturation (Azari and Feeney 1958).

Ovotransferrin from egg albumen inhibits gram-negative bacteria by depriving bacteria of the iron source that is essential for their growth and survival (Lock and Board 1992). Ovotransferrin belongs to the family of transferrins. Transferrins are a metal-binding transport protein family in an in vivo preference for iron and widely distributed in physiological fluids. The antimicrobial activity of transferrins can result from a direct effect on the membranes: interaction of the cationic ovotransferrin with the anionic outer membrane of gram-negative bacteria (Valenti et al. 1986).

Ovomucin

Ovomucin is a macromolecule and heavily glycosylated glycoprotein, consisting of peptide-rich α-subunit and a carbohydrate-rich β-subunit. Ovomucin is a minor egg white glycoprotein (3.5 % w/w) with a molecular mass of approximately 165 kDa. It contains O-linked carbohydrate moieties that, upon formation of extensive hydrogen bonds with water, can give rise to a characteristic gel-like structure. Ovomucin

serves physical functions such as maintaining the structure and viscosity of egg white albumen, thus serving to prevent the spread of microorganisms (Ibrahim 1997), and possessing good foaming and emulsifying properties. Ovomucin plays a role in the decrease of the viscosity of thick white during storage of the egg.

Ovomucin has been shown to possess virus hemagglutination inhibition activity (Tsuge et al. 1996), antitumor activity (Oguro et al. 2001), immunomodulation activity (Tanizaki et al. 1997), and cholesterol uptake inhibition activity (Nagaoka et al. 2002).

Ovomucoid

Ovomucoid is a highly glycosylated protein (20–25 % carbohydrates, w/w) of 28 kDa. It constitutes 11 % of egg white proteins. It is a highly glycosylated protein consisting of 186 amino acids. It is also known to exhibit trypsin inhibitor activity passed from the albumen to the embryo during incubation. The molecule consists of three structurally independent tandem homologous domains (Domains Gal d 1.1, 1.2, and 1.3) possesses nine entities. Gal d 1.3 was reported as the immunodominant fraction (Rupa and Mine 2006). A large proportion of the weight of the ovomucoid molecule is carbohydrate, which is attached to asparagine in the sequences Asn-X-thr/ser in up to five places on the sequences. Ovomucoid is a serine proteinase inhibitor. Ovomucoid consists of three domains defined by the amino acid sequences 1–68, 69–130, and 131–186, each domain being cross-linked by three disulfide bridges (Kato et al. 1987). The carbohydrate moiety consists of three oligosaccharide units bound to protein through asparagine residues (Montgomery and Wu 1963). The polypeptide chain is composed of 26 % α-helix, 46 % β-structure, 10 % β-turns, and 18 % random coil (Watanabe et al. 1981). Ovomucoid is highly resistant to heat owing to its high cystine content. Under acidic conditions, ovomucoid can resist to heat treatments up to 100 °C, but it is rapidly denaturated at 80 °C and pH 9.0 in the presence of lysozyme (Matsuda et al. 1982).

Lysozyme

Lysozymes can be found in egg albumen, the shell, and the vitelline membrane, belonging to a class of enzyme that lyses the cell walls of gram-positive bacteria. They, also known as muramidase or *N*-acetylmuramic hydrolase, are a relatively small secretory enzyme that catalyzes the hydrolysis of specific polysaccharides contained in cell walls of bacteria. The main activity of the lysozymes is to catalyze the hydrolysis of beta 1,4 bonds between *N*-acetylmuramic acid and *N*-acetylglycosamine. These bonds stabilize the glycans in the cell walls of gram-positive bacteria. Since lysozyme has four disulfide bounds, this small protein molecule is unusually compact and heat stable. The thermal denaturation temperature of lysozyme is around 75 °C but depends on pH and medium conditions. However, it is rapidly inactivated by thiol compounds. In egg white, lysozyme is much more heat sensitive than when present alone (Donovan et al. 1975). Egg albumen is the most plentiful source of this enzyme to form biologically important complexes with other albumen proteins, especially ovomucin. Egg yolk inhibits lysozyme activity as a

result of electrostatic-complex formation with the yolk granules. Lysozyme from hen's egg white is a polypeptide of 129 amino acid residues having a molecular weight of 14.3 kDa. It is an elementary protein with the isoelectric point (pI) of 10–11 and is a strongly basic protein in egg white, well known for bacteriostatic, bacteriolytic, and bactericidal activity particularly against gram-negative bacteria. The protein represents only 3.4 % of the egg protein's total content. It is a good example for naturally occurring enzymes used in the food industry as preservative to maintain product quality and reduce the incidence of spoilage.

Penalbumin

The molecular weight of penalbumin is 61.6 kDa larger than ovalbumin (47.1 kDa). This protein has several features related to ovalbumin. In terms of the composition of penalbumin, it has more carbohydrate and lacks phosphate. The amino acid compositions are significantly different, but the differences could be explained if penalbumin is an extended form of ovalbumin.

Ovoglobulins

The composition of ovoglobulin contains 13.6 % of hexose, 13.8 % of hexosamine, and 3 % of sialic acid. Hexose occurs as mannose and galactose in the ratio 2:1, hexosamine as glucosamine, and sialic acid as *N*-acetylneuraminic acid. It has a minimum molecular weight, calculated from the tryptophan content, of 24,400 Da. The term ovoglobulin refers to ovoglobulins G2 and G3, each constituting about 4 % of egg albumen proteins. Ovoglobulins G2 and G3 are similar in many properties, including their molecular weight (49 kDa). In chickens, ovoglobulin G2 shows polymorphism.

Ovoglobulins are also of interest for commercial applications of albumen because they denature rapidly and may therefore have more effect on the initial foaming of the albumen than the more plentiful albumen proteins.

Minor Proteins of Albumen

Ovoinhibitors

Ovoinhibitor is a glycoprotein in egg white, composed of 447 amino acids with a molecular weight of 48 kDa. The sequence of ovoinhibitors contains seven Kazal-like domains involving 21 disulfide bonds. Like the ovomucoid, this protein is a proteinase inhibitor. It inhibits the activities of trypsin, chymotrypsin, and some proteinases of microbial origin. The oxidation of methionine residues of ovoinhibitor results in a loss of its inhibitory activity against chymotrypsin and elastase (Schechter et al. 1977). The domain I is a potent inhibitor of trypsin but is devoid of inhibitory activity against chymotrypsin, elastase, or proteinase K. Domains I to IV contain an arginine at the P1 position and are thought to inhibit trypsin-like enzymes. Domain V has a phenylalanine at P1, which is consistent with anti-chymotrypsin activity, and domains VI and VII possess a methionine at P1, making them likely to inhibit chymotrypsin and elastase.

The ovoinhibitor has been found to prevent the development of rotavirus-induced gastroenteritis in a mouse model and to inhibit the formation of active oxygen species by human polymorphonuclear leukocytes, which are associated with inflammatory diseases, mutagenicity, and carcinogenicity.

Ovomacroglobulin

The ovomacroglobulin is the largest globular protein in eggs, featuring a wide spectrum of immunological cross-reactivity among different avian species and inhibiting a wide range of proteases by physically engulfing the enzyme. This protein, also known as ovostatin, is composed of four subunits, each having a molecular weight of 175 kDa, with pairs of the subunits joined by disulfide bonds. Ovomacroglobulin inhibits hemagglutination, possesses anti-collagenase activity, and has inhibitory activity against diverse proteolytic enzymes representing serine, thiol, and metal protease (Li-Chan and Nakai 1989). It has demonstrated broad-spectrum inhibitory activity against various types of proteases, including serine proteases, cysteine proteases, thiol proteases, and metalloproteases (Molla et al. 1987). Enzyme inhibitory action by this protein is quite different from that of the low molecular weight inhibitors, such as ovomucoid. The hen's ovomacroglobulin differs from macroglobulins in the blood in that it does not contain a thiol ester. The protein denatures and precipitates on heating to 62–64 °C, pH 7.6. It undergoes a thermal transition at about 60 °C.

Cystatin

A member of a "superfamily" of cystatins, egg white cystatin belongs to the type 2 cystatin, which has about 115 amino acids and two disulfide bonds, but no carbohydrates. Secreted cystatin has a theoretical molecular weight of 13,147 Da. The major isoelectric forms differing by the occurrence of phosphorylation on serine 8 have been identified. The non-phosphorylated and phosphorylated forms display a pI of 6.5 and 5.6, respectively. Cystatin inhibits most of the cysteine proteases with 1:1 stoichiometry, including ficin; papain; cathepsins B, H, and L; and papaya peptidase. Cystatin is stable to heat but the effects of high temperatures are unusual. For example, heating at 100 °C for 3 min decreased the activity against ficin by 64 %, but further heating did not cause a further decrease.

