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Effects of emulsion coatings on the internal quality and shelf life of eggs

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EFFECTS OF EMULSION COATINGS ON THE INTERNAL QUALITY AND SHELF LIFE OF EGGS

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ABSTRACT

Mineral oil (MO) is currently used for coating eggs to preserve quality. Chitosan possesses inherent antimicrobial and film-forming properties. Chitosan coating (CH) is dried much faster than MO when applied on egg shell. Information on synergistic effects of MO:CH emulsion coatings on egg quality does not exist. We developed MO:CH emulsion coatings, and evaluated their effects on internal quality and shelf-life of eggs during storage. In the first study, MO, CH and three emulsions (MO:CH = 75:25, 50:50, and 25:75) were evaluated during 5 weeks at 25°C. Haugh unit (HU) and yolk index values decreased whereas weight loss increased during storage. Noncoated eggs changed from AA to C grade after 3 weeks. However, all emulsion-coated eggs maintained an A-grade for 4 weeks. All emulsion-coated eggs had weight losses <1.5%. Only 25:75 MO:CH emulsion-coated eggs were not sensorially glossier than noncoated eggs. All emulsion-coated eggs had >80% positive purchase intent and were negative for *Salmonella* spp. In the second study, 25:75 MO:CH emulsions prepared with four different emulsifier types were evaluated during 5 weeks at 25 °C and 20 weeks at 4 °C. All emulsion-coatings minimized weight loss (<1.5%) and preserved internal quality of eggs for at least 3 weeks longer than observed for noncoated eggs at 25 °C. At 4 °C, all coated eggs changed from AA to A grade after 5 weeks and maintained this grade up to 10 weeks with weight losses <2% at refrigeration. The emulsifier type generally did not insert significant effect on the internal quality. In the third study, MO and 25:75 MO:CH emulsion were evaluated during 5 weeks at 25°C using eggs from three different albumen qualities, expresed as HU, before coating: 'High'=87.8 HU, 'Medium'=75.6 HU and 'Low'=70.9 HU. MO and/or 25:75 MO:CH coatings could preserve the internal quality for at least 4 more weeks for 'High' HU eggs; all with weight losses <0.92%). This study demonstrated that MO:CH emulsion coatings could preserve the internal quality of eggs and prolong their shelf life.

CHAPTER 1. INTRODUCTION

Eggs are a rich source of protein and other nutrients (Watkins 1995). Furthermore, eggs are consumed globally and thus their production has represented an important segment of the world food industry (Stadelman 1995c). The production of table eggs in the United States in 2009 was 6.48 billion dozen with a value of approximately 4.24 billion dollars (USDA 2010). However, eggs are highly susceptible to internal quality deterioration and microbial contamination during storage. These conditions can cause serious economic losses to the poultry industry (Wong and others 1996).

Factors associated with the level of quality loss are time, temperature, humidity, air movement, and handling (Stadelman 1995b). Interior quality deterioration of eggs can be delayed significantly by maintaining storage temperature near the freezing point (Zeidler 2002b). Nonetheless, in some developing regions of the world where refrigeration of eggs is seldom practiced, coating materials are effective methods to preserve the internal quality of eggs and prevent microbial contamination. Numerous food-grade coating materials have been proven to be efficient in reducing interior quality deterioration of eggs. These materials include chitosan, whey protein, waxes, mineral and vegetable oils (Meyer and Spencer 1973; Obanu and Mpieri 1984; Wong and others 1996; Caner 2005; Jirangrat and others 2010).

Chitosan, a natural biopolymer derived by deacetylation of chitin, generates a semi-permeable coating that modifies the internal atmosphere and decreases transpiration rates in food products (Nisperos-Carriedo 1994). Despite the fact that chitosan films are efficient barriers against permeation of oxygen, these films act as low water barriers due to their strong hydrophilic properties (Butler and others 1996). Mineral oil is another coating material currently used to preserve the internal quality of eggs (Waimaleongora-Ek and others 2009; Jirangrat and

others 2010). Even so, a problem associated with mineral oil coating is that oil dries very slowly compared with chitosan solution when applied on the surface of the eggshell without wiping it dry. Thus, coating of eggs with emulsion of mineral oil and chitosan solution may considerably reduce the drying time. To date, there is no information available on the effects of emulsions of mineral oil and chitosan solution on the internal quality and shelf life of eggs during long term storage. This provides a sound justification for the development of this work.

This thesis is divided into six chapters. Chapter one provides a summarized introduction and discusses the research's justification. Chapter two presents a literature review with concepts associated with this thesis work. Chapter three presents the evaluation of three mineral oil:chitosan emulsion-coatings (MO:CH = 75:25, 50:50, and 25:75) on the internal quality and shelf life of eggs. Chapter four discusses the effects of four 25:75 MO:CH emulsion-coatings (by using different emulsifier types) and two storage temperatures (room and refrigerated). Chapter five discusses the effects of MO and 25:75 MO:CH emulsion coatings on egg quality as affected by initial albumen qualities before coating. Chapter six consists of a brief summary of composite findings of this work. Appendices containing the questionnaire form, the research consent form, and other tables and figures are included. Finally, the VITA of the author of this work is provided on the last page of this thesis work.

CHAPTER 2. LITERATURE REVIEW

2.1 Shell Eggs

2.1.1 Egg Production

Eggs are one of the few foods that are widely consumed throughout the world; thus eggs represent an important segment of the world food industry and an important commodity in international trades (Stadelman 1995b). According to the Food and Agriculture Organization (FAO), the production of egg worldwide in 2009 totaled 67.4 million metric tons that represented an increase in the production of 1.97% compared with that of previous year (2008) (FAO 2010). Of this total egg production in 2009, China comprised about 41%, making it the largest egg production worldwide (Figure 2.1).

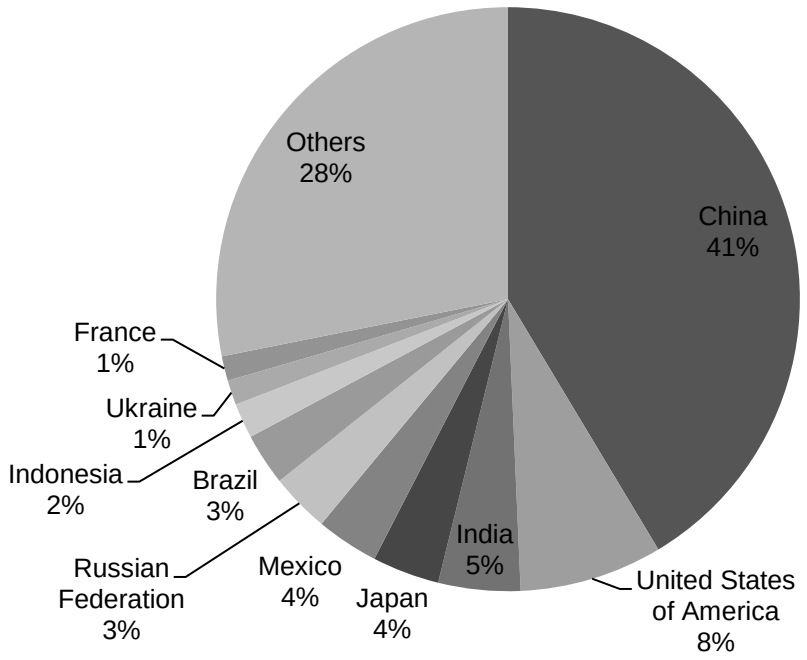

Figure 2.1 Percentage (%) of the Worldwide Egg Production per Country (FAO 2010)

The percentage (%) of the worldwide egg production per country in Figure 2.1 shows that the United States of America (USA) is the second largest egg production industry, and represents

about 8% (5.3 million metric tons) of the total worldwide production, followed by India (5%), Japan (4%), and Mexico (4%) (FAO 2010). A large proportion of the egg production market belongs to developing countries (more than 50%), and this can be explained by the necessity for these countries to meet their protein needs. However, limited technology and feed supplies combined with the low production of native chickens hamper their progress (Stadelman 1995c).

Within the US, the production of eggs (Figure 2.2) has been possessing a constant growth during the last 20 years as reported by the United States Department of Agriculture (USDA) (USDA 2010). In 2009, the production of table eggs in the US totaled 77.7 billion eggs (6.48 billion dozen) with a value of approximately 4.24 billion dollars.

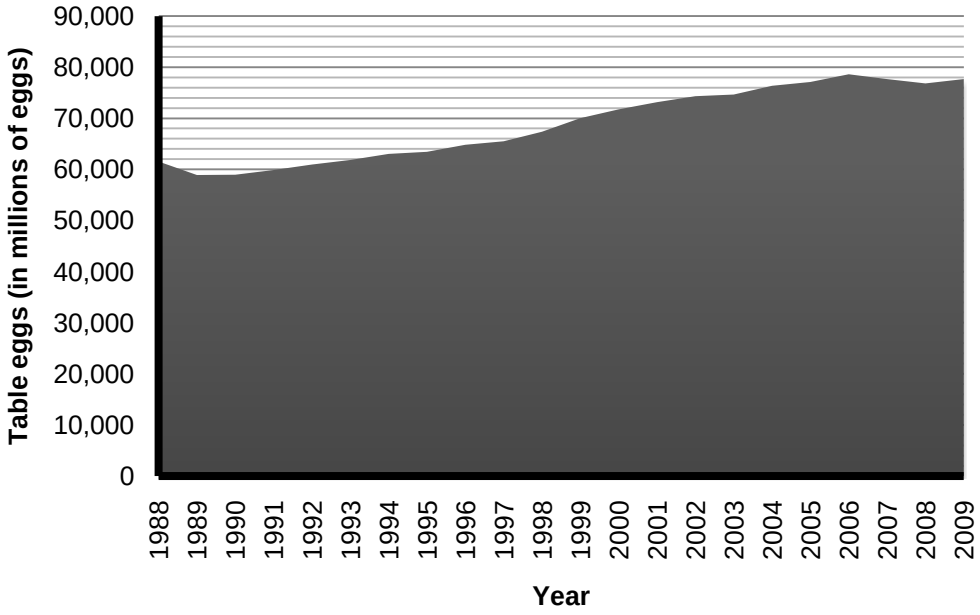

Figure 2.2 Production of Table Eggs in the US (in Millions of Eggs) from 1988 to 2009 (USDA 2010)

This 2009 production represented an increment of 26% compared with that of 1988 (USDA 2010). The table egg industry in the US during the past 25 years has experienced

somewhat of a revolution in structure, technology, and fluctuating economics. Moreover, improvements of the productivity of laying hens together with a stable demand of consumers during these years have leaded to a continuous growing of this industry (Bell 2002).

2.1.2 Egg Structure and Composition

The structure of the hen's egg is shown in Figure 2.3. It is generally accepted that hens form an egg in about two weeks (Stadelman 1995a). A hen egg is composed of three main parts: shell, albumen (egg white) and yolk, and their distributions out of the total egg weight are 9-11%, 60-63%, and 28-29%, respectively (Okubo and others 1997). The egg shell is composed of a thin film of cuticle, a calcium carbonate layer (composed by a vertical crystal layer, palisade layer, and mammillary knob layer), and two shell membranes (inner and outer membranes). Moreover, egg shells contain a large number of pores (in excess of 7,500 per egg) that allow permeation of water and gases (Zeidler 2002a; Okubo and others 1997). The cuticle protects the egg from moisture loss and invasion of microorganisms to a certain extent but it can be easily removed by washing with water in industrial processes (Board and Hall 1973; Belyavin and Boorman 1980).

The egg albumen occurs in four layers: the chalaziferous or inner thick white, the inner thin white (inner liquid), the outer thick white (dense) and the outer thin (outer liquid) layer (Figure 2.3) (Stadelman 1995a). The egg yolk consists of a surrounding yolk membrane (vitelline) and concentric rings of white and yellow materials. These layers are the result of the alternating deposition of yolk components which occur during the day and the night, and cannot be seen with the naked eye (Okubo and others 1997).

The main components of a hen egg are lipids (12%), proteins (12%) and water (75%) with additional small amounts of carbohydrates and minerals (Sugino and others 1997).

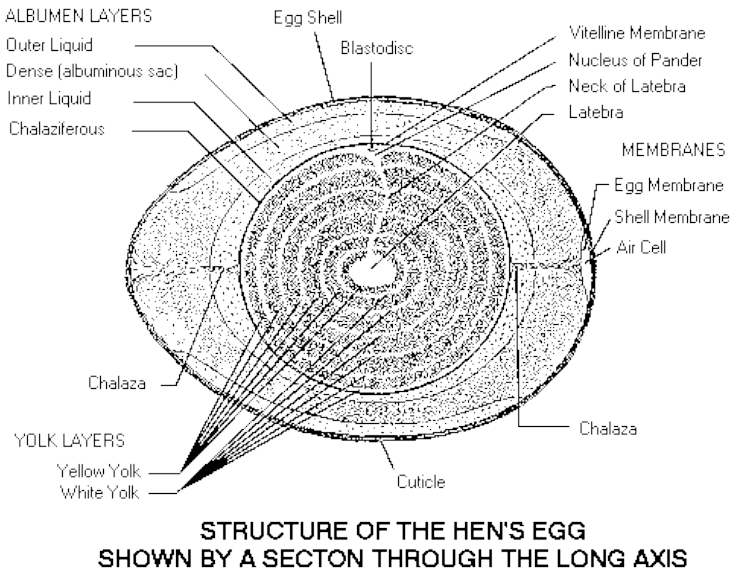

Figure 2.3 Structure of the Hen's Egg (Anonymous 2010)

The egg albumen contains approximately 12% of solid matter, which is predominantly protein with small amounts of minerals, sugars and only traces of fat. Conversely, egg yolk contains about 50% of solids in which, nearly two-thirds are fat and one-third is protein; the latter being generally of a very different composition compared with that of the egg white protein. Major proteins present in egg albumen are ovoalbumin (54% of the total albumen solids), conalbumin (13%), ovomucoid (11%), lysozyme (3.5%) and ovomucin (1.5%). Besides, ovomucin is found in a much greater concentration in the thick layer than in the thin layer.

On the other hand, egg yolk is constituted by lipids (46 % of total yolk solids, mainly triglycerides, phospholipids and sterols), proteins (4-10%, mainly phosphoproteins and lipoproteins), carbohydrates (2%), minerals (2%) and traces of vitamins (Parkinson 1966). Egg shell is composed of about 95% minerals, of which calcium is more than 98%; other inorganic components include phosphorus, magnesium and trace contents of iron and sulfur (Sugino and others 1997).

With regards to their human nutritional values, eggs are classified into the protein food group due to their proteins having an ideal balance of indispensable amino acids. Low caloric content, blandness, and ease of digestibility are other characteristics that make eggs ideal for young or old people, healthy or convalescent (Gutierrez and others 1997).

2.1.3 Shell Egg Quality

Egg quality is based on those characteristics of a shell egg that affect its acceptability by final consumers (Stadelman 1995a). Quality control is an essential part of the marketing process for any product, and it can be defined as the maintenance of the characteristics of a product level and tolerances acceptable to end users. Regarding shell egg quality, grading (a process of identification, classification and separation) is a form of quality control that divides a variable commodity or product into a number of classes (Overfield 1987). In the US, the USDA has established standards for quality of individual shell eggs based on a grading system (AA, A and B) by using quality factors of the shell, air cell, egg white and yolk (Table 2.1). The Haugh unit, an expression relating egg weight and height of the thick albumen, is a measurement of the albumen quality. This expression is an important tool for measuring the internal quality of eggs, and it is related to the USDA egg-quality grades as follows: AA (above 72 units), A (72-60 units), B (59-31 units), and C (below 30 units, inedible or loss) (Lee and others 1996).

