Study of Chicken Egg Qualities Related to Different Protein Level Dietaries and Various Types Of Layers

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 6, 2012

Key words: egg, nutrition, foaming ability,
electrophoresis, protein, poultry

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Abstract

To determine if strain (brown and white egg shell color type) or diet (16% crude protein, 15% crude protein and 14% crude protein) would affect the component portions of egg, protein composition of albumen, and whether the composition or the treatment changes would affect the foaming quality of the albumen, albumen samples were measured in the present study. Electrophoresis of egg albumen, whole egg and component weight, total solids, fats and protein content of albumen, total solids, fats and protein content of albumen of yolk, albumen whipping height, angel food cake volume and cake texture analysis were measured and analyzed. If the consumer preference is a large and firm cake, eggs from any of all three brown shell type and the 16% crude protein white treatments should be used. However, if the consumer preference is a large and soft cake, eggs from the 15% white treatment should be used.

Acknowledgments

I would like to express sincere appreciation to my graduate committee, Drs. David Roland and Tung-shi Huang from Auburn University, Dr. Hong Lin from Ocean University of China, with special thanks going to Dr. Pat Curtis. I would also like to thank the staff of Auburn University Egg Quality lab- Lesli Blair Kerth, Stacy Wright, Braden Cook and Jessica Butler- for their help and kind advice.

I would like to thank Drs. Clinton Rowe, Zhanjiang Liu and Sang-jin Suh for their aid and support throughout the protein study. To Auburn University Poultry Farm staff, Laura Bauermeister, Mary Bryant, Marcia Kloepper and Ashley Sears, thank you for all the friendly help.

I would like to extend many thanks to my family and friends. To all my American families, Yuan, Yun, Christina, Nan, Suihan, Ziyan and Fan, Jeremy and Joshua, for all the understanding and love, which help me go though all my dark days, guide me to the light. To my father, mother and grandparents, I love you all and thank you for the opportunities you created in my life.

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REVIEW OF LITERATURE

Production and maintenance of egg quality can be considered in 12 aspects, according to the USDA Egg Grading Manual. They are breeding, hatching, brooding and raising, housing, feeding, care of eggs on the farm, egg-processing facilities, building and premises, washing, grading and packing operations and equipment, cooling facilities, health and cleanliness of employees and distribution (USDA, 2000).

In the present study, two types of eggs were separated by shell color from 14 strains (seven brown shell egg layer strains and seven white shell egg layer strains) and three dietary treatments (16% crude protein level, 15% crude protein level and 14% crude protein level) were used. The differences in egg quality between shell types under different diet treatments condition will be discussed in the following chapters.

Structure and Composition of the Egg

The average weight of table eggs is about 62.1 grams (Bell, 2001), which includes the rough weights of shell, yolk and albumen. Yolk constitutes approximately 27.5% of the total weight of the egg, while the albumen constitutes about 63% of the total egg weight. The shell makes up about 9.5% of the total egg weight (Cotterill and Geiger, 1977). These three component parts of the egg are separated from each other by membranes. The shell is separated from the albumen by the shell membranes, and the yolk is separated from the albumen by the vitelline membrane (Bellairs, et al., 1963).

Yolk (Ovum)

The yolk is developed in the chicken ovary, which is a part of the reproductive system of the hen (Roth and Porter, 1964). Unlike most female animals, the right ovaries of hens are not functional, only the left ovaries and oviducts are used for reproduction. However, the left ovary contains approximately 3,600 to 4,000 tiny ova, which will develop within its own sac into yolk (USDA, 2000).

The yolk first slowly develops by protein and lipids of yolk fluid; the pigments (fat soluble) then are transferred from the digestive tract to the yolk through blood stream. As the yolk matures, the germ stays at the surface of the yolk when enough fluid is added. This action leaves latebra extending to the center of the yolk, which is tube-like (USDA, 2000).

The release of a mature yolk is controlled by the stimulating hormone of the pituitary gland in the ovary (Nalbandov, 1966). Development of yolk is usually enclosed in a yolk sac and is released from a blood vessel free area (stigma). However, if the yolk is ruptured and released in any place other than stigma, there is a high chance that blood spots will occur on the yolk or cause the white to be bloody (Jeffrey, 1945; Nalbandov and Card, 1944). Several factors can cause blood spots, such as age, gene, and stress. One possible cause of blood spots is a *Campylobacter jejuni* infection, since this bacteria can result in the breakage of the blood vessels (Neill, et al., 1985).

There is a small white spot, which is the germinal disk, about 2 mm in diameter on the surface of the yolk. If the egg is infertile, the germinal disk contains the genetic material only from the hen. However, if the egg is fertile, the germinal disk contains genetic material from both parents. Yolk material supplies nutrients to the embryonic development when the fertilized egg starts to develop (Klein, et al., 2002).

Proteins and lipids are the main components of yolk. Triglycerides make up 65.5% of fatty substances in the yolk, while 28.3 % of the fatty substance is phospholipid and the rest (5.2%) is cholesterol (Privett, et al., 1962). The pH of the yolk in a freshly laid egg is about 6.0 and then generally increases to 7.0 during storage (USDA, 2000). The yolk contains iron, phosphorus, sulphur, copper, potassium, sodium, magnesium, calcium, chlorine, and manganese, all of which are essential elements to human health (Stadelman and Cotterill, 1995).

The yolk contains most of the known vitamins with the exception of vitamin C. The vitelline membrane is similar to the shell membranes and is fairly permeable to water (USDA, 2000). During the storage, the water from the albumen may penetrate into the yolk. The excess of water can cause a size increase of the yolk and a less viscous texture of the yolk due to the lower concentration of the solids in the yolk (Jacob, et al., 2000; Karoui, et al., 2006; Obi and Igbokwe, 2009).

Albumen

The albumen is produced by the oviduct. There are four types of albumen (USDA, 2000). The outer thin albumen is the narrow fluid layer closest to the shell membrane, which comprises 23.2% of the total albumen (Stadelman and Cotterill, 1995). The viscous or thick layer of albumen is the gel that forms the center of the albumen. The gel acts as a pocket to hold the inner thin albumen and yolk, and accounts for 57.3 % of the albumen (Stadelman and Cotterill, 1995; USDA, 2000). The inner thin albumen is the fluid layer located next to the inner thick layer, which is surrounded by thick layer. The inner thick albumen (chalaziferous layer) is a dense, matted, fibrous capsule of albumen, which includes the chalazas and makes up 2.7% of the total albumen.

The inner thick albumen is also the layer most close to the vitelline membrane of the yolk (Stadelman and Cotterill, 1995; USDA, 2000).

The pH of albumen from a fresh laid egg is between 7 and 8.5, and will rise during storage due to the loss of carbon dioxide (USDA, 2000). Ovomucin, ovalbumin, conalbumin, ovoglobulin, lysozyme, and ovomucoid are the main proteins in the thick albumen. The thin albumen is made up of the same proteins in thick albumen except the ovomucin, which acts as the structure material in the thick albumen (USDA, 2000).

Shell

The egg shell is composed of three layers; they are mammillary layer, the spongy layer and cuticle layer (Baker and Balch, 1962). Pores perforate the eggshell. The pores are like funnel tubes, they connect some of the space between the mammilla and the surface, and help the transferring of gases, water and small molecules (Paganelli, 1980; Spotila, et al., 1981).

Calcium carbonate is the main component of eggshell. It composes 94% of the weight of dry shell. A laying hen may use as much as 47% of her skeletal calcium for the formation of eggshells. Vitamin D involves in absorption and bone deposition, while the excess of phosphate and magnesium decreases absorption of calcium. In the meantime, the ratio between calcium and phosphorus should be 2:1, since their metabolisms interact with each other during adsorption and bone deposition (USDA, 2000).

Shell Membranes

The eggshell has both inner and outer membranes. The main functions of these membranes are retaining the albumen and preventing bacteria infection (Nakano, et al., 2003). The shell

membranes are made of tough and fibrous lace-like nitrogenous compounds. The inner membrane is slightly thinner than the outer membrane, but the difference is only about 0.00609 mm (USDA, 2000).

Quality Factors

There are various quality factors affecting the shell, yolk, and white condition. Quality factors of eggs can be divided into two general groups: Exterior and Interior (USDA, 2000).

Exterior quality factors are identified by direct external observation, which should be the first point of evaluation. Interior quality factors involve the contents of the egg as they appear in front of a candling light, or when the egg is broken out and measured by the Haugh unit method plus visual examination of the yolk. Interior quality should be considered the second point of evaluation (USDA, 2000).

The most basic level of egg grading is examination of the egg by "candling" to determine the condition of the egg. Candling is done by placing the egg in the path of a bright light, which reveals conditions such as excessive debris on the shell, cracks, leakers, blood spots or mold growth within the shell. The interior egg quality is judged based on the apparent condition of the interior contents of the egg as it is turned in front of the candling light. During candling, the operator rejects eggs that do not meet standards based on their observations (Stadelman and Cotterill, 1995). It is also suggested to break out an egg occasionally. By determining the Haugh unit value and comparing the broken-out and candled appearance, the correlating between candling and broken-out appearance can be made (Stadelman and Cotterill, 1995).

Although the shell color does not affect the quality of the egg and is not a factor in the United States standards and grades, eggs are usually sorted by shell color and sold as either "whites" or

"browns" (USDA, 2000). From a scientific point of view, there's no difference between white and brown shell eggs (Bell, et al., 2001). However, the interior quality of brown eggs is more difficult to classify because the color of the shell makes it more difficult for light to pass through during candling. There is also a significantly higher incidence of meat spots found in brown shell eggs than in white shell eggs, due to the genetic selection of white shell layers during past 60 years (Jeffrey and Walker, 1950; USDA, 2000).

External Quality Factors

The external factors like egg shape, texture, cleanliness can be determined without using the candling light (USDA, 2000). However, the soundness, which means wholesomeness, of the shell should be verified by candling (De Ketelaere, et al., 2004; USDA, 2000).

Shell Shape. A "normal" chicken egg is oval in shape with one end larger than another, and the smaller end is more cone-shaped than the larger end (USDA, 2000). The unusual eggs are long and narrow, round, or flat-sided and do not fit well into cartons (Jacob, et al., 2000; Stadelman and Cotterill, 1995). There is more of a chance for the misshaped eggs to be broken during transportation than the normal shaped ones.

Shell Texture. An eggshell that is smooth is preferred for both retail and processing markets. The unusual egg may have ridges, rough areas, or thin spots. Rough-shelled eggs fracture more easily, have poor appearance and more risk of contamination (Musgrove, et al., 2009). Those abnormal shells may have several causes, like disease, improper nutrition, or the physical condition of the hen (USDA, 2000). Some eggs have a rough pimply appearance. The pimples

are distortions to the shell due to the calcium deposits (Jacob, et al., 2000). Infection is not responsible for the pimples, since they can also occur in disease-free flocks and may be partly hereditary (Jacob, et al., 2000).

Shell Soundness. The shell of an egg may be sound, checked/cracked, or leaking. Sound means the whole eggshell is unbroken. Check/crack means the egg that has a broken shell or crack in the shell but its shell membranes are intact and the contents are not leaking. A leaking egg has a crack or break in the shell and shell membranes to the extent that the egg contents are exposed or are exuding or free to exude through the shell (USDA, 2000).

A checked egg is an unavoidable problem in the marketing of eggs due to the breakage during assembling, grading, packing, transporting and processing (Bain, et al., 2006; USDA, 2000). Another reason to have a standard grade for eggs is to limit the loss by sorting them by size, shape and soundness (USDA, 2000).

"Body checks" are eggs with shells that have been cracked during shell calcification before the egg is laid and have had a layer of calcium deposited over the cracked area in the hen (Roland, 1984). If the layer of calcium is thick, it is hard to detect unless the egg is candled. If the body checks are only covered by a thin layer before being laid, they are easily detected (USDA, 2000). The incidence of body checks will increase if hens are excited in the afternoon or early evening just as the egg shell begins to form in the oviduct (Roland, 1984; USDA, 2000). It is important, therefore, to keep hens as calm as possible during shell formation (Jacob, et al., 2000).

Practically normal. A practically normal shell approximates the usual shape and is sound and free from thin spots. Ridges and rough areas that do not materially affect the shape and strength of the shell are permitted in AA or A quality eggs (USDA, 2000).

Abnormal. A shell that may be somewhat unusual or decidedly misshapen or faulty in soundness or strength is considered abnormal (USDA, 2000). Abnormal eggs usually occur when the hens are sick, aged or stressed (Appleby and Hughes, 1991; Hughes and Black, 1976). Blood spots, meat spots, soft-shell and thin shell are the main types of abnormal eggs.

Blood spots are mostly caused by a rupture of one or more small blood vessels during the ovulation. The detection of small blood spots can be difficult during candling due to the cloudiness of albumen of newly laid eggs (Jeffrey, 1945; Sherwood, 1958; USDA, 2000). Some spots have been demonstrated to be blood spots changed color in chemical reaction (Jeffrey, 1945). However, most of the meat spots are from sources other than blood spots. The tissue sloughed off from the reproductive organs of the layer can also cause meat spots (USDA, 2000; Vaclavik and Christian, 2008). Leghorn (white shell egg type) strains vary in the number of eggs they lay with blood spot (Lucas, 1946). Brown eggs will usually show a higher incidence of blood and meat spots than white eggs (Jacob, et al., 2000; USDA, 2000).

Soft-shelled eggs usually occur when an egg is laid prematurely. The insufficient time in the uterus interrupts the deposit of the shell (USDA, 2000).

Thin-shelled eggs may be caused by either dietary deficiencies, such as calcium, phosphorus, magnesium, copper, zinc, manganese, Vitamin A and Vitamin D, or disease (Clunies, et al., 1992; Roberts, 2004; USDA, 2000).

Shell Cleanliness. Most eggs are clean when they are laid, but they can become contaminated with manure or other foreign material. In industrial processing, the examination for the cleanliness is most often done immediately following the washing operation, during or after the mass scanning for interior quality prior to packaging (USDA, 2000).

If the shell is free from readily visible foreign material and from stains or discolorations, the egg is clean. An egg may be considered clean if it has only very small specks, stains, or if cage marks are not of sufficient number or intensity to detract from the generally clean appearance of the egg. Eggs that show traces of processing oil on the shell are considered clean unless otherwise soiled. An egg should be considered dirty, when the shell is unbroken and has dirt or foreign material adhering to its surface, has prominent stains, or has moderate stains covering more than 1/32 of the shell surface if localized, or 1/16 of the shell surface if scattered (USDA, 2000).

In the United States, an egg with manure or adhering material on the shell cannot be marketed, since it has a higher risk on contamination by salmonella and E. coli. It is classified as dirty and cannot be used for human consumption. Eggs with stained shells are unattractive in appearance and cause eggs to be downgraded to B quality or dirty depending on the severity of the stain (Jacob, et al., 2000; USDA, 2000).

Interior Quality Factors

Interior egg quality is based on air cell size, albumen quality, yolk quality, and the presence of blood or meat spots (USDA, 2000). The eggs are either examined in front of candling light, or broken out for visual evaluation.

Air Cell Size. Further increase in the size of the air cell is related to the storage temperature and time, due to the evaporation of the water from the egg. However, shell texture and humidity will also influence the air cell size (USDA, 2000).

Albumen Quality. The albumen has a major influence on overall interior egg quality. Thinning of the albumen is a sign of quality loss (De Ketelaere, et al., 2004; Honkatukia, et al., 2005). When a fresh egg is carefully broken out onto a smooth flat surface, the round yolk is in a central position surrounded by thick albumen (De Ketelaere, et al., 2004). When a stale egg is broken out, the yolk is flattened and often displaced to one side and the surrounding thick albumen has become thinner, resulting in a large area of albumen collapsed and flattened to produce a wide arc of liquid (Honkatukia, et al., 2005; USDA, 2000).

During the last decades, several types of sensors were developed and have been used to replace human candling (De Ketelaere, et al., 2004). There are generally three classes of sensors. The first class is developed based on mechanical techniques, which allows investigation of the physical shell quality, such as the presence of cracks and shell strength. The second class is based upon the principles of spectroscopy. This type of sensor allows the operator to “see” through the eggshell in order to determine the internal quality of the eggs. It can be used to detect albumen pH and viscosity, and the presence of inclusions such as blood and meat spots. The third class of sensors aims at mimicking the activity of a human eye by means of a camera and a software platform (“computer vision”). Besides these types of sensors, some others based on ultrasonic, magnetic resonance and electronic nose principles have been investigated (De Ketelaere, et al., 2004).

Standards for Quality of Shell Eggs

United States Standards for Quality of Individual Shell Eggs is applicable only to eggs that are the product of the domesticated chicken hen and are in the shell (USDA, 2000). The eggs are classified into AA quality, A quality, B quality, dirty and check. Detail standards are sorted in the following table:

Table 1. United States Standards for Quality of Individual Shell Eggs

Egg Class	Standards
AA Quality	The shell must be clean, unbroken, and practically normal. The air cell must not exceed 1/8 inch (3.2 mm) in depth, may show unlimited movement, and may be free or bubbly. The albumen must be clear and firm so that the yolk is only slightly defined when the egg is twirled before the candling light. The yolk must be practically free from apparent defects.
A Quality	The shell must be clean, unbroken, and practically normal. The air cell must not exceed 3/16 inch (4.8 mm) in depth, may show unlimited movement, and may be free or bubbly. The albumen must be clear and at least reasonably firm so that the yolk outline is only fairly well defined when the egg is twirled before the candling light. The yolk must be practically free from apparent defects.
B Quality	The shell must be unbroken, may be abnormal, and may have slightly stained areas. Moderately stained areas are permitted if they do not cover more than 1/32 inch (0.8 mm) of the shell surface if localized, or 1/16 inch (1.6 mm) of the shell surface if scattered. Eggs having shells with prominent stains or adhering dirt are not permitted. The air cell may be over 3/16 inch (4.8 mm) in depth, may show unlimited movement, and may be free or bubbly. The albumen may be weak and watery so that the yolk outline is plainly visible when the egg is twirled before the candling light. The yolk may appear dark, enlarged, and flattened, and may show clearly visible germ development but no

	blood due to such development. It may show other serious defects that do not render the egg inedible. Small blood spots or meat spots (aggregating not more than 1/8 inch (3.2 mm) in diameter) may be present.
Dirty	An individual egg that has an unbroken shell with adhering dirt or foreign material, prominent stains, or moderate stains covering more than 1/32 inch (0.8 mm) of the shell surface if localized, or 1/16 inch (1.6 mm) of the shell surface if scattered.
Check	An individual egg that has a broken shell or a crack in the shell, but its shell membranes intact and its contents do not leak.