Egg white cystatin has been shown to possess antibacterial activity, preventing the growth of group A streptococcus (Bjock et al. 1989), *Salmonella typhimuriu*m (Nakai 2000), and the periodontitis-causing *Porphyromonas gingivalis* (Travis et al. 1997).

Cystatin, a naturally occurring protease inhibitor, has fewer side effects than other synthetic protease inhibitors used in medical treatments. This protein can also induce the synthesis of various cytokines, resulting in upregulated nitric oxide release in vitro using mouse peritoneal macrophages (Verdot et al. 1996). It has also been shown in vivo, greatly reducing parasite numbers in a mouse model of visceral leishmaniasis (Korant et al. 1985).

Increased levels of cysteine proteases and the concomitant decrease of cystatin have been observed in various cancers; cystatin has been shown to inhibit tumor

invasion in Ras-transformed breast epithelial cells (Premzl et al. 2001). This would suggest that chicken cystatin may have a role in cancer therapy.

Riboflavin-Binding Protein

Ovoflavoprotein, also referred to as flavoprotein or riboflavin-binding protein, is a phosphoglycoprotein that is responsible for binding most of the riboflavin (vitamin B_2) in egg white. Flavoprotein is considered to have the highest Se content (1,800 ng/g) among egg white proteins. Ovoflavoprotein could serve as a useful food ingredient from egg white as it is abundant as a low-cost egg processing by-product. Ovoflavoprotein binds riboflavin at pH above 4.3 with an association constant of 7.9×10^8 M. It is composed of 219 amino acids (Hamazume et al. 1984) with a molecular weight of 32 kDa. The carbohydrate content is about 15 %, consisting of mannose, galactose, glucosamine, and sialic acid.

Chicken egg white riboflavin-binding proteins are the prototypes of a family that includes other riboflavin- and folate-binding proteins. One unusual characteristic of these molecules is their high degree of cross-linking by disulfide bridges. Another is in the case of avian proteins, where there are stretches of highly phosphorylated polypeptide chain. Each mole of riboflavin-binding apoprotein (apo-RBP) binds one mole of riboflavin with high-affinity constant (1.4 nM), causing loss of the characteristic riboflavin fluorescence.

Avidin

The avidin protein constitutes a maximum of 0.05 % of the total protein content of egg white. Avidin is an alkaline (pI 10.5), highly stable, tetrameric glycoprotein that is best known for its biotin-binding properties. Each of the four monomers binds one molecule of biotin and the avidin–biotin interaction, with dissociation constant of 10^{-15} M, as the strongest non-covalent interaction reported between protein and ligand. Each avidin chain, composed of 128 amino acid residues, is arranged in an eight-stranded antiparallel β-barrel, whose inner region defines the D-biotin binding site. A fairly rigid binding site is readily accessible in the apoprotein structure, making it sterically complementary to the shape and polarity of biotin.

Both chicken egg white avidin and its bacterial relative streptavidin are widely used as tools in a number of affinity-based separations, diagnostic assays, and a variety of other applications. Other applications include the potential of avidin as an insecticide and antimicrobial agent. Due to its high proportion of tryptophan residues, avidin is unstable under oxidizing conditions in strong light.

Thiamin-Binding Protein

Thiamin-binding protein can be isolated from the hen's egg albumen by using affinity chromatography. In terms of function, the protein binds thiamine (vitamin B_1) in a 1:1 ratio and is similar with avidin in that it is a vitamin scavenger. In terms of structure, the protein has a molecular weight of 38 kDa and does not contain carbohydrate. It is not usual in that it forms a stoichiometric complex with the albumen riboflavin-binding protein. An identical protein is present in egg yolk.

Vitamin B_2-Binding Protein

Vitamin B_2-binding protein has a molecular weight of 98 kDa. The vitamin-binding ability of this protein was heat labile in 2 h at 80 °C, but the complex was stable for 6 h at this temperature. Due to this difference, this albumen protein is distinguished from a B_{12}-binding protein in egg yolk.

Ovoglycoprotein

Ovoglycoprotein is a protein of the lipocalin family present in egg white. It represents about 1 % of the egg white protein. It is an acidic glycoprotein (pI 3.9) with a theoretical molecular weight of 20.3 kDa and a sugar content of 30 %. Despite the information just previously given, very little more is known about this protein. Currently, ovoglycoprotein is mainly used as a chiral selector to separate drug enantiomers by high-performance liquid chromatography or capillary electrophoresis.

Enzymes in the Albumen

In addition to lysozyme, albumen contains many enzymes. These enzymes include those with phosphatase, catalase, and glycosidase activity (Sugino et al. 1997). An aminopeptidase has been isolated from albumen. It acts with broad specificity, hydrolyzing aliphatic, aromatic, and basic aminoacyl-2-naphthylamides, di- to hexapeptides, with a preference for methionine at the NH_2 end, and basic or bulky hydrophobic residues at the penultimate position (Skrtić and Vitale 1994). The enzyme is a hydrophilic, acidic glycoprotein with molecular weight of ~180 kDa and optimal activity at pH 7.0–7.5 and 50 °C. Amastatin, bestatin, and *o*-phenanthroline were found to be strong inhibitors, while Co^{2+} activated the enzyme (Skrtić and Vitale 1994). A dimeric glycoprotein containing one molecule of FAD per 80 kDa subunit was isolated from chicken egg white and found to have sulfhydryl oxidase activity on a range of low molecular weight thiols, generating hydrogen peroxide in aerobic solution.

Lipids of the Egg Yolk

Lipids are the main components (32–36 %) of the egg yolk solids. The composition of yolk lipid is generally about 65 % triglyceride, 28–30 % phospholipids, and 4–5 % cholesterol. However, the composition of yolk lipids can be affected by various factors including hen's age, genotype, and changes in the diet of the hens.

Fatty Acids

The predominant saturated fatty acids in eggs are palmitic (C16:0) and stearic (C18:0). The content of these two fatty acids in chicken eggs may range from 22 % to 26 % and 8–10 %, respectively. In addition to these two fatty acids, there are also other minor amounts of C14 and C20. The total saturated fatty acids may constitute up to 30–35 % of the total fatty acids in egg yolks.

Monounsaturated fatty acids (MUFA) in eggs are C16:1 and C18:1, which constitutes to 42–46 % of total fatty acids. Oleic acid (C18:1) is the major monounsaturated fatty acid in chicken eggs. Oleic acid has been reported to be hypolipidemic, reducing both cholesterol and triacylglycerol without decreasing high-density lipoprotein cholesterol in human patients (Mattson and Grundy 1985). Dietary lipids affect monounsaturated fatty acids in egg content. Addition of high oleic acid sunflower seeds in feed has been reported to increase the content of oleic acid in eggs up to 3.2 g, compared with 1.9 g in regular egg (Cherian and Sim 1993). Recent studies reported a significant decrease in egg oleic acid when diets contained conjugated linoleic acids (CLA) (Aydin et al. 2001; Cherian et al. 2002).

In comparison of polyunsaturated and monounsaturated fatty acids, the polyunsaturated fatty acid contents were significantly higher for eggs laid by 39-week-old hens compared with older hens, while monounsaturated fatty acid contents were significantly higher for eggs laid by 93-week-old hens. The contents of long-chain (20 and 22) omega-6 and omega-3 polyunsaturated fatty acids (PUFA) were 20 % and 25 %, respectively, higher in egg yolks from 21-year-old hens than 57-week-old hens. Egg size did not significantly affect yolk lipid or fatty acid concentration. However, lipid levels were lower while linoleic acid level was higher in yolks of eggs from hens older than 47 weeks of age than in those produced by younger birds. The unsaturated to saturated fatty acid ratio for yolk produced at 27 and 39 weeks of age was lower than that for yolk produced at 51 weeks.