Eggs are highly susceptible to internal quality deterioration and microbial contamination since the moment of lay (Hinton 1968). During storage, a thinning of the albumen and an increase in the size of the air cell is observed mainly due to water loss. Carbon dioxide (CO_2) migration throughout the egg shell leads to an increase in albumen pH and a decrease in the vitelline (yolk) membrane strength, thus causing interior quality deteroration (Figure 2.4) (Zeidler 2002b; Stadelman 1995a).

Table 2.1 United States Quality Standards for Shell Eggs (USDA 2000)

Quality Factor	AA Quality	A Quality	B Quality
Shell	Clean	Clean	Clean to slightly stained[a]
	Unbroken	Unbroken	Unbroken
	Practically normal	Practically normal	Abnormal
Air cell	1/8 inch or less in depth	3/16 inch or less in depth	Over 3/16 inch in depth
	Unlimited movement and free or bubbly	Unlimited movement and free or bubbly	Unlimited movement and free or bubbly
White	Clear	Clear	Weak and watery, small blood and meat spots present[b]
	Firm	Reasonably firm	
Yolk	Outline slightly defined, practically free from defects	Outline, fairly well defined, practically free from defects	Outline plainly visible
			Enlarged and flattened
			Clearly visible germ development but no blood
			Other serious defects
Haugh unit[c]	Above 72 units	72-60 units	59-31 units

[a]Moderately stained areas permitted (1/32 of surface if localized, or 1/16 if scattered)
[b]If they are small (aggregating not more than 1/8 inch in diameter)
[c]Stadelman 1995a; Lee and others 1996

Moreover, bacteria such as *Pseudomonas* spp. and *Proteus* spp. can penetrate the egg shell and cause spoilage during the handling and storage (Hinton 1968). *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium may contaminate the internal content of eggs and become a serious health hazard for final consumers (Padron 1990; Berrang and others 1999). Other important factors that affect internal quality of eggs during storage are differences in their initial quality, size and storage conditions (Muller 1958; Goodwin and others 1962; Silversides and Scott 2001).

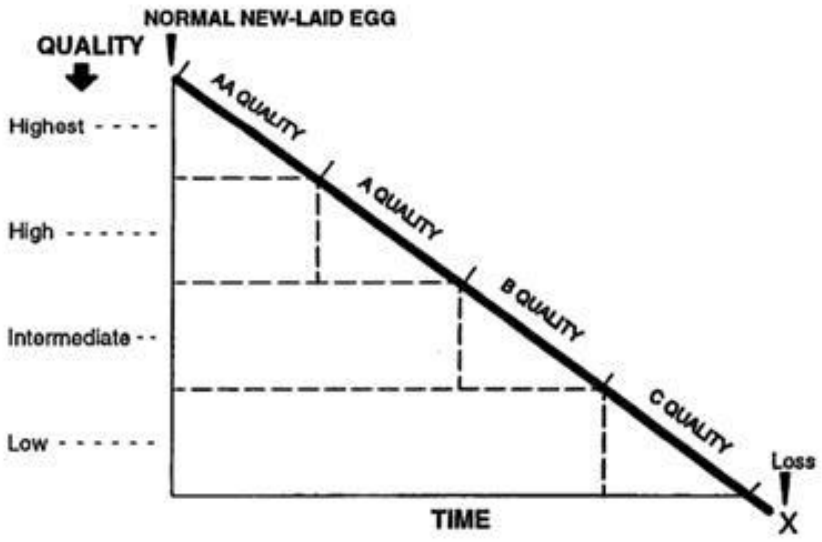

Figure 2.4 Egg Quality Standards and Albumen Quality Affected by Time (Stadelman 1995a)

2.1.3.1 Egg Shell Quality

Pimpled, windowed, misshapen and soft-shelled eggs are some of the common defects related with egg shells. Strength, texture, porosity, shape, cleanliness, soundness, and color are factors used for determining shell quality. Of these, shell soundness is the most important, economically. It is estimated that about 10% of all eggs produced are cracked or broken between oviposition and retail sale, which represents a significant financial loss for the egg industry (Zeidler 2002b). According to USDA, the production of table eggs in the US in 2008 was 6.40 billion dozen with a value of approximately 6.23 billion dollars. Of this production volume, broken eggs totaled 2.05 billion dozen that represents an increment of 2.54% compared with that in 2007 (USDA 2010). Moreover, cracked eggs are more susceptible to interior quality deterioration and microbial contamination even under refrigeration conditions.

As far as the consumer is concerned, the eggshell is the first point for evaluation but it is not possible to produce entirely clean eggs. Thus, methods for cleaning must be employed. With

water washing, nonetheless, there is a possibility of bacterial penetration throughout the egg shell due to the possible removal of the natural cuticle, resulting in rotten eggs (Stadelman 1995b).

2.1.3.2 Weight Loss

Evaporation of water and, to a much lesser extent, loss of CO_2 from the albumen through the approximately 7,500 pores of the egg shell lead to an overall weight loss of the whole egg (Obanu and Mpieri 1984). In warm and dry regions, eggs can lose moisture and weight rapidly. However, keeping eggs under refrigeration substantially reduces this moisture loss. Likewise, this moisture loss reductions can be achieved by increasing the relative humidity (RH) of the storage room. RH in between 75 and 80% of egg storage rooms can prevent moisture loss and an equal loss of egg weight (Zeidler 2002c). Storage time, air movement and handling are factors that also affect overall weight loss of eggs.

Experimentally, the percentage (%) of weight loss of the whole egg is calculated as {[initial whole egg weight (g) at day 0 – whole egg weight (g) after storage]/initial whole egg weight (g) at day 0} × 100 (Bhale and others 2003). In an experiment using eggs from hens of two different ages (6 to 7-week-old hens against 18 to 19-week-old hens), Bornstein and Lipstein (1962) reported that weight loss (%), when expressed as a percentage of initial weight, is independent of egg size and age of hens laying the eggs; however, increases in weigh loss are linearly correlated with increases in storage time periods. Previous investigations (Waimaleongora-Ek and others 2009; Jirangrat and others 2010) indicated that percentage of weight loss in control noncoated eggs progressively increased to 8.78% at 25 °C and to 4.11% at 4 °C after 5 weeks of storage period (RH of 70%). Bhale and others (2003) reported that weight loss of noncoated eggs increased from 1.36% after 1 week to 4.71% and 7.84% after 3 and 5 weeks of storage, respectively, at 25°C. According to FAO (2003), a weight loss of 2-3% is

common in marketing eggs and is hardly noticeable to consumers. This indicates that noncoated eggs are not suitable for the market after approximately 3 weeks (if stored at 25 °C) and 5 weeks (if stored at 4 °C) of storage according with previous studies (Bhale and others 2003; Waimaleongora-Ek and others 2009; Jirangrat and others 2010).

2.1.3.3 Albumen Quality

During the storage of shell eggs, changes in physical, chemical, biological, and functional characteristics of egg albumen constituents may occur principally due to storage conditions such as time, temperature, and relative humidity (Li-Chan and Nakao 1989). The albumen pH can be used as an indicator of the albumen quality of eggs (Scott and Silversides 2000). Freshly laid eggs contain 1.44-2.05 mg CO_2/g of albumen (Keener and others 2001; Biladeau and Keener 2009) and have an albumen pH value of 7.6-8.7 (Goodwin and others 1962; Rhim and others 2004; Waimaleongora-Ek and others 2009). During storage, carbon dioxide escapes via eggshell pores, resulting in increased albumen pH value up to 9.6-9.7 (Li-Chan and Nakao 1989; Kemps and others 2007).

Another major change is the physical deterioration of the gelatinous structure of the thick albumen, leading to thin albumen (thinning). Several hypotheses have been proposed involving the polydisperse sulfated glycoprotein ovomucin in this deterioration mechanism (Li-Chan and Nakao 1989). Exposure to alkaline pH was suggested to cause thinning or viscosity decrease as a result of alkali-catalyzed hydrolysis of disulfide bonds (Tomimatsu and Donovan 1972; Sato and others 1976). Howthorne (1950) suggested that the interaction of the basic protein lysozyme with ovomucin may cause structural changes and slow insolubilization of ovomucin, which may contribute to thinning. Conversely, Robinson (1972) proposed that natural thinning of egg albumen may be due to structural modifications of the ovomucin itself, particularly β-ovomucin

components, and may not be due to either the breakdown or the formation of lysozyme-ovomucin complexes.

The Haugh unit, an expression that measures the egg internal quality, is calculated as 100 log (H – 1.7 $W^{0.37}$+ 7.57), where H is the albumen height (mm) and W is the weight (g) of egg (Haugh 1937). According to Table 2.1, the higher the Haugh unit value, the better the albumen quality of eggs (Stadelman 1995a). Previous investigations (Waimaleongora-Ek and others 2009; Jirangrat and others 2010) reported that Haugh unit of control noncoated eggs significantly decreased with increased storage periods; however, this decrease progressed at a much slower rate for eggs stored at refrigerated temperature than at room temperature during 5 week of storage. Besides storage time and temperature, differences in initial egg quality, egg size, and other storage conditions (humidity, air movement and handling) may negatively affect albumen pH and Haugh unit (Muller 1958; Goodwin and others 1962; Sabrani and Payne 1978).

2.1.3.4 Yolk Quality

Several characteristics of the egg yolk affect its quality including color, spherical condition, and strength of the vitalline membrane. In a freshly laid egg, the yolk is nearly spherical, and when the egg is broken out onto a flat surface, the yolk stands high with only a little change in shape (Zeidler 2002b). During storage of shell eggs, the yolk index value (an indicator of the spherical nature of egg yolk) declines as a result of a progressive weakening of the vitelline membranes, reduction of the total solid, and liquefaction of the yolk caused mainly by the osmotic diffusion of water from the albumen (Obanu and Mpieri 1984; Stadelman 1995a). Caner and Cansiz (2007) reported that yolk index of control noncoated eggs decreased from an initial value of 0.45 to 0.25 and 0.16 after 2 and 4 weeks of storage at 25 °C. In addition to this observed decrease of the yolk index, increases of water content, pH, furosine, pyroglutamic acid,

and urdine were also reported during storage of egg as well as a progressive transition of egg yolk rheological properties from pseudoplastic to Newtonian behavior. The decrease in apparent viscosity of egg yolk was also observed (Hidalgo and others 1996).

2.1.4 Shell Egg Quality Preservation

2.1.4.1 Refrigeration

The most profound factor that affects quality deterioration rate of eggs is storage temperature. The rate of quality declining slows down when the storage temperature is closer to the freezing point (Hinton 1968; Stadelman 1995b). The best conditions for storage are at a temperature of about –1 °C and relative humidity between 80 and 85 percent. On the other hand, freezing eggs practically ruins the internal content [albumen freezes at 31 °F (-0.4 °C)]. At a temperature of 10° C, lower relative humidity is needed between 75 and 80 percent. However, at all temperatures, there is a risk of mould spoilage where the relative humidity is too high (FAO 2003). By law in the US, eggs are required to be refrigerated at 45 °F (7 °C) or below to retard the growth of *Salmonella enterica* which was found to be directly proportional to the temperature at which eggs are stored. Researchers observed that holding eggs at temperatures of 4 to 8 °C (39 to 46 °F) reduces the heat resistance of *Salmonella enteriditis*, and also reduces the level of microbial multiplication in shell eggs (Zeidler 2002c).

Recent studies have been showing that refrigeration can effectively reduce by half the weight loss of eggs compared with that observed at room temperature, and refrigerated eggs can maintain a quality grade of AA for at least 4 weeks of storage (Jirangrat and others 2010; Biladeau and Keener 2009). During storage, albumen pH of refrigerated eggs (5 °C) decreased while that of eggs stored at 21 °C or 29 °C increased as observed in Samli and others (2005). The decrease in albumen pH during storage may be due to the continuing breakdown of the

constituents in egg white and/or a change in the bicarbonate buffer system (Obanu and Mpieri 1984; Biladeau and Keener 2009).

Concerning the egg yolk quality, Samli and others (2005) reported that yolk indices of eggs from old laying hens decreased with increased storage time; however, this decrease was slower at 5 °C than at 21 °C or 29 °C. This entails that migration of water from the albumen to the yolk is a function of storage temperatures with a faster migration rate occurring at higher temperatures (Stadelman 1995b).

2.1.4.2 Coating Materials

Low temperature refrigeration is considered the single most important treatment for preserving eggs. In the United States, eggs are required to be refrigerated at 45 °F (7 °C) or below. Nonetheless, in some developing regions of the world where refrigeration of eggs is seldom practiced, coating of eggs is thus an alternative and effective way to preserve the internal quality. Various coating materials have been applied to the surface of egg shells for preserving the internal quality of eggs. These include synthetic polymers (Meyer and Spencer 1973), polysaccharides (Bhale and others 2003; Kim and others 2006), proteins (Xie and others 2002; Rhim and others 2004) and oils (Obanu and Mpieri 1984; Waimaleongora-Ek and others 2009; Jirangrat and others 2010).

Table 2.2 shows comparisons among several different coating materials [mineral oil, coconut oil, chitosan, chitosan+sorbitol, cellulose and whey protein isolate (WPI)] in their abilities to extend the shelf life of shell eggs compared with that observed for noncoated eggs after 4 weeks of storage at room temperature (25 °C). Weight losses and grades of the control noncoated and coated eggs after 4 weeks are also shown in Table 2.2.

Table 2.2 Comparisons of Different Studies Regarding Coating Materials and their Effects on Internal Eggs Quality of After a Storage Period of 4 Weeks at 25 °C

Coatings	WL (%)[a]	Grade[a]	Shelf life[a]	Reference
Control	9.30±0.64	–	3 weeks	Obani and Mpieri 1984[b]
Coconut oil	0.64±0.14	–	longer	
Control	5.66	B	2 weeks	Caner 2005[e]
WPI	3.63	B	longer	
Control	10.46±2.31	C	3 weeks	Kim and others 2008[c]
Chitosan + Sorbitol	5.25±1.10	B	longer	
Control	9.87±1.50	C	3 weeks	Kim and others 2009[c]
Chitosan	5.34±1.08	B	longer	
Control	7.56±1.04	C	3 weeks	Waimeleongora-Ek
Mineral oil	0.75±0.24	B	longer	and others 2009
Control	8.83±0.12	B	2 weeks	Suppakul and others 2010[d]
Cellulose	4.28±0.07	A	longer	

[a]WL(%) = Percentage of weight loss. Based on the Haugh unit values; AA, above 72; A, 71 to 60; B, 59 to 31; C, below 30. Extended shelf life imparted by coating materials was based on weight loss, Haugh units and yolk index.
[b]Haugh unit was not calculated in this experiment. The extended shelf life was based on weight loss, albumen pH and yolk index.
[c]Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid. Sorbitol used as a platicizer at 2% (w/v).
[d]WPI=Whey protein isolate at 12% (w/w protein) using glycerol as a platicizer.
[e]Methylcellulose (2.00% w/v) and hydroxypropyl methylcellulose (1.00% w/v) powders mixed in ethanol and distilled water (2:1)

One important contrast observation is that oil (mineral oil and/or coconut oil) coated eggs (0.64-0.75%) had a lower weight loss compared with that of (3.63%) protein (whey protein isolate, WPI) or (4.28-5.34%) polysaccharide (chitosan and/or cellulose) coated eggs. Chitosan films are efficient barriers against permeation of oxygen but act as low water barriers due to their strong hydrophilic nature (Butler and others 1996). Methylcellulose is also a hydrophilic material; however, the incorporation of fatty acids enhances its water vapor barrier properties as a coating film (Suppakul and others 2010).