*From USDA, 2000.

United States Standards for Quality of Individual Shell Eggs are also classified on weight basis. In America, shell eggs are generally sold by dozen in retail chains, unlike in Asian and European markets. Eggs are sorted into narrow weight range groups, and the high majority of the eggs must meet the minimum single egg requirements. In the meantime, a minimum carton or case requirement needs to be achieved as well. United States Consumer Grades and U.S. Nest-Run Grade on Weight Classes for Shell Eggs are the main standards followed by the industry (Stadelman and Cotterill, 1995) as shown in Table 2 and 3.

Table 2. United States Consumer Grades for Shell Eggs

Weight Class	Minimum net weight per dozen	Minimum net weight on lot basis 30-dozen	Minimum weight for individual eggs at rate per

	(ounces/gram)	(pound/Kg)	dozen (ounce/gram)
Jumbo	30 / 850.5	56 / 25.4	29 / 822.1
Extra La	27 / 765.4	50.5 / 22.9	26 / 737.1
Large	24 / 680.4	45 / 20.4	23 / 652.0
Medium	21 / 595.3	39.5 / 17.9	20 / 567.0
Small	18 / 510.3	34 / 15.4	17 / 481.9
Peewee	15 / 425.2	28 / 12.7	--

*From USDA, 2000.

United States Consumer Grades for Shell Eggs apply to all consumer grades, however, large and above grades are more common in the retail market. A lot basis average tolerance of 3.3% weight difference for individual eggs in the next lower weight class is permitted as long as no individual case within the lot exceeds 5% (USDA, 2000).

Table 3. Weight Classes for U.S. Nest Run Grade for Shell Eggs

Weight Class	Minimum average net weight on lot basis 30-dozen (pound/Kg)
Class XL	51 / 23.1
Class 1	48 / 21.8
Class 2	45 / 20.4
Class 3	42 / 19.1
Class 4	39 / 17.7

*From USDA, 2000.

The weight classes for the U.S. Nest-Run Grade for Shell Eggs apply to Nest-Run Grade (Table 3). No individual sample case may vary more than 2 pounds (plus or minus) from the lot average

net weight (USDA, 2000). Although United States Consumer Grades for Shell Eggs apply to a wider range of eggs, the Nest-Run Grade focuses more on the retail sizes. The majority of the eggs under weight classes for the U.S. Nest-Run Grade for Shell Eggs are under the classes between Jumbo to Medium.

Nutrition of Feedstuff

The most important issues in relation to the feedstuffs for the poultry industry are price and availability of the ingredients, which are also issues in aquaculture and cattle industries (Agudu, 1969; Jacob, et al., 2000; Waldroup, 2004). Use of lower-priced feedstuffs that maintains good conditions of growth, feather renewal, egg production and the production of the meat is the major method used to lower the production cost. The major feed ingredients include cereal grain, protein and fat supplement, certain mill by-products and major minerals (Bell, et al., 2002). The nutrient requirements for poultry have been well established due to both the rapid growth of the poultry industry and the fact that the chick is a good experimental animal for nutritional studies (Moore, et al., 1946; Nelson, et al., 1971). So far, 13 vitamins, 13 to 16 inorganic elements, 13 amino acids, and one essential fatty acid have been established for chick nutritional requirements (Pesti, et al., 2005).

Proteins in Albumen and Functions

Proteins in Albumen

Albumen, commonly called egg white, is the nutritive and protective gelatinous substance surrounding the yolk. The majority of albumen is water-soluble. Because albumen proteins posses multiple functional properties, such as enzymes, enzyme inhibitors, foaming agents and gelling functions, they are a desirable ingredient in the food industry, especially for bakery products, meringues and meat products.

Table 4. Some properties of proteins in albumin

Protein	Percentage of total proteins	Denaturation temperature (°C)	Molecular weight (KDa)	Isoelectric point (pH)	Characteristics
Ovalbumin	54[1,2]	84.5[2]	45[1], 44.5[2]	4.5[1,2]	
Conalbumin (Ovotransferrin)	12-13[1], 12[2]	61.5[2]	77.7[1], 76[2]	6.0[1], 6.1[2]	Binds iron and other metal ions[1,2]
Ovomucoid	11[1,2]	70[2]	28[1,2]	4.1[1,2]	Inhibits serine proteinases[1,2]
Lysozyme	3.4-3.5[1], 3.4[2]	75[2]	14.3[1,2]	10.7[1,2]	Lysis of bacterial cell walls[1], N-acetylemuramidase[2]
Ovomucin	1.5-3.5[1], 3.5[2]		220-270000[1], 5500000-8300000[2]	4.5-5.0[1,2]	Interacts with lysozyme[1], inhibits viral hemagglutination[2]
Ovoglobulin G2	1.0[1], 4[2]	92.5[2]	47[1], 30-45[2]	4.9-5.3[1], 5.5[2]	Good foam builders[2]
Ovoglobulin G3	1.0[1], 4[2]		50[1], 30-45[2]	4.8[1], 5.8[2]	Good foam builders[2]
Ovoflavoprotein	0.8[1,2]		32[1,2]	4.0[1,2]	Binds riboflavin[1,2]

Ovomacroglobulin (Ovostatin)	0.5[1,2]	760-900[1,2]	4.5-4.7[1], 4.5[2]	Inhibits serine and cysteine proteinases[2]
Cystatin	0.05[1,2]	12[1], 12.7[2]	5.1[1,2]	Inhibits cysteine proteinases[1,2]
Avidin	0.05[1,2]	68.3[1,2]	10[1], 9.5[2]	Binds biotin[1,2]
Thiamine-binding protein	-	38[1]	-	Binds thiamine[1]
Glutamylaminopeptidase	-	320[1]	4.2[1]	
Minor glycoprotein 1	-	52[1]	5.7[1]	
Minor glycoprotein 2	-	52[1]	5.7[1]	
Ovoglycoprotein	1.0[2]	24[2]	3.9[2]	
Ovoinhibitor	0.1[2]	49[2]	5.1[2]	Proteinases inhibitor[2]

[1] from (Awade, 1996)

[2] from (Belitz, et al., 2009a)

Ovalbumin. Ovalbumin was one of the first proteins purified in the 19th century by Hofmeister. It is also the main albumen protein (54%, Table 4). Ovalbumin is a monomeric phosphoglycoprotein with 3.2% carbohydrates and 0-2 moles of serine-bond phosphoric acid per protein. The complete amino acid sequence of hen ovalbumin with 385 amino acid residues and its crystal structure have been reported (Nisbet, et al., 1981). The phosphoric acid groups are at Ser-68 and Ser-344 (Nisbet, et al., 1981). During storage, they may be replaced by sulfate through thiol-disulfide exchange to form more heat-stable S-ovalbumin (Nakamura and Ishimaru, 1981; Smith and Back, 1962). The carbohydrate moiety is bound to Asn-292 in the sequence (Nisbet, et al., 1981).

Ovalbumin has long been the subject of physical and chemical studies as a convenient protein model, but its function remains largely unknown. The interphase denaturation occurs through unfolding (whipping) and aggregation (heating) of the protein molecule. These coagulation, gelation and foaming properties (Johnson and Zabik, 1981a; Johnson and Zabik, 1981c; Vachier, et al., 1995) make it a widely used ingredient in the food industry.

Traditionally, ovalbumin is purified from albumen by salt precipitation: ammonium sulfate (Sorensen and Hoyrup, 1915) and sodium sulfate (Kekwick and Cannan, 1936) are two commonly used salts for this application. However, ion exchange chromatography has become a more and more popular method for the purification of proteins, although the cost is relatively high.

Conalbumin (Ovotransferrin). Conalbumin constitutes 12% (Table 4) of the albumen protein. It has been identified as the iron-binding protein from hen egg albumen and it is very similar to the serum transferring in animals. Chicken conalbumin is a monomeric glycoprotein containing 686

amino acid residues and has 15 disulfide bridges (Shen, et al., 2010). It binds with metallic ions at pH 6 or above (2 moles Mn^{3+}, Fe^{3+}, $Cu2^{+}$ or Zn^{2+} per mole of protein) and is not denatured at interphase but coagulates at low temperatures (Belitz, et al., 2009b; Tan and Woodworth, 1969; Vachier, et al., 1995; Warner and Weber, 1953).

Conalbumin has one peptide chain and contains one oligosaccharide unit made of four mannose and eight N-acetylglucosamine residues (Shen, et al., 2010). The metal complex occasionally gives pink white discoloration of the egg products during processing if Fe^{3+} is in a conalbumin-iron complex, and is fully dissociated at a pH less than 4 (Evans, et al., 1960). Tyr and His are involved at the binding site in this reaction (Dewan, et al., 1993; Windle, et al., 1963). Its iron-binding ability is partially or completely abolished when 10 to 14 histidine residues with bromoacetate is alkylated; the nitration of tyrosine residues with tetranitromethane also abolishes transferrin iron-binding ability (Belitz, et al., 2009b; Line, et al., 1967). The iron sequestering properties of transferrin is responsible for conalbumin's bacteriostatic and bactericidal properties (Valenti, et al., 1983). Pathogenic organisms such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio cholera* are impacted by conalbumin's bactericidal and bacteriostatic effects (Boesman-Finkelstein and Finkelstein, 1985; Ko, et al., 2009).

Ovomucoid. Chicken ovomucoid was first reported to inhibit general trypsin in 1903 (Delezenne and Pozerski). It was later corrected as highly inhibits bovine, but not human, trypsin (Figarella, et al., 1975). Electrophoresis reveals 2 or 3 forms of this protein, which apparently differ in their sialic acid contents (Melamed, 1967). A large proportion of carbohydrate exists in this glycoprotein (about 25%). The carbohydrate moiety consists of 3 oligosaccharide units bound to protein through Asp residues, and 9 disulfide bonds have been reported (Swint-Kruse and

Robertson, 1996; Yamashita, et al., 1984). This reportedly helps to stabilize the protein against coagulation during heating. It can be heated at 100 ^{o}C under acidic conditions for long periods without any apparent changes in its physical or chemical properties (Gu, et al., 1989; Konishi, et al., 1985).

Chicken ovomucoid inhibits only trypsin, while turkey ovomucoid inhibits both trypsin and chymotrypsin (Feeney, et al., 1963). Ovomucoid may play a more important role in the pathogenesis of allergic reactions to egg albumen than other albumen proteins (Bernhisel-Broadbent, et al., 1994; Urisu, et al., 1997). It is also a water-soluble glycoprotein that is antigenic even in boiled shell eggs (Urisu, et al., 1997).

Lysozyme. Lysozyme is a globular enzyme that is widely distributed in animal and human tissues and secretions (Fange, et al., 1976; Levitt and Chothia, 1976; Mason and Taylor, 1975). It constitutes approximately 3.5% egg albumen (Table 4). Its peptide chain has 129 amino acid residues and 4 disulfide bonds (Ibrahim, et al., 2002). The four disulfide bonds stabilize lysozyme during thermal drying and freeze-drying (Perry and Wetzel, 1987). This protein is an N-acetymuramidase enzyme that hydrolyzes the cell walls of Gram-positive bacteria, and also interacts strongly with outer membrane lipopolysaccharide of Gram-negative bacteria (Bilej, et al., 2001).

Due to its basic character, lysozyme can bind to ovomucin, conalbumin and ovalbumin in egg albumen (Sugino, et al., 1997). It functions with ovomucin on increasing the viscosity of albumen, which has weak proteolytic activity (Fucci, et al., 1983). Lysozyme also is reported as having antiviral and antitumor functions (Sava, et al., 1989). The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives

declared that lysozyme was safe to be used in food in 1992 (Kijowski and Lesnierowski, 1999). Lysozyme only shows antimicrobial activity to a certain range of bacteria and fungi. However, its enzyme activity can be enhanced by substances such as ethylenediaminetetraacetic acid (EDTA), butylparaben, and tripolyphosphate (Johnson, 1994).

Ovoglobulin G2 and G3. Ovoglobulin was considered as one of the six globulin fractions in earlier studies. However, a later study found ovoinhibitor and ovoglobulin G1 should be identified as lysozyme (Sugino, et al., 1997). The globulin proteins have proven to be the main proteins in albumen that impact good foaming ability. They can quickly unfold and spread at the interface between air and liquid, reducing the interfacial tension between the air and water and helping to stabilize the foam (Johnson and Zabik, 1981a; Kinsella, 1981).

Ovomucin. There are two subunits found for ovomucin (alpha and beta). The alpha subunit consists of a peptide chain with 2,087 amino acid residues and is a low-carbohydrate fraction, while the beta subunit has 827 amino acid residues and is a high-carbohydrate fraction (Watanabe, et al., 2004). Ovomucin can form fibrillar structures with lysozyme and impacts the viscosity of albumen during the storage of eggs (Rabouille, et al., 1990; Sugino, et al., 1997). This structure acts to prevent the spread of microorganisms (Ibrahim, 1997). Ovomucin is heat stable (Donovan, et al., 1975; Garibaldi, et al., 1968), and has been reported to inhibit the growth of tumors (Oguro, et al., 2001; Watanabe, et al., 1998).

Ovoflavorprotein. Ovoflavorprotein binds firmly with riboflavin (Vitamin B_2) and acts as a carrier and store of riboflavin in albumen in a 1:1 ratio for chicken embryo (Adiga and

Dakshinamurti, 1994; Sugino, et al., 1997). It contains 14% carbohydrates, which are basically mannose, galactose and glucosamine (Farrell Jr, et al., 1969).

Ovoinhibitor. This protein functions like ovomucoid and is a proteinase inhibitor. As the proteinase inhibitor, it has a wide range of specificities: trypsin, chymotrypsin, elastase, subtilisin and alkaline proteinases (Gertler and Feinstein, 1971; Shechter, et al., 1977).

Avidin. Avidin is a basic glycoprotein, whose biological function is not yet known (Elo, et al., 1980). It is also a homotertramer consisting of four identical subunits, each of them binds one or more biotins (Green and Toms, 1973). It may deprive the biotin essential for bacterial growth, and act bacteriostatically (White III, 1985).

Cystatin. Cystatin is also called Ficin-papain inhibitor (Nicklin and Barrett, 1984). It consists of one peptide with 116 amino acid residues and has a high thermal stability (Sen and Whitaker, 1973). This protein binds tightly to and inhibits a variety of thiol proteases including ficin, papain, and cathepsins B, H, and L (Kondo, et al., 1990). However, it doesn't act on serine proteinases including trypsin, chymotrypsin and microbial enzymes (Hines, et al., 1991).

Ovomacroglobulin. Ovomacroglobulin is also called ovostatin (Nagase, et al., 1986). It can inhibit all four classes of proteinases by forming unique cleavage sites on the peptide stretch. When a proteinase cleaves the bait region, a conformational change is induced in the protein, then the proteinase is trapped (Sottrup-Jensen, et al., 1989).

Ovoglycoproteins. Ovoglycoproteins contain hexoses (13.6%), glucosamine (13.8%) and N-acetylneuraminic acid (3%) (Ketterer, 1965). Their biological functions are still unclear.

Proteins Responsible for Foaming

Foam can be defined as a two-phase system in which the gas (air) is diffused into the protein solution (water) by a blending process where they are trapped by unfolded proteins at the interface and stabilized with associated cohesive films around the air cells (Kinsella, 1981). Several factors can affect the foaming property, which are mainly related to the energy input intensity. Hydrophobicity and viscosity are two of the main factors, since they can determine the energy needed to unfold the proteins and build the new network (Townsend and Nakai, 1983).

Albumen is one of the best foaming agents, mainly due to its high protein content. It also contains a mixture of proteins and is a heterogeneous system. Globulin G2 and G3 can unfold rapidly for air trapping. They are the only protein fractions of egg white with good foaming properties (Raeker and Johnson, 1995). However, it is also believed globulins can help to slow down drainage by the high viscosity produced by the globulin-ovomucoid complex. The large quantity of ovalbumin helps assists foaming. Ovomucoid and ovomucin do not show good foaming properties due to the higher ratios of disulfide bonds in the molecule. The surface basic and acid protein complex also helps with the viscosity but weaker (Lyklema, 2005).

In 1981, Johnson and Zabik (1981a; 1981c) published two articles in the Journal of Food Science, which discussed the protein interaction in angel food cake system. In the same year,

they published two other articles about ultrastructure examination of egg albumen protein foams and gelation properties of albumin proteins (Johnson and Zabik, 1981b; Johnson and Zabik, 1981d). Johnson and Zabik observed some differences in membrane thickness: globulin and ovalbumin film looked very thin; conalbumin film seemed slightly thicker and less flexible while lysozyme film was considerably thicker (Johnson and Zabik, 1981a; Zayas, 1997). Johnson and Zabik (1981a) also believe ovomucin, lysozyme, ovomucoid, and conalbumin had little or no foaming capacity. The combination of ovomucin and globulin is believed to favor the foam formation (whipping), but not the cake volume (Johnson and Zabik, 1981a; Johnson and Zabik, 1981c). Lysozyme in the presence of ovomucin was apparently associated with formation of ovomucin-lysozyme complex, which seemed to alter the viscous nature of ovomucin (Johnson and Zabik, 1981c; Johnson and Zabik, 1981d).