There are two families of PUFA in egg, namely, n-6 and n-3 fatty acids. The predominant n-6 PUFA in egg lipids is C18:2n-6 (linoleic acid). Other n-6 fatty acids in eggs may include C20:4n-6, C22:4n-6, and C22:5n-6. The content of long-chain n-6 PUFA (LCPUFA) (>20-carbon) may vary from 1 % to 2 % and is reflected by the type of laying hen diet (Cherian and Sim 1991). The content of n-3 fatty acids in eggs is made of α-linoleic acids (18:3n-3) and docosahexaenoic acids (DHA, 22:6n-3). Among these, DHA is the major n-3 fatty acid in the egg. The α-linoleic content in regular eggs is under 1 % of the total lipids; DHA may constitute between 1 % and 3 %. The content of n-3 PUFA is a reflection of dietary fat. Addition of flax, fish oil, and marine algae in laying hen diet leads to significant increases in α-linoleic acid and DHA in eggs (Cherian and Sim 1991).

Sim (1998) described the development of "designer egg" rich in omega-3 PUFA such as α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which have been associated with beneficial effects for human health. The beneficial effects include reduction of triglyceride level, lowering of blood pressure, decrease in platelet aggregation, and a decrease in tumor growth. Many studies have investigated the incorporation of different feed ingredients such as fish oil, vegetable oils (including flaxseed or linseed), soy oil, canola oil, and microalgae into the diet of hens, in order to optimize the omega-3 to omega-6 and PUFA to saturated fatty acid ratios of eggs for human health.

Products enriched with PUFA are prone to oxidation and the enrichment with antioxidants is necessary in order to prevent the risk of oxidative damage. Grune et al. (2001) suggested supplementation of feed with least 80 IU vitamin E/kg to prevent increase in cytotoxic aldehydic lipid peroxidation during production and

storage of omega-3 PUFA-enriched eggs. Dietary vitamin E resulted in a decrease of PUFA, SFA, and total lipids in fresh yolk lipids, whereas MUFA did not change. Also, dietary E supplement slowed down the process of oxidation of egg yolk fatty acid during storage.

Several studies have also investigated the effect of dietary conjugated linoleic acid (CLA) on the composition of egg yolk lipids. The levels of CLA incorporated into the lipid of the egg yolk were proportional to the levels of CLA in the diet, although more CLA was incorporated in the triacylglycerol than were phospholipid components. The incorporation rate of differing CLA isomers in different classes of lipids was significantly different. Furthermore, the inclusion of CLA in the diet also influenced the metabolism of polyunsaturated fatty acids. The amount of arachidonic acid was decreased by CLA added to linoleic acid and linolenic acid-rich diet, but EPA and DHA were increased in the linolenic-rich diet, indicating that synthesis or deposition of long-chain n-3 fatty acids was accelerated after CLA feeding. Despite this, increases in saturated fatty acids in yolk and decreases in MUFA and PUFA by dietary CLA have also been reported (Watkins et al. 2003). Feeding conjugated linoleic acid-enriched diets resulted in gradually increasing deposition of CLA isomers in egg yolk lipids, while feeding CLA led to accumulation of isomer in polar and neutral lipids of the egg yolk that migrated into the egg albumen (Watkins et al. 2003).

Aydin et al. (2001) reported that olive oil prevented CLA-induced increases in 16:0 and 18:0 and decrease in 18:1 (ω-9) in yolk and also prevented CLA-induced abnormal changes in the pH of albumen and yolks. Hur et al. (2003) indicated that lipid oxidation of the egg yolk during cold storage could be inhibited by dietary CLA due not only to the change in fatty acid composition but also to the high concentration of CLA in egg yolk. Szymczyk and Pisulewski (2005) reported that dietary vitamin E increased the rate of laying and egg production per hen and may also exert alleviating effects on fatty acid composition of CLA-enriched eggs.

Phospholipids

The major components of egg yolk phospholipids (PL) are phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which may make up ~81 % and 12 % of egg yolk lecithin; lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and sphingomyelin are also known components of yolk PL. The major fatty acids in egg PC are palmitic, oleic, stearic, and linoleic acids, represented 32 %, 26 %, 16 %, and 13 %, respectively; arachidonic and docosahexaenoic acids (4.8 % and 4 %, respectively) are also present in significant amounts.

Yolk phospholipid content, expressed in relation to weight of egg oil or whole egg, was reported to be positively related to hen's age. Egg from hens receiving low-dose chitosan treatment contained a 1.8-fold increase in yolk phospholipid level (Vrzhesinskaia et al. 2005).

Kivini et al. (2004) studied the influence of oil-supplemented feeds (containing 15 % vegetable-based or fish oils) on the concentration of the phospholipid content

and their composition in hen eggs. Also, the total phospholipid contents and proportions of PC, PE, and sphingomyelin were similar for all feeding groups. The supplemented feeds had a significant ($p < 0.05$) effect on the fatty acid composition of phosphatidylcholines. Furthermore, supplements decreased the proportion of saturated fatty acids in total fat, but not in the phospholipids.

Shimizu et al. (2001) investigated effects of feeding dietary fish oil to hens on the fatty acid composition of eggs. A variation of fatty acid composition in egg yolks was found in the acyl groups of PC and PE rather than in TG. The results showed that the supplementation of the diet of hens with fish oil altered essential fatty acid composition. In particular, increasing DHA and decreasing arachidonic acid in egg yolk phospholipids did this.

Nakane et al. (2001) reported growth factor-like lipids in hen egg yolk and white, which were associated with high amounts of lysophosphatidic acid (acyl LPA) and small amounts of the lysoplasmanic acid (alkyl LPA). The levels of acyl LPA in hen egg yolk (44.23 nmol/g tissue) and white (8.81 nmol/g tissue) were on the same order as or higher than the levels of acyl LPA containing predominantly saturated fatty acids as the acyl moiety and egg white acyl LPA containing primarily PUFA.

Many studies have been conducted on methods for extraction and separation of phospholipids or lecithins from egg, as well as preparation of lysolecithin by the enzymatic action of phospholipase A2 (PLA2), including immobilized PLA2. In addition to providing sources of purified phospholipids for basic research, these methods have been established to meet the demand to produce purified egg lecithin for pharmaceuticals, nutraceutical, and food applications. Examples of beneficial properties of yolk phospholipids, with potential industrial applications as nutraceuticals and functional food ingredients, include anti-oxidative activity (Sugino et al. 1997) and inhibition of cholesterol absorption.

Fat-Soluble Vitamins

Most egg vitamins, especially the fat-soluble vitamins, are contained in the yolk. Hen egg is considered a source of most vitamins necessary for human nutrition, except vitamin C. One egg may supply almost 12 % vitamin A, more than 6 % of vitamin D, 9 % riboflavin, and 8 % pantothenic acid of the recommended daily allowance in the United States. Only fish contains more vitamin D than eggs.

The antitumor activity of carotenoids and retinoids plays an important role in scavenging peroxide radicals. Modified eggs enriched in α-tocopherol, β-carotene, and retinol are obtained by supplementing these substances in the hen's feed and are highly regarded nutritionally.

Pigments in Yolk

The color of the yolk is an important factor of the consumer acceptability of commercial eggs. The natural pigments in egg yolk are carotenoids that are

conjugated isoprene derivatives. Among carotenoids, lutein and zeaxanthin are incorporated to a larger extent than *b*-carotene and astaxanthin. A large proportion of the yolk pigments is transported through the blood from the intestine by lipoproteins, which are normally deposited in the yolk. The functions of pigments are not known clearly, but the health of the chick after hatching may be improved by these carotenoids.

Proteins of the Egg Yolk

Yolk lipoprotein precursors such as very low-density lipoproteins (VLDL) and vitellogenin are synthesized in the laying hen's liver and are transported in the blood to the oocyte. VLDL consist of apoVLDL II and apolipoprotein-B (Burley et al. 1984). ApoVLDL II is the only apoprotein from blood lipoproteins to be transferred to the yolk without any modification and is called apovitellenin I (Dugaiczyk et al. 1981). The source of yolk low-density lipoproteins (LDL) is VLDL. During the transfer from the blood to yolk, apolipoprotein-B is cleaved into several fragments, referred as apovitellenins I–VI (Burley et al. 1993). Vitellogenin consists of three species designated vitellogenins I, II, and III (Wang and Williams 1980; Wang et al. 1983). Vitellogenin is cleaved into the yolk granule proteins lipovitellin I and II and the phosphoprotein phosvitin. Amino acid analysis indicated the presence of a highly phosphorylated phosvitin in vitellogenins I and II and small phosvettes derived from vitellogenin III (Wallace and Morgan 1986). Schmidt et al. (1956) separated the granules by subjecting yolk to a centrifugal force of 20,000 g and granules consisted of 11–13 % of the solids in yolk and contained both lipoprotein and phosphoprotein, and most of the iron and calcium of the yolk. This was confirmed in a more detailed investigation by Burley and Cook (1961), who reported that the granules represent about 19–23 % of the yolk solids on a dry weight basis and consisted of 70 % high-density lipoproteins (HDL), 16 % phosvitin, and 12 % LDL. These authors concluded that LDL is the structural constituents of the granules. Granules are mainly constituted by HDL and phosvitin, and HDL–phosvitin complex is the basic unit of granules linked by phosphocalcic bridges between the phosphate groups of their phosphoseryl residues (Causeret et al. 1991). Plasma forms the aqueous phase where yolk particles are in suspension. It comprises 77–81 % of yolk dry matter and is composed of 85 % LDL and 15 % livetins (Burley and Cook 1961).