All coated eggs possessed at least B-grade (A-grade in the case of cellulose) compared with a C-grade observed in most of the cases in noncoated eggs after 4 weeks of storage. Moreover, all coating materials could extend shelf life of eggs by at least 2 weeks longer compared with noncoated at 25 °C, based on weight loss, Haugh units and yolk index (Table 2.2). Differences in weight loss and grades of eggs among these studies may be due to different temperatures, egg sizes, shell porosities, relative humidities, hens' ages, and initial albumen qualities of eggs expressed as the Haugh unit (Muller 1958; Williams 1992).

2.1.4.2.1 Chitosan

Polysaccharides are the most extensively distributed group of nature compounds that generate industrial interests due to their unique physical, biochemical and technological applications. Unlike other polysaccharides, chitin (β-(1→4)-N-acetyl-D-glucosamine, Figure 2.5) and chitosan (depending of the degree of acetylated polymers of glucosamine) have basic characteristics that provide them unique properties such as their solubility in various media, the viscosity of their solutions, their polyelectrolyte behavior, membrane-forming ability and polyoxysalt formation (Ruiz-Herrera 1978). After cellulose, chitin is the second most abundant organic compound on earth where structural cell wall of fungi represents its main source.

Furthermore, chitin is the main structural polysaccharide of most invertebrates which belongs to the *Protostomia*. Arthropods constitute the most important chitin-producing animals and their cuticles can contain up to 80% of chitin in terms of dry organic matter. Moreover, arthropod shells (exoskeletons) are the most easily accessible sources of chitin, and major commercial productions of chitin emerge from shells of marine crustaceans such as crabs and shrimps that are available as waste from the seafood processing industry (Ruiz-Herrera 1978).

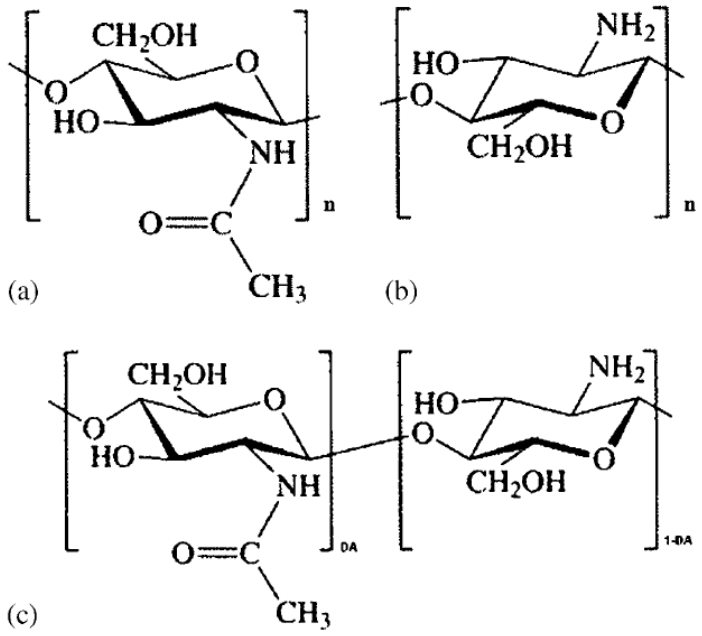

Figure 2. 5 Chemical Structure (a) of Chitin (Poly N-Acetyl-β-D-Glucosamine) and (b) of Chitosan (Poly(D-Glucosamine) Repeat Units. (c) Structure of Partially Acetylated Chitosan, a Copolymer Characterized By Its Average Degree of Acetylation DA (Rinaudo 2006).

Chitosan is a collective name given to a group of polymers deacetylated from chitin [composed by β-(1→4)-linked D-glucosamine and β-(1→4)-N-acetyl-D-glucosamine molecules, Figure 2.5]. Generally, the reaction of deacetylating chitin in an alkaline solution (by using 50% NaOH) cannot reach completion even under harsh treatment. The degree of deacetylation usually ranges from 70% to 95%, depending on the method used (Li and others 1992).

One of the most useful properties of chitosan is its chelation. Chitosan can selectively bind desired materials such as cholesterol, fats, metal ions, proteins, and tumor cells. Besides, this property has been applied to areas of food preparation, health care, water improvement, and pharmaceutics (Li and others 1992). Chitosan is also a good cationic polymer for membrane formation, and it is currently used for water clarification, filtration, food coating, surgical dressing, and controlled release (Li and others 1992).

Shahidi and others (1999) published an extensive review of food applications of chitin, chitosan and their derivatives in the food industry (Table 2.3). These applications include, among others, the antimicorbial capacity, edible film formation and several additive, nutritional and chelating functionalites. As a biodegradable nontoxic film, chitosan generates a semi-permeable coating that modifies the internal atmosphere and decreases transpiration rates in food products (Nisperos-Carriedo 1994). Recent studies (Bhale and others 2003; No and others 2005; Kim and others 2007, 2008) revealed that chitosan coating preserved the internal quality of eggs and extended their shelf life for at least 3 weeks longer than observed for noncoated eggs at 25 °C. Bhale and others (2003) reported that at a concentration of 2% (w/w), lower molecular weight chitosan coating could effectively prevent weight loss and preserved albumen and yolk quality of eggs up to 5 weeks of storage at 25 °C. Moreover, based on external quality parameters (such as surface smoothness, glossiness, odor and overall difference), consumer could not differentiate the chitosan coated eggs from the control noncoated eggs, and their acceptability scores were not significantly different (Bhale and others 2003). However, as is shown in Table 2.2, chitosan coatings are not as effective as oil coatings in maintaining the weigh loss of eggs lower that 1% after 4 weeks of storage, due to its highly hydrophilic nature.

Table 2.3 Food Applications of Chitin, Chitosan and Their Derivatives in the Food Industry*

Area of application	Examples
Antimicrobial agent	Bactericidal
	Fungicidal
	Measure of mold contamination in agricultural commodities
Edible film industry	Controlled moisture transfer between food and surrounding environment
	Controlled release of antimicrobial substances
	Controlled release of antioxidants
	Controlled release of nutrients, flavours and drugs
	Reduction of oxygen partial pressure
	Controlled rate of respiration
	Temperature control
	Controlled enzymatic browning in fruits
	Reverse osmosis membranes
Additive	Clarification and deacidification of fruits and beverages
	Natural flavour extender
	Texture controlling agent
	Emulsifying agent
	Food mimetic
	Thickening and stabilizing agent
	Colour stabilization
Nutritional quality	Dietary fibre
	Hypocholesterolemic effect
	Livestock and fish feed additive
	Reduction of lipid absorption
	Production of single cell protein
	Antigastritis agent
	Infant feed ingredient
Recovery of solid materials from food processing wastes	Affinity flocculation
	Fractionation of agar
Purification of water	Recovery of metal ions, pesticides, phenols and PCB's
	Removal of dyes
Other applications	Enzyme immobilization
	Encapsulation of nutraceuticals
	Chromatography
	Analytical reagents

*Source: Shahidi and others 1999

2.1.4.2.2 Mineral Oil

White mineral oil is a pretroleum-based product, being a mixture of liquid paraffinic and naphthenic hydrocarbons. In the US, mineral oil is approved for use as a food-release agent and

as a protective coating for fresh foods (Baldwin 1999). Mineral oil used for egg coating must be odorless, colorless, and free of fluorescent materials (Stadelman 1995b).

Oiling has been proved to reduce the rate of carbon dioxide and moisture loss of eggs (Stadelman 1995b). Moreover, Waimaleongora-Ek and others (2009), in a study using different viscosities of mineral oil (from 7 to 26 mPa s) as coating materials of eggs, reported that mineral oil with the highest viscosity (26 mPa s) was more effective in preventing weight loss and in preserving albumen quality of eggs compared with that observed for other lower viscosity mineral oil coatings during storage. Moreover, coating with mineral oil (26 mPa s) reduced the weight loss of eggs by more than 10 times (0.75% against 7.56%, Table 2.2) and extended the shelf life of eggs by at least 3 more weeks compared with noncoated eggs during 4 weeks of storage at 25 °C (Waimaleongora-Ek and others 2009). However, shell color and visual appearance of eggs may be altered by oil used as a coating material. Wong and others (1996) reported that eggshells coated with mineral oil possessed a higher L^* value (lightness) than noncoated eggs (87.05 vs. 83.90), possibly due to glossier surface. Moreover, preliminary studies in our laboratory showed that mineral oil coating dries very slowly (one day or longer without forced-air blowing) compared with chitosan solution (less than 15 min) when applied on the surface of the eggshell without wiping it dry.

2.1.4.2.3 Emulsion Coatings

Some composite films made by combining hydrophilic and hydrophobic materials have been studied for their potential as food bio-films. Wong and others (1992) evaluated different composite films of chitosan with various fatty acids in which a film made of chitosan and lauric acid showed to have lower water permeability that chitosan itself. Moreover, it was observed that chitosan polymers (of a cationic and hydrophilic nature) can interact with water molecules on the

film matrix and increase its permeation rates. Gennadios and others (1993) observed that a modification of a wheat gluten-based film by incorporation of mineral oil produced a lower water vapor permeability compared with that of the non changed wheat gluten-based film; however, both films exhibited good oxygen barrier properties. To date, there is no information available on the effects of emulsion of mineral oil and chitosan solution on the internal quality and shelf life of eggs during room temperature storage. Emulsions of mineral oil and chitosan may act differently as a protective barrier against transfer of moisture and carbon dioxide through the shell surface of eggs, compared with mineral oil and chitosan.

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CHAPTER 3. A NOVEL EMULSION COATING AND ITS EFFECTS ON INTERNAL QUALITY AND SHELF LIFE OF EGGS DURING ROOM TEMPERATURE STORAGE*

3.1 Introduction

Eggs are an excellent source of high quality protein (Watkins, 1995). Furthermore, eggs hold important functional properties such as coagulation, solidification, aeration, emulsification, coloration, and texturization (Stadelman, 1999). According to USDA, the production of table eggs in the United States in 2008 was 6.40 billion dozen with a value of approximately 6.23 billion dollars. Of this production volume, broken eggs totaled 2.05 billion dozen that represent an increment of 2.54% compared with that in 2007 (USDA, 2009). In addition to the breakage of shell eggs, interior quality deterioration and microbial contamination during storage cause a serious economic loss to the poultry industry (Stadelman, 1995b; Wong *et al*., 1996). Certain microorganisms such as *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium that exist on the shell surface can penetrate into the interior of eggs and contaminate the internal content (Padron, 1990; Berrang *et al*., 1999). During storage, the loss of moisture and carbon dioxide via the shell pores causes quality changes in albumen and yolk as well as weight loss of eggs (Stadelman, 1995b). Thus, a protective barrier against the loss of moisture and carbon dioxide through the shell is necessary to preserve the egg quality.

Low temperature refrigeration is considered the single most important treatment for preserving eggs. In United States, eggs are required to be refrigerated at 45 °F (7 °C) or below.

*Torrico, D.D., Jirangrat, W., No, H.K., Prinyawiwatkul, W., Ge, B. & Ingram, D. (2010a). A novel emulsion coating and its effects on internal quality and shelf life of eggs during room temperature storage. *International Journal of Food Science and Technology*, doi:10.1111/j.1365-2621.2010.02396.x

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Nonetheless, in some developing regions of the world where refrigeration of eggs is seldom practiced, coating of eggs is thus an alternative way to preserve the internal quality. Various coating materials have been applied to the surface of egg shells for preserving the internal quality of eggs. These include synthetic polymers (Meyer & Spencer, 1973), polysaccharides (Xie *et al*., 2002; Bhale *et al*., 2003; No *et al*., 2005; Kim *et al*., 2006; Caner & Cansiz, 2008), proteins (Herald *et al*., 1995; Cho *et al*., 2002; Xie *et al*., 2002; Rhim *et al*., 2004) and oils (Knight *et al*., 1972; Kamel *et al*., 1980; Obanu & Mpieri, 1984; Waimaleongora-Ek *et al*., 2009). Chitosan is a natural biopolymer derived by deacetylation of chitin, a major component of shells in crustacean such as crab, shrimp, and crawfish. Chitosan generates a semi-permeable coating that modifies the internal atmosphere and decreases transpiration rates in food products (Nisperos-Carriedo, 1994). Recent studies (Bhale *et al*., 2003; No *et al*., 2005; Kim *et al*., 2007; 2008) revealed that chitosan coating preserved the internal quality of eggs and extended the shelf life for at least 3 weeks longer than noncoated eggs at 25 °C. Butler *et al*. (1996) reported that chitosan films are efficient barriers against permeation of oxygen but act as low water barriers due to their strong hydrophilic properties.

Oil is another coating material currently used to preserve the internal quality of eggs. Waimaleongora-Ek *et al*. (2009) reported that coating with mineral oil (26 mPa s) reduced the weight loss of eggs by more than 10 times (0.85% vs. 8.78%) and extended the shelf life of eggs by at least 3 more weeks compared with noncoated eggs during 5 weeks of storage at 25 °C. Homler & Stadelman (1963) also proved that oil-coated eggs had higher Haugh units and lower weight loss than noncoated eggs after 3 weeks of storage at 22 °C. Oils used for egg coating must be odorless, colorless, and free of fluorescent materials (Stadelman, 1995b). However, shell color and visual appearance of eggs may be altered by oil used as a coating material. Wong *et al*.

(1996) reported that eggshells coated with mineral oil possessed a higher *L** value (lightness) than noncoated eggs (87.05 vs. 83.90), possibly due to glossier surface.

A problem associated with mineral oil coating is that oil dries very slowly (one day or longer without forced-air blowing) compared with chitosan solution (less than 15 min) when applied on the surface of the eggshell without wiping it dry. Thus, coating of eggs with emulsion of mineral oil and chitosan solution may considerably reduce the drying time. However, the emulsion may act differently as a protective barrier against transfer of moisture and carbon dioxide through the shell surface of eggs, compared with mineral oil and chitosan. To date, there is no information available on the effects of emulsion of mineral oil and chitosan solution on the internal quality and shelf life of eggs during room temperature storage.

The objectives of the present research were to evaluate the effects of mineral oil (MO), chitosan solution (CH) and their three emulsions (MO:CH = 75:25, 50:50 and 25:75 ratios) as coating materials in preserving the internal quality (weight loss, Haugh unit, yolk index, albumen pH) of coated eggs during 5 weeks of storage at 25 °C, and to evaluate consumer perception and purchase intent of freshly coated eggs by a sensory discrimination test. Total plate count and *Salmonella* detection of the coated eggs were also evaluated before and after 5 weeks of storage.

3.2 Materials and Methods

3.2.1 Materials

Mineral oil (viscosity = 26 mPa s; transparent, odorless and food-grade) was obtained from Penreco® (Karns city, PA, USA). Chitosan (molecular weight = 223 kDa), acid soluble and white-colored powder prepared from crab leg shell, was purchased from Biotech (Mokpo, Korea). Emulsifier Tandem® 552K (a mixture of mono- and diglycerides, polysorbate, water and proply gallate) was obtained from Caravan® ingredients (Lenexa, KS, USA). Unwashed, feces-free,

white-shell eggs (from 51-weeks old, Hyline W-36 hens; a weight range of 50-70 g) were obtained from Cal-Maine Foods (Jackson, MS, USA). Immediately after collected from the farm and screened for defects and desirable weight range, eggs were stored in the cold room (approximately 7 °C) before the next day coating. Before coating, eggs were kept at room temperature (approximately 25 °C) for 2 h to avoid water condensation on the egg surface that could interfere with coating.