Poole et al (1984) believes that lysozyme, as a basic protein, interacts with acid proteins in egg albumen. The interaction is believed to enhance the foaming properties at the pH value between the isoelectric points. This basic-acid protein complex theory was also observed to help with the foam viscosity but it is weaker than the reaction of sucrose (Lyklema, 2005).

Protein concentration and temperature are also important in the foaming system. The phase angle of the angel food cake batter containing albumen protein continually decreases as temperature is increased which produces a continuous increase in elastic behavior. This is not directly related to the final cake volumes as the phase angle transitions for both egg white concentrations are similar. However, this affects the texture of the cake since a 10% protein content is stable while a 5% protein cake will collapse due to the lack of a network (Pernell, et al., 2002).

Literature review Conclusion

Egg quality can be evaluated by external and internal factors. The shell quality and egg shape are the main focuses of the exterior examination, while the albumen quality and air cell are the main focuses of the interior examination. Three standards were concluded in this literature review for the grading of the shell egg equality. Foaming property and angel food cake as the evaluation of egg quality are mainly controlled by the quality of albumen. However, temperature is also important in the foaming system. The portion of albumen, fat contamination present and protein content are the most important factors influencing the foaming functions. Ovalbumin and ovoglobulin G2, G3 are mainly responsible for foaming. In the angel food cake system, conalbumin and ovomucin are the major proteins stabilizing the foaming structure during the heating procedure.

Evaluation of the Albumen Protein Production responding to the influence of Diet Crude Protein and Strain Treatments

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ABSTRACT

To determine if strain (brown and white egg shell color type) or diet (16% crude protein, 15% crude protein and 14% crude protein) would affect the protein composition of albumen, and whether the composition or the treatment changes would affect the foaming function of the eggs, albumen samples were measured in the present study. Electrophoresis, whole egg and component weight, total solids, fats and protein content of albumen were measured and analyzed. No significant percentage differences were found across the six treatments for all the identified proteins in the albumen. Thus, the protein mass difference in the albumen apparently took responsibility for the difference in foaming quality. Since the albumen production was positively correlated to the whole egg weight ($r^2 = 0.874$), the heavier eggs had higher potential to yield more albumen and have better foaming performance. The brown shell type hens yielded significantly heavier whole egg weight than the white shell type hens ($P < 0.05$). Except the 14 % CP diet, the white shell eggs produced significantly heavier yolks than the brown shell eggs ($P < 0.05$). The brown shell type hens with 16% CP treatment should be used for maximum egg size and albumen production, while the white shell type hens with 14% CP diet should be used to produce more marketable large size eggs.

Key words: egg, albumen, protein, SDS-PAGE, avidin

INTRODUCTION

According to American Egg Board (**AEB**), approximately 30.8% (30.1 thousand metric tons.) of the eggs produced were processed by breaking plants in 2009. The breaking plants break eggs and separate the shell, white (albumen) and yolk. The albumen parts are then widely used in the food industry, especially bakery industry due to their multi functions. Foaming function is one of the most important functions of albumen in the manufacture of bakery products. The average weight of a whole egg is about 62.1 g, which includes the weights of shell, yolk and albumen (Bell, 2001). Yolk constitutes approximately 31% of the total weight of the egg, while the albumen constitutes about 58% of the total egg weight. The shell composes about 11% of the total egg weight. Although most of the proteins in mammals are produced in the liver (Kita and Okumura, 1993), the proteins in egg albumen are mainly produced in the oviduct (Thibodeau, et al., 1978). This difference brings the competition for the amino acids in substrate level for the protein productions. The differences of the turnover time for the protein synthesis also appears to be highly dependent on energy input, protein level and available amino acid composition (Carew and Hill, 1961; Macleod, 1997; Muramatsu, et al., 1986). To determine whether the protein content in the dietary will affect the albumen protein production, and thus affect the foaming function of the albumen, 3 diets and 2 shell color types of hens were used in this study. The 3 diets were designed with different crude protein levels, corn and soybean meal were used as the main protein source and the energy input levels were statistically equal among the diet treatments.

Less than 20 proteins have been identified from the hen egg albumen so far and some of them remain uncharacterized (Desert, et al., 2001). The physical properties of egg albumen proteins,

like the size (molecular weight), charge, isoelectric point, etc. can be used to identify the specific proteins (Smith, 2010). Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (**SDS-PAGE**) followed by Coomassie blue detection is one of the techniques, which have commonly been used to identify the different components in the albumen (Desert, et al., 2001, Ong, et al., 2002). However, western blot was usually applied after the SDS-PAGE for the accurate protein identify and quantity analysis.

For the egg albumen, ovalbumin and ovoglobulin G2, G3 are mainly responsible for foaming. Ovalbumin (egg albumin) helps with the foaming due to the highest quantity among all albumen proteins (Awade, 1996). Globulin G2 and G3 can unfold rapidly for air trapping in a foaming system. They are also the only protein fractions in the egg albumen with good foaming properties (Raeker and Johnson, 1995). In the angel food cake system, conalbumin and ovomucin are the major proteins stabilizing the foaming structure during the heating procedure (Johnson and Zabik, 1981a; Johnson and Zabik, 1981b). However, it is also believed globulins can help slow down the drainage by the high viscosity produced by globulin-ovomucoid complex (Johnson and Zabik, 1981a; Kinsella, 1981). Ovomucoid and ovomucin do not have good foaming properties due to the higher ratios of disulfide bonds in the molecule (Johnson and Zabik, 1981a; Johnson and Zabik, 1981c). Conalbumin has the second largest quantity in the egg albumen, but do not show good foaming property.

Since the foaming functions of the hen eggs related to the albumen proteins were the main focus in the present study, a homogenous egg sample pool of twelve eggs was used to simulate the small bakery production. And only SDS-PAGE was used to identify and analysis the quantities of the albumen proteins, as an attempt to substitute for the SDS-PAGE / Western blot method, for a non-specific but simpler method in this study.

MATERIALS AND METHODS

A total of 2,016 eggs were collected over 4 sampling wks (wk 73, 75, 79 and 80) from 14 strains of hens (7 brown shell type egg strains and 7 white shell type egg strains) from Auburn University Poultry Farm. Hens were fed with 3 different protein level diets: 16% CP, 15% CP and 14% CP (Table 1-1). In each sampling period, whole egg weights were recorded on the collecting day. All the eggs were stored at 4 °C for the duration of the study. Albumens and yolks were separated with an egg separator and the yolks were rolled on a paper towel to remove excess albumen on the next day. Shells were then carefully washed to remove any leftover albumen. Yolk weights were recorded during the breaking procedure and shell weights were recorded after the shells were completely air dried under room temperature. For the chemical analysis, the experimental unit was the pooled sample of yolk or albumen from 12 eggs from the same diet and strain. Each sample pool was packed in a stomacher bag with minimum air and homogenized at 230 rpm for 30 sec (Deckers, et al., 2008). Total solids, fats and protein content of albumen were measured. Samples for protein analysis (SDS-PAGE) were reserved from albumen sample pool in a 1-mL micro-tubes and stored at – 20 °C. The brown shell control eggs were the Eggland's Best® Brown Large Organic Eggs (Jeffersonville, PA) and the white control eggs were the Sunny Meadow® large grade A eggs. All control eggs were purchased two days in advance from Walmart, Auburn, AL. Individual eggs were broken following the same procedure above; only the egg white was collected for analysis. The size of the control sample pool was four large A eggs from the same brand.

Chemical Analyses

Total Solids, Fat and Protein of Albumen Measurement. The CEM Microwave Technology Smart Trac® System in conjunction with the CEM Microwave Phoenix® system (CEM Corp., Matthews, NC) was used to measure the total solids, lipids and protein content. Total solids were calculated as the percentage of whole weight excluding the total moisture content. Approximately 2.0 to 2.5 g albumen from the sample pool was used for the test. Total moisture was calculated automatically by the CEM, based on the weight difference before and after the microwave-drying procedure operated by AOAC Official Method 2008.06 (Leffler, et al., 2008). The dried sample was then placed into the fat analysis tube and compressed to the height of approximate 1 mm before being placed into the Smart Trac® system. Percentage of total fat was calculated using nuclear magnetic resonance signal, following the AOAC official method 2008.06 (Leffler, et al., 2008).

The ash content was measured using the CEM Phoenix Microwave Muffle Furnace (CEM Corp., Matthews, NC). Approximately 5.0 to 6.0 g albumen from the sample pool was heated in the furnace at a constant of 550 °C for 35 min (Marshall, 2010). After cool down, the weight difference was quickly measured to obtain the amount of ash. The results for % total moisture and % total fat were automatically calculated and printed out by the Smart Trac® System, however, the following formulas were needed to calculate the percentage of ash, total solid and protein:

$$\% \text{ Ash} = \text{Ash Weight} / \text{ Initial Weight of the sample}$$

$$\% \text{ Total Solid} = 100\% - \% \text{ Total Moisture}$$

$$\% \text{ Protein} = 100\% - \% \text{ Total Moisture} - \% \text{ Total Fat} - \% \text{ Ash}$$

Protein Extraction. After thawing micro-tubes with reserved samples from sample pools at room temperature (18 ^{o}C) for 15 min, the micro-tubes were vortexed and the 1 mL of albumen samples were placed into VWR® high-performance centrifuge 15-mL tubes (Atlanta, USA). Then 1 mL of 10% trichloroacetic acid (**TCA**, Alfa Aesar®, Ward Hill, MA) was added to each 15-mL tube. The tubes were then vortexed and placed on ice for 30 min for mild precipitation. After the mild precipitation, tubes were centrifuged (BeckMan Coulter Allegra® X-15R, Brea, CA) at 1,500 xg at 4 °C for 15 min, and the supernatants were discarded. The pellet left in the tube then was vortexed with 1mL of diethyl ether (Alfa Aesar®, Ward Hill, MA) and 1mL of ethanol (Alfa Aesar®, Ward Hill, MA). The mixed samples were then centrifuged at 1,500 xg at 4°C for 15 min, and the supernatants were discarded. The precipitates were washed twice with 5 mL standard salt solutions [100 mM KCl, 10 mM KH_2PO_4, 10 mM K_2HPO_4, 2 mM $MgCl_2$, 2 mM EGTA, 1 mM NaN_3 (pH 6.8)] to remove the excess TCA and organic solvent. After the washes, 5 mL standard salt solution was added into each tube for protein concentration measurement (Raikos, et al., 2006).

Sample Preparation for SDS-PAGE. Protein concentrations from the exacted samples were determined from a standard curve using biuret procedure. The standard curve was constructed by plotting the concentrations of known protein (x-axis) versus the spectrophotometer readings (y-axis). The bovine serum albumin (**BSA**) solutions of 0, 1, 2, 3, 4, 5, 7 and 9 g/mL were used and the solutions were measured (Thermo® Eletron Corporation Original Multiskan EX) at 540 nm, due to the detectable color reaction between protein and Biuret reagent. For each sample or standard curve solution, triplicate measurements were used and the mean reading was used for calculating the sample protein concentration. The volume of sample solution needed for 4 mg protein, which was calculated from the calculated protein concentration, was pipetted into the

testing micro-tubes. The testing micro-tubes were centrifuged at 1,500 xg at 4 ℃ for 10 minutes, and the supernatants were discarded. Then 1 mL of SDS-PAGE sample buffer (ROCKLAND® 2X, VWR) without dithiothreitol (**DTT**) or beta-mercaptoethanol (**β-MeOH**) and 50 µL β-MeOH (Fisher-Biotech, Wembley, WA, Australia) were added into the tubes separately. Testing tubes were then vortexed and boiled in a water bath for 10 min, then were cooled to room temperature and stored at -20 ℃. A total of 16 gels and 184 wells load were used for this analysis.

SDS-PAGE. Protein standard (Novex® sharp unstained, Invitrogen) was loaded into the left terminal lane of a 12 wells precast SDS gel (ClearPAGE™ 4-12 % 12W). The rest wells on the precast SDS gel were loaded with 20 µg protein sample (5 µL). SDS reducing buffer (ClearPAGE™) was diluted 20 times, then was added into both the upper and lower chambers for the electronic flow. Power supply of 75 V was used through the stacking gel phase, and then was raised to 150 V through the running gel phase until the dye front migrated to the end of the precast gel. After turned off the power, and gels were carefully removed from the cassette. Immediately, the gels were fixed with 25 % cold TCA for 15 min in plastic containers, then the gels were rinsed twice with distilled water before the use of the rapid Coomassie blue stain (NuSep® coomassie blue stain). After staining with Coomassie blue for overnight, destain solution (20% Methanol, 7% acetic acid and 73% ddH_2O) was applied until the gel background was clear.

Gel Analysis. Gels were scanned using Biospectram® 300 Image System, bands abundances were automatically measured by the software associated with the System. The original volume of band abundances were used for the statistical analysis, but the gel images were corrected using Excel® 14.1.0 (Microsoft®) for better visual measurement (Figure 1-2). In the present study, the

mobility of sample proteins differed from the standard protein marker, which may due to several reasons. Thus, the protein or proteins (if their molecular weights are similar) were mainly identified by their relatively percentages than the calculated molecular weights. The relatively percentages and molecular weight were based on Awade, A. C (1996), Miguel, et al. (2005), Ahlborn, et al. (2006) and Omana and Wu (2009). The protein standard marked the relative migration distances (**rf**) for molecular weights of 3, 5, 10, 15, 20, 30, 40, 50, 60, 80, 110, 160 and 260 kDa, while the identity of the sample protein bands were particularly judged by the molecular weight calculated from the standard marker rf standard curve. The software output of the abundance percentage was the other reference for the identification. An example table of the software output was shown as Figure 1-1. Background values were excluded from the band abundances, and it was crucial to stain the gels overnight to visualize all detectable proteins in the gel. The sum of volumes of all detectable bands, except the front dye, was the approximate total protein contents. The percentage of protein or proteins identified from one band in the loading sample was calculated by the following formula:

$$\%\ of\ identifiable\ proteins\ \text{in}\ band_k = \frac{I-Mass_k * 100}{\sum I-Mass}$$, where I-Mass = percentage from total column mass (background excluded) and K = rank of the band.

The part per thousand (**ppt**) of protein or proteins identified from one band in the sample pool (12 eggs) was calculated by the following formula:

$$ppt\ of\ proteins_k = \frac{\%\ of\ identifiable\ proteins\ \text{in}\ band_k * \%\ protein}{1000}$$

Statistical Analysis

Statistical analyses were performed using JMP 9.0 (SAS Institute Inc., Cary, NC). Data were analyzed by two way ANOVA appropriately for a randomized complete block design with 28 blocks (4 sampling wk x 7 strains in each shell type) in a 2 x 3 factorial arrangement of 2 shell color types of eggs (brown and white) and 3 diet treatments (16% CP, 15% CP and 14% CP). The effects of diet treatments were also analyzed separately by shell type. The 12-egg sample pool was considered as an experimental unit for chemical analysis. The unequal error variability by blocks, unequal error variability by treatments and the block-treatment interaction were examined graphically. Least Squares means were found to be significantly different using student's t-test or Tukey HSD. Bivariate fits of egg weight measurements were analysis using the whole sample population (n = 2016). Capability analysis was used respectively to answer logical questions.

RESULTS AND DISCUSSION

No visible degradations were found by comparing the control sample gel bands to the experimental sample gel bands in the present study. However, the pre-stained molecular weight markers migrated slightly different from predicted, which may due to the presence of differences of the attached dye molecules. The similar migration difference was also observed by Raikos, which provided a reference for this study (Raikos, et al., 2006).

The number of bands slightly differ from gel to gel, however, four bands were clearly identified from all the samples (Figure 1-2). Band 4, 5 and 6 were identified by rf (Ahlborn, et al., 2006; Miguel, et al., 2005 Omana and Wu, 2009). The proteins with the major quantities were conalbumin, ovalbumin and ovoglobulin G2 & G3 in this study, and one uncertain band occasionally showed around the molecular weight 87 to 100 kDa. Since the small sample size for the ovomucin, uncertain band 1, avidin and lysozyme, only the occurrences and mean were reported in Table 1-2. The following transformations were used to normalize the distributions for both the results from band 2 and 4: $Y= Log_{10}$ (band 2) ^ 2 and Y= Arcsine (- Log_{10}(band 4)), where Y = transformed data.

Significant differences were found on ovoglobulin G2 & G3 (band 2) content by diet (Table 1-3). However, the standard errors were greater than the least squares mean. Thus, the significant is questionable and treated as insignificant for the rest of discussion. The percentage for band 4 was also questionable due to the high standard error of means. Band 5 was identified as avidin and other uncertain proteins due to the rf, however, avidin was used to consider as a low abundance protein in albumen unlike showing in Table 1-2.