Lipovitellin Apoproteins

Yolk proteins in the water phase consist of lipoproteins (30 %) and soluble proteins (8 %). High-density lipoprotein consists of α- and β-lipovitellins, which differ in amino acid composition as well as bound phosphorus and carbohydrates. The proportion of α- and β-lipovitellins in yolk granules appears to be genetically based. The protein content of lipovitellin is about 80 % while lipid content is

about 20 %, including phospholipids (60 % of the lipid, primarily a lecithin), triacylglycerols (40 %), and small amounts of cholesterol, sphingomyelin, and other lipids. Both lipovitellins are glycoconjugates with mannose, galactose, glucosamine, and sialic acid, but α-lipovitellin contains much higher sialic acid content than does β-lipovitellin, explaining its relatively acidic nature (Juneja and Kim 1997). The apoprotein form of lipovitellins, sometimes referred to as vitelline, is present in a dimeric form linked through hydrophobic interactions; delipidation of lipovitellin has been reported to result in loss of solubility (Juneja and Kim 1997).

Yamamoto and Omori (1994) studied anti-oxidative activity of egg yolk lipoproteins and apoproteins in a linoleic acidic emulsion system. High-density lipoprotein was a more effective antioxidant than LDL, and apo-HDL was more effective than apo-LDL. The lipid moiety of HDL also had an anti-oxidative effect on linoleic acid directly or in emulsion and possibly enhanced the anti-oxidative activity of the lipoproteins.

Kassaify et al. (2005) conducted in vitro experiments using confluent Caco-2 cell monolayers to investigate adhesion elimination, adhesion prevention, and antimicrobial properties of various extracted granule and plasma fractions against *Salmonella enteritidis*, *S. typhimurium*, and *Escherichia coli* O157:H7. The result revealed that the granule component HDL was the yolk fraction with protective effect against the foodborne pathogens, and this protective activity was confirmed to remain intact despite peptic and tryptic enzymatic digestion. Thus, the granule component has an anti-adhesive effect but no antimicrobial effect.

The low-density lipoprotein of the egg yolk is the most abundant non-water phase of the egg yolk. It is about 60 % of the dry weight of egg yolk. The lipoproteins can be separated by high-speed centrifuging, gel chromatography, and ion-exchange chromatography. Yolk low-density lipoprotein from egg yolks contains about 12 % of protein, the rest being neutral and phospholipid.

Apovitellenins

The only apoprotein from blood lipoproteins to be transferred to yolk in large amount without any modifications is apoVLDL II, called apovitellenin I in the yolk. Apovitellenin I is a small protein of low molecular weight that lacks histidine with a small homodimer with disulfide-linked subunits of 9 kDa. Apovitellenin II is also isolated from egg yolk low-density lipoprotein. The protein's molecular weight is 20 kDa with polysaccharide residues of glucosamine, hexose, and sialic acid. The functions of both apovitellenins are not clearly understood, but their properties appear to be an essential part of the lipoprotein structure. Apovitellenins III and IV with molecular weight more than 60 kDa are isolated from the total apoprotein mixture of egg yolk low-density lipoprotein by gel and hydrophobic chromatography.

Livetin

Livetin is water-soluble protein that accounts for 30 % of the plasma proteins and is composed of α-livetin (serum albumin), β-livetin (α2-glycoprotein), and γ-livetins

[γ-globulin immunoglobulin Y (IgY)] (Sugino et al. 1997). The mean molecular weights of α-, β-, and γ-livetins are reported to be 80,000, 45,000, and 170,000 Da, respectively. The relative proportion of the three livetins in the yolk is 2:5:3, respectively.

α-Livetin

Egg yolk α-livetin and chicken serum albumin are identical. It has a molecular weight of 70 kDa and isoelectric point of 4.3 and 5.7. Chicken serum albumin (α-livetins) has been implicated as the causative allergen of the bird egg syndrome. Chicken serum albumin is partially heat-labile inhalant. IgE reactivity to chicken serum albumin was reduced by nearly 90 % by heating for 30 min at 90 °C.

β-Livetin

β-livetin has been identified as a 45 kDa α2-glycoprotein. Chemically, its composition includes 14.3 % nitrogen and 7 % hexose. Unfortunately, not much information is available about this protein and the existing data available is ambiguous.

γ-Livetin (IgY)

The γ-livetins in yolk are transported from the blood serum of hens. Of the three immunoglobulins (IgM, IgA, and IgG) found in the serum, the laying hens transfer IgG to yolk at concentration of ~25 mg/ml, whereas IgM and IgA are transferred to egg white at concentrations of 0.15 and 0.7 mg/ml, respectively. Morrison et al. (2002) identified several regions within the antibody molecule important for its uptake into the egg yolk. Intact Fc and hinge regions, but not the Fc-associated carbohydrate, are required for transport.

The basic structure of all immunoglobulin molecules is a unit consisting of two identical light polypeptide chains and two identical heavy polypeptide chains. These chains are linked together by disulfide bonds. Typically avian plasma contains IgY plus IgA and IgM, which are evolutionary different from five distinct classes of mammalian immunoglobulins: IgG, IgA, IgM, IgD, and IgE. Although IgY antibody is functionally equivalent to mammalian IgG, they have profound structural differences.

The γ-globulins or γ-livetins in yolk are referred to as immunoglobulin Y (IgY) to distinguish them from mammalian IgG. Although IgY is derived from hen serum IgG, it differs in many chemical and structural features from mammalian IgG (Kovacs-Nolan and Mine 2004). Both IgG and IgY contain Asn-linked oligosaccharides, despite the composition of the oligosaccharides being different. Like IgG, yolk IgY contains two heavy chains (H) and two light chains (L), but the molecular weight of the H chains of the IgY is greater than those of mammalian IgG, yielding an overall molecular weight of 180 kDa compared to 150–160 kDa for mammalian IgG. Furthermore, IgY H chain lacks hinge region and processes four constant regions and one variable domain, whereas the IgG H chain contains a hinge region between the first two of three constant domains, which lead to flexibility of the Fab fragments. The average molecular weights of IgY, heavy-chain, and Fab fragments are 167, 65, and 45 kDa, respectively. Peptic digestion degrades IgY into Fab

Table 4 Comparison of chicken IgY and mammalian IgG

Character	IgY	IgG
Molecular weight	180 kDa	150 kDa
Isoelectric point	>acidic	<acidic
Heat stability	>sensitive	<sensitive
pH stability	>sensitive	<sensitive
F_c receptor-binding activity	Low	High
Protein A/protein G binding	No	Yes
Interference with mammalian IgG	No	Yes
Interference with rheumatoid factor	No	Yes
Complement activation	No	Yes

fragments, in contrast to disulfide-linked F(ab')2 fragments generated from IgG. IgY is relatively heat stable even after heating to 65 °C for 30 min. It remained stable over the pH 5–11 range, but the antigen-binding activity was rapidly lost at pH 2–3 or lower, probably because of conformational changes. Table 4 provides a comparison between avian IgY and mammalian IgG.

The separation of IgY from the egg yolk involves various chemical reactions and a simple water extraction process. The water-soluble fractions (WSF) of egg yolks can be obtained by using the water dilution method based on the aggregation of yolk lipoproteins at low ionic strengths. Centrifugation or filtration is subsequently used to fractionate the WSF from water-insoluble lipid components of egg yolk. Acidic conditions change the integrity of the egg yolk granules and lead to increases in the lipid-binding ability. Therefore, lowering pH results in not only increasing the recovery of IgY but also decreasing the amount of LDL in the supernatant. The WSF was almost devoid of lipids at mild acidic conditions and the highest yield of IgY was obtained between pH 5.0 and 5.2. To obtain a great purity of the IgY fraction collected from the WSF, a variety of methods have been used. These purification steps involved isolation of IgY from other water-soluble proteins by further concentration using specific salts or acids. Further purification by gel permeation chromatography, ultracentrifugation, and ultrafiltration resulted in a 95 % pure IgY diagnostic agent.