3.2.2 Preparation of Mineral Oil/chitosan Solution Emulsions

Chitosan coating solution was prepared by dissolving chitosan in 1% (v/v) acetic acid at 2% (w/v) concentration (Kim *et al*. 2009). Three emulsions of mineral oil (MO) and chitosan solution (CH) were prepared by adding 1% of emulsifier Tandem® 552K to three different ratios of MO and CH (MO:CH = 75:25, 50:50 and 25:75) and mixing using an ultrasonic processor (CPX 500, Cole Parmer, Vernon Hills, IL, USA) for 8 min at 10°C. The CH solution and all emulsions were prepared on the day of the coating experiment.

3.2.3 Coating Treatment and Storage of Eggs

Eggs were individually weighed with a balance (TS400, Ohaus Corp., Florham Park, NJ, USA), coated with MO, CH or one of the three emulsions using a sponge brush, and dried overnight at room temperature (25 ± 2 °C). Seven coating treatments were evaluated throughout the storage period: Control = noncoated eggs; MO (U) = unwiped after coating with 100% MO; MO (W) = wiped after coating with 100% MO; 75:25 MO:CH = coating with MO:CH emulsion at a ratio of 75:25; 50:50 MO:CH = coating with MO:CH emulsion at a ratio of 50:50; 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75; CH = coating with 100% CH. All eggs (50 eggs/treatment) were placed in a small-end down position (Kim *et al*., 2009) in cardboard egg racks and stored at room temperature (25 ± 2 °C) and averaged 60% RH for 5

weeks. Ten eggs per each treatment were taken at 1 week intervals for determination of weight loss, Haugh unit, yolk index, and albumen pH.

3.2.4 Determination of Weight Loss

Weight loss (%) of the coated whole egg during storage was calculated as {[initial whole egg weight (g) after coating at day 0 – whole egg weight (g) after storage]/initial whole egg weight (g) after coating at day 0} × 100. Weight loss (%) of the control noncoated whole egg was calculated as {[initial whole egg weight (g) at day 0 – whole egg weight (g) after storage]/initial whole egg weight (g) at day 0} × 100. The weight of whole eggs was measured with a balance (TS400S, Ohaus Corp., Florham Park, NJ, USA). Ten measurements per treatment were taken.

3.2.5 Determination of Haugh Unit and Yolk Index

The height of albumen and yolk was measured with a tripod micrometer (Model S-6428, B.C. Ames Inc., Melrose, MA, USA). The yolk width was measured with a digital caliper (General Tools & Instruments, New York, NY, USA). The Haugh unit was calculated as 100 log (H – 1.7 $W^{0.37}$+ 7.57), where H is the albumen height (mm) and W is the weight (g) of egg (Haugh, 1937). The yolk index was calculated as yolk height/yolk width (Stadelman, 1995a; Lee *et al*., 1996). Ten measurements per treatment were taken.

3.2.6 Measurement of Albumen pH

After measurement of Haugh unit and yolk index, the albumen was separated from the yolk. The thin and thick albumen were mixed thoroughly prior to measuring pH with a pH meter (IQ150, IQ Scientific Instruments, San Diego, CA, USA). Ten measurements per treatment were taken.

3.2.7 Sensory Discrimination and Consumer Purchase Intent

Consumers (n = 109) were recruited from Baton Rouge, Louisiana to participate in the sensory discrimination of the coated eggs [with MO (unwiped or wiped), CH, and/or one of the three emulsions] compared with the control noncoated eggs at Day 0. Consumers were first presented with the labeled control egg, followed by six unlabeled coated eggs and one unlabeled control (to ascertain the "noise" level). The unlabeled eggs were individually compared to the labeled control for specified attributes. For surface glossiness, consumers were asked to indicate whether the unlabeled coated and unlabeled control eggs were perceived as "more," "the same," or "less" in the specified attribute compared with that of the labeled control, and whether they were "sure" or "unsure" about their decision; in this case, as the direction of a given attribute was of interest, the bipolar R-index was used.

For surface odor and color, and overall surface appearance, consumers were asked if the unlabeled coated and unlabeled control eggs were "different from" or "the same as" the labeled control, and whether their decision was "sure" or "unsure"; in this case, as the direction of a given attribute was not measured, the unipolar R-index was used. The test protocol followed that of Bhale *et al.* (2003). Consumers self-paced their evaluation (but not longer than 30 min.). Afterward, these consumers evaluated purchase intent for all eggs on a yes/no scale, and reported as % positive purchase intent.

3.2.8 Microbiological Analysis

The control noncoated eggs and eggs coated with MO (unwiped or wiped), CH, and one of the three emulsions were analyzed for total plate count (TPC) and *Salmonella* at Day 0 and after 5 weeks of storage. The internal content (yolk and albumen) of egg samples was homogenized using a stomacher (STO-400, Tekmar Company, Cincinnati, OH, USA) in a

dilution of 1:10 of 0.1% buffered peptone water (BD Difco™, Sparks, MD, USA). For TPC, viable cells (CFU/g of egg) were enumerated on plate count agar (PCA) (BD Difco™, Sparks, MD, USA) by the pour plate and spread plate methods followed by incubation at 35 °C for 24 h.

For *Salmonella spp.* detection, homogenates of egg samples were enriched by using Tetrathionate broth (BD Difco™, Sparks, MD, USA) and incubated at 35 °C for 24 h. Following enrichment, subcultures were plated onto XLT4 agar (BD Difco™, Sparks, MD, USA) at 35 °C for 24 h prior to detection. All microbiological assays were done in duplicate for each treatment.

3.2.9 Statistical Analysis

For internal quality (weight loss, Haugh unit, yolk index and albumen pH) of eggs, mean ± standard deviation values were reported based on 10 measurements (eggs) per treatment. Data were analyzed using Analysis of Variance, followed by the Tukey's studentized range test (α = 0.05) using the SAS software (SAS, 2003).

The data obtained from the sensory discrimination test were converted into frequency counts, and then the R-index was calculated for each attribute and expressed as a percentage of sensory discrimination. The bipolar R- index for surface glossiness and the unipolar R-index for odor, color and overall surface appearance were computed from the equations as in Bhale *et al*. (2003). The significance of the R-index was determined using the table provided by Bi and O'Mahony (2007). At the significance of 5%, the observed R-index value was significant if it exceeded the critical R-index of 56.65% for the unipolar R-index test. For the bipolar R-index, the result was significant if it exceeded the critical R-index of 57.89% for R-index more, or fell behind the critical R-index of 42.11% for R-index less.

3.3 Results and Discussion

3.3.1 Effects of Mineral Oil, Chitosan Solution, and Their Emulsions as a Coating Material on Weight Loss

Differences in the weight loss among the control noncoated eggs and those coated with mineral oil (MO), chitosan solution (CH) and/or their three emulsions (MO:CH = 75:25, 50:50, and 25:75) were found (interaction between coating treatments * storage periods, $P < 0.0001$) during 5 weeks of storage at 25 °C (Table 3.1). Overall, the weight loss progressively increased with increased storage periods. Without exception, all eggs coated with MO (unwiped or wiped) and/or three MO:CH emulsions had significantly ($P < 0.05$) lesser weight loss than noncoated and CH-coated eggs throughout the 5 weeks of storage period. However, there were no significant differences ($P > 0.05$) in weight loss observed among five treatment groups of eggs coated with MO (unwiped or wiped) and three emulsions throughout 5 weeks of storage. After 5 weeks, eggs coated with MO (unwiped or wiped) and/or three emulsions had at least 7 times lesser weight loss (%) compared with that of the control eggs (0.69-1.03% vs. 7.14%).

Evaporation of water and, to a much lesser extent, loss of CO_2 from the albumen through the shell leads to overall weight loss of the whole egg (Obanu & Mpieri, 1984). Table 3.1 shows that the weight loss of eggs coated with MO (unwiped or wiped) (0.69-0.70%) and three emulsions (0.88-1.03%) after 5 weeks of storage was lower than that (1.43%) of noncoated eggs after 1 week of storage. Similarly, Waimaleongora-Ek *et al*. (2009) reported that, at 25 °C storage, the weight loss (0.85%) of eggs coated with MO (wiped; 26 mPa s) after 5 weeks was lower than that (1.97%) of noncoated eggs after 1 week. Moreover, Obanu & Mpieri (1984) reported that vegetable oil coatings significantly reduced (11 times less) the weight loss (0.013-0.016 g) of coated eggs, compared to that (0.186 g) of noncoated eggs after 35 days of storage at 25-32 °C.

Table 3.1 Weight Loss (%)* of Eggs Coated with Mineral Oil, Chitosan and/or Three Emulsions during 5 Weeks of Storage at 25 °C

Coating**	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	$1.43 \pm 0.22^{E,a}$	$3.09 \pm 0.38^{D,a}$	$4.71 \pm 1.13^{C,a}$	$5.77 \pm 0.70^{B,a}$	$7.14 \pm 0.51^{A,a}$
MO (U)	$0.22 \pm 0.04^{B,b}$	$0.41 \pm 0.21^{AB,b}$	$0.47 \pm 0.11^{AB,b}$	$0.72 \pm 0.47^{A,b}$	$0.69 \pm 0.26^{A,b}$
MO (W)	$0.32 \pm 0.07^{B,b}$	$0.51 \pm 0.20^{AB,b}$	$0.55 \pm 0.20^{A,b}$	$0.50 \pm 0.19^{AB,b}$	$0.70 \pm 0.19^{A,b}$
75:25 MO:CH	$0.23 \pm 0.05^{D,b}$	$0.40 \pm 0.07^{CD,b}$	$0.46 \pm 0.14^{BC,b}$	$0.67 \pm 0.14^{AB,b}$	$0.88 \pm 0.35^{A,b}$
50:50 MO:CH	$0.21 \pm 0.04^{C,b}$	$0.37 \pm 0.09^{BC,b}$	$0.47 \pm 0.12^{B,b}$	$0.83 \pm 0.32^{A,b}$	$0.90 \pm 0.30^{A,b}$
25:75 MO:CH	$0.34 \pm 0.21^{B,b}$	$0.52 \pm 0.23^{B,b}$	$0.74 \pm 0.30^{AB,b}$	$0.73 \pm 0.38^{AB,b}$	$1.03 \pm 0.64^{A,b}$
CH	$1.44 \pm 0.13^{D,a}$	$2.97 \pm 0.31^{C,a}$	$4.28 \pm 0.85^{B,a}$	$6.05 \pm 1.01^{A,a}$	$6.82 \pm 0.83^{A,a}$

*Means ± standard deviations of 10 measurements. $^{A-E}$ Means with different superscripts within a row indicate significant differences ($P < 0.05$). $^{a-b}$ Means with different superscripts within a column indicate significant differences ($P < 0.05$).
**Control = noncoated eggs; MO (U) = unwiped after coating with 100% mineral oil (MO); MO (W) = wiped after coating with 100% MO; 75:25 MO:CH = coating with MO:CH emulsion at a ratio of 75:25; 50:50 MO:CH = coating with MO:CH emulsion at a ratio of 50:50; 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.

Slight differences in weight loss among these studies may be due to different coating materials used, storage period and temperature, egg size and shell porosity (Muller, 1958). In our present study (Table 3.1), no significant difference ($P > 0.05$) in weight loss was observed between noncoated (7.14%) and CH-coated (6.82%) eggs after 5 weeks. These values are similar to those reported for noncoated (7.84%) and CH-coated (6.69-7.66%) eggs after 5 weeks of storage at 25°C by Bhale *et al*. (2003). It was obvious that CH-coating was less effective in minimizing weight loss than mineral oil and MO:CH emulsion coating (Table 3.1). Since chitosan films are cationics, water molecules can interact with the matrix and increase the water vapor permeability rate due to their highly hydrophilic nature (Wong *et al*., 1992; Butler *et al*., 1996), thus reducing the film's water barrier capability. According to FAO (2003), a weight loss of 2-3% is common in marketing eggs and is hardly noticeable to consumers. This study demonstrated that MO (unwiped or wiped) and MO:CH emulsion (irrespective of the MO:CH ratio) coatings can equally ($P > 0.05$) offer a protective barrier against the loss of moisture through the eggshell, thus minimizing weight loss (< 1.03%, Table 3.1).

3.3.2 Effects of Mineral Oil, Chitosan Solution, and Their Emulsions as a Coating Material on Haugh Unit

The Haugh unit, an expression relating egg weight and height of the thick albumen, is a measurement of the albumen quality. The higher the Haugh unit value, the better the albumen quality of eggs (Stadelman, 1995a). Changes in the Haugh unit of noncoated and coated eggs during 5 weeks of storage at 25 °C were observed (interaction between coating treatments * storage periods, $P < 0.0001$) (Table 3.2). Overall, the Haugh unit significantly decreased with increased storage periods; however, this decrease progressed at a much slower rate for eggs coated with MO (wiped or unwiped) and/or MO:CH emulsions than for noncoated and CH-coated eggs. Compared with noncoated eggs, eggs coated with MO (wiped or unwiped) and three

emulsions had significantly higher Haugh unit throughout 5 weeks of storage ($P < 0.05$). No significant differences in Haugh unit were observed among five treatment groups of eggs coated with MO and/or MO:CH emulsions throughout the 5 weeks of storage. The Haugh unit of CH-coated eggs was significantly higher than that of the control eggs during 2-4 weeks of storage, but was comparable to that of the control eggs after 5 weeks (Table 3.2).

The Haugh unit of noncoated eggs decreased from an initial value of 83.79 to 58.79 after 1 week and to 37.00 after 2 weeks of storage. The Haugh unit (53.23-59.12) of eggs coated with MO (unwiped or wiped) and/or three emulsions after 5 weeks of storage was higher than that (37.00) of noncoated eggs after 2 weeks of storage; this implies that coating with MO (unwiped or wiped) or MO:CH (irrespective of the ratio) could preserve the albumen quality for at least 3 more weeks compared with noncoated eggs at 25 °C (Table 3.2). On the other hand, the Haugh unit (38.16) of CH-coated eggs after 4 weeks of storage was comparable to that (37.00) of noncoated eggs after 2 weeks of storage; this implies that CH coating was also effective in preserving the albumen quality of eggs for at least 2 more weeks compared with noncoated eggs at 25 °C. These results were substantiated by previous observations for MO-coated eggs (Homler & Stadelman, 1963; Kamel *et al*., 1980; Waimaleongora-Ek *et al*., 2009) and CH-coated eggs (Lee *et al*., 1996; Bhale *et al*., 2003; Kim *et al*., 2007, 2008). Based on the Haugh unit, eggs can be classified into four grades: AA (above 72), A (72-60), B (59-31), and C (below 30) (Lee *et al*., 1996). Changes in classified egg grade during 5 weeks of storage at 25 °C are shown in Table 3.2. The grade of noncoated eggs decreased rapidly from AA to B and C after 1 and 3 weeks, respectively. However, eggs coated with MO (unwiped or wiped) and three emulsions changed from AA to B grade after 5 weeks, thus preserving the A grade quality up to 4 weeks. The CH-coated eggs changed from AA to B grade after 3 weeks and to C grade after 5 weeks.