Avidin is a basic glycoprotein, which biological function is still under discussion. It is also a homotertramer consisting of four identical subunits and may deprive the essential biotin for bacterial growth, and acts bacteriostatically (Laitinen, et al., 2001). It is an extremely stable protein under extreme pH, elevated temperature, chemical denatures and proteolytic enzymes hydrolyze (Green, 1963; Hytönen, et al., 2004). Coupe reasons may cause this statistical increase of avidin. One of the reasons is the increasing portion of soybean meal in the poultry feeding. Streptavidin is found in nitrogen-fixing symbiotic bacteria (Kaneko et al. 2002), which can commonly form root nodules on soybeans (Banfalvi, et al., 1988; Nordlund, et al., 2005). This protein and chicken egg albumen avidin exhibit nearly identical secondary, tertiary and quaternary structures, and in the same manner of respective binding site residues of protein acquire analogous positions and function similar in their natural hosts (Gitlin, et al., 1990; Green, 1990; Hendrickson, et al., 1989; Pugliese, et al., 1993; Weber, et al., 1989). The avidin-like protein may enter the chick with the soybean meal in the diet. Since the structure of this protein is extremely stable under the similar conditions of chicken digestion system, it may eventually enter the bloodstream. After slight modification, it may be present in egg albumen. The injury or the condition of the poultry farm may also stimulate the production of avidin, since it acts bacteriostatically and functions to protect the embryo. Carter (1968) also thinks the avidin may be a necessary component for the transport or storage of biotin.

The percentages of the foaming responsible proteins (ovomucin, conalbumin, ovalbumin and maybe ovoglobulin G2, G3) in albumen were not significantly affected by the diet or the shell type factor in this study. Thus, heavier albumen with more protein mass rinsed to the next key to improve the albumen quality and foaming function. To study the correlation and regression between the egg component weights and albumen weight, the bivariate fits were tested for all

egg samples (n = 2016) and for the white shell type (n = 1008) and brown shell type (n = 1008) separately. After removing the outliners due to the recording and operating mistake, the slopes for the white shell type and the brown shell type were not significantly different within the predict interval. Thus, only the figures for the all egg samples were listed (Figure 1-3a and 1-3b). The regressions between egg components and the whole egg weight were analyzed, since the whole egg weight measurement was the most practical measurement. The albumen weight was significantly positively correlated with the whole egg weight ($r^2 = 0.874$); however, there were no evidences for other component correlations (Figure 1-3a and 1-3b). Thus, for a better foaming function, heavier eggs are needed.

The dietary CP level and shell type interaction significantly influenced the whole egg and yolk production (Table 1-4, Figure 1-4a and 1-4b). The shell type responded differently when the diet protein content dropped from 15% CP to 14% CP, the white shell type had a significantly sharper response than the brown shell type on the whole egg and yolk production. The white shell eggs had significantly greater yolk percentage, but less shell and albumen percentage than the brown shell eggs ($P < 0.01$, Table 1-5). The brown shell eggs had significantly heavier albumen and shell, but lower yolk / albumen ratio than the white shell eggs ($P < 0.01$, Table 1-5). Scott (2000) and Bean (2003) observed similar results on the weight changes with the nutrition input difference. However, Bean (2003) found the brown shell egg hens produced heavier yolk than the white shell egg hens. In fact, Fletcher et al (1983) found the increasing flock age could also cause the increasing yolk yield in grams. He also found the weight gain of albumen and shell was positively dependent on the whole egg weight, but also negatively related to the flock age. This may apply to this present study, since the hens were over 60 weeks age. The difference could also be caused by strain difference.

The higher CP level diet yielded heavier albumen portion significantly ($P < 0.01$, Table 1-5). However, the percentage of albumen in the whole egg weight didn't increase as quickly as the actual albumen weight gain since the 15% CP and 14% CP did not significantly differ the means for the percentage of albumen but the albumen weight. The percentage of shell weight decreased with higher CP level when the actual shell weight increased is also noticeable from Table 1-5. As seen in Figure 1-3a, the confidence coefficient of shell weight is low comparing to the other two egg components. The whole egg weight could increase faster than the shell production, which caused the decrease of percentage of shell weight as the whole egg weight increase (Figure 1-3b). The lower percentage of shell weight may cause problem during the egg transportation, since the thinner shell eggs may crack. Thus, the grades of eggs in this study were studied.

Almost all of the whole shell eggs weights sized large or above (greater than 56.7 gram/egg, 94.6% for brown and 91.1% for white) in this study. However, the white 14% CP treatment had a major downgrade according to the percentage in the grade of median or below (Table 1-6, Figure 1-4a). When the diet protein level reached to 16%, both the brown or white shell type hens tend to produce more extra large instead of large eggs (Table 1-6). The white 16 % CP and the brown 14 % CP treatments had similar amount of large eggs.

The diet factor affected differently on the albumen weight, shell weight, percentage of albumen and percentage of shell weight significantly within the different shell type ($P < 0.05$, Table 1-7). Only the white shell type eggs were influenced on shell weight by the diet treatment. Since the diet calcium contents were balanced among the treatments, this can be explained by the shell type difference (Table 1-5) and the smaller eggs produced (Table 1-6). On the other hand, although the diet treatment significantly affected the percentage of albumen generally ($P < 0.01$, Table 1-5), only the brown shell type was significantly influenced ($P = 0.037$). The brown and

white shell types also showed different patterns on the shell percentage and albumen weight gain (Table 1-7). As discussed for the shell weights, the egg size increase and strain difference can be the cause. The high CP diet promoted both the albumen and yolk production, but more related to the albumen production (Table 1-7 and Figure 1-3).

No treatment interaction was found for the all the albumen chemical analysis measurements. The brown shell eggs had significantly heavier albumen solids and more albumen protein than the white shell eggs ($P < 0.01$). The high CP diet contributed the heaviest total solids, fats and protein content significantly ($P < 0.01$, Table 1-8). However, only the white shell type reacted on all the measurement as main effect. The brown shell type only significantly responded on the total solids ($P < 0.05$, Table 1-8). The white shell type of hens was more sensitive than the brown shell type of hens to the dietary protein level change. However, the brown shell type hens yielded constantly heavier shell egg, albumen portion and total solids than the white shell type hens fed the same CP treatment. Thus, the brown shell type with 16% CP treatment should be used for maximum egg size and albumen production, although the white shell type with 14% CP diet produced more marketable large size eggs.

Table 1-1. Composition and nutrient content of laying hen diets with different levels of CP

Ingredients	CP (%)		
	16	15	14
Corn	646.00	679.21	711.38
Soybean meal	224.16	194.60	167.76
DL methionine	0.66	0.35	0.06
Limestone	52.40	50.67	50.75
Di-Calcium Phosphate	12.94	16.32	16.44
Salt	3.63	3.63	3.63
AU vitamin premix	2.50	2.50	2.50
AU mineral premix	2.50	2.50	2.50
AU poultry oil	15.24	10.24	5.00
Hard shell	40.00	40.00	40.00
		Nutrients	
Fat (%)	4.218	3.831	3.419
ME (kcal/ kg)	2866.02	2866.02	2867.07
Calcium (%)	4.000	4.000	4.000
Phosphorus, Avail. (%)	0.400	0.400	0.400
Sodium (%)	0.170	0.170	0.170
Lysine-total (%)	0.828	0.747	0.674
Met. + Cys-total (%)	0.621	0.560	0.505
Crude Protein (%)	15.966	14.795	13.746
Lysine-avail. (%)	0.776	0.699	0.630
Tryptophan-avail. (%)	0.183	0.166	0.151
Methionine-avail. (%)	0.367	0.320	0.278
Threonine-avail. (%)	0.615	0.568	0.526
Isoleucine-avail. (%)	0.775	0.709	0.649
Met. + Cys-avail. (%)	0.618	0.554	0.496
Choline-total (mg/ kg)	1171.00	1104.19	1046.30

Table 1-2. Occurrences and means in part per thousand of proteins in the sample pools

	Occurrence				Mean ± SD			
	Ovom[1]	Band[2]	Avidin	Lysozyme	Ovom[1]	Band[2]	Avidin	Lysozyme
Brown 16% CP	1	5	3	2	0.056	0.085 ± 0.021	0.057 ± 0.012	0.065 ± 0.029
Brown 15% CP		3	2	3		0.068 ± 0.033	0.053 ± 0.000	0.035 ± 0.005
Brown 14% CP	1	1	4	2	0.020	0.137	0.068 ± 0.021	0.044 ± 0.010
White 16% CP	1	2	5	3	0.005	0.045 ± 0.006	0.080 ± 0.015	0.093 ± 0.014
White 15% CP		2	4	4		0.057 ± 0.017	0.077 ± 0.012	0.038 ± 0.004
White 14% CP		3	5	3		0.106 ± 0.027	0.093 ± 0.022	0.099 ± 0.007

[1]Ovom = Ovomucin

[2]Band = Uncertain Band 1

Table 1-3. Part per thousand of identified proteins in albumen sample pool (n = 28)

	Brown	White	Pooled SEM	CP (%)			Pooled SEM		Shell Type	Diet	Diet x Shell type
				16	15	14					
Conalbumin	0.303	0.300	0.005	0.303	0.304	0.298	0.007	P-value	NS	NS	NS
Ovoglobulin G2 & G3	0.136	0.138	0.752	0.146[a]	0.134[a]	0.130[b]	0.730		NS	<0.05	NS
Ovalbumin	0.460	0.454	0.008	0.455	0.449	0.466	0.010		NS	NS	NS
Ovomucoid & Ovoglycoprotein	0.137	0.143	0.937	0.141	0.139	0.140	0.920		NS	NS	NS

[a, b]Means within a row and under each main effect with no common superscripts differ significantly ($P < 0.05$).

* NS = not significant

Table 1-4. Effect of shell type and dietary interactions on weights of egg components

	Egg Component Weight (g)				Egg Component (%)			
	Whole	Yolk	Albumen	Shell	Yolk	Albumen	Shell	Yolk: Albumen
Brown 16% CP	66.94[a]	16.82[bc]	44.17	5.95	25.13	65.98	8.89	0.381
Brown 15% CP	65.70[ab]	16.70[cd]	43.03	5.95	25.45	65.47	9.06	0.389
Brown 14% CP	64.54[bc]	16.35[d]	42.27	5.88	25.34	65.48	9.11	0.387
White 16% CP	65.12[bc]	17.29[a]	42.07	5.76	26.58	64.58	8.85	0.414
White 15% CP	64.02[c]	17.16[ab]	41.19	5.66	26.81	64.34	8.84	0.417
White 14% CP	61.09[d]	16.35[d]	39.18	5.54	26.78	64.15	9.79	0.418
Pooled SEM	0.320	0.096	0.268	0.048	0.142	0.147	0.072	0.003
P-value	< 0.01	< 0.05	0.056	0.300	0.9448	0.6440	0.3804	0.8683

[a-d]Means within a column without a common superscript differ significantly ($P < 0.05$)

Table 1-5. Treatment effects on weight of egg components (n = 28)

	Egg Component Weight (g)				Egg Component (%)			
	Whole	Yolk	Albumen	Shell	Yolk	Albumen	Shell	Yolk: Albumen
Brown	65.73[a]	16.62[b]	43.16[a]	5.93[a]	25.30[b]	65.64[a]	9.02[a]	0.386 [b]
White	63.41[b]	16.93[a]	40.82[b]	5.65[b]	26.72[a]	64.36[b]	8.92[b]	0.415 [a]
Pooled SEM	0.185	0.056	0.155	0.021	0.074	0.085	0.042	0.002
Diet CP (%)								
16	66.03[c]	17.05[c]	43.12[c]	5.85[c]	25.85	65.28[c]	8.87[d]	0.396
15	64.86[d]	16.93[c]	42.11[d]	5.80[cd]	26.13	64.90[d]	8.95[cd]	0.403
14	62.81[e]	16.35[d]	40.73[e]	5.71[d]	26.06	64.81[d]	9.09[c]	0.402
Pooled SEM	0.226	0.068	0.189	0.034	0.094	0.103	0.051	0.002
	P-value							
Shell type	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.0835	< 0.01
Diet	< 0.01	< 0.01	< 0.01	< 0.01	0.1294	< 0.01	< 0.01	0.0576

[a, b]Means within a column without a common superscript differ by shell type significantly ($P < 0.05$)

[c-e]Means within a column without a common superscript differ by diet CP significantly ($P < 0.05$)

Table 1-6. Percentage of eggs by the United States Weight Classes for Consumer Grades

	Medium or below ≤ 56.7 g	Large 56.7 – 63.8 g	Extra Large or up ≥ 63.8 g
	%		
Brown 16% CP	2.68	25.89	71.43
Brown 15% CP	5.67	31.94	62.39
Brown 14% CP	7.74	37.20	55.06
White 16% CP	4.46	38.99	56.55
White 15% CP	9.52	47.33	43.15
White 14% CP	12.80	50.30	36.90

Table 1-7. Effect of dietary treatment within the shell type on weight of egg components

	Egg Component Weight (g)				Egg Component (%)			
	Whole	Yolk	Albumen	Shell	Yolk	Albumen	Shell	Yolk: Albumen
Brown 16% CP	66.94[a]	16.82[a]	44.17[a]	5.95	25.12	65.98[a]	8.89[b]	0.381
Brown 15% CP	65.70[b]	16.70[a]	43.03[b]	5.95	25.45	65.47[b]	9.06[a]	0.389
Brown 14% CP	64.54[c]	16.35[b]	42.27[b]	5.88	25.34	65.48[b]	9.11[a]	0.387
Pooled SEM	0.318	0.088	0.257	0.034	0.144	0.164	0.055	0.003
White 16% CP	65.12[d]	17.29[d]	42.07[d]	5.76 [d]	26.78	64.58	8.86[e]	0.411
White 15% CP	64.02[e]	17.16[d]	41.19[e]	5.66 [de]	26.81	64.34	8.85[e]	0.417
White 14% CP	61.09[f]	16.36[e]	39.19[f]	5.54 [e]	26.78	64.15	9.09[d]	0.418
Pooled SEM	0.268	0.091	0.214	0.040	0.121	0.128	0.058	0.003
	P-value							
Diet within the Brown Shell	< 0.01	< 0.01	< 0.01	0.3936	0.2805	< 0.05	< 0.05	0.1690
Diet within the White Shell	< 0.01	< 0.01	< 0.01	0.01	0.3375	0.0733	< 0.01	0.2602

[a-c]Means within a column without a common superscript differ significantly within the brown shell ($P < 0.05$)

[d-f]Means within a column without a common superscript differ significantly within the white shell ($P < 0.05$)

Table 1-8. Means for egg quality measurements

	Total Solids of Albumen (w/w, %)	Total Fats of Albumen (w/w, %)	Protein Content of Albumen (w/w, %)
Brown 16% CP	11.669	0.209	11.014
Brown 15% CP	11.520	0.183	10.912
Brown 14% CP	11.458	0.184	10.884
White 16% CP	11.546	0.207	10.904
White 15% CP	11.324	0.168	10.743
White 14% CP	11.192	0.177	10.540
Pooled SEM	0.069	0.011	0.068
Brown	11.549[a]	0.192	10.937[a]
White	11.354[b]	0.184	10.729[b]
Pooled SEM	0.040	0.007	0.040
16% CP	11.608[c]	0.208[c]	10.959[c]
15% CP	11.422[d]	0.176[d]	10.827[cd]
14% CP	11.325[d]	0.180[d]	10.712[d]
Pooled SEM	0.049	0.008	0.049
White 16% CP	11.546[e]	0.207[e]	10.904[e]
White 15% CP	11.324[f]	0.168[f]	10.743[ef]
White 14% CP	11.192[f]	0.177[ef]	10.591[f]
Pooled SEM	0.063	0.011	0.072
Brown 16% CP	11.669[g]	0.209	10.989
Brown 15% CP	11.505[gh]	0.180	10.897
Brown 14% CP	11.458[h]	0.184	10.884
Pooled SEM	0.056	0.116	0.057

[a, b]Means within a column without a common superscript differ for shell type ($P < 0.01$) ($n = 84$)

[c, d] Means within a column without a common superscript differ for diet ($P < 0.01$) ($n = 56$)

[e, f] Means within a column without a common superscript differ for diet within the white shell eggs: total fat ($P < 0.05$), total solid ($P < 0.01$) and protein content ($P = 0.0118$) ($n = 28$)

[g, h]Means within a column without a common superscript differ for diet within the brown shell eggs ($P < 0.05$) ($n = 28$)

Band ID	MW	Rf	I-Vol	I-Mass	Background Correction
A1	260	0.08	18025330	2.72	65535
A2	160	0.16	13645264	2.06	65535
A3	110	0.28	18405617	2.77	65535
A4	80	0.38	13136015	1.98	65535
A5	60	0.44	30177669	4.55	65535
A6	50	0.5	28867045	4.35	65535
A7	40	0.61	24327443	3.66	65535
A8	30	0.67	53999870	8.13	65535
A9	20	0.73	11479745	1.73	65535
A10	15	0.81	13301602	2	65535
A11	10	0.87	20976742	3.16	65535
A12	3.5	0.94	26662441	4.02	65535
B1	71.23	0.4	106691480	14.63	65535
B2	42.95	0.58	49265898	6.76	65535
B3	29.2	0.67	129200264	17.72	65535
B4	17.51	0.76	22789399	3.13	65535
B5	2.91	0.95	27288103	3.74	65535

Figure 1-1. Typical gel scanning example for SDS-PAGE analysis

*From the left: Band ID = the position on the gel (A-Z is for the sample well, 1-n is for the band from the same sample from top to bottom)

MW = molecular weight calculated from the standard curve basing on rf

Rf = relative migration distance

I-Vol = Total volume (not exclude background correction)

I-Mass = Percentage from total column mass (exclude background correction)

Background correction = Volume of background

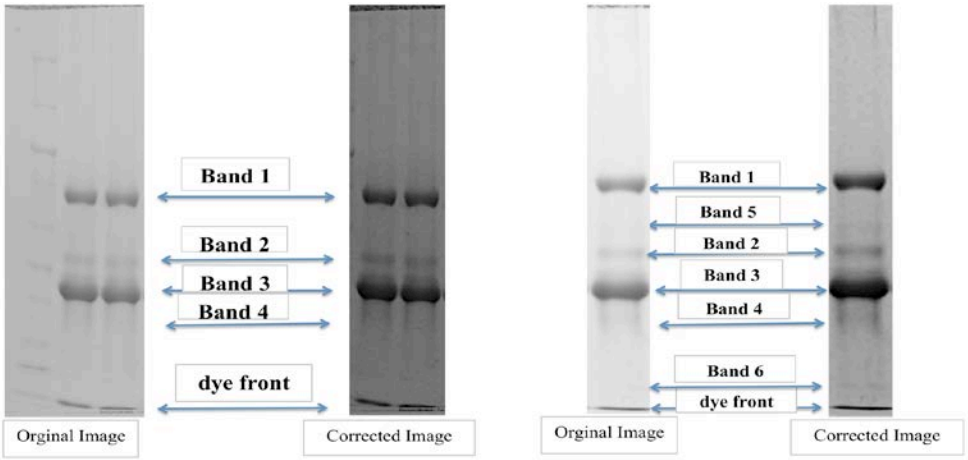

Figure 1-2. Typical SDS-PAGE analysis on hen egg albumen proteins

* Band 1 was identified as conalbumin (ovotransferrin)
* Band 2 was identified as ovoglobulin G2 and ovoglobulin G3 combination.
* Band 3 was identified as ovalbumin.
* Band 4 was identified as ovomucoid and possible ovoglycoproteins combination.
* Band 5 was identified as avidin and other uncertain proteins.
* Band 6 was identified as lysozyme.