Microbial foodborne diseases are responsible for serious health problems in humans and animals due to pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., enteropathogenic *E. coli*, viruses, and parasites. IgY studies have demonstrated that specific IgY against *E. coli* O157:H7 and *Salmonella* is able to inhibit the growth of pathogens, eventually resulting in bacterial death. This research offers many advantages over traditional antibiotics and provides the basis of a highly effective means of producing inexpensive antibodies in egg yolks as functional food and nutraceutical ingredients for the prophylactic treatment of humans and animals against enteric diseases. Among these, oral passive immunotherapy may be of value due to the advantages of reduced cost, ease of administration, and potential to treat localized conditions in

the gastrointestinal tract (GIT). Chicken egg yolk immunoglobulin (IgY) is ideal for passive immunotherapy, as it may be readily obtained in large quantities from egg yolk, presenting a more cost-effective, convenient, and hygienic alternative to mammalian antibodies. IgY antibody has been proved to neutralize disease-causing pathogens, i.e., *Rotavirus*, *E. coli* O157:H7, *Salmonella enteritis*, *Clostridium perfringens*, and toxic gluten for celiac disease (Gujral et al. 2012).

Using chicken as an antibody producer brings a number of advantages over conventional mammalian antibody and recombinant antibody production and serves as an alternative to antibody sources (Box 1). Combined with the egg industry's capacity to produce thousands of eggs per day and an existing technology for the efficient fractionation and purification of IgY, it is conceivable that kilogram quantities of antibodies could be produced on a daily basis. Thus, IgY has been widely used as a passive immunization therapy to treat enteric infections in humans and animals. Another application is the use of IgY as an immunological tool in the field of diagnostics as well as biomedical research. In this presentation, we summarize published data on properties and applications of IgY in immune-power eggs for prophylactic, therapeutic, and diagnostic uses and suggest directions for its future use.

Box 1: Advantages of IgY

- Maintenance of a large flock of laying hens is inexpensive and practical because large-scale feeding of hens and the collection of eggs are less labor intensive and well integrated.
- Eggs as the source of IgY can be collected from laying hens by the noninvasive method, which is compatible with animal protection regulations, as compared to mammal's sera from which IgG is separated.
- Also, immunization of hens (vaccination) has long been applied to prevent hens from infectious diseases, indicating that immunization of hens is much more systematized to be effective than doing it for animals.
- A laying hen produces an average of 285 eggs in a year with a yolk of approximately 15 g, whereas an immunized rabbit provides about 40 ml of sera. One gram of egg yolk contains about 10 mg of IgY, whereas 1 ml of rabbit serum yields about 35 mg of IgG. An immunized hen produces about 43 g of antibodies per year.
- As egg yolk is known as a perfect food package, the isolation of IgY from the yolk is much easier than that of IgG from animal blood sera. For separation of IgY, a large-scale method is now applicable by automatic separation of the egg yolk with a machine.
- The immune response of chickens could be maintained for a long period of more than 20 weeks with two injections.
- The conventional method inevitably sacrifices animals which have produced the specific IgG in their circulating blood. On the other hand, the method of using hens is sufficient only to collect the eggs laid by super immunized hens.

Phosvitin

Phosvitin is a phosphoglycoprotein that contains about 10 % phosphorus, with α- and β-phosvitin containing about 2–9 % phosphorus, respectively. It is therefore one of the most highly phosphorylated proteins occurring in nature. About 80 % of protein-bound phosphorus in egg yolk is located in phosvitin. Serine residues are predominant in the protein, many of which are phosphorylated and occur consecutively in the primary sequence of the molecule. In addition to phosphorus, the phosvitin molecule contains 2.5 % hexose, 1 % hexosamine, and 2 % sialic acid. However, unlike many of the other yolk proteins, it does not contain any lipid.

Phosvitin is constituted by 217 amino acid residues that comprise a core region of 99 amino acids, grouped in runs of maximally 14 Ser residues interspersed by Arg, Lys, and Asp (Byrne et al. 1984; Van Het Schip et al. 1987). The relative abundance of phosphoseryl groups in the phosvitin amino acid sequence confers to the protein a large central hydrophilic portion surrounded by two small hydrophobic parts at the N-terminal and C-terminal. Owing to its polyanionic character (pI = 4), phosvitin possesses a very strong metal-chelating property (Castellani et al. 2004). The major site of phosvitin binding to carbohydrate is the Asn residue at position 169, and the carbohydrate moiety is a branched oligosaccharide, consisting of mannose, galactose, *N*-acetylneuraminic, and *N*-acetylglucosamine acid, and linked to protein by *N*-glycosidic bond (Shainkin and Perlmann 1971). Fourier transform infrared spectroscopy showed that the secondary structure of phosvitin is composed of 0 % α-helix, 50 % β-sheet, 7 % β-turns, and 43 % random coil (Losso et al. 1993). However, factors such as pH can affect its secondary structure and shift the percentages of β-sheets to α-helices and random coils (Renugopalakrishnam et al. 1985; Prescott et al. 1986).

Due to its structure, phosvitin is resistant to heat treatments. No precipitate is observed after heating phosvitin solutions with a range of different pH (4–7) for several hours at 100 °C (Mecham and Olcott 1949). Since native phosvitin has a very stable conformation, once iron is bound it is not easily released. After a heat treatment at 90 °C for 60 min, no decrease in the iron-binding capacity could be detected (Castellani et al. 2004). The unique chemical characteristic of phosvitin conferred by its high proportion of ionizable phosphorylated serine residues is accompanied by properties such as high water solubility and resistance to heat denaturation (Anton et al. 2000) and proteolytic attack (Juneja and Kim 1997). Because of the phosphate groups, phosvitin is one of the strongest naturally occurring metal-binding biomolecule. Under low ionic strength and acidic conditions, phosvitin forms soluble complexes with Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Fe^{2+}, and Fe^{3+}. Heating to 90 °C and high pressure up to 600 MPa did not lead to a loss of Fe-binding capacity (Castellani et al. 2004). Nielsen et al. (2000) reported that the addition of ascorbic acid and ascorbic acid 6-palmitate gave rise to an increase in the amount of free iron Fe (II) in egg yolk dispersions, possibly owing to reaction with phosvitin–Fe (III), which subsequently propagated lipid oxidation. The iron-chelating activity of phosvitin has been associated with protection against oxidative damage. Katayama et al. (2006) reported that oligophosphopeptides from hen egg

yolk phosvitin have novel anti-oxidative activity against oxidative stress in intestinal epithelial cells and that both phosphorus and peptide structure have key roles in the activity. The protective effects of phosphopeptide structure against H_2O_2-induced oxidative stress were almost the same as that of glutathione, and egg phosvitin phosphopeptide with high content of phosphorus exhibited higher protective activity than those without phosphorous. Yet, phosphoserine did not show any significant anti-oxidative stress activity.

Novel hen egg phosvitin phosphopeptide with molecular masses of 1–3 kDa was prepared; phosvitin phosphopeptide with 35 % retention was effective for enhancing calcium-binding capacity and inhibiting the formation of insoluble calcium phosphate. Jiang and Mine (2001) reported that 1–3 kDa fragments of these peptides derived from partially dephosphorylated phosvitin by tryptic digestion showed a higher ability than did commercial casein phosphopeptides to solubilize calcium in a calcium phosphate precipitate, while Feng and Mine (2006) reported that phosvitin phosphopeptide from partially dephosphorylated phosvitin increased iron uptake in a Caco-2 cell monolayer model. Choi et al. (2004) demonstrated high Ca solubilization in the presence of phosvitin or its tryptic peptides when incubated under conditions simulated those of the ileum, while Choi et al. (2005) found that phosvitin peptides improved bioavailability of Ca and thus increased incorporation of Ca into the bones of rats.

Choi et al. (2004) reported that phosvitin and its peptides exhibited antibacterial and DNA leakage effects against *E. coli* under thermal stress at 50 °C and suggested that phosvitin peptides disrupt the bacterial cells by chelating with metals in the outer cell membranes. Antibacterial activity was dramatically reduced by treatment with α-chymotrypsin, although the chelating effect remained.