Table 3.2 Haugh Unit* and Grade** of Eggs Coated with Mineral Oil, Chitosan and/or Three Emulsions during 5 Weeks of Storage at 25 °C

Coating***	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	83.79 ± 4.05^{A} AA	58.79 ± 12.46^{B,b} B	37.00 ± 9.38^{C,c} B	29.13 ± 8.77^{CD,c} C	26.27 ± 7.10^{CD,c} C	24.06 ± 4.82^{D,b} C
MO (U)	83.79 ± 4.05^{A} AA	72.79 ± 5.18^{B,a} AA	66.55 ± 11.92^{BC,ab} A	62.08 ± 7.42^{BC,a} A	62.11 ± 8.58^{BC,a} A	58.05 ± 10.01^{C,a} B
MO (W)	83.79 ± 4.05^{A} AA	73.10 ± 7.24^{B,a} AA	73.15 ± 5.82^{B,a} AA	58.66 ± 7.37^{C,ab} B	61.34 ± 6.72^{C,a} A	56.50 ± 10.41^{C,a} B
75:25 MO:CH	83.79 ± 4.05^{A} AA	70.84 ± 8.19^{B,a} A	71.18 ± 7.05^{B,ab} A	63.86 ± 4.56^{BC,a} A	60.41 ± 8.88^{C,a} A	59.12 ± 9.11^{C,a} B
50:50 MO:CH	83.79 ± 4.05^{A} AA	75.70 ± 7.25^{AB,a} AA	69.83 ± 5.40^{B,ab} A	60.26 ± 8.21^{C,a} A	60.32 ± 7.99^{C,a} A	53.83 ± 5.39^{C,a} B
25:75 MO:CH	83.79 ± 4.05^{A} AA	72.94 ± 6.00^{B,a} AA	68.61 ± 7.78^{BC,ab} A	62.65 ± 4.79^{CD,a} A	60.54 ± 4.71^{CD,a} A	53.23 ± 13.97^{D,a} B
CH	83.79 ± 4.05^{A} AA	68.59 ± 10.40^{B,ab} A	60.84 ± 7.71^{BC,b} A	48.12 ± 12.89^{CD,b} B	38.16 ± 11.41^{DE,b} B	30.12 ± 15.15^{E,b} C

*Means ± standard deviations of 10 measurements. $^{A-E}$ Means with different superscripts within a row indicate significant differences ($P < 0.05$). $^{a-c}$ Means with different superscripts within a column indicate significant differences ($P < 0.05$).
**Based on the Haugh unit values; AA, above 72; A, 71 to 60; B, 59 to 31; C, below 30.
***Control = noncoated eggs; MO (U) = unwiped after coating with 100% mineral oil (MO); MO (W) = wiped after coating with 100% MO; 75:25 MO:CH = coating with MO:CH emulsion at a ratio of 75:25; 50:50 MO:CH = coating with MO:CH emulsion at a ratio of 50:50; 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.

3.3.3 Effects of Mineral Oil, Chitosan Solution, and Their Emulsions as a Coating Material on Yolk Index

The spherical nature of egg yolk can be expressed as a yolk index value, an indication of freshness, by measuring the yolk height and width (Stadelman, 1995a). Generally, the yolk index values decreased with increased storage periods (Table 3.3). This decrease was affected by the coating treatments and storage period at 25 °C (interaction between coating treatments * storage periods, $P < 0.0001$) (Table 3.3), and indicated a progressive weakening of the vitelline membranes and liquefaction of the yolk caused mainly by the diffusion of water from the albumen (Obanu & Mpieri, 1984). All eggs coated with MO (unwiped or wiped) and three emulsions had significantly higher yolk index values (0.33-0.37) than noncoated (0.24) and CH-coated eggs (0.27) after 5 weeks of storage ($P < 0.05$). Under similar storage time and temperature, Waimaleongora-Ek *et al*. (2009) reported a higher yolk index value (0.37) of MO (26 mPa s)-coated eggs compared with that (0.21) of noncoated eggs. In our present study (Table 3.3), the yolk index values (0.33-0.37) of eggs coated with MO (unwiped or wiped) and/or three emulsions after 5 weeks of storage were all higher than that (0.30) of noncoated eggs after 2 weeks of storage. Data from Tables 3.2 (Haugh unit) and 3.3 (yolk index) imply that coating with MO (unwiped or wiped) or MO:CH (irrespective of the ratio) could preserve the albumen and yolk quality of eggs for at least 3 more weeks compared with noncoated eggs at 25 °C.

3.3.4 Effects of Mineral Oil, Chitosan Solution, and Their Emulsions as a Coating Material on Albumen pH

Besides the Haugh unit, albumen pH can also be used as an indicator for the albumen quality of eggs (Scott & Silversides, 2000). Freshly laid eggs contain 1.44-2.05 mg CO_2/g of albumen (Biladeau & Keener, 2009; Keener *et al*., 2001) and have an albumen pH value of 7.6-

8.7 (Goodwin *et al*., 1962; Rhim *et al.*, 2004; Waimaleongora-Ek *et al*., 2009). In this study, the albumen pH values of all noncoated and coated eggs ranged from 7.91 to 8.76 (Table 3.4).

During storage, carbon dioxide escapes via eggshell pores, resulting in thinning of the albumen and an increased albumen pH value up to 9.6 (Knight *et al*., 1972; Heath, 1977; Kemps *et al*., 2007). The albumen pH values of all eggs coated with MO (unwiped or wiped) and/or three emulsions were significantly lower than that of noncoated and CH-coated eggs throughout the 5 weeks of storage (Table 3.4). This implies that MO and MO:CH emulsions as coating materials could retard a loss of carbon dioxide through eggshell pores by acting as a gas barrier. No significant differences in albumen pH values were observed among five treatment groups of eggs coated with MO or MO:CH emulsions after 5 weeks of storage.

The pattern for changes in albumen pH during 5 weeks of storage somewhat differed with coating treatments. The albumen pH of noncoated and CH-coated eggs gradually increased from an initial value of 8.28 to 8.66 and 8.63, respectively, after 5 weeks of storage. However, the opposite was observed for the albumen pH of eggs coated with MO (unwiped or wiped) and/or three emulsions, with the pH decreasing from 8.28 to 7.91-8.04 (Table 3.4). Kamel *et al.* (1980) reported that the albumen pH of noncoated eggs increased from the initial value of 8.64 to 9.51 after ca. 5 weeks of storage at 25 °C. Biladeau & Keener (2009) observed that the albumen pH of MO-coated eggs decreased from the initial value of 8.35 to 7.96 after 12 weeks of storage at 7 °C. Jirangrat *et al.* (2010) observed that the albumen pH of noncoated eggs markedly ($P < 0.05$) increased from 8.71 to 9.42 while that of MO-coated eggs slightly decreased (but not significant, $P \geq 0.05$) from 8.71 to 8.64 after 5 weeks of storage at 25 °C. The decrease in albumen pH during storage may be due to the continuing breakdown of the constituents in egg white and/or a change in the bicarbonate buffer system (Sharp & Powell, 1931; Obanu & Mpieri, 1984;

Biladeau & Keener, 2009). However, differences in initial egg quality, egg size, and storage conditions (temperature and period) may affect albumen pH before and after storage (Muller, 1958; Goodwin *et al*., 1962; Sabrani & Payne, 1978; Scott & Silversides, 2000; Silversides & Scott, 2001).

Results from Tables 3.1 to 3.4 collectively indicate that coating with MO (unwiped or wiped) and/or MO:CH emulsions (irrespective of the MO/CH ratios) effectively reduced weight loss and preserved the albumen and yolk quality of eggs for at least 3 weeks longer than observed for the noncoated eggs at 25 °C.

3.3.5 Sensory Discrimination and Purchase Intent of Noncoated and Coated Eggs

The R-index (%) was used to measure the degree of difference between the control noncoated eggs and freshly coated eggs (Table 3.5). A value of 100% indicates perfect discrimination, whereas a chance value of 50% indicates that the two samples cannot be differentiated (Bhale *et al*., 2003). As shown in Table 3.5, more consumers indicated that the coated eggs were perceived to be significantly ($P < 0.05$) glossier than the noncoated control, except for eggs coated with emulsion of MO:CH = 25:75 (not significantly different from the control, $P > 0.05$, with the R-more of 56.93%).

For the surface odor and color, and overall surface appearance, the unipolar R-index values for all coated eggs fell between 37.38 and 55.16, indicating that consumers could not significantly ($P > 0.05$) differentiate the coated eggs from the control noncoated eggs. Table 3.5 also shows that the purchase intent of the MO:CH-coated eggs was above 80% compared with 67% for the CH-coated eggs. The purchase intent of MO-coated was not determined due to its less practicality because of the longer drying time.

Table 3.3 Yolk Index* of Eggs Coated with Mineral Oil, Chitosan and/or Three Emulsions during 5 Weeks of Storage at 25 °C

Coating**	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	0.45^{A}	$0.39^{B,ab}$	$0.30^{C,d}$	$0.28^{CD,c}$	$0.27^{DE,c}$	$0.24^{E,c}$
MO (U)	0.45^{A}	$0.42^{AB,a}$	$0.39^{BC,ab}$	$0.36^{CD,ab}$	$0.37^{CD,a}$	$0.35^{D,ab}$
MO (W)	0.45^{A}	$0.40^{BC,ab}$	$0.42^{B,a}$	$0.37^{C,a}$	$0.37^{C,a}$	$0.37^{C,a}$
75:25 MO:CH	0.45^{A}	$0.40^{B,ab}$	$0.40^{B,ab}$	$0.37^{BC,a}$	$0.37^{BC,a}$	$0.35^{C,ab}$
50:50 MO:CH	0.45^{A}	$0.41^{B,a}$	$0.37^{CD,bc}$	$0.38^{BC,a}$	$0.37^{CD,a}$	$0.33^{D,ab}$
25:75 MO:CH	0.45^{A}	$0.38^{B,ab}$	$0.38^{B,bc}$	$0.37^{B,a}$	$0.37^{B,a}$	$0.33^{C,b}$
CH	0.45^{A}	$0.37^{B,b}$	$0.35^{BC,c}$	$0.33^{CD,b}$	$0.30^{DE,b}$	$0.27^{E,c}$

*Means of 10 measurements with the standard deviation range of 0.01-0.04. $^{A-E}$ Means with different superscripts within a row indicate significant differences ($P < 0.05$). $^{a-d}$ Means with different superscripts within a column indicate significant differences ($P < 0.05$).

**Control = noncoated eggs; MO (U) = unwiped after coating with 100% mineral oil (MO); MO (W) = wiped after coating with 100% MO; 75:25 MO:CH = coating with MO:CH emulsion at a ratio of 75:25; 50:50 MO:CH = coating with MO:CH emulsion at a ratio of 50:50; 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.

Table 3.4 Albumen pH* of Eggs Coated with Mineral Oil, Chitosan and/or Three Emulsions during 5 Weeks of Storage at 25 °C

Coating**	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	8.28 ± 0.06^{D}	$8.70 \pm 0.03^{BC,a}$	$8.76 \pm 0.03^{A,a}$	$8.71 \pm 0.03^{ABC,a}$	$8.72 \pm 0.04^{AB,a}$	$8.66 \pm 0.04^{C,a}$
MO (U)	8.28 ± 0.06^{A}	$8.18 \pm 0.10^{B,c}$	$8.14 \pm 0.06^{B,c}$	$8.02 \pm 0.06^{CD,c}$	$8.04 \pm 0.07^{C,b}$	$7.95 \pm 0.06^{D,b}$
MO (W)	8.28 ± 0.06^{AB}	$8.32 \pm 0.10^{A,b}$	$8.18 \pm 0.10^{BC,c}$	$8.06 \pm 0.10^{C,bc}$	$8.13 \pm 0.10^{C,b}$	$7.91 \pm 0.08^{D,b}$
75:25 MO:CH	8.28 ± 0.06^{A}	$8.34 \pm 0.08^{A,b}$	$8.14 \pm 0.12^{B,c}$	$8.13 \pm 0.08^{B,bc}$	$8.08 \pm 0.07^{B,b}$	$7.94 \pm 0.12^{C,b}$
50:50 MO:CH	8.28 ± 0.06^{A}	$8.24 \pm 0.13^{AB,bc}$	$8.18 \pm 0.10^{AB,c}$	$8.17 \pm 0.06^{AB,b}$	$8.13 \pm 0.08^{BC,b}$	$8.03 \pm 0.11^{C,b}$
25:75 MO:CH	8.28 ± 0.06^{A}	$8.32 \pm 0.06^{A,bc}$	$8.22 \pm 0.09^{AB,c}$	$8.14 \pm 0.11^{BC,b}$	$8.13 \pm 0.06^{BC,b}$	$8.04 \pm 0.15^{C,b}$
CH	8.28 ± 0.06^{C}	$8.57 \pm 0.13^{AB,a}$	$8.53 \pm 0.09^{B,b}$	$8.63 \pm 0.09^{AB,a}$	$8.70 \pm 0.14^{A,a}$	$8.63 \pm 0.10^{AB,a}$

*Means ± standard deviations of 10 measurements. $^{A-D}$ Means with different superscripts within a row indicate significant differences ($P < 0.05$). $^{a-c}$ Means with different superscripts within a column indicate significant differences ($P < 0.05$).
**Control = noncoated eggs; MO (U) = unwiped after coating with 100% mineral oil (MO); MO (W) = wiped after coating with 100% MO; 75:25 MO:CH = coating with MO:CH emulsion at a ratio of 75:25; 50:50 MO:CH = coating with MO:CH emulsion at a ratio of 50:50; 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.

Table 3.5 R-Index (% Sensory Discrimination)***** Comparing Noncoated Eggs with Freshly Coated Eggs and Their Purchase Intent

Coating**	Surface glossiness		Surface odor	Surface color	Overall surface appearance	Purchase intent (%)
	R-index more	R-index less	R- index	R-index	R-index	
MO (U)	*65.47****	57.83	45.03	49.37	46.72	-****
MO (W)	*75.21****	57.65	39.46	53.42	42.71	-
75:25 MO:CH	*71.69****	47.94	38.33	51.04	43.53	83
50:50 MO:CH	*69.34****	52.23	45.28	54.52	45.33	81
25:75 MO:CH	56.93***	47.67	50.53	55.16	44.97	81
CH	*74.78****	58.01	37.38	48.34	37.44	67

*Based on 109 consumers. At α=0.05, the critical R-index value for a bipolar test is 57.9% for R-index-more and 42.1% for R-index-less, and the critical R-index value for a unipolar test is 56.65%. Italicized R-index values indicates significant difference ($P< 0.05$).
**Control = noncoated eggs; MO (U) = unwiped after coating with 100% mineral oil (MO); MO (W) = wiped after coating with 100% MO; 75:25 MO:CH = coating with MO:CH emulsion at a ratio of 75:25; 50:50 MO:CH = coating with MO:CH emulsion at a ratio of 50:50; 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.
***More responses were selected by consumers. Therefore, the R-Index less was not considered.
****Not determined due to its less practicality because of the longer drying time.

Based on Tables 3.1-5, the MO:CH = 25:75 emulsion would have more potential as a coating material for eggs because it was more cost effective, yet performed similarly in preserving the internal quality of eggs, compared to other MO and MO:CH coatings.

3.3.6 Microbiological Analysis

Total plate count (TPC) is a quality indicator of the raw material before processing (ICMSF, 1986). Bacteria including *Salmonella* can readily penetrate the shell and membranes of an intact hatching egg (Berrang *et al*., 1999; Messens *et al*., 2005). Results of TPC and *Salmonella* detection for internal noncoated eggs and eggs coated with MO (unwiped or wiped), CH, and/or MO:CH emulsions before and after 5 weeks of storage at 25 °C are shown in Table 3.6. At Day 0, TPC of noncoated eggs (control) was not detectable (ND) by the pour plate method but detected at 3.2×10^2 CFU/g by the spread plate method. After 5 weeks of storage, TPC of noncoated eggs and eggs coated with MO (unwiped or wiped), CH, and/or MO:CH emulsions ranged from ND to 3.5×10^1 CFU/g by the pour plate method and from ND to 2.2×10^2 CFU/g by the spread plate method.