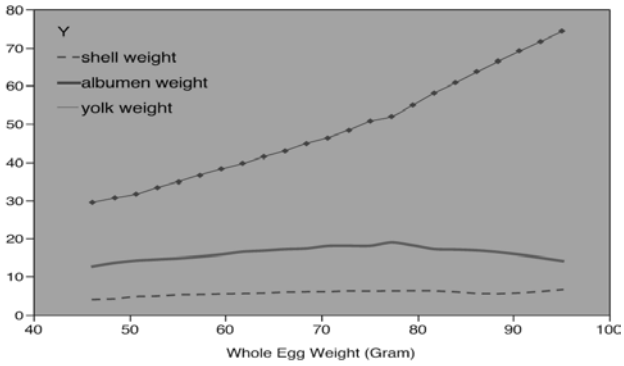

Figure 1-3a. Bivariate fits of shell weight, albumen weight and yolk weight by whole egg weight

*Albumen Weight (g) = -7.617 + 0.768 * Whole Egg Weight (g) (r^2 = 0.874, n = 1994)

*Shell Weight (g) = 2.137 + 0.057 * Whole Egg Weight (g) (r^2 = 0.219, n = 2007)

*Yolk Weight (g) = 5.500 + 0.175 * Whole Egg Weight (g) (r^2 = 0.285, n = 1995)

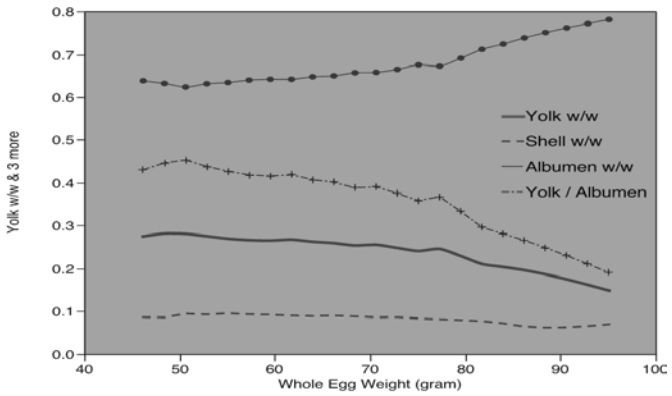

Figure 1-3b. Bivariate fits of % shell, % albumen, % yolk weight and yolk/albumen ratio by whole egg weight

*% Albumen (w/w) = 53.424 + 0.178 * Whole Egg Weight (g) (r^2 = 0.137, n = 1994)

*% Shell (w/w) = 12.240 - 0.050 * Whole Egg Weight (g) (r^2 = 0.084, n = 2007)

*% Yolk (w/w) = 34.367 - 0.129 * Whole Egg Weight (g) (r^2 = 0.084, n = 1995)

*% Yolk/Albumen = 0.601- 0.003 * Whole Egg Weight (g) (r^2 = 0.100, n = 1994)

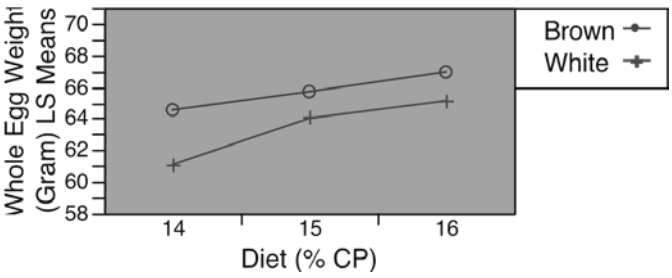

Figure 1-4a. Least square mean plot for interactions on whole egg weight

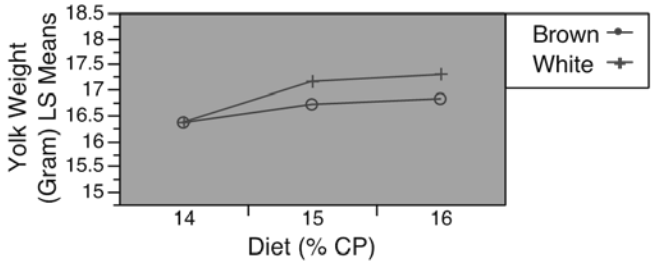

Figure 1-4b. Least square mean plot for interactions on yolk weight

Functionality Evaluation

Study of Albumen Foaming Quality Related to Different Protein Levels

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ABSTRACT

To determine if strain (brown and white egg shell) or diet (16% crude protein, 15% crude protein and 14% crude protein) would affect the chemical compositions of egg yolk and albumen, the foaming quality of albumen and the bakery product quality, albumen samples were measured in the present study. Total solids, fats, protein content and pH of albumen and yolk, whipping height of albumen, volume and texture profile analysis of angel food cake were measured to evaluate the quality of the eggs. No significant treatment interaction was found on all egg quality measurements. The brown shell eggs produced significantly more total solids ($P < 0.01$) and protein content ($P < 0.01$) in the albumen than the white shell eggs. Diet factor significantly influenced the albumen composition ($P < 0.01$) that the 16% crude protein treatment yielded significantly more total fats and solids in the albumen than 15% CP and 14% CP diet treatments. Shell type and dietary treatment interaction significantly affected the angel food cake springiness ($P < 0.05$). The crude protein level in the dietary significantly influenced the angel food cake volume ($P < 0.05$). The theory of density of network was discussed and proved by angel food cake measurement and egg quality measurement. The cake volume was negatively correlated with the cake hardness ($P < 0.01$), while the hardness of the cake was also negatively related to the cohesiveness and resilience of the cake ($P < 0.01$).

Key words: egg, albumen, foaming, protein, angel food cake, texture profile analysis

INTRODUCTION

According to American Egg Board (AEB), approximately 30.8% (30.1 thousand metric tons.) of the eggs produced were processed by breaking plants in 2009. The breaking plants break eggs and separate the shell, white (albumen) and yolk. The albumen parts are then widely used in the food industry, especially bakery industry due to their multi functions. Foaming function is one of the most important functions of albumen proteins in the manufacture of bakery products. In the present study, eggs were manually broken and the compositional component of albumen and yolk were studied. Albumen generally contains 87.57% water, 10.90% protein and 0.17% total lipids; and for the raw egg yolk, the major components are water (52.31%), total lipids (26.54%) and protein (15.86%) (USDA, 2010). Yolk contains most of the fatty material in the egg, and these fat-protein complexes give yolk special functional properties, such as emulsifying, foaming, and coagulating (Kamat, et al., 1973; Paraskevopoulou and Kiosseoglou, 1997; Stang, et al., 1994; Tsutsui, 1988).

Since both the metabolism and reproduction of hens highly depend on feed intake. Feed ingredients' protein content and their protein compositions become the main factors impact the egg protein content and protein composition (Whitehead, 2002). To test the foaming functional differences of eggs from different shell type hens with different protein level dietary, albumen and yolk qualities were evaluated. Albumen quality can be evaluated by measuring foaming abilities as in whipping height, volume of angel food cake, moisture and lipid content and pH measurement as in the present study (USDA, 1984). Yolk quality can be partially evaluated by pH measurement, total moisture, fats and solids.

Texture profile analysis can also be used to evaluate the foaming functionality of the albumen. Foam can be defined as a two-phase system in which the air is diffused into the protein solution

by a blending process. The gas bubbles are trapped by unfolded proteins at the interface and stabilized with associated cohesive films around the air cells (Kinsella, 1981). For the egg albumen, ovalbumin and ovoglobulin G2, G3 are mainly responsible for foaming. Ovalbumin (egg albumin) helps with the foaming due to the highest quantity among all albumen proteins (Awade, 1996). Globulin G2 and G3 can unfold rapidly for air trapping in a foaming system. They are also the only protein fractions in the egg albumen with good foaming properties (Raeker and Johnson, 1995). In the angel food cake system, conalbumin and ovomucin are the major proteins stabilizing the foaming structure during the heating procedure (Johnson and Zabik, 1981a; Johnson and Zabik, 1981b). However, it is also believed globulins can help slow down the drainage by the high viscosity produced by globulin-ovomucoid complex (Johnson and Zabik, 1981a; Kinsella, 1981). Ovomucoid and ovomucin do not have good foaming properties due to the higher ratios of disulfide bonds in the molecule (Johnson and Zabik, 1981a; Johnson and Zabik, 1981c). Conalbumin has the second largest quantity in the egg albumen, but do not show good foaming property. Poole et al (1984) studied the role of protein and protein interaction in the foaming system, especially the role of lysozyme. Poole believes that lysozyme, as a basic protein, could interact with acid proteins in egg albumen. The interaction is believed to enhance the foaming properties at the pH value between the isoelectric points.

According to Pernell et al. (2002), the phase angle of the angel food cake batter containing albumen proteins continually decreases as temperature increasing, this produces a continuous increase in the elastic behavior. Pernell reported that cake with a 10% or higher albumen protein content was stable and had a high final volume, while a cake with 5% albumen protein would collapse and had a low final volume. Optimal protein content for the angel food cake was also discussed as 5 to 10% albumen protein, when the cake reached a high final volume but still had a

soft texture. Thus, cakes with 10% or higher albumen protein contents could reach similar high final volume but harder texture. Or cakes with 10% or higher albumen proteins could have a similar stable network but higher final volume.

The texture profile analysis (TPA) can be used to test this hypothesis. TPA mimics the human bite behavior that compresses the network bonds until they reach their surface extension limits and break permanently (Wilkinson, et al., 2000). It can be used to indicate the toughness and richness of the network, and evaluate the protein functionality in the angel food cake system when the test causes the moisture-balanced object to deform with small force. Hardness, springiness, cohesiveness, gumminess, chewiness and resilience can be measured. Hardness is the peak force in the 1st compression curve. Springiness is the distance recovered by the sample during the time between the end of the 1st compression and the start of the 2nd compression; here the bite means the 4 cm distance. Cohesiveness is the ratio of the positive areas under the 2nd compression to the 1st compression. Gumminess is the product of hardness and cohesiveness, and chewiness is the product of gumminess and springiness, while resilience is the ratio of positive area under 1-2 to 2-3 in the first compression. The red areas in the Figure 1 are the positive areas under 1st compression and 2nd compression. A typical TPA curve obtained for the angel food cake sample was list (Figure 2-1).

MATERIALS AND METHODS

A total of 2,016 eggs were collected over 4 sampling weeks (wk 73, 75, 79 and 80) from 14 strains of hens (7 brown shell type egg strains and 7 white shell type egg strains) from Auburn University Poultry Farm. Hens were fed with 3 different protein levels diet: 16% CP, 15% CP and 14% CP (Table 2-1). In each sampling period, whole egg weights were recorded on the collecting day. All the eggs were stored at 4 °C for the duration of the study. Albumens and yolks were separated with an egg separator and the yolks were rolled on a paper towel to remove excess albumen on the next day. Shells were then carefully washed to remove any leftover albumen. Yolk weights were recorded during the breaking procedure and shell weights were recorded after the shells were completely air dried under room temperature. The albumen weights were calculated by excluding the above two weights from the whole egg weights. For the chemical analysis, the experimental unit was the pooled sample of yolk or albumen from 12 eggs from the same diet and strain. Each sample pool was packed in a stomacher bag with minimum air and homogenized at 230 rpm for 30 sec (Deckers, et al., 2008). Protein analyses samples were reserved from albumen sample pool (>1 mL) in 1-mL micro-tubes and stored at –20 ^{o}C. Total solids, fats and protein content measurements were measured, as well as pH measurement, whipping height, angel food cake volume, angel food cake TPA.

Chemical Analyses

The pH of each sample pool was measured using an Accumet® excel XL 20 pH / Conductivity meter (Fisher Scientific, Pittsburg, PA). The meter was calibrated using solutions of pH 4, pH 7, and pH 10.

Total Solids, Fats and Protein Content Measurement. The CEM Microwave Technology Smart Trac® System in conjunction with the CEM Microwave Phoenix® system (CEM Corp., Matthews, NC) was utilized to measure the total solids, fats and protein content. The total solids were calculated as the percentage of whole weight excluding the total moisture content. Approximately 2.0 - 2.5 g albumen or yolk from sample pool was used for this measurement. The percentage of total moisture was calculated automatically by the CEM, based on the weight difference before and after the microwave-drying procedure operated by AOAC Official Method 2008.06 (Leffler, et al., 2008).

The dried sample was then placed into the fat analysis tube and compressed to the height of approximate 1 cm before being placed into the Smart Trac® system. Percentage of total fats was also calculated automatically by the CEM, using nuclear magnetic resonance signal, following the AOAC official method 2008.06 (Leffler, et al., 2008).

To test the protein content of the sample, the ash content was measured using the CEM Phoenix Microwave Muffle Furnace (CEM Corp., Matthews, NC). Approximately 5.0 - 6.0 g albumen or yolk from sample pool was heated in the furnace at constant 550°C for 35 min (Marshall, 2010). After cool down, the weight difference was quickly measured to obtain the amount of ash. The following formula was used to calculate the percentage of protein:

% Protein Content = 100% - % Total Moisture - % Total Fat - % Ash

Whipping Height. Approximately 200 mL albumen from sample pool was measured by a graduated cylinder and transferred into a 5-quart mixing bowl for the whipping height measurement. Samples were whipped by a Kitchen Aid Artisan Series® (Kitchen Aid, Inc., St. Joseph, MI) mixer using a balloon whisk attachment for 45 s at speed 6, then for an additional 45

s at speed 10. Once foam had formed, both the whisk and the bowl were unlocked from the mixer. The leftover foam on the whisk was shaken back to the bowl, and the foam in the bowl was gently leveled off with a spatula. A clear plastic ruler was then inserted to the bottom of the bowl from the center to measure the level of the foam. The whipping height was recorded in centimeters and three observations were averaged for a sample.

Angel Food Cake Volume. Angel food cakes were prepared using a modification from the procedures from Jones (2007) to accommodate the mixers and oven utilized. Viking® (Viking Corp., Greenwood, MS) ovens were pre-heated to 350°F (181 ^{o}C) for the baking. During the preparing, 180 mL of homogenous liquid albumen was measured and poured in a 5 quart mixing bowl, then 0.9 g of table salt and 2.7 g of McCormic® cream of tartar (Hunt Valley, MD) mixture were added into it. A Kitchen Aid Artisan Series® (Kitchen Aid, Inc., St. Joseph, MI) mixer with a balloon whisk attachment was used for mixing. The albumen and salt / cream of tartar mixture was first whipped for on speed 10 for 45 s, and then 138 g of GreatValue™ (Bentoville, AR) pure cane sugar were added in 3 increasingly larger portions by mixing at speed 6 for 4 s after each addition. Then, 2 mL of GreatValue™ (Bentoville, AR) vanilla was added to the mixture and homogenized for another 4 s at speed 6. Then the bowl was removed from the mixer, a well sifted mixture of 46 g of GreatValue™ pure cane sugar (Bentoville, AR) and 66 g of Swans Down® cake flour (New Orleans, LA) were added to the batter in 3 equal portions. After gingerly folded well by a hand whisk for less than 20 strokes per flour addition, batter was then divided into 3 individual replicate cake pans. These volumes pre-determined pans were placed onto a balance and 75 ± 1 grams of batter weighed into the tarred pans. Then the pans with batters were placed into the pre-heated ovens and baked for 14 min. Baked cakes were then

removed from the oven after cooking and placed upside down on cooling racks to cool down for at least 2 h post bake. Each cake was dusted with flour to prevent seeds from sticking to the cake surface before the rapeseed displacement measurements for the cake volume. Cake volumes were measured by differences between empty pan volumes and the volumes of rapeseed required to fill the pan level up to the top as the formula below:

Cake Volume (mL) = Pre-determined Pan Volume (mL) - Rapeseed Volume (mL)

Angel Food Cake TPA. A Stable Micro System's TA.XT Plus Texture Analyzer (Texture Technologies Corp., Marietta, GA) was used in the present study. The cakes were covered by foil during the overnight storage, and the measurement was finished in less than 12 h after the baking for balanced moisture. After the device was calibrated, in-pan cake was placed under the central force of the texture analyzer. The cakes were compressed twice to 30% of their original height for 0.50 s using a cylindrical probe 2.5 cm in diameter and test speeds for all pre-, actual and posttest were set as 5.00 mm/s. The trigger force is 5.0 g and triggered automatically. Hardness, springiness, cohesiveness, gumminess, chewiness and resilience were measured, and the averages of the 3 replicates were used to statistical analysis.