Chemical Composition of Egg Products

Egg products can be defined as processed forms of chicken eggs for commercial, foodservice, and customers. Egg products include refrigerated liquid, frozen yolk, and specialty egg components. Most egg products can be added with desirable flavors, enhanced with nutrients and functional properties. Convenience foods such as cake and pudding mixes, pasta, ice cream, mayonnaise, candies, and bakery goods are based on egg products. Egg products are widely used by commercial bakers, food processing manufacturers, and the foodservice industry due to their convenience, minimal storage requirements, ease of portion control, product safety, long-term stability, and nutritional uniformity.

Liquid Egg Products

Shell eggs from chicken farms are collected and delivered to the egg-breaking plant. Before breaking, eggs are washed in warm water, spray-rinsed with a sanitizing agent, and dried with cool air. Eggs are broken by automatic breaking

machine. The egg yolk and white are separated, if necessary, and the liquid eggs can be shipped in a container refrigerated at 4 °C to bakeries or other outlets for immediate use or to other plants for further processing.

Liquid egg products allow a consumer to have the nutrition from eggs without the hassle of having to crack some shells. One manufacturer that specializes in liquid egg products includes Burnbrae Farms. Naturegg Omega Plus Liquid Eggs are said to be lower in cholesterol and fat than regular eggs, including more omega-3 polyunsaturates. One hundred milliliters of Naturegg Omega Plus Liquid Eggs contains 40 % of the daily requirement of omega-3 polyunsaturated fatty acids, making them a healthy alternative as a low fat source of protein compared to natural eggs. The product is made with 100 % real egg whites and contains 125 mg of DHA omega-3, 125 mg of EPA omega-3, and 0.5 mg of lutein. While omega-3 polyunsaturated fatty acids have been promoted as being "heart-healthy," lutein is considered an antioxidant that is important for eye health. It also boasts no fat or cholesterol, reinforcing the heart-healthy image the manufacturer is trying to convey. By being a source of vitamin B_{12}, vitamin D, vitamin E, folate, and protein as well as the ability to be frozen for up to 3 months before the expiry date, Burnbrae Farms has produced quite the consumer product. The manufacturer also suggests that Naturegg Omega Plus Liquid Eggs are actually healthier than consuming Omega Plus Shell Eggs. The reason for this is that the Liquid Eggs product contains 90 % of longer chain fatty acids, while the Omega-3 shell eggs only provide 25 %. This would suggest that consuming Naturegg Omega Plus Liquid Eggs is similar to eating a serving of salmon or other cold-water fish.

Frozen Egg Products

Frozen egg products include separated whites and yolks, whole eggs, blends of whole eggs and yolks, or whole eggs and milk and these same blends with sugar, corn syrup, or salt added. Salt and carbohydrates are sometimes added to yolks and whole eggs in order to prevent gelation during freezing. In terms of preservation, frozen egg products should be kept frozen or refrigerated until used. When it is time for use, they should be thawed under refrigeration or under cold running water in unopened containers. After defrosting, they should be refrigerated and used within 3 days.

Specialty Egg White Products

Egg white products are free of fat and cholesterol and even come as a liquid product that can be poured from a container. They can be used as a substitute for eggs and therefore be prepared scrambled or as omelets, egg patties, and quiche, or they could even be used as a protein drink. Indeed, there are products online where egg whites come separated and in containers with pumps. There is a growing trend in the use of egg whites in bodybuilding. Because there is no fat and cholesterol, and

very little in terms of carbohydrates, egg white as a source of protein is viable. Many protein powder shakes have a high amount of fat and other components, whereas egg whites have zero fat and a very small amount of carbohydrates. A benefit of specialty liquid egg white powder would be the average consumer would not have to buy a dozen eggs, crack them, separate them, and throw away waste to get egg whites. Prepackaged egg whites would save time and energy. There are many major commercial producers that produce egg white products. Major nutrient and supplement and health stores both online and in stores also have egg white products. Aside from specialty egg white products, many cooking recipes use egg whites. Egg yolk is popular in cooking, more so than egg whites and often egg whites are leftover. However, if one were to substitute egg whites instead of using a traditional egg, one would decrease calorie intake along with removing the cholesterol and fat intake that would have occurred due to the egg yolk components. Dieting this way, by substituting egg whites for the entire egg, is also possible. There are many uses for egg whites that can be easily found online. Egg white smoothies and egg white salads are common. Allergies to eggs may be due to the egg yolk and not the egg white. It may be possible for people with egg allergies to substitute egg whites into their recipes that call for the use of eggs and not have allergic reaction. Egg white as a meat alternative is also available due to its capability as a protein source. Because the fat of the egg is located in the yolk, a liquid egg white product that is fat and cholesterol free while providing an excellent source of protein appears to be a viable product.

Egg Yolk Products

By being the component that houses most of the fat in an egg, egg yolks have found many uses in culinary pursuits, especially as an emulsifying agent. Egg yolk products allow manufacturers to provide restaurants and bakeries with an easy source of egg yolks without the worry of what to do with the separated egg whites. They can be used in mayonnaise, salad dressing, and cream-style sauces. Bakery products derive their beautiful color from an egg yolk wash, and the lecithin can prevent moisture loss and tenderize a crumb. They have also found a place in ice cream and frozen custard by imparting richness and flavor. These applications of egg yolk, mainly egg lecithin (phospholipid), are used as an emulsifier and binder and also as an additive to improve or to hold the freshness of foods and act as a flavor-active ingredient.

Egg lecithin is widely used as a component in body care products and cosmetics. The customer prefers more natural ingredients which egg lecithin represents in an excellent combination with a cell membrane phospholipids and a carrier function for vitamins and pigments. Egg lecithin acts as an emulsifier to form a layer on the scalp and on the hair by enhancing the fluidity of the hairstyle, whereas the egg yolk protein stabilizes the foam. The application range of egg lecithin in cosmetic products includes emulsifier, stabilizer function in creams and lotions, skin nutrient as metabolic active and membrane-forming lipid, maintenance in moisture

regulation of the skin, improvement of skin permeation, protection of hair and skin against deformation by making the skin become smooth, and pigment incorporation in creams, lotions, or sticks for the skin colorization. A hydrogenated egg lecithin can form liposomes that are already used as a carrier of other active ingredients in moisturizers, sunscreens, tanning agents, vitamins, and fragrances.

Egg Specialty Products

There are many specialty egg products in the market. Examples of such include pickled eggs, fermented eggs, balut eggs, and powdered eggs. Pickled eggs come in jars or containers and are cured in vinegar or brine in order for the eggs to be preserved for months. In the past, pickled eggs were common in bars where one would enjoy beers and pickled eggs. The hard-boiled eggs have their shells removed and then are pickled in the pickling solution. Fermented eggs, or century eggs, are cured in a special mixture that covers the outside of the egg for up to months in order to break down flavorless compounds into smaller flavorful ones. It comes from a Chinese background. The yolk becomes a dark green or gray color and has a sulfurous odor, while the egg white becomes brown and has a salty flavor. Balut eggs are embryos that have been developed and then boiled alive so that it can be eaten in the shell. They are also known as 14-day eggs due to the act of allowing the embryo to develop for around 14 days. All of the inside of the egg can be eaten. This product is already available in many major commercial chain stores to be bought and eaten. Powdered egg is common due to its price, nature, and shelf life. Dehydrated egg is useful in that it can be stored without refrigeration and also it is very common in camping purposes. The dehydrated egg can be rehydrated in order for culinary purposes; rehydrated egg can be used to make scrambled eggs and omelets. There are many other kinds of ways eggs can be prepared in restaurants. For example, there are tea eggs, smoked eggs, soy eggs, and many more. As listed above, there are several different kinds of egg products already available to the public, aside from separated forms of egg whites and egg yolks.

Conclusion and Future Directions

Eggs are a most reliable food and encompass all the nutrient elements essential for developing new life of chickens. Hen eggs are considered to be a storage container and safe house for nutrients and growth-promoting factors needed for the continuing life cycle of chickens. In particular, the nature of albumen, egg white, is best simply understood if its main function is thought of as to keep microorganisms away from the egg yolk, where it was shown that most of the essential nutrients and vitamins are. This is apparently achieved by the bactericidal activity, which is the removal of nutrients and the possible inhibition of proteolytic enzymes that are likely produced by microorganisms.

In terms of what is possible in the future, a potential avenue for future projects and research is the fact that albumen successfully resists microorganisms over a long period of time without metabolizing. In contrast with the immune systems of animals, it is a passive system that actively produces new antimicrobial agents whenever they are needed. It is probable that not all of the antimicrobial systems in eggs have been uncovered; this means that albumen remains a useful subject for antimicrobial protein research and undiscovered potential.