No *Salmonella* colonies were detected in all noncoated and coated eggs before and after 5 weeks of storage. According to Ricke *et al*. (2001), eggs products should meet the specification of less than 2.5×10^4 CFU/g for aerobic plate count (APC) and a negative presence of *Salmonella*. ICMSF (1986) establishes a presence between 5.0×10^4 and 1.0×10^6 CFU/g for APC and zero tolerance for *Salmonella* as limits for egg products. Thus, our present results (Table 3.6) indicate that noncoated and coated eggs were all microbiologically safe throughout the 5 weeks of storage at 25 °C.

Table 3.6 Microbiological Analysis of Eggs Coated with Mineral Oil, Chitosan and/or Three Emulsions Before and After 5 Weeks of Storage at 25 °C

Treatments*	TPC** (Pour plate)	TPC (Spread plate)	*Salmonella* spp. detection
	CFU/g of egg	CFU/g of egg	
D0 - Control	ND	317	Negative
W5 - Control	5	25	Negative
W5 - MO (U)	35	75	Negative
W5 - MO (W)	20	175	Negative
W5 - 75:25 MO:CH	33	ND	Negative
W5 - 50:50 MO:CH	ND	ND	Negative
W5 - 25:75 MO:CH	5	225	Negative
W5 - CH	15	75	Negative

*D0 and W5 indicate 0 day (fresh) and 5 weeks of storage, respectively. Control = noncoated eggs; MO (U) = unwiped after coating with 100% mineral oil (MO); MO (W) = wiped after coating with 100% MO; 75:25 MO:CH = coating with MO:CH emulsion at a ratio of 75:25; 50:50 MO:CH = coating with MO:CH emulsion at a ratio of 50:50; 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.
**TPC = Total plate count. Values represent the average of two replicates. ND = Not detectable.

3.4 Conclusions

This study demonstrated that MO (unwiped or wiped) and three MO:CH emulsions (irrespective of the MO:CH ratio) were more effective than CH as a coating material in preserving the internal quality of eggs. MO (unwiped or wiped) and three MO:CH emulsions coating reduced weight loss and preserved the albumen and yolk quality of eggs for at least 3 weeks longer than observed for the noncoated eggs during 5 weeks of storage at 25 °C. A major problem associated with MO coating is drying time. Three emulsions, especially emulsion at the ratio of MO:CH = 25:75, and CH solution require much less drying time than MO (wiped or unwiped) when applied on the surface of the eggshell, which is an obvious advantage in a large-

scale egg production. According to the sensory discrimination, surface glossiness of eggs coated with emulsion of MO:CH = 25:75 was not significantly different from the control (noncoated) whereas eggs coated with other treatments including two MO (unwiped or wiped) and two other emulsions (MO:CH = 75:25 and 50:50) exhibited greater surface glossiness than the control. Eggs coated with three emulsions were safe for human consumption, all with at least 80% positive purchase intent. Collectively, coating of eggs with emulsion of MO:CH = 25:75 would be most effective in view of preservation of the internal egg quality, sensory perception, purchase intent, and drying time.

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CHAPTER 4. MINERAL OIL-CHITOSAN EMULSION COATING AND EMULSIFIER TYPES AFFECT QUALITY AND SHELF-LIFE OF COATED EGGS DURING REFRIGERATED AND ROOM TEMPERATURE STORAGE

4.1 Introduction

Eggs are consumed globally and thus their production has represented an important segment of the world food industry (Stadelman 1995c). The production of table eggs in the United States in 2009 was 6.48 billion dozen with a value of approximately 4.24 billion dollars (USDA 2010). Eggs are highly susceptible to internal quality deterioration and microbial contamination since the moment of lay (Hinton 1968). During storage, the loss of moisture and carbon dioxide via the shell pores causes quality changes in albumen and yolk as well as weight loss of eggs (Stadelman 1995b). Bacteria such as *Pseudomonas* spp. and *Proteus* spp. can penetrate the egg shell and cause spoilage during the handling and storage (Hinton 1968). *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium may contaminate the internal content of eggs and become a serious health hazard for final consumers (Padron 1990; Berrang and others 1999).

The most profound factor that affects quality deterioration rate of eggs is storage temperature. The rate of declining quality slows down when the storage temperature is closer to the freezing point (Hinton 1968; Stadelman 1995b). Quality deterioration of eggs stored for 10 days at 27 °C was comparable to that of eggs stored for several months at -1 °C (FAO 2003). Nevertheless, in some developing regions of the world where refrigeration of eggs is seldom practiced, surface coating is an alternative method to preserve the internal quality of eggs and to prevent microbial contamination.

Among these coating materials, chitosan, a natural biopolymer derived by deacetylation of chitin, generates a semi-permeable coating that modifies the internal atmosphere and

decreases transpiration rates in food products (Nisperos-Carriedo 1994). Recent studies (No and others 2005; Kim and others 2006, 2007, 2008) revealed that chitosan coating preserved the internal quality and extended the shelf-life of eggs for at least 3 wk longer than noncoated eggs at 25 °C. Chitosan films are efficient barriers against permeation of oxygen but act as low water barriers due to their strong hydrophilic nature (Butler and others 1996). Mineral oil is another coating material currently used to preserve the internal quality of eggs. Waimaleongora-Ek and others (2009) reported that mineral oil coating reduced the weight loss of eggs by more than 10 times (0.85% against 8.78%) and extended the shelf-life of eggs by at least 3 more weeks compared with noncoated eggs during 5 wk of storage at 25 °C.

A problem associated with mineral oil (MO) coating is that oil dries very slowly (1 d or longer without forced-air blowing) compared with chitosan solution (CH) (less than 15 min) when applied on the surface of the eggshell without wiping it dry. Therefore, coating of eggs with emulsion of MO and CH may considerably reduce drying time (Torrico and others 2010). More recently, Torrico and others (2010) found that three emulsions of MO and CH (MO:CH = 75:25, 50:50 and 25:75 ratios) were as equally effective as MO but were more effective than chitosan solution as a coating material in preserving the internal quality of eggs at room temperature storage. Among these emulsions, only MO:CH = 25:75 emulsion-coated eggs were not sensorially glossier than noncoated eggs. Presently, various water miscible or oil miscible commercial emulsifiers are available with different compositions. Different types of emulsifiers may influence mechanical and permeation properties of emulsion coating and thus affect the internal quality of emulsion-coated eggs. To date, there is no information available on the effect of MO:CH emulsion coating prepared with different types of emulsifiers on the internal quality and shelf-life of eggs during room temperature and refrigerated storage.

The objective of this study was to evaluate and compare the effects of four MO:CH emulsion coatings (at a ratio of 25:75; prepared with different emulsifiers) as well as mineral oil coating in preserving the internal quality (weight loss, Haugh unit, yolk index, albumen pH) of eggs during 5 wk of storage at 25 °C and during 20 wk of storage at 4°C. Total plate count and *Salmonella* spp. detection of all eggs were evaluated before and after 5 wk of storage at 25 °C.

4.2 Materials and Methods

4.2.1 Materials

Mineral oil (viscosity = 34 mPa s; transparent, odorless and food-grade) was obtained from Ste Oil Company® Inc. (San Marcos, Tex., U.S.A.). Chitosan (prepared from crab leg shell; acid soluble and white-colored powder; molecular weight = 223 kDa) was purchased from Biotech (Mokpo, Korea). Four emulsifiers used were: (1) Tandem® 552K (a mixture of mono and diglycerides, polysorbate, water and proply gallate; Caravan® ingredients, Lenexa, Kans., U.S.A.), (2) Tic Pretested®Ticaloid®210 S Powder (gum acacia and xanthan gum; Tic Gums®, Inc., White Marsh, Md., U.S.A.), (3) Tween 80 (Polyoxyethylene-20-sorbitan monooleate, reagent grade; Amresco® Inc., Solon, Ohio, U.S.A.), and (4) Eficacia XE (Acacia gum purified and instantized; Colloides Naturels International, Rouen cedex, France). These emulsifiers were previously screened among others in their ability to form a stable emulsion between mineral oil (MO) and chitosan (CH) at a ratio of MO:CH = 25:75. Feces-free, white-shell eggs (from 48-wk old, Hyline W-36 hens; a weight range of 50-70 g) were obtained from Cal-Maine Foods (Jackson, Miss., U.S.A.). Immediately after collected from the farm and screened for defects and desirable weight range, eggs were stored in a cold room (approximately 7 °C) before the next day coating. Before coating, eggs were kept at room temperature (approximately 25 °C) for 2 h to avoid water condensation on the egg surface that could interfere with coating.

4.2.2 Preparation of Mineral Oil/Chitosan Solution Emulsions

Chitosan solution was prepared by dissolving chitosan in 1% (v/v) acetic acid at 2% (w/v) concentration (Kim and others 2009). Four MO:CH emulsions were prepared at a fixed ratio of 25:75 (Torrico and others 2010) by adding 1% of each of the four different emulsifiers (Tandem® 552K, Tic Pretested®Ticaloid®210 S Powder, Tween 80 and Eficacia XE) as further described below. Emulsifiers Tandem® 552K and Tween 80 (oil miscible) were added to MO and mixed using a hand blender (Model # 59780R, Hamilton Beach® Brands Canada, Inc., Picton, Onratio, Canada) at a low speed for 2 min at 25 °C. The mixture stood for 30 min at room temperature, and subsequently CH was added and mixed by using the hand blender at a high speed for 6 min at 25 °C. Conversely, Tic Pretested®Ticaloid®210 S Powder and Eficacia XE (water miscible) were added to CH, mixed at a low speed for 2 min, stood for 30 min, and mixed with MO at a high speed for 6 min using a hand blender at 25 °C. The CH and all MO:CH emulsions were prepared on the day of the coating experiment.

4.2.3 Coating Treatment and Storage of Eggs

Eggs were individually weighed with a balance (TS400, Ohaus Corp., Florham Park, N.J., U.S.A.), coated with MO or one of the four MO:CH (25:75) emulsions using a sponge brush, and dried overnight at room temperature (25 ± 2 °C). Six coating treatments were evaluated throughout the storage periods: (1) CONTROL = noncoated eggs, (2) MO = coating with 100% MO, (3) TANDEM = coating with emulsion containing Tandem® 552K, (4) TIC = coating with emulsion containing Tic Pretested® Ticaloid®210 S Powder, (5) TWEEN = coating with emulsion containing Tween 80, and (6) EFICACIA = coating with emulsion containing Eficacia XE. Coating treatment with 100% CH was excluded from this study because CH was found to be less effective than MO:CH emulsion as a coating material in preserving the internal

quality of eggs (Torrico and others 2010). All eggs were placed in a small-end down position (Kim and others 2009) in cardboard egg racks and stored at 25 ± 2 °C and/or at 4 ± 2 °C, both at 60-65% of RH. For determination of weight loss, Haugh unit, yolk index, and albumen pH, ten eggs per each treatment were taken weekly for 5 wk at 25 °C and at 5-wk intervals for 20 wk at 4 °C.

To simplify the results, only data from wk 0, 1, 3 and 5 at 25 °C, and wk 5, 10 and 20 at 4 °C were presented for weight loss, Haugh unit and yolk index. Noncoated eggs after 3 wk of storage at 25 ± 2 °C were discontinued due to their extremely low albumen quality (Haugh units below 25; C grade).

4.2.4 Determination of Weight Loss

Weight loss (%) of the coated whole egg during storage was calculated as {[initial whole egg weight (g) after coating at day 0 – whole egg weight (g) after storage]/initial whole egg weight (g) after coating at day 0} × 100. Weight loss (%) of the control (noncoated) whole egg was calculated as {[initial whole egg weight (g) at day 0 – whole egg weight (g) after storage]/initial whole egg weight (g) at day 0} × 100. The weight of whole eggs was measured with a balance (TS400S, Ohaus Corp.). Ten measurements per treatment were taken.

4.2.5 Determination of Haugh Unit, Yolk Index, and Albumen pH

The height of albumen and yolk was measured with a tripod micrometer (Model S-6428, B.C. Ames Inc., Melrose, Mass., U.S.A.). The yolk width was measured with a digital caliper (General Tools & Instruments, New York, N.Y., U.S.A.). The Haugh unit was calculated as 100 log (H – 1.7 $W^{0.37}$+ 7.57), where H is the albumen height (mm) and W is the weight (g) of egg (Haugh 1937). The yolk index was calculated as yolk height/yolk width (Stadelman 1995a; Lee and others 1996). After measurement of Haugh unit and yolk index, the albumen was separated

from the yolk. Both thin and thick albumen were mixed thoroughly prior to measuring pH with a pH meter (IQ150, IQ Scientific Instruments, San Diego, Calif., U.S.A.). Ten measurements per treatment were taken.

4.2.6 Microbiological Analysis

The internal content of the control noncoated eggs and eggs coated with MO and/or four MO:CH emulsions were analyzed for total plate count (TPC) and *Salmonella* spp. at wk 0 and after 5 wk of storage at room temperature (25 °C). The yolk and albumen of egg was homogenized using a stomacher (STO-400, Tekmar Company, Cincinnati, Ohio, U.S.A.) in a dilution of 1:10 of 0.1% buffered peptone water (BD Difco™, Sparks, Md., U.S.A.). For TPC, viable cells (CFU/g of egg) were enumerated on plate count agar (PCA) (BD Difco™) by the pour plate method followed by incubation at 35 °C for 24 h. For *Salmonella* spp. detection, homogenate of egg was enriched by using Tetrathionate broth (BD Difco™) and incubated at 35 °C for 24 h, then the subculture was plated onto XLT4 agar (BD Difco™) at 35 °C for 24 h prior to detection. All microbiological assays were done in duplicate for each treatment.

4.2.7 Statistical Analysis

For internal quality (weight loss, Haugh unit, yolk index and albumen pH) of eggs, mean ± standard deviation values were reported based on ten measurements (eggs) per treatment. ANOVA was used to determine differences among the noncoated and all coated eggs, considering the main effects of coating, storage time, and the 2-way interaction between the two main effects at $\alpha = 0.05$. When main effects were significant, the Tukey's studentized range test was performed for post-hoc multiple comparisons. Pearson correlation coefficients (r) among the weight loss, Haugh unit, yolk index and albumen pH were calculated. The statistical software (SAS 2003) was used to analyze the data.

4.3 Results and Discussion

4.3.1 Effects of MO and MO:CH Emulsion Coating on Weight Loss

Overall, the weight loss progressively increased with increased storage periods; however, the extent was lesser at 4 °C than at 25 °C after 5 wk of storage (Table 4.1). Without exception, all eggs coated with MO and/or MO:CH emulsions, irrespective of emulsifier types, had significantly ($P < 0.05$) lesser weight loss than noncoated eggs throughout 5 wk of storage period at 25 °C and 20 wk of storage period at 4 °C (interaction between coating treatments * storage periods, $P < 0.0001$). However, there were no significant differences ($P > 0.05$) in weight loss among five (MO and MO:CH) coated eggs, and neither were among four MO:CH emulsion-coated eggs during the entire storage period at 25 °C and 4 °C. Results indicated that the weight loss of eggs coated with emulsions was not affected by emulsifier types under the present experimental conditions.