Statistical Analysis

Statistical analyses were performed using JMP 9.0 (SAS Institute Inc., Cary, NC). Data were analyzed by two way ANOVA appropriately for a randomized complete block design with 28 blocks (4 sampling wk x 7 strains in each shell type) in a 2 x 3 factorial arrangement of 2 shell color types of eggs (brown and white) and 3 diet treatments (16% CP, 15% CP and 14% CP). The effects of diet treatments were also analyzed separately by shell type. The 12-egg sample

pool was considered as an experimental unit. The unequal error variability by blocks, unequal error variability by treatments and the block-treatment interaction were examined graphically. Least squares means were found to be significantly different using student's t-test or Tukey HSD. Capability analysis was used respectively to answer logical questions. Pearson Correlation Coefficients were applied to study the correlations between variables. The distributions of pH measurements were abnormal and failed to transfer. Thus the results from the questionable sampling week (wk. 75) were excluded for the mean calculation. However, the ranges were reported as for the original sample pool, and relative statistical test were used to answer logical questions.

RESULTS AND DISCUSSIONS

No significant treatment interaction was found on all egg quality measurements (Table 2-2). The brown shell eggs had significantly more total solids ($P = 0.0024$) and protein content ($P = 0.0049$) in the albumen than the white shell eggs. Diet factor significantly influenced the albumen composition ($P < 0.01$,Table 2-3) that the 16% CP treatment yielded significantly more total fats and solids in the albumen than the other 2 diet treatments. However, only the white shell type reacted on all the measurement as main effect in Table 2-3. The brown shell type only significantly responded on the total solids ($P < 0.05$). This may indicate the white shell type of hens was more sensitive than the brown shell type of hens to the dietary protein level change.

The pH of albumen ranged from 8.18 to 9.16, which mean was 8.44 with a standard deviation of 0.190. The pH of yolk ranged from 5.69 to 6.57 with a mean at 6.014 (SD = 0.111). The pH ranges for both the albumen and yolk indicated the eggs were fresh at the timing of testing, according to Brake et al. (1997), Hidalgo et al. (1996) and Waimaleongora Ek et al. (2009). This means the foaming properties of the sample pools could reach their best performances if the experiment environment was cooperated (Lomakina and Mikova, 2006). Although both the ranges of the pH of albumen and yolk indicated the freshness, significant unusual greater variations from the sampling wk 75 were also noticeable ($P < 0.01$ by Levene test). To understand whether these greater variations were caused by the system error (pH meter) or other factors, all the albumen quality measurements were separated by sample week to discover the interactions. Significant different variations were found on pH of albumen ($P < 0.01$), pH of yolk ($P < 0.01$), total fats of albumen ($P < 0.01$), protein content of albumen ($P < 0.05$) and albumen whipping height ($P < 0.01$). However, no correlation was found among all the measurements

within each sampling week. Thus, these abnormal variations can be ignored. And for more reliable analysis, the results of pH from the questionable sampling week (wk. 75) were excluded.

The values of the yolk quality measurement in Table 2-2 (total solids ranged from 57.55% to 59.19%, total fats ranged from 38.01% to 39.37 % and protein content ranged from 17.40% to 17.85%) are significantly higher than values in 2010 USDA National Nutrient Database by t-test ($P < 0.01$). This may due to the extra large size eggs and dietary difference in the present study than the sample eggs used for the database. Traditionally, yolk generally contains 47.5% water (w/w) and 16.6% proteins (w/w) (Marion, et al., 1964; Romanoff and Romanoff, 1949). However, the improvements in the industry over the past 60 years had undoubtedly changed egg quality and its composition (Tharrington, et al., 1999).

The histogram and residual plot of pH of albumen indicated the new sample population could yield reliable results (Figure 2-2a, 2-2b and 2-2c). Diet significantly impacted the pH of albumen ($P = 0.0228$, $n = 126$, block = 21); the mean from the 16% CP diet (8.34 ± 0.126) was significantly lower than the values from the 15% and 14% CP diets (8.36 ± 0.126 and 8.40 ± 0.126). A significant influence was found only on the white shell type ($P = 0.0167$), that the hens fed 14% CP diet produced higher pH albumen eggs than the other two diet treatments. Both the shell type eggs had higher pH values when the diet CP level dropped to 14%, however, the white shell type responded more sharply than the brown shell type ($P < 0.01$). As discussed above, unlike the brown shell type, the white shell type hens were fully influenced by the diet treatments on the albumen composition. Thus, the significant decrease on the albumen protein content would be one of the reasons to cause the significant pH change. Instead of the low CP level caused higher pH, the phenomenon can be explained by the heavier load of proteins trap more free OH^- in the solution and significantly lower the pH since the low isoelectric point of most

proteins in the egg albumen (Belitz, et al., 2009a). It might also be caused by the egg size reduction. As discussed in the protein paper, the albumen weight was highly correlated with the whole egg weight ($r^2 = 0.874$). Thus, the low CP level treatment, which significantly yielded smaller eggs, might also yielded lighter albumens with less albumen solutions. Since there was no significant difference on total albumen solids and protein production between 15% CP diet and 14% CP diet, but a significant difference on the whole egg weight ($P < 0.01$), it is reasonable to believe the egg size decrease might indirectly increased the pH also.

The TPA indicated the physical properties of the angel food cakes. As seen in Figure 2-1, the angel food cake had a relatively low breaking strength. The structure of the cake was also relatively flexible under 30% stain, which gave only one peak under the first positive area rather than two peaks. The texture of angel food cake is equally important due to its role on product acceptability. An angel food cake with a firm texture (high hardness) requires more work to break due to the higher breaking strength caused by internal bonding. And if an angel food cake has a high springiness, possesses a higher elasticity and high chewiness, it will become more difficult to eat and not market favorable. However, the hardness of the cake may increase during storage as the water evaporates out of the cake (Baik, O., et al. 2000). To avoid this bias in the present study, the TPA measurement was applied less than 12 hours after the cooking and sealed with foil in the pan. Thus, the moisture transporting would not significantly affect the hardness of the cake and the cool down procedure would yield a solid structure with balanced moisture for the texture analysis.

Cake springiness was significantly influenced by the diet and shell type treatment interaction ($P = 0.0244$, Figure 2-4). Cake springiness relates to the rapidity and degree of recovery from a deforming force (Di Monaco, et al., 2008). The mean springiness ranged from 0.788 to 0.819,

this indicated a rich and flexible network, and a relatively high moisture content to support the flexible hydrogen bonds (Hara, et al., 2003). Thus, the significant lower springiness did not cause significant quality degradation.

The mean of cake volume of the 16% CP diet (365.05 mL) was significantly greater than the mean of the 14% CP diet (352.35 mL), but none of them separated significantly from the mean of 15% CP diet (P = 0.0366). However, this significant increase of cake volume due to the higher CP level was only shown on the brown shell eggs when analyzed by shell type separately (P = 0.0157). Although the increase of cake volume may answer the question raised in the introduction, that cakes with 10% or higher albumen proteins could have a similar stable network but higher final volume. The shell type obviously affected the cake volume performance and by the defining of stable network with a consideration of the consumers' consumption, the measurements of hardness (initial bite feel), springiness (effort needed for second bite, flexibility) and cohesiveness (chewing time) should also be considered.

Diet also affected the cake gumminess and chewiness significantly within the white shell type (P = 0.0196 and P = 0.0123, Figure 2-5b and 2-5c). Since chewiness is a product of gumminess and springiness, and gumminess is a product of cohesiveness and hardness from the 2^{nd} compression, cohesiveness and hardness from the 2^{nd} compression should be discussed as the direct factors. However, the second hardness was not available. Thus, the cake cohesiveness should be considered as the indicator parameter in this study, although the difference was not significant (P = 0.0556). To test whether cohesiveness could be used to represent the data, the mean plots for cake gumminess; chewiness and cohesiveness within the white shell type were compared (Figure 2-5a, 2-5b and 2-5c). The three figures looked identical, and the mean of the 15% CP diet did was significantly lower than at least one of the other 2 diet treatments on the

cake gumminess, chewiness and possibly cohesiveness. Thus, the means of cake cohesiveness was treated as significantly different in this study.

The measurement of cohesiveness indicated the structure lost after the 1st compression (Figure 2-1) as it measured the difficulty of permanently breaking the internal structure (Han, et al., 2005). The means of cake cohesiveness arranged from 0.607 to 0.633, which can be understood as less 40% internal structure was unable to recover after the 1st compression. Thus, high cohesiveness indicates a rich internal network, although this network wasn't just built by the egg proteins. And the lower means of cohesiveness reflected less recovery response, which indicates lack of network and less stable disulfide bonds in the network.

As the protein evaluation article didn't observe a solid significance on the albumen protein composition change, in this case, treatment yielded albumens with more protein should build a stronger and richer angel food cake network. This stronger and richer network should be indicated by high hardness, high springiness, high cohesiveness, high gumminess and high volume respectively. However, no significant cake hardness and albumen protein content difference was observed cross all six treatments; and a significant increase on cake volume with the 16% CP diet within the brown shell type and significant decreases of cake cohesiveness and springiness with the 15% CP of cakes were observed within the white shell type. From Table 2-3, the brown shell type had significantly more total solids and higher protein content in the albumen than the white shell type. And as mentioned earlier, only the white shell type was sensitively influenced by the diet CP level that the albumen protein content from 14% CP diet was significantly lower than the 16% CP diet, but none of them were separated from the 15% CP diet. And for the brown shell type, the total solids of the 16% CP was significantly higher than both the 15% CP and 14% CP diet treatment. Thus, the significant higher protein content in the 16%

CP brown treatment can explain the cake volume differences. Meanwhile, due to the high protein content (Table 2-2), the brown shell type had more stable cake foaming performance than the white shell type. On the other hand, the significant decrease of cake cohesiveness and springiness from the 15% CP white eggs can be explained by the special volume difference. If dividing the albumen protein contents of the treatments by their cake volumes, the protein content per cake unit could be calculated. Thus, the 15% CP white shell eggs produced cakes with similar high volumes of 16% CP white shell eggs (Table 2-4) but less protein content (Table 2-2) should build weaker structure than both the 16% white and 14% CP white treatment cakes as observed in Table 2-4. In addition, the cake volume was negatively correlated with the cake hardness ($P < 0.01$, Table 5), which also proved the special volume explanation.

Based on the above discuss, the albumen protein could be considered the main factor affected the cake foaming performance in this study. The albumen proteins worked as the air holding structure during the volume expansion. When the cake volume got larger, the density of the network got relatively lower, which weakened the hardness of the cake and made it easier to chew on. The hardness of the cake was also negatively related to the cohesiveness and resilience of the cake (Table 5), which proved the above theory about the density of network. This theory can also explain the abnormal decrease of cake chewiness, gumminess and cohesiveness for the 15% CP diet within the white shell eggs as discussed above. From this study, the increase of albumen protein content affected on the cake volume first but not the cake hardness. Similar results was observed by Pernell et al. (2002) as low protein content cake would collapse. However, low-density angel food cake was not market unfavorable, indeed lower chewiness and hardness but high springiness cakes are preferred in some origins. Thus, to find a treatment will

produce eggs with suitable albumen protein content that will produce light texture but high volume cake is needed.

Total fats of albumen were positively related to the cake hardness, and were negatively correlated with the springiness, cohesiveness and resilience of the angel food cake (Table 5). The fats are more competitive on the air and water interface than the albumen proteins and cause the air bubble to burst during the beating and heating. They can increase the surface extension needed to support the structure, creating thicker protein connection. But the lower volume and less air bubbles could reduce the elasticity of cake and cause the low cake volume and poor quality. The fats in the albumen may be leaked from the yolk (Wang and Wang, 2009), caused by the operation error or the age of the hens since older hens may lay eggs with weaker vitelline membranes.

The total solids of albumen and the protein contents of albumen were highly correlated (Table 5). This can be explained by the method of calculating the protein content. The protein content in this study was calculated from subtracting the moisture, fats and ashes weight out off the initial albumen weight. This directly caused the correction between the total solids and protein content. The protein content value was the value of total solids with ashes and fats removed, while weights of ashes and fats from individual eggs are relatively light and similar.

Table 2-1. Composition and nutrient content of laying hen diets with different levels of CP

Ingredients	CP (%)		
	16	15	14
Corn	646.00	679.21	711.38
Soybean meal	224.16	194.60	167.76
DL methionine	0.66	0.35	0.06
Limestone	52.40	50.67	50.75
Di-Calcium Phosphate	12.94	16.32	16.44
Salt	3.63	3.63	3.63
AU vitamin premix	2.50	2.50	2.50
AU mineral premix	2.50	2.50	2.50
AU poultry oil	15.24	10.24	5.00
Hard shell	40.00	40.00	40.00
		Nutrients	
Fat (%)	4.218	3.831	3.419
ME (kcal/ kg)	2866.02	2866.02	2867.07
Calcium (%)	4.000	4.000	4.000
Phosphorus, Avail. (%)	0.400	0.400	0.400
Sodium (%)	0.170	0.170	0.170
Lysine-total (%)	0.828	0.747	0.674
Met. + Cys-total (%)	0.621	0.560	0.505
Crude Protein (%)	15.966	14.795	13.746
Lysine-avail. (%)	0.776	0.699	0.630
Tryptophan-avail. (%)	0.183	0.166	0.151
Methionine-avail. (%)	0.367	0.320	0.278
Threonine-avail. (%)	0.615	0.568	0.526
Isoleucine-avail. (%)	0.775	0.709	0.649
Met. + Cys-avail. (%)	0.618	0.554	0.496
Choline-total (mg/ kg)	1171.00	1104.19	1046.30

Table 2-2. Means for egg quality measurements by the treatment interaction (n = 28)

	Brown CP			White CP			Pooled SEM	P > F
	16%	15%	14%	16%	15%	14%		
Total Solids of Yolk (w/w, %)	58.21	59.19	58.03	57.83	57.85	57.55	0.755	0.7800
Total Fats of Yolk (w/w, %)	38.63	39.37	38.03	38.59	38.13	38.01	0.617	0.5239
Protein Content of Yolk (w/w, %)	17.44	17.63	17.85	17.19	17.70	17.40	0.228	0.5138
Total Solids of Albumen (w/w, %)	11.67	11.52	11.46	11.55	11.32	11.19	0.069	0.5922
Total Fats of Albumen (w/w, %)	0.209	0.183	0.184	0.207	0.168	0.177	0.113	0.8380
Protein Content of Albumen (w/w, %)	11.01	10.91	10.88	10.90	10.74	10.54	0.697	0.2199
Whipping Height (cm)	6.69	6.84	7.11	6.73	7.01	6.95	0.142	0.4937

Table 2-3. Means for egg quality measurements by main factors

	Brown	White	Pooled SEM	P-value	16% CP	15% CP	14% CP	Pooled SEM	P-value
Total Solids of Yolk (w/w, %)	58.47	57.74	0.433	NS	58.02	58,52	57.79	0.534	NS
Total Fats of Yolk (w/w, %)	38.68	38.24	0.3538	NS	38.61	38.75	38.02	0.436	NS
Protein Content of Yolk (w/w, %)	17.64	17.43	0.130	NS	17.32	17.67	17.62	0.160	NS
Total Solids of Albumen (w/w, %)	11.55[a]	11.35[b]	0.040	< 0.01	11.61[c]	11.42[d]	11.33[d]	0.049	< 0.01
Total Fats of Albumen (w/w, %)	0.19	0.18	0.007	NS	0.21[c]	0.18[d]	0.18[d]	0.008	< 0.01
Protein Content of Albumen (w/w, %)	10.94[a]	10.73[b]	0.040	< 0.01	10.96[c]	10.83[cd]	10.71[d]	0.049	< 0.01
Whipping Height (cm)	6.88	6.89	0.081	NS	7.03	6.92	6.71	0.100	0.0718

*n = 84 for shell type and 56 for diet treatment

[a, b] Means without a common superscript in a row differ by shell type factor ($P < 0.05$).

[c. d] Means without a common superscript in a row differ by dietary treatment ($P < 0.05$).

Table 2-4. Means for quality measurements of angel food cakes

	Cake Volume (mL)	Hardness (g)	Gumminess	Chewiness	Springiness	Cohesiveness	Resilience
Brown 16% CP	373.21	543.34	331.21	271.96	0.819[a]	0.612	0.275
Brown 15% CP	359.73	539.32	326.93	267.48	0.815[ab]	0.607	0.272
Brown 14% CP	357.69	523.78	320.36	258.64	0.805[ab]	0.614	0.277
White 16% CP	356.88	551.78	338.71	276.67	0.813[ab]	0.618	0.282
White 15% CP	356.12	510.77	303.33	240.50	0.788[b]	0.611	0.276
White 14% CP	347.01	524.55	333.36	274.01	0.818[a]	0.633	0.302
Pooled SEM	3.830	18.361	9.539	9.043	0.007	0.007	0.008

[a, b] Means within a column without a common superscript differ in a column ($P < 0.05$)

Table 2-5. Correlation coefficients among the measurements

	Total Solids of Albumen	Total Fats of Albumen	Total Solids of Yolk	Total Fats of Yolk	Cake Volume	Hardness	Springiness	Cohesiveness	Resilience
Whipping Height		0.29197 0.0001 168					-0.22769 0.003 168	-0.25058 0.0011 168	-0.31285 <. 0001 168
Protein Content of Albumen	0.92528 <. 0001 167				0.20731 0.0072 167				
Total Solids of Albumen					0.21874 0.0044 168				
Total Fats of Albumen			0.23369 0.0024 166	0.28494 0.0002 166		0.22156 0.0039 168	-0.19681 0.0106 168	-0.19560 0.0111 168	-0.33808 <. 0001 168
Protein Content of Yolk			0.65430 <. 0001 166	0.44886 <. 0001 166	0.16087 0.0384 166				
Total Solids of Yolk				0.96547 <. 0001 166	0.17921 0.0209 166				
Total Fats of Yolk					0.15737 0.0429 166		-0.18129 0.0194 166		
Cake Volume						-0.26422 0.0005 168			
Hardness							0.15583 0.0437 168	-0.45191 <. 0001 168	-0.47455 <. 0001 168
Springiness								0.50884 <. 0001 168	0.51325 <. 0001 168
Cohesiveness									0.89641 <. 0001 168

* Results in each cell from top to bottom are Pearson correlation coefficients, P value and number of observations.