An important feature of egg yolk is that it preserves essential nutrients against oxidation and against the enzyme activities of the yolk itself. However it is not understood why this is and it would be of value to know, at the molecular level, details about how this is achieved. Also, it would be of interest to know if the livetins are quite independent in unbroken yolk or are associated with the lipoproteins and thus help to maintain their integrity. Furthermore, egg yolk contains fascinating physiologically and immunologically functional carbohydrates and proteins of which biotechnology industries develop the processing technology and explore new applications in nutraceutical and other fields. For example, chicken egg yolk IgY antibodies are convenient and inexpensive to produce at a large scale. The antibody has been known as a replacement of antibiotic agents to prevent food pathogens and antibiotic-resistant diseases. The IgY antibody can be also considered a safe natural health product, nutraceutical and function food supplements, or veterinary biologics. Another example of egg lipid is omega-3 fatty acids in egg yolks. Omega-3 fatty acids must become incorporated into foods rather than be used solely as dietary supplements. The development of various omega-3-rich foodstuffs would allow increased dietary intakes of the nutrient. Among the foodstuffs, omega-3 fatty acid-enrich egg products may attract health-conscious population. This would necessitate the development of research for the nutritional evaluation of the various egg products and the education of the public.

In the near future, innovative egg products such as ultra-pasteurized liquid egg, free-flowing frozen egg pellets, and modified atmosphere packaging for hard-cooked eggs are expected to become available.

Cross-References

- ▶ Bioactive Substances of Animal Origin
- ▶ Chemical Composition of Fat and Oil Products
- ▶ General Properties of Major Food Components
- ▶ General Properties of Minor Food Components

References

Anton M, Le Denmat M, Gandemer G (2000) Thermostability of hen egg yolk granules: Contribution of native structure of granules. J Food Sci 65(4):581–584.

Aydin R, Pariza MW et al (2001) Olive oil prevents the adverse effects of dietary conjugated linoleic acid on chick hatchability and egg quality. J Nutr 131:800–806

Azari PR, Feeney RE (1958) Resistance of metal complexes of ovotransferrin and transferrin to proteolysis and to thermal denaturation. J Biol Chem 232:293–302
Bjock L, Akesson P et al (1989) Bacterial growth blocked by a synthetic peptide based on the structure of a human proteinase inhibitor. Nature 337:385–386
Bourgeois Adragna O (1994) Valuer nutritionnelle del'oeuf. In: Thapon JL, Bourgeois WG (eds) L'oeuf et les ovoproduits. Laviosier, Paris, pp 6–26
Burley RW, Cook WH (1961) Isolation and composition of avian egg yolk granules and their constituents α- and β-lipovitellins. Can J Biochem Physiol 39:1295–1307
Burley RW, Sleigh RW, Shenstone FS (1984) Lipoproteins from the blood and egg yolk of the hen. Eur J Biochem 142:171–176
Burley RW, Evans AJ, Pearson JA (1993) Molecular aspects of the synthesis and deposition of hen's egg yolk with special reference to low-density lipoprotein. Poult Sci 72(5):850–855
Byrne BM, Van Het Schip AD, Van de Klundert JAM, Arnberg AC, Gruber M, Geert AB (1984) Amino acid sequence of phosvitin derived from the nucleotide sequence of part of the chicken vitellogenin gene. Biochemistry 23:4275–4279
Castellani O, Guérin-Dubiard C, David-Briand E, Anton M (2004) Influence of physicochemical conditions and technological treatments on the iron binding capacity of egg yolk phosvitin. Food Chem 85:569–577
Causeret D, Matringe E, Lorient D (1991) Ionic strength and pH effects on composition and microstructure of yolk granules. J Food Sci 56:1532–1536
Cherian G (2006) Egg biology. In: Hui YH (ed) Handbook of food science, technology, and engineering. CRC Press/Taylor & Francis Group, Boca Raton, pp 153-4–154-5
Cherian G, Sim JS (1991) Effect of feeding full fat flax and canola seeds to laying hens on the fatty acid composition of eggs, embryos and newly hatched chicks. Poult Sci 70:917–922
Cherian G, Sim JS (1993) Omega-3 fatty acid and cholesterol content of newly hatched chicks from alpha linolenic acid enriched eggs. Lipids 27:706–710
Cherian G, Goeger MP et al (2002) Dietary conjugated linoleic acid with fish oil alters yolk n-3 and trans fatty acid content and volatile compounds in raw, cooked, and irradiated eggs. Poult Sci 81:1571–1577
Choi I, Jung C, Seog H, Choi H (2004) Purification of phosvitin from egg yolk and determination of its physicochemical properties. Food Sci Biotechnol 13(4):434–437.
Choi I, Jung C et al (2005) Effectiveness of phosvitin peptides on enhancing bioavailability of calcium and its accumulation in bones. Food Chem 93:577–583
Donovan JW, Mapes CJ (1976) A differential scanning calorimetric study of conversion of ovalbumin to *S*-ovalbumin in eggs. J Sci Food Agric 27:197–204
Donovan JW, Mapes CJ, Davis JG, Garibaldi JA (1975) A differential scanning calorimetric study of the stability of egg white to denaturation. J Sci Food Agric 26:73–83
Dugaiczyk A, Inglis AS, Strike PM, Burley RW, Beattie WG, Chan L (1981) Comparison of the nucleotide sequence of cloned DNA coding for an apolipoprotein (apo-VLDL II) from avian blood and the amino acid sequence of an egg yolk protein (apovitellenin I): equivalence of the two sequences. Gene 14:175–182
Etches RJ (2008) Production of novel proteins in chicken eggs. In: Mine Y (ed) Egg Bioscience and Biotechnology. Wiley, Hoboken, p 293
Feng F, Mine Y (2006) Phosvitin phosphopeptides increase iron uptake in a Caco-2 monolayer model. Int J Food Sci Tech 41:455–458
Grune T, Krämer K et al (2001) Enrichment of eggs with n-3 polyunsaturated fatty acids: effects of vitamin E supplementation. Lipids 36:833–838
Gujral N, Loebenberg R et al (2012) In-vitro and in-vivo binding activity of chicken egg yolk immunoglobulin Y (IgY) against gliadin in food matrix. J Agric Food Chem 60:3166–3172
Hamazume Y, Mega T, Ikenaka T (1984) Characterization of hen egg white and yolk riboflavin binding proteins and amino acid sequence of egg white riboflavin binding protein. J Biochem 95:1633–1644