Evaporation of water and, in much lesser extent, loss of CO_2 from the albumen through the pores of shell leads to overall weight loss of the whole egg (Obanu and Mpieri 1984). After 5 wk of storage at 25 °C, the weight loss of eggs coated with MO (0.72%) and MO:CH emulsions (0.86-1.20%) was lower than that (6.73%) of noncoated eggs after 3 wk of storage. At 4 °C, the weight loss of eggs coated with MO (1.17%) and MO:CH emulsions (1.27-1.63%) after 20 wk of storage was lower than those (4.17% and 9.78%, respectively) of noncoated eggs after 5 and 20 wk of storage. Similarly, Waimaleongora-Ek and others (2009) stated that MO (viscosity = 26 mPa s) coating significantly reduced the weight loss (0.85%) of coated eggs, compared to that (8.78%) of noncoated eggs, after 5 wk of storage at 25 °C. Jirangrat and others (2010) reported that at 4 °C storage, the weight loss of eggs coated with MO (viscosity = 26 mPa s) after 15 wk was lower than that of noncoated eggs after 5 wk of storage (1.19% against

4.11%). The weight loss of MO-coated eggs was 9 times lesser than that of noncoated eggs (0.35 g against 3.40 g) after 12 wk of storage at 7 ± 2 °C (Biladeau and Keener 2009). Slight differences in weight loss among these studies may be due to different coating materials used, storage period, temperature, egg size, shell porosity, relative humidity, hens' age, and initial albumen quality of eggs expressed by the Haugh unit (Muller 1958; Williams 1992).

According to FAO (2003), a weight loss of 2-3% is common in marketing eggs and is hardly noticeable to consumers. In our present study (Table 4.1), the weight loss (0.72%-1.20%) of eggs coated with MO and four MO:CH emulsions after 5 wk of storage at 25 °C was significantly ($P < 0.05$) lower than that (4.17%) of noncoated eggs after 5 wk of storage at 4 °C. This indicated that during the first 5 weeks of storage, refrigeration alone was not sufficient to keep the weight loss below the FAO's recommended level where as the MO or MO:CH coating without refrigeration was. Kamel and others (1980) also reported that eggs coated with MO and stored at 25 °C showed a lower weight loss compared with that of noncoated eggs stored at 5 °C after the same storage period of 40 days (0.6% against 2.3%). This study demonstrated that MO and MO:CH emulsion (irrespective of emulsifier types) coatings can equally ($P > 0.05$) offer a protective barrier against the transfer of moisture through the eggshell, thus minimizing weight loss for at least 5 wk at 25 °C (< 1.20%, Table 4.1) and, in a synergistic effect with refrigeration, at least 20 wk at 4 °C (<1.63%, Table 4.1).

4.3.2 Effects of MO and MO:CH Emulsion Coating on Haugh Unit and Egg Grade

During storage of shell eggs, the gelatinous structure of the thick albumen gradually deteriorates, changing into thin albumen (thinning), which is associated with either ovomucin-lysozyme interactions, disulfide bonds of ovomucin, carbohydrate moieties of ovomucin, or interrelations between α and β ovomucins (Li-Chan and Nakai 1989; Stevens 1996). The Haugh

unit, an expression relating egg weight and height of the thick albumen, is a measurement of the albumen quality. The higher the Haugh unit value, the better the albumen quality of eggs.

Significant changes in the Haugh unit of all eggs during 5 wk of storage at 25 °C and during 20 wk at 4 °C were observed (interaction between coating treatments * storage periods, $P < 0.0001$) (Table 4.2). Generally, the Haugh unit significantly decreased ($P < 0.05$) with increased storage periods; however, this decrease progressed at a much slower rate for eggs coated with MO and four MO:CH emulsions than for noncoated eggs, and likewise at 4 °C than at 25 °C. Compared with noncoated eggs, eggs coated with MO or MO:CH emulsions had significantly higher Haugh units ($P < 0.05$) throughout 5 wk of storage at 25 °C, except for eggs coated with emulsion containing TWEEN at 1 wk of storage, in which its Haugh unit was not significantly different ($P > 0.05$) from the CONTROL. At 4 °C, only eggs coated with emulsions containing TIC, TWEEN and EFICACIA had significantly higher ($P < 0.05$) Haugh units than noncoated eggs after 10 wk of storage. Nonetheless, Haugh units of noncoated eggs, and eggs coated with MO and four emulsions were not significantly different after 20 wk of storage ($P > 0.05$).

The Haugh unit of noncoated eggs decreased from an initial value of 75.62 to 57.01 after 1 wk (Table 4.2) and to 39.34 after 2 wk of storage at 25 °C (data not shown). The Haugh unit (37.02-42.70) of eggs coated with MO and/or four MO:CH emulsions after 5 wk of storage was comparable to that (39.34) of noncoated eggs ($P > 0.05$) after 2 wk of storage; this implies that, based on the Haugh unit, coating with MO or emulsions (regardless of emulsifier types) could preserve the albumen quality of coated eggs for at least 3 more wk compared with noncoated eggs at 25 °C). These results were substantiated by previous observations for MO-coated eggs (Kamel and others 1980; Waimaleongora-Ek and others 2009; Jirangrat and others 2010).

Based on the Haugh unit, eggs can be classified into four grades: AA (above 72), A (72-60), B (59-31), and C (below 30) (Lee and others 1996). At 25 °C, the grade of noncoated eggs decreased rapidly from an initial AA to B and C grade after 1 and 3 wk of storage, respectively (Table 4.2). However, all coated eggs maintained AA or A grade after 1 wk and B grade after 5 wk. At 4 °C, the grade of noncoated eggs decreased from AA to A and B after 5 and 10 wk, respectively. Biladeau and Keener (2009) reported that noncoated egg at refrigeration maintained AA-grade for 4 wk at 7 ± 2 °C.

In our study, eggs coated with MO and/or four MO:CH emulsions changed from AA to A grade after 5 wk and maintained this grade up to 10 wk (5 wk longer than that of noncoated eggs) at 4 °C. Hence, this demonstrates the existence of a synergistic effect between coating treatment (MO and/or four emulsions) and refrigeration on the albumen quality of eggs.

4.3.3 Effects of MO and MO:CH Emulsion Coating on Yolk Index

During storage of shell eggs, the yolk index value (an indicator of freshness) declines as a result of a progressive weakening of the vitelline membranes, reduction of the total solid and liquefaction of the yolk caused mainly by the osmotic diffusion of water from the albumen (Obanu and Mpieri 1984; Stadelman 1995a).

In our study, the yolk index values of noncoated eggs and eggs coated with MO and four MO:CH emulsions decreased with increased storage periods (interaction between coating treatments * storage periods, $P < 0.0001$) (Table 4.3). This decrease was more evident at 25 °C than at 4 °C, and was retarded by coating treatments. The yolk index values (0.29-0.32) of all coated eggs after 5 wk of storage at 25 °C were all higher than that (0.27) of noncoated eggs after 2 wk of storage (data not shown).

Table 4.1 Weight Loss (%)* of Noncoated and Coated Eggs during 5 wk of Storage at 25 °C and 20 wk at 4 °C

Coating**	25 °C			4 °C		
	1wk	3wk	5wk	5wk	10wk	20wk
CONTROL	$2.18\pm0.3^{D,a}$	$6.73\pm1.0^{B,a}$	-***	$4.17\pm0.8^{C,a}$	$7.06\pm0.7^{B,a}$	$9.78\pm1.3^{A,a}$
MO	$0.27\pm0.1^{D,b}$	$0.45\pm0.2^{CD,b}$	$0.72\pm0.3^{BC,a}$	$0.56\pm0.2^{CD,b}$	$1.07\pm0.5^{AB,b}$	$1.17\pm0.6^{A,b}$
TANDEM	$0.35\pm0.2^{C,b}$	$0.67\pm0.4^{BC,b}$	$0.87\pm0.4^{BC,a}$	$0.78\pm0.5^{BC,b}$	$0.98\pm0.5^{B,b}$	$1.63\pm0.7^{A,b}$
TIC	$0.34\pm0.2^{D,b}$	$0.56\pm0.2^{CD,b}$	$0.94\pm0.2^{B,a}$	$0.55\pm0.3^{CD,b}$	$0.72\pm0.2^{BC,b}$	$1.30\pm0.4^{A,b}$
TWEEN	$0.34\pm0.1^{D,b}$	$0.96\pm0.4^{B,b}$	$1.20\pm0.6^{AB,a}$	$0.43\pm0.1^{CD,b}$	$0.86\pm0.3^{BC,b}$	$1.63\pm0.5^{A,b}$
EFICACIA	$0.24\pm0.1^{B,b}$	$0.87\pm0.5^{A,b}$	$0.86\pm0.3^{A,a}$	$0.34\pm0.1^{B,b}$	$0.92\pm0.6^{A,b}$	$1.27\pm0.5^{A,b}$

*Means ± standard deviations of 10 measurements. Chitosan solution (CH) at 2% (w/v) was prepared in 1% of acetic acid (v/v).
**CONTROL = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); TANDEM = coating with MO:CH (25:75) emulsion by using emulsifier Tandem® 552K; TIC = coating with MO:CH (25:75) emulsion by using emulsifier Tic Pretested®Ticaloid®210 S Powder; TWEEN = coating with MO:CH (25:75) emulsion by using emulsifier Tween 80; and EFICACIA = coating with MO:CH (25:75) emulsion by using emulsifier Eficacia XE.
***Not determined as the Haugh unit was below 25 (C grade).
$^{A-D}$For each storage temperature, means with different superscripts in a row indicate significant differences ($P < 0.05$) by Tukey's Studentized Range (HSD) test.
$^{a-b}$Means with different superscripts in a column indicate significant differences ($P<0.05$) by Tukey's Studentized Range (HSD) test.

Table 4.2 Haugh Unit* and Grade** of Noncoated and Coated Eggs during 5 wk of Storage at 25 °C and 20 wk at 4 °C

Coating***	0wk	25 °C			4 °C		
		1wk	3wk	5wk	5wk	10wk	20wk
CONTROL	75.62±3.4^{A}	57.01±8.3^{B,c}	23.78±9.1^{C,b}	-****	60.22±6.3^{B,b}	54.64±7.5^{B,c}	50.84±7.4^{B,a}
	AA**	B	C		A	B	B
MO	75.62±3.4^{A}	66.71±6.7^{AB,ab}	43.62±7.8^{D,a}	38.08±9.5^{D,a}	69.61±6.5^{A,a}	60.31±4.4^{BC,bc}	56.79±6.8^{C,a}
	AA	A	B	B	A	A	B
TANDEM	75.62±3.4^{A}	67.06±6.5^{B,ab}	45.96±7.1^{D,a}	37.02±6.0^{E,a}	64.35±6.2^{BC,ab}	60.84±3.9^{BC,bc}	58.43±7.3^{C,a}
	AA	A	B	B	A	A	B
TIC	75.62±3.4^{A}	72.79±5.2^{A,a}	51.28±4.9^{B,a}	38.89±7.3^{C,a}	70.44±3.6^{A,a}	71.73±3.5^{A,a}	55.34±10.1^{B,a}
	AA	AA	B	B	A	A	B
TWEEN	75.62±3.4^{A}	62.42±6.2^{BC,bc}	50.41±9.5^{D,a}	37.52±13.2^{E,a}	70.16±5.0^{AB,a}	63.37±6.4^{BC,b}	59.44±7.0^{CD,a}
	AA	A	B	B	A	A	B
EFICACIA	75.62±3.4^{A}	69.69±6.6^{AB,ab}	49.87±5.4^{D,a}	42.70±8.2^{D,a}	67.25±5.6^{B,ab}	65.44±5.2^{BC,ab}	58.86±3.8^{C,a}
	AA	A	B	B	A	A	B

*Means ± standard deviations of 10 measurements. Chitosan solution (CH) at 2% (w/v) was prepared in 1% of acetic acid (v/v).
**Quality grades of eggs based on the Haugh unit values where AA is above 72; A, 71 to 60; B, 59 to 31 and C is below 30.
***CONTROL = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); TANDEM = coating with MO:CH (25:75) emulsion by using emulsifier Tandem® 552K; TIC = coating with MO:CH (25:75) emulsion by using emulsifier Tic Pretested®Ticaloid®210 S Powder; TWEEN = coating with MO:CH (25:75) emulsion by using emulsifier Tween 80; and EFICACIA = coating with MO:CH (25:75) emulsion by using emulsifier Eficacia XE.
****Not determined as the Haugh unit was below 25 (C grade).
$^{A-E}$For each storage temperature, means with different superscripts in a row indicate significant differences ($P < 0.05$) by Tukey's Studentized Range (HSD) test.
$^{a-c}$Means with different superscripts in a column indicate significant differences ($P < 0.05$) by Tukey's Studentized Range (HSD) test.

Under similar storage temperature (25 °C), Waimaleongora-Ek and others (2009) also observed a higher yolk index value (0.37) of MO (26 mPa s)-coated eggs after 5 wk compared with that (0.31) of noncoated eggs after 2 wk of storage. Data from Tables 4.1 (weight loss), 4.2 (Haugh unit) and 4.3 (yolk index) collectively imply that coating with MO or MO:CH emulsion (regardless of emulsifier types) can preserve both albumen and yolk quality for at least 3 more wk compared with observed for noncoated eggs at 25 °C.

At 4 °C, the decline of the yolk index values was less obvious throughout 20 wk of storage. The yolk index value of noncoated eggs was not significantly different ($P > 0.05$) from those of eggs coated with MO and four MO:CH emulsions after 20 wk (0.38 against 0.38-0.42). A similar trend was observed by Jirangrat and others (2010) in that the decline of the yolk index was slight throughout 15 wk of storage at 4 °C, and that the yolk index values of noncoated and MO-coated eggs were not significantly different ($P > 0.05$) after 10 wk of storage. Kamel and others (1980) also reported comparable yolk index value between noncoated and MO-coated eggs after 75 days of storage at 5 °C (0.40 against 0.39). All these confirm that the migration of water from the albumen to the yolk is a function of storage temperatures with a faster migration rate occurring at higher temperatures, which was observed in this study (Table 4.3).

4.3.4 Effects of MO and MO:CH Emulsion on Albumen pH

The albumen pH can also be used as an indicator of the albumen quality of eggs (Scott and Silversides 2000). Freshly laid eggs contain 1.44-2.05 mg CO_2/g of albumen (Keener and others 2001; Biladeau and Keener 2009) and have an albumen pH value of 7.6-8.7 (Goodwin and others 1962; Rhim and others 2004; Waimaleongora-Ek and others 2009). During storage, carbon dioxide escapes via eggshell pores, resulting in thinning of the thick albumen and an increased albumen pH value up to 9.6-9.7 (Li-Chan and Nakao 1989; Kemps and others 2007).

In our present study, albumen pH values of eggs coated with MO and/or four MO:CH emulsions were significantly ($P < 0.05$) lower than those of noncoated eggs throughout 5 wk of storage at 25 °C and 20 wk of storage at 4 °C (Figure 4.1). This implies that MO and MO:CH emulsions as coating materials could retard loss of carbon dioxide through eggshell pores by acting as a gas barrier (Obanu and Mpieri 1984; Stadelman 1995b). There were no significant differences ($P > 0.05$) in albumen pH among five (MO and MO:CH) coated eggs, and neither were among four MO:CH emulsion-coated eggs after 5 wk of storage at 25 °C and after 20 wk of storage at 4 °C.

The pattern for changes in albumen pH during the storage periods differed between noncoated and coated eggs (interactions between coating treatments * storage periods, $P < 0.0001$) as well as between storage temperatures (25 °C against 4 °C) (Figure 4.1). The albumen pH of noncoated eggs slightly increased from an initial value of 9.20 to 9.28 after 3 wk of storage at 25 °C. However, the opposite was observed for the albumen pH of eggs coated with MO and/or four MO:CH emulsions, with the pH gradually decreasing from 9.20 to 8.58-8.69 after 5 wk of storage at 25 °C (Figure 4.1).