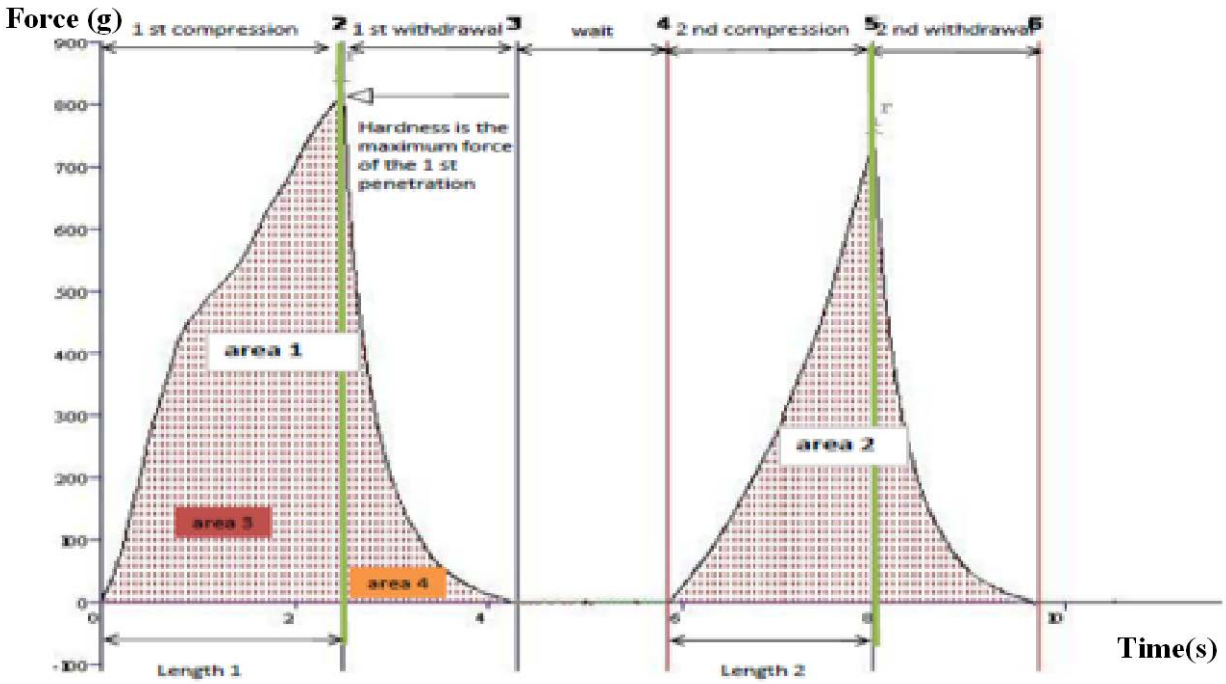

Figure 2-1. Typical TPA force-by-time plot for angel food cake sample

* Springiness= Length 2/ Length 1

* Resilience= Area 4/ Area 3

* Cohesiveness= Area 2/ Area 1

* Gumminess= Cohesiveness * Hardness from 2nd compression

* Chewiness= Gumminess * Springiness

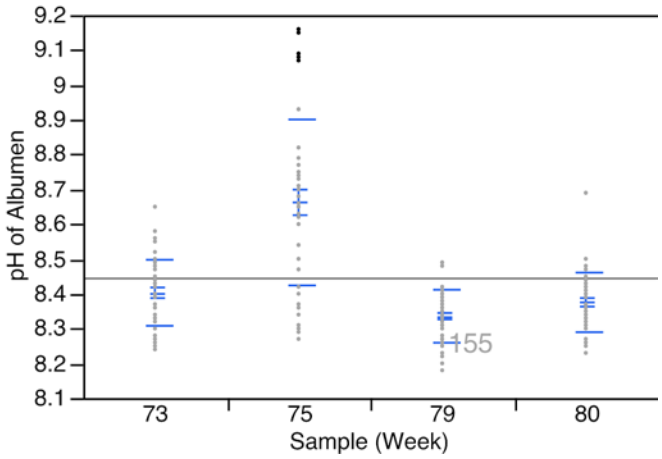

Figure 2-2a. One-way analysis of pH of albumen by sampling week ($P < 0.01$)

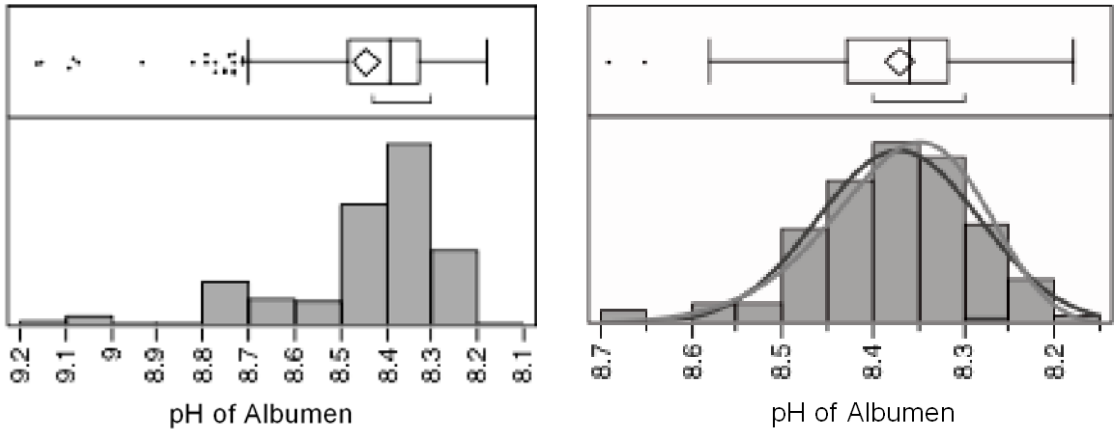

Figure 2-2b and 2-2c. The histograms before and after exclude the data from week 75

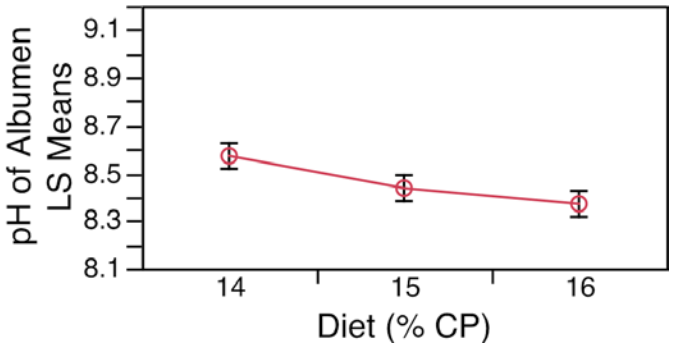

Figure 2-3a. Least squares mean for white shell type by diet treatments on pH of albumen

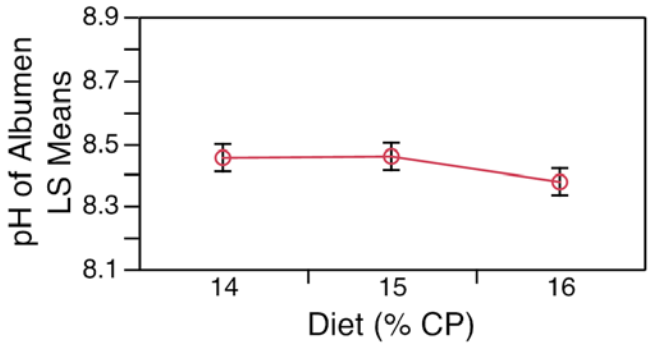

Figure 2-3b. Least squares mean for brown shell type by diet treatments on pH of albumen

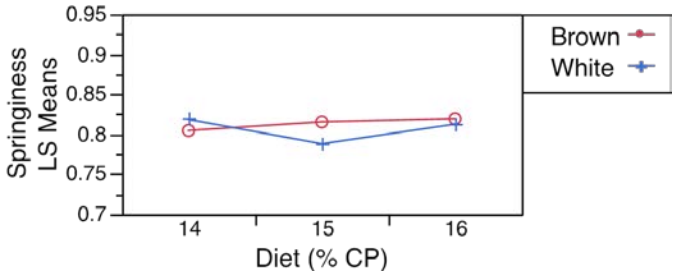

Figure 2-4. Least squares mean for shell type and diet interaction on cake springiness

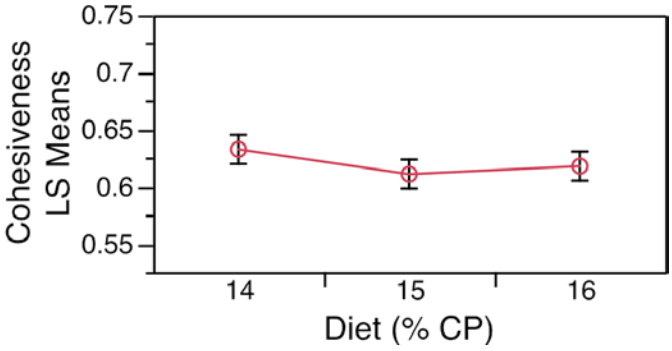

Figure 2-5a. Least squares mean for white shell type by diet treatments on cake cohesiveness

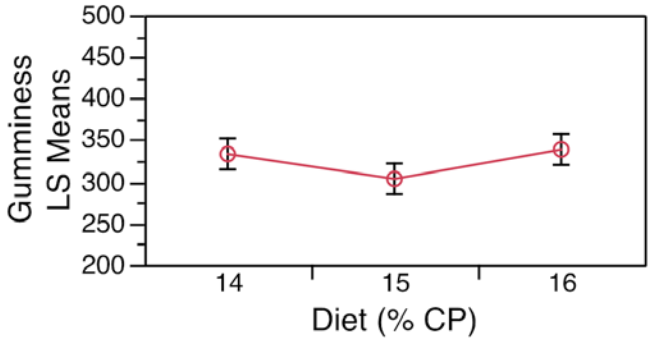

Figure 2-5b. Least squares mean for white shell type by diet treatments on cake gumminess

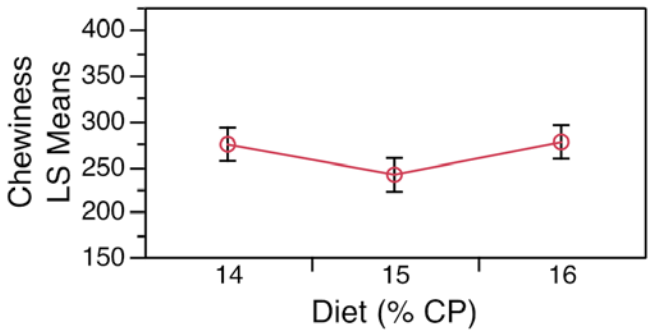

Figure 2-5c. Least squares mean for white shell type by diet treatments on cake chewiness

CONCLUSION

No significant percentage differences were found across the six treatments for all the identified proteins in the albumen and yolk quality measurements. Albumen protein was the main factor influencing the angel food cake foaming performance, while the albumen production was positively correlated to the whole egg weight ($r^2 = 0.874$). No significant interaction was found across the diet and shell type treatments on the angel food cake texture analysis, except the cake springiness. The albumen proteins worked as the air holding structure during the volume expansion. As the cake volume increased, the density of the cake decreased, which weakened the hardness of the cake and made it more tender. The cake volume was negatively correlated to the hardness ($P < 0.01$), while the hardness was negatively related to the cohesiveness ($P < 0.01$) and resilience ($P < 0.01$) of the angel food cake. The brown shell type produced heavier eggs ($P < 0.01$) with higher protein content albumen ($P < 0.01$), thus produced higher volume stable firm angel food cakes. The white shell type was more sensitive to the diet CP change: the 16% CP diet produced the highest volume stable firm angel food cake due to the higher protein content ($P = 0.0118$) and the heaviest whole shell egg ($P < 0.01$); the 15% CP diet produced a similar large but more brittle and loose angel food cake and the 14% CP diet produced the smallest but rich and springy angel food cake. If the consumer preference is a high volume, rich and firm cake, then eggs from any of all three brown shell type and the 16% CP white treatments should be used. However, if the consumer preference is a high volume and soft cake, then the eggs from the 15% white treatment should be used.

Besides the albumen protein content, the fat contamination may also affect the cake quality. The total fats of albumen were positively correlated to the cake hardness, and were negatively

correlated with the springiness, cohesiveness and resilience of the angel food cakes. Thus, the fat contamination from the yolk leaking, older hens and operation errors could cause dramatically decrease of egg foaming performance.

If the eggs were produced for direct egg consumption, the eggs from both the shell type fed a lower CP diet had higher pH values would have a longer the storage time. The brown shell type hens had significantly greater responses on the whole egg, albumen and shell weight gain than the white shell type hens to the increasing protein levels ($P < 0.05$). Except the 14 % CP diet, the white shell eggs produced significantly heavier yolks than the brown shell eggs with the same diet treatment ($P < 0.05$). The brown shell type with 16% CP treatment should be used for maximum egg size and albumen production, although the extra large size may cause transportation issues. The white shell type with high CP level also had the transportation issues, but mainly due to the thinner shells than the brown shell type. The white shell type with 14% CP diet produced more marketable large size eggs, but had a major downgrade on all the egg components measurement and albumen quality measurements.

Percentages of identified avidin in the albumen samples were found higher in this study than the past reports. This may be caused by the more widely use of soybean meal in the current poultry feeding, hen injury or metabolism requirement.

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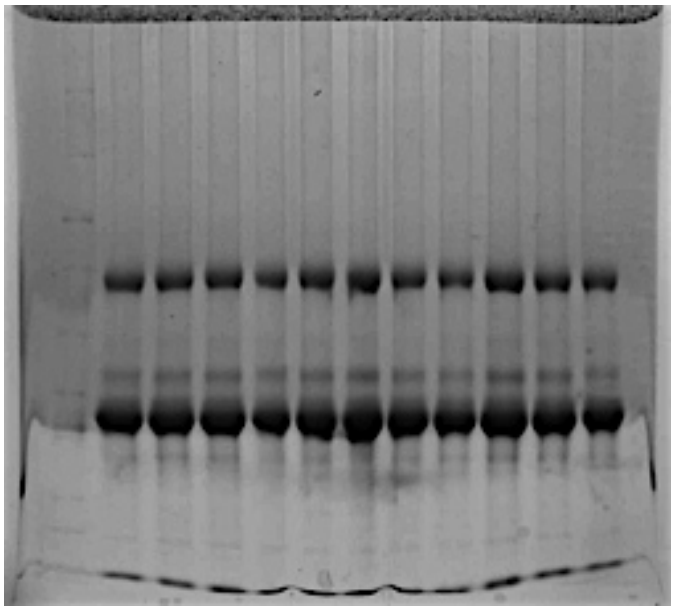
GEL SCAN FROM PROTEIN ANALYSIS



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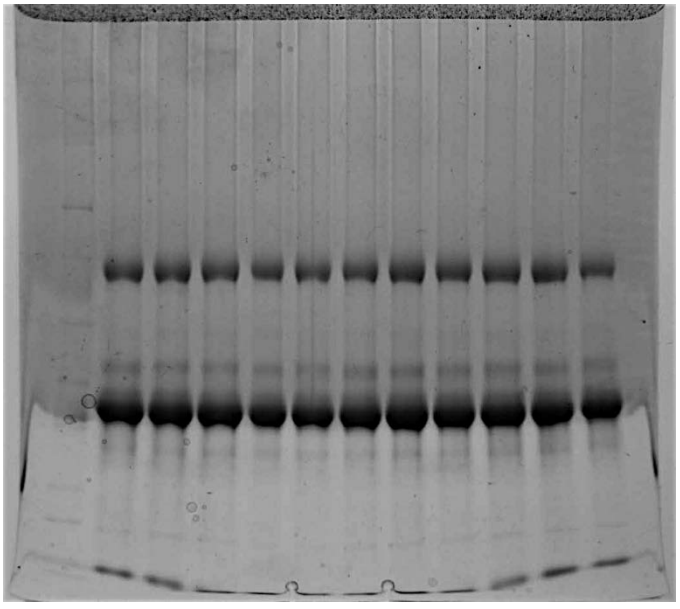

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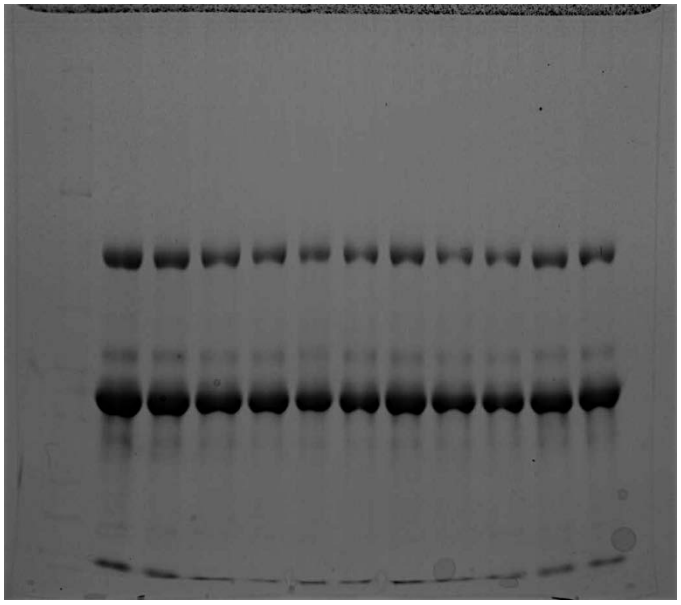