Hur S-J, Kang G-H et al (2003) Effect of dietary conjugated linoleic acid on lipid characteristics of egg yolk. Asian-Australas J Anim Sci 16:1165–1170
Ibrahim HR (1997) Insights into the structure-function relationships of ovalbumin, ovotransferrin, and lysozyme. In: Yanamoto T, Juneja LR et al (eds) Hen eggs, their basic and applied science. CRC Press, Boca Raton, pp 37–56
Jiang B, Mine Y (2001) Phosphopeptides derived from hen egg yolk phosvitin: effect of molecular size on the calcium-binding properties. Biosci Biotechnol Biochem 65:1187–1190
Juneja LR, Kim M (1997) Egg yolk proteins. In: Yamamoto T, Juneja LR et al (eds) Hen eggs: their basic and applied science. CRC Press, New York, pp 57–71
Kassaify ZG, Li EW et al (2005) Identification of antiadhesive fraction(s) in nonimmunized egg yolk powder: in vitro study. J Agric Food Chem 53:4607–4614
Katayama S, Xu X et al (2006) Antioxidative stress activity of oligophosphopeptides derived from hen egg yolk phosvitin in Caco-2 cells. J Agric Food Chem 54:773–778
Kato I, Schrode J, Kohr WJ, Laskowski M Jr (1987) Chicken ovomucoid: determination of its amino acid sequence, determination of trypsin reactive site, and preparation of all three of its domain. Biochemistry 26:193–201
Kivini H, Järvenpää EP et al (2004) Qualitative and quantitative liquid chromatographic analysis methods for the determination of the effects of feed supplements on hen egg yolk phospholipids. J Agric Food Chem 52:4289–4295
Korant BD, Brzin J et al (1985) Cystatin, a protein inhibitor of cysteine proteases alters viral protein cleavages in infected human cells. Biochem Biophys Res Commun 127:1072–1076
Kovacs-Nolan J, Mine Y (2004) Avian egg antibodies: basic and potential applications. Avian Poult Biol Rev 15:25–46
Li-Chan E, Nakai S (1989) Biochemical basis for the properties of egg white. Crit Rev Poult Biol 2:21–58
Lin LN, Mason AB et al (1994) Calorimetric studies of serum transferring and ovotransferrin. Estimates of domain interactions and study of the kinetic complexities of ferric ion binding. Biochemistry 33:565–581
Lock JL, Board RG (1992) Persistence of contamination of hen's egg albumen in vitro with Salmonella serotypes. Epidemiol Infect 108:389–396
Losso JN, Bogumil R, Nakai S (1993) Comparative studies of phosvitin from chicken and salmon egg yolk. Comp Biochem Physiol 106:919–923
Matsuda T, Watanabe K, Nakamura R (1982) Immunochemical studies on thermal denaturation of ovomucoid. Biochim Biophys Acta 707:121–128
Matsudomi N, Takasaki M, Kobayashi K (1991) Heat-induced aggregation of lysozyme with ovotransferrin. Agric Biol Chem 55(1651):1653
Mattson FH, Grundy SM (1985) Comparison of the effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 26:194–205
Mecham DK, Olcott HS (1949) Phosvitin, the principal phosphoprotein. J Am Chem Soc 71:3670–3679.
Molla A, Matsumura Y et al (1987) Pathogenic capacity of proteases from Serratia marcescens and Pseudomonas aeruginosa and their suppression by chicken egg white ovomacroglobulin. Infect Immun 55:2509–2517
Montgomery R, Wu YC (1963) The carbohydrate of ovomucoid. J Biol Chem 238:3547–3554
Morrison SL, Mohammed MS et al (2002) Sequences in antibody molecules important for receptor-mediated transport into the chicken egg yolk. Mol Immunol 38:619–625
Naber EC, Squires MW (1993) Vitamin profiles of eggs as indicators of nutritional status in the laying hen: diet to egg transfer and commercial flock survey. Poult Sci 72:1046–1053
Nagaoka S, Masaoka M et al (2002) Egg ovomucin attenuates hypercholesterolemia in rats and inhibits cholesterol absorption in Caco-2 cells. Lipids 37:267–272
Nakai S (2000) Molecular modification of egg proteins for functional improvement. In: Sim JS, Nakai S (eds) Egg nutrition and biotechnology. CAB International, Oxon, pp 205–217

Nakamura R, Ishimaru M (1981) Changes in the shape and surface hydrophobicity of ovalbumin during its transformation to *S*-ovalbumin. Agric Biol Chem 45:2775–2780

Nakane S, Tokumura A et al (2001) Hen egg yolk and white contain high amounts of lysophosphatidic acids, growth factor-like lipids: distinct molecular species compositions. Lipids 36:413–419

Nakano T, Ikawa N et al (2001) Extraction of glycosaminoglycans from chicken eggshell. Research notes. Poult Sci 80:681–684

Nielsen JH, Kristiansen GH et al (2000) Ascorbic acid and ascorbic acid 6-palmitate induced oxidation in egg yolk dispersions. J Agric Food Chem 48:1564–1568

Oguro T, Ohaki Y et al (2001) Ultrastructural and immunohistochemical characterization on the effect of ovomucin in tumor angiogenesis. Jpn J Clin Electron Microsc 33:89–99

Premzl A, Puizdar V et al (2001) Invasion of ras-transformed breast epithelial cells depends on the proteolytic activity of cysteine and aspartic proteinases. Biol Chem 382:853–857

Prescott B, Renugopalakrishnam V, Glimcher MJ, Bhusham A, Thomas GJ Jr (1986) A raman spectroscopic study of hen egg yolk phosvitin: structures in solution and in solid state. Biochemistry 2:2792–2798

Renugopalakrishnam V, Horowitz PM, Glimcher MJ (1985) Structural studies of phosvitin in solution and in the solid state. J Biol Chem 260:11406–11413

Romanoff AL, Romanoff AJ (1949) The avian egg, Core historical literature of agriculture: animal science and health. Wiley, New York

Rupa P, Mine Y (2006) Engineered recombinant ovomucoid third domain can modulate allergenic response in Balb/c mice model. Biochem Biophys Res Commun 342:710–717

Schechter Y, Burstein Y et al (1977) Effect of oxidation of methionine residues in chicken ovoinhibitor in its inhibitory activities against trypsin, chymotrypsin and elastase. Biochemistry 16:992

Schmidt G, Bessman MJ, Hickey MD, Thannhauser SJ (1956) The concentration of some constituents of egg yolk in its soluble phase. J Biol Chem 223:1027–1031

Shainkin R, Perlmann GE (1971) Phosvitin, a phosphoglycoprotein: composition and partial structure of carbohydrate moiety. Arch Biochem Biophys 145:693–700

Shimizu Y, Arai K et al (2001) Dietary fish oil for hens affects the fatty acid composition of egg yolk phospholipids and gives a valuable food with an ideal balance of n-6 and n-3 essential fatty acids for human nutrition. J Oleo Sci 50:797–803

Sim JS (1998) Designer eggs and their nutritional and functional significance. World Rev Nutr Diet 83:89–101

Skrtić I, Vitale L (1994) Methionine-preferring broad specificity aminopeptidase from chicken egg-white. Comp Biochem Physiol Biochem Mol Biol 107:471–478

Smith MB (1964) Studies on ovalbumin: denaturation by heat and the heterogeneity of ovalbumin. Aust J Biol Sci 17:261–270

Sugino H, Nitoda T et al (1997) General chemical composition of hen eggs. In: Yammamoti T, Juneja LR et al (eds) Hen eggs: their basic and applied science. CRC Press, Boca Raton, pp 13–24

Suguro N, Horiike S et al (2000) Bioavailability and commercial use of eggshell calcium, membrane proteins and yolk lecithin products. In: Sim S, Nakai N et al (eds) Egg nutrition and biotechnology. CAB International, Oxon, pp 219–232

Szymczyk B, Pisulewski PM (2005) Effect of conjugated linoleic acid and vitamin E on fatty acid composition and cholesterol content of hen egg yolks. J Anim Feed Sci 14:109–123

Tanizaki H, Tanaka H et al (1997) Activation of macrophages by sulfated glyco-peptides in ovomucin, yolk membrane and chalazae in chicken eggs. Biosci Biotechnol Biochem 61:1883–1889

Travis J, Pike R et al (1997) Porphyromonas gingivalis proteinases as virulence factors in the development of periodontitis. J Periodontal Res 32:120–125

Tsuge Y, Shimoyamada M et al (1996) Differences in hemagglutination inhibition activity against bovine rotavirus and hen Newcastle disease virus based on the subunits in hen egg white ovomucin. Biosci Biotechnol Biochem 60:1503–1504

Tung MA, Richards JF et al (1970) Rheology of fresh, aged and gamma-irradiated egg white. J Food Sci 35:872–874
Valenti P, Visca P et al (1986) Interaction between lactoferrin and ovotransferrin and Candida cells. FEMS Microbiol Lett 33:271–275
Van Het Schip FD, Samallo J, Broos J, Ophuis J, Mojet M, Gruber M, Geert AB (1987) Nucleotide sequence of a chicken vitellogenin gene and derived amino acid sequence of the encoded yolk precursor protein. J Mol Biol 196:245–260
Verdot L, Lalmanach G et al (1996) Cystatins up-regulate nitric oxide release from interferon-gamma-activated mouse peritoneal macrophages. J Biol Chem 271:28077–28081
Vrzhesinskaia OA, Filimonova IV et al (2005) Influence of chitosan feeding of laying hens on egg vitamin and cholesterol content. Vopr Pitan 74:28–31
Wallace RA, Morgan JP (1986) Isolation of phosvitin: retention of small molecular weight species and staining characteristics on electrophoretic gels. Anal Biochem 157:256–261
Wang SY, Williams DL (1980) Identification, purification and characterization of two distinct avian vitellogenins. Biochemistry 19:1557–1563
Wang SY, Smith DE, Williams DL (1983) Purification of avian vitellogenin III: comparison with vitellogenins I and II. Biochemistry 22:6206–6212
Watanabe K, Matsuda T, Sato Y (1981) The secondary structure of ovomucoid and its domain as studied by circular dichroism. Biochim Biophys Acta 667:242–250
Watkins BA, Feng AK et al (2003) Conjugated linoleic acids alter the fatty acid composition and physical properties of egg yolk and albumen. J Agric Food Chem 51:6870–6876
Yamamoto Y, Omori M (1994) Antioxidative activity of egg yolk lipoproteins. Biosci Biotechnol Biochem 58:1711–1713