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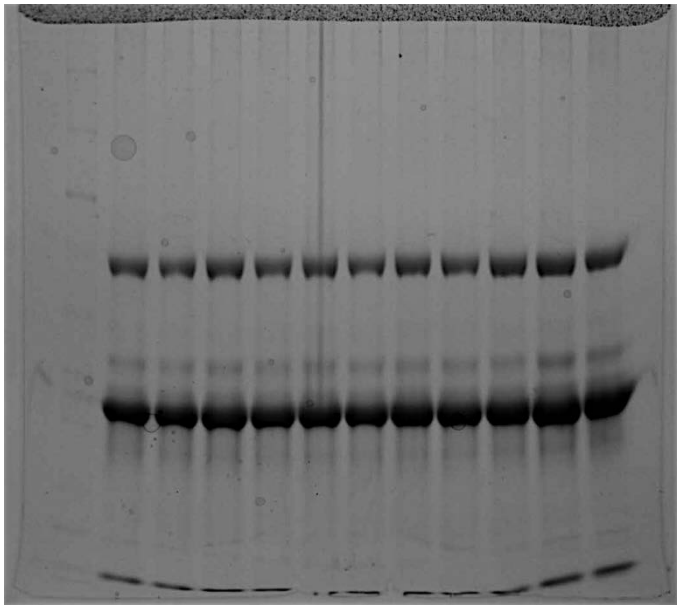

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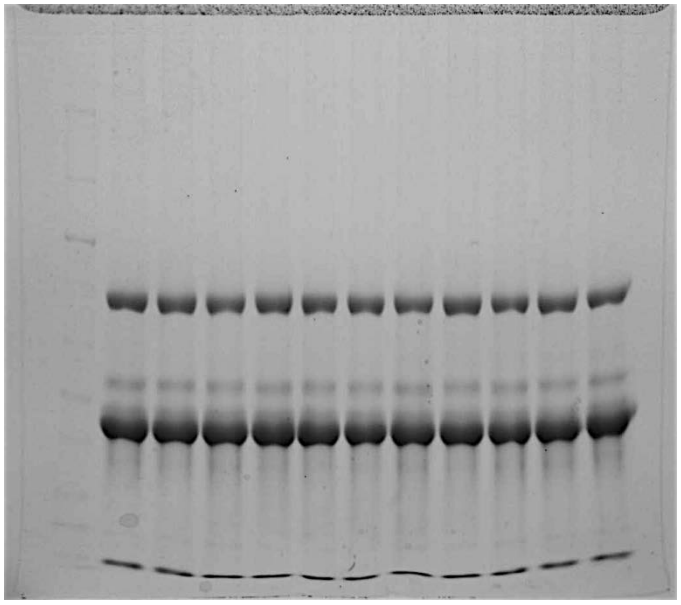

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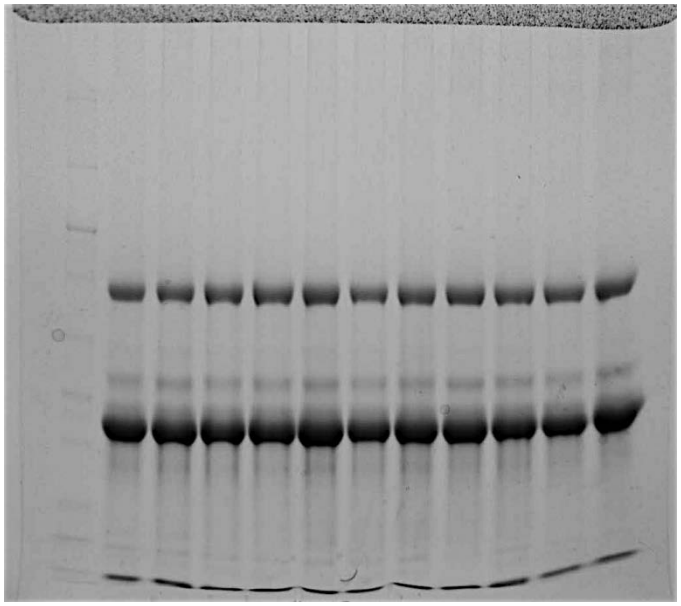

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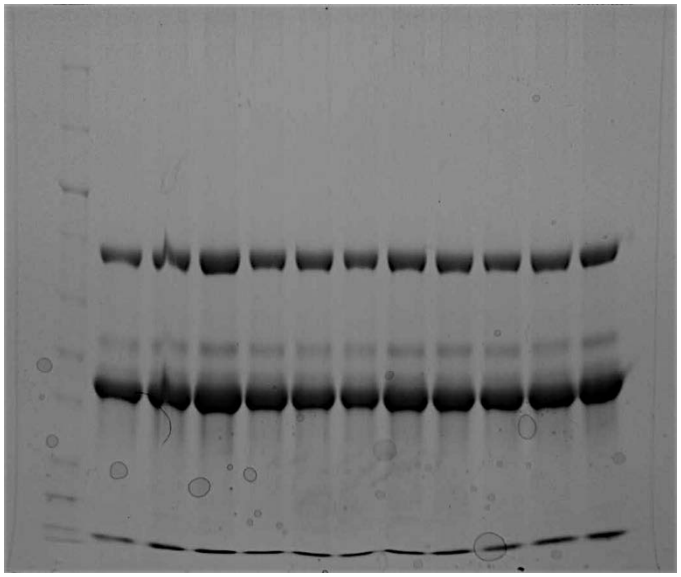

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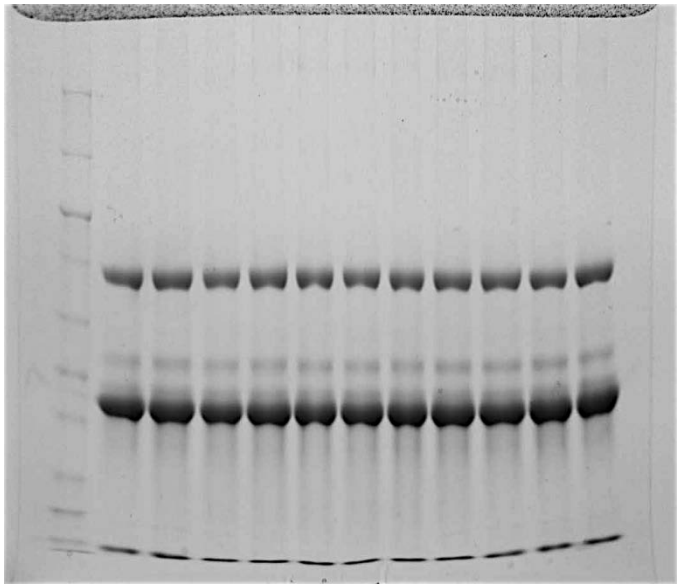

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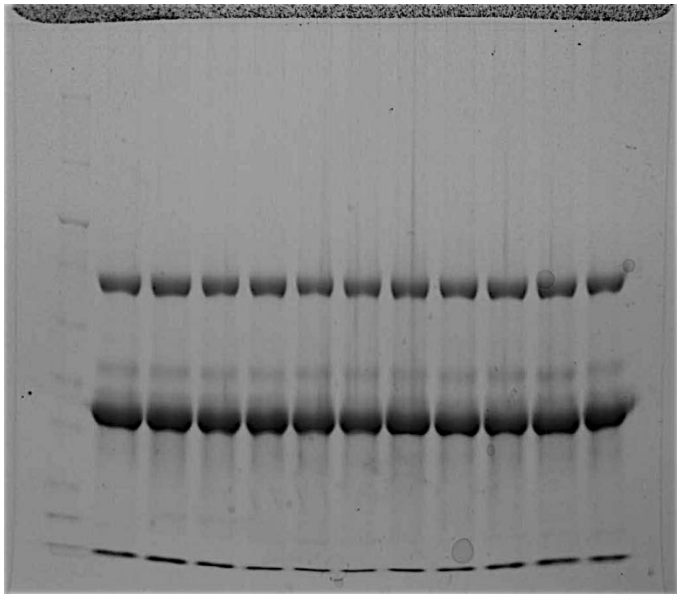

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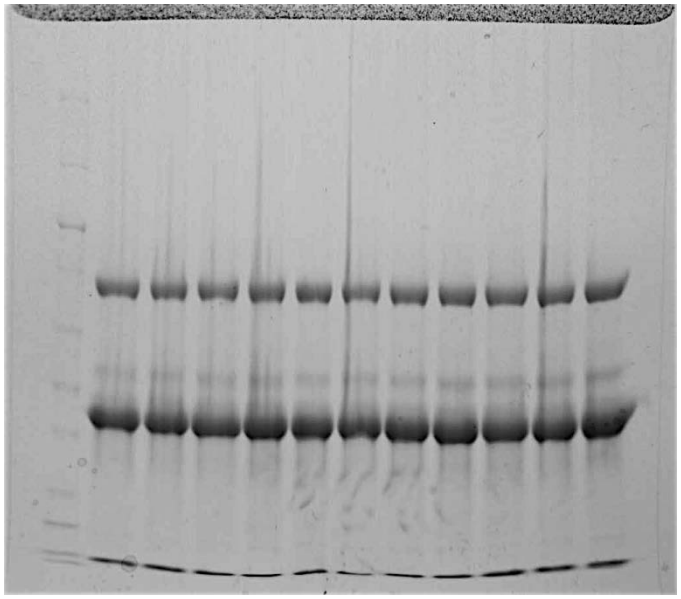

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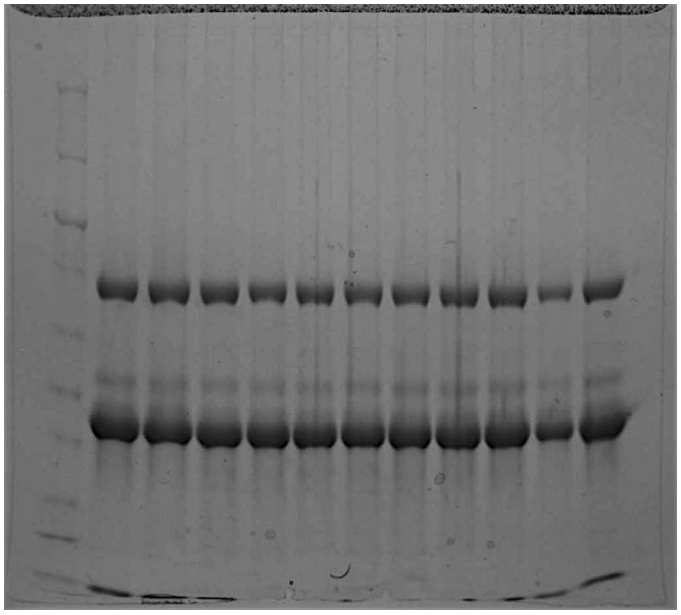

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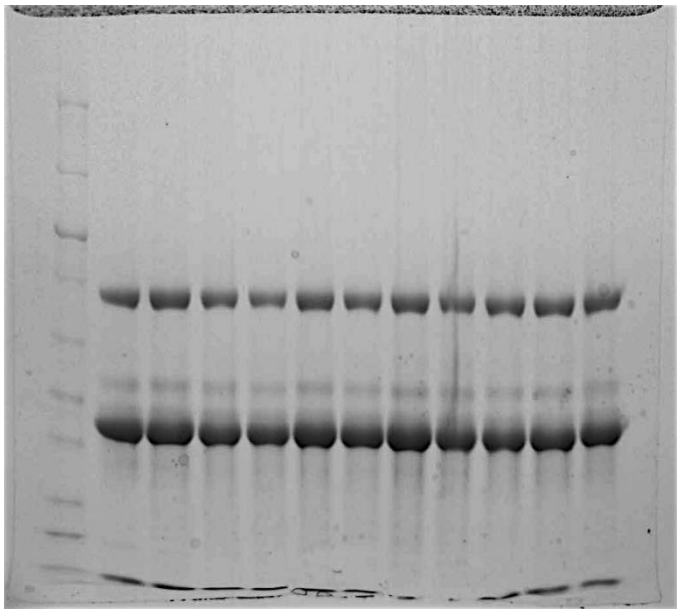

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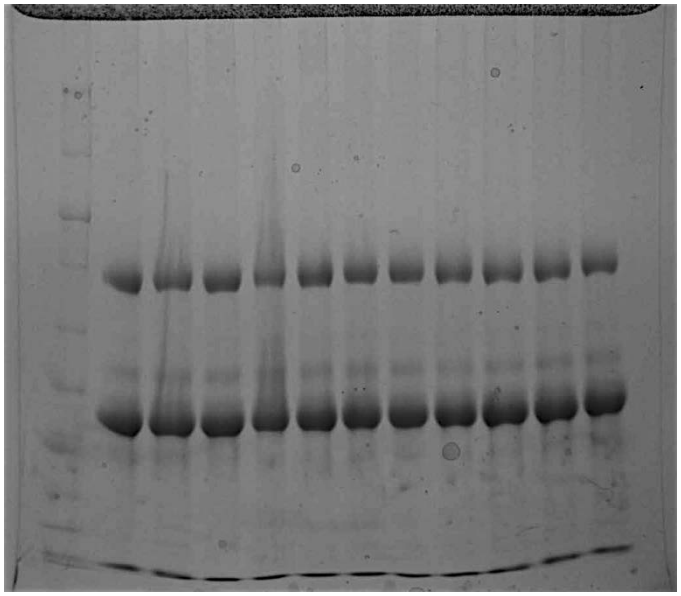

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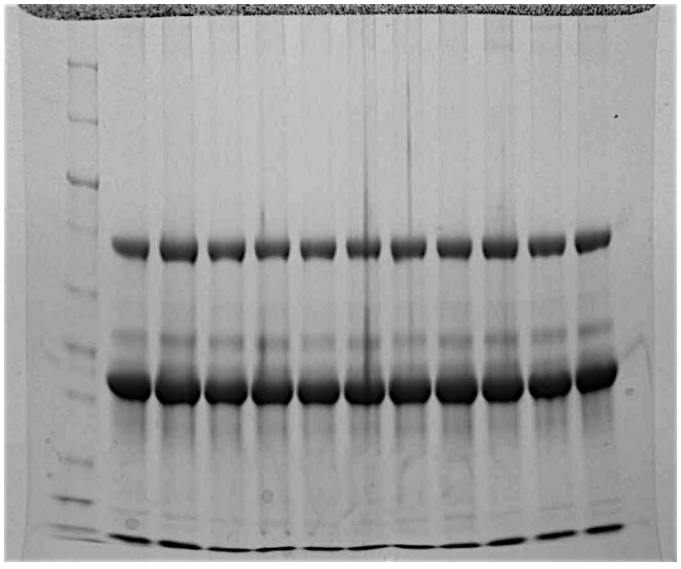

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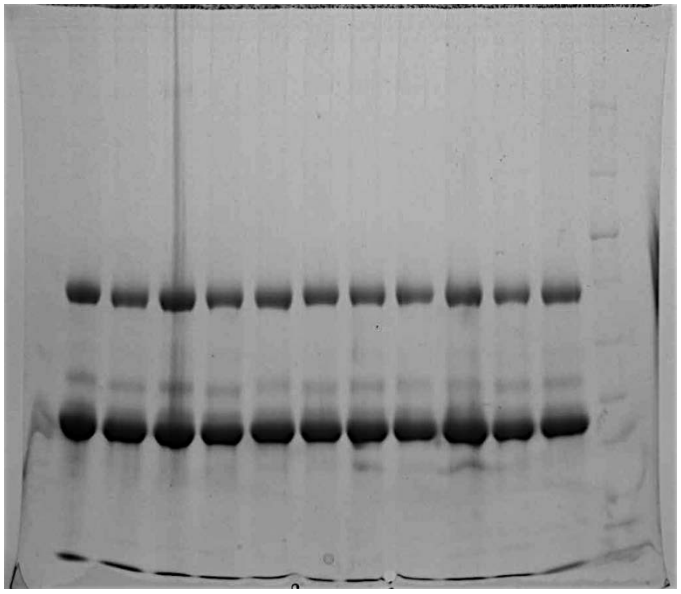

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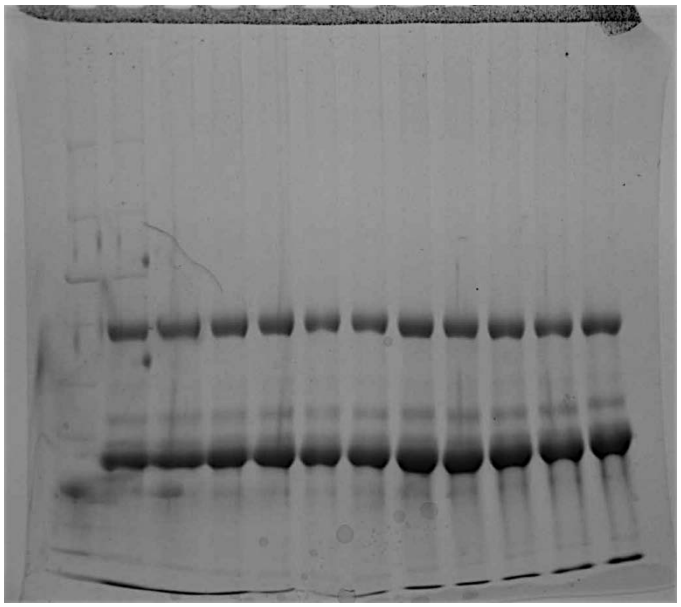

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