Similarly, Kamel and others (1980) reported the increased albumen pH of noncoated eggs from an initial value of 8.64 to 9.51 after 5 wk of storage at 25 °C. Jirangrat and others (2010) observed that the albumen pH of noncoated eggs markedly ($P < 0.05$) increased from 8.71 to 9.42 while that of MO-coated eggs slightly decreased (but not significant, $P > 0.05$) from 8.71 to 8.64 after 5 wk of storage at 25 °C. In contrast, at 4 °C (Figure 4.1), the albumen pH of noncoated eggs decreased from an initial value of 9.20 to 9.15 after 5 wk and to 9.05 after 20 wk of storage. On the other hand, the albumen pH of eggs coated with MO and/or four MO:CH emulsions decreased from 9.20 to 8.84-8.90 after 5 wk and to 8.58-8.62 after 20 wk.

Table 4.3 Yolk Index* of Noncoated and Coated Eggs during 5 wk of Storage at 25 °C and 20 wk at 4 °C

Coating**	0wk	25 °C			4 °C		
		1wk	3wk	5wk	5wk	10wk	20wk
CONTROL	0.43^{A}	$0.36^{C,c}$	$0.23^{D,b}$	-***	$0.40^{AB,a}$	$0.36^{C,b}$	$0.38^{BC,a}$
MO	0.43^{A}	$0.39^{A,abc}$	$0.33^{B,a}$	$0.29^{B,a}$	$0.41^{A,a}$	$0.39^{A,ab}$	$0.40^{A,a}$
TANDEM	0.43^{A}	$0.38^{C,bc}$	$0.32^{D,a}$	$0.29^{D,a}$	$0.40^{ABC,a}$	$0.39^{BC,ab}$	$0.42^{AB,a}$
TIC	0.43^{A}	$0.42^{A,a}$	$0.33^{B,a}$	$0.30^{B,a}$	$0.41^{A,a}$	$0.41^{A,a}$	$0.39^{A,a}$
TWEEN	0.43^{A}	$0.38^{B,bc}$	$0.33^{C,a}$	$0.32^{C,a}$	$0.41^{AB,a}$	$0.40^{AB,a}$	$0.38^{B,a}$
EFICACIA	0.43^{AB}	$0.40^{AB,ab}$	$0.32^{C,a}$	$0.32^{C,a}$	$0.44^{A,a}$	$0.40^{B,a}$	$0.40^{AB,a}$

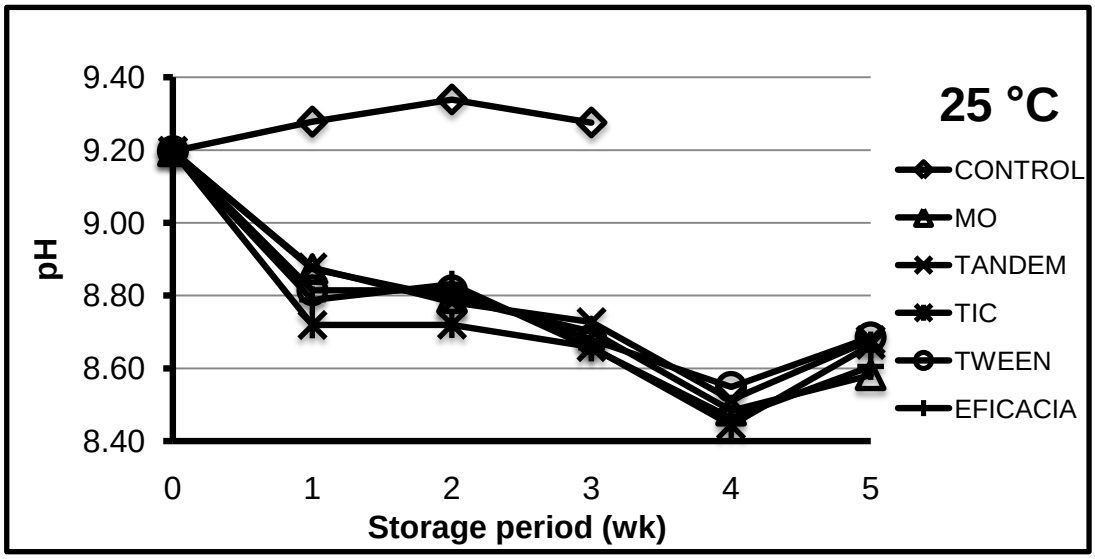
*Means of 10 measurements. Standard deviations for all yolk-index values ranged from 0.01 to 0.04. Chitosan solution (CH) at 2% (w/v) was prepared in 1% of acetic acid (v/v).

**CONTROL = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); TANDEM = coating with MO:CH (25:75) emulsion by using emulsifier Tandem® 552K; TIC = coating with MO:CH (25:75) emulsion by using emulsifier Tic Pretested®Ticaloid®210 S Powder; TWEEN = coating with MO:CH (25:75) emulsion by using emulsifier Tween 80; and EFICACIA = coating with MO:CH (25:75) emulsion by using emulsifier Eficacia XE.

***Not determined as the Haugh unit was below 25 (C grade).

$^{A-D}$For each storage temperature, means with different superscripts in a row indicate significant differences ($P < 0.05$) by Tukey's Studentized Range (HSD) test.

$^{a-c}$Means with different superscripts in a column indicate significant differences ($P < 0.05$) by Tukey's Studentized Range (HSD) test.



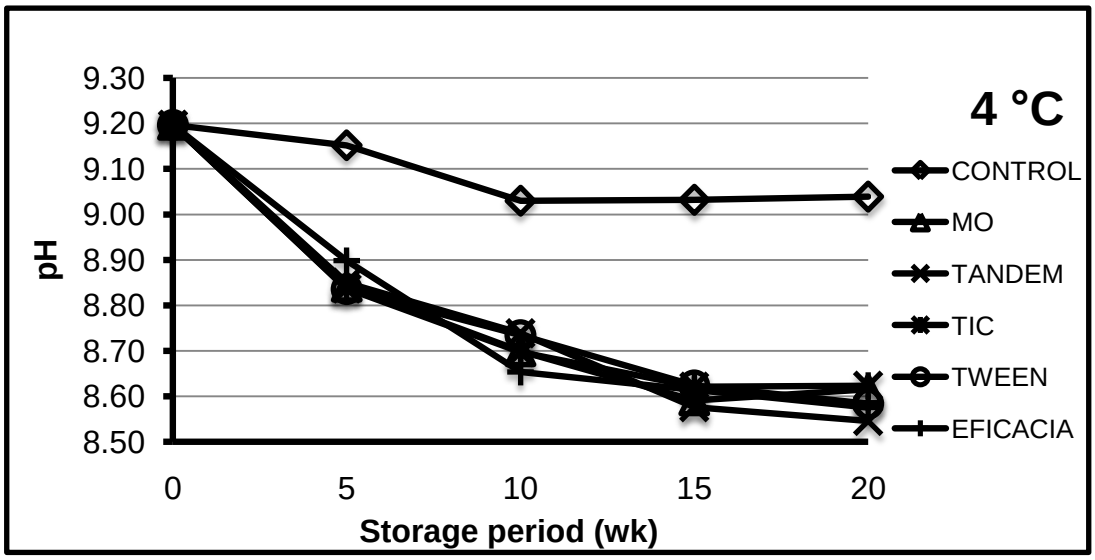


Figure 4.1 Variations in Albumen pH* of Noncoated and Coated Eggs** during 5 wk of Storage at 25 °C and 20 wk at 4 °C.

*Each point represents an average value of 10 measurements. For all data, standard deviation values ranged from 0.03 to 0.22.
**CONTROL = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); TANDEM = coating with MO:CH (25:75) emulsion by using emulsifier Tandem® 552K; TIC = coating with MO:CH (25:75) emulsion by using emulsifier Tic Pretested®Ticaloid®210 S Powder; TWEEN = coating with MO:CH (25:75) emulsion by using emulsifier Tween 80; and EFICACIA = coating with MO:CH (25:75) emulsion by using emulsifier Eficacia XE.

Jirangrat and others (2010) also reported that the albumen pH of noncoated and MO-coated eggs decreased from 8.71 to 8.53 and 7.96, respectively, after 15 wk of storage at 4 °C. Biladeau and Keener (2009) observed that the albumen pH of MO-coated eggs decreased from an initial value of 8.35 to 7.96 after 12 wk of storage at 7 ± 2 °C. The decrease in albumen pH during storage may be due to the continuing breakdown of the constituents in egg white and/or a change in the bicarbonate buffer system (Obanu and Mpieri 1984; Biladeau and Keener 2009). However, differences in initial egg quality, egg size, and storage conditions (temperature, humidity, and period) may affect albumen pH before and after storage (Muller 1958; Goodwin and others 1962; Sabrani and Payne 1978; Scott and Silversides 2000; Silversides and Scott 2001).

4.3.5 Person Correlation Coefficients (R) Among the Internal Quality Parameters

The correlation matrix of four internal quality parameters (weight loss, Haugh unit, yolk index and albumen pH) is presented in Table 4.4. At 4 °C, less significant correlations ($P < 0.01$) were found among albumen pH, Haugh unit and yolk index compared with those at 25 °C (Table 4.4). At 25 °C, a significant negative correlation ($P < 0.01$) was found between the weight loss and Haugh unit, and was higher in the noncoated eggs group (-0.89) compared with those in the MO and four MO:CH emulsion coated eggs groups (-0.46 to -0.65). This can be explained by the ability of MO and MO:CH as coating materials to decrease the loss of water and, in much lesser extent, the loss of CO_2 from eggs and to decrease their rate of albumen deterioration as shown in Tables 4.1 and 4.2. At 25 °C, the yolk index was significantly positively correlated ($P < 0.01$) with the Haugh unit for noncoated eggs and all coated eggs (0.68 to 0.93). Between albumen pH and Haugh unit, a significant negative correlation (-0.56, $P < 0.01$) was observed for noncoated eggs while significant positive correlations (0.38-0.57, $P < 0.01$) was observed for all coated eggs at 25 °C.

Table 4.4 Pearson Correlation Coefficients (R) Among Internal Quality Parameters of Noncoated and Coated Eggs during 5 wk of Storage at 25 °C and 20 wk at 4 °C

Coating*		25 °C			4 °C		
		Haugh unit	**Yolk index**	**Albumen pH**	**Haugh unit**	**Yolk index**	**Albumen pH**
CONTROL	Weight Loss (%)	-0.89**	-0.89**	0.44**	-0.81**	-0.65**	-0.76**
	Haugh unit		0.93**	-0.56**		0.70**	0.55**
	Yolk index			-0.66**			0.44**
MO	Weight Loss (%)	-0.60**	-0.63**	-0.53**	-0.16	-0.27	-0.50**
	Haugh unit		0.81**	0.57**		0.34	0.30
	Yolk index			0.54**			0.07
TANDEM	Weight Loss (%)	-0.47**	-0.36	-0.45**	-0.32	0.07	-0.44**
	Haugh unit		0.82**	0.46**		0.28	0.31
	Yolk index			0.33			-0.13
TIC	Weight Loss (%)	-0.65**	-0.67**	-0.27	-0.45**	-0.18	-0.43**
	Haugh unit		0.86**	0.38**		0.50**	0.50**
	Yolk index			0.36			0.13
TWEEN	Weight Loss (%)	-0.46**	-0.50**	-0.22	-0.21	-0.08	-0.49**
	Haugh unit		0.68**	0.38**		0.51**	0.38
	Yolk index			0.41**			0.45**
EFICACIA	Weight Loss (%)	-0.51**	-0.49**	-0.45**	-0.40	-0.39	-0.30
	Haugh unit		0.82**	0.48**		0.45**	0.36
	Yolk index			0.32			0.39

*CONTROL = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); TANDEM = coating with MO:CH (25:75) emulsion by using emulsifier Tandem® 552K; TIC = coating with MO:CH (25:75) emulsion by using emulsifier Tic Pretested®Ticaloid®210 S Powder; TWEEN = coating with MO:CH (25:75) emulsion by using emulsifier Tween 80; and EFICACIA = coating with MO:CH (25:75) emulsion by using emulsifier Eficacia XE.

**Significant at $P < 0.01$ for the null hypothesis (Ho): r = zero.

4.3.6 Microbiological Analysis

Results of total plate count (TPC) and *Salmonella* spp. detection for internal content of noncoated eggs and eggs coated with MO and four MO:CH emulsions before and after 5 wk of storage at 25 °C are shown in Table 4.5. At wk 0, TPC of noncoated eggs (CONTROL) was not detectable. After 5 wk of storage, TPC of all noncoated and coated eggs ranged from 0 to 2.2×10^2 CFU/g. No *Salmonella* spp. colonies were detected in all noncoated and coated eggs before and after 5 wk of storage at 25 °C. According to Ricke and others (2001), eggs products should meet the specification of less than 2.5×10^4 CFU/g for aerobic plate count (APC) and a negative presence of *Salmonella* spp. ICMSF (1986) establishes a presence between 5.0×10^4 and 1.0×10^6 CFU/g for APC and zero tolerance for *Salmonella* spp. as limits for egg products. Thus, our present results (Table 4.5) indicate that noncoated and coated eggs were all microbiologically safe throughout the 5 wk of storage at 25 °C.

4.4 Conclusions

Although refrigeration is considered as the single most important treatment for preserving eggs and retarding their internal quality deterioration, in this study, a synergistic effect between refrigeration and coating (MO and/or MO:CH, irrespective of the emulsifier types) was evidenced in maintaining lower weight losses (<2%) and albumen and yolk quality during 5 wk storage at 25 °C and 20 wk at 4°C. At 25 °C, MO and/or four MO:CH emulsions coatings minimized the weight loss and preserved the albumen and yolk quality of eggs for at least 3 wk longer than those observed for noncoated eggs. At 4 °C, the grade of noncoated eggs decreased from AA to A and B after 5 and 10 wk, respectively. Nonetheless, all coated eggs changed from AA to A grade after 5 wk and maintained this A grade up to 10 wk, that is, 5 wk longer than that of nonocoated eggs).

Table 4.5 Microbiological Analysis of Noncoated and Coated Eggs Before and After 5 wk of Storage at 25 °C

Treatments*	Total Plate count (Pour Plate)	*Salmonella* spp. detection
	CFU/g of egg	
Wk 0 - CONTROL	Not detectable	Negative
Wk 5 - CONTROL	<250	Negative
Wk 5 - MO	<250	Negative
Wk 5 - TANDEM	<250	Negative
Wk 5 - TIC	<250	Negative
Wk 5 - TWEEN	<250	Negative
Wk 5 - EFICACIA	<250	Negative

*CONTROL = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); TANDEM = coating with MO:CH (25:75) emulsion by using emulsifier Tandem® 552K; TIC = coating with MO:CH (25:75) emulsion by using emulsifier Tic Pretested®Ticaloid®210 S Powder; TWEEN = coating with MO:CH (25:75) emulsion by using emulsifier Tween 80; and EFICACIA = coating with MO:CH (25:75) emulsion by using emulsifier Eficacia XE.

Our preliminary work on sensory discrimination of eggs coated with MO indicated that for the surface odor and color, consumers could not significantly ($P > 0.05$) differentiate the MO-coated eggs from the control noncoated eggs (49.52 and 48.96% for unipolar R-index values, respectively; critical R-index value = 56.35%, one tailed test, $\alpha = 0.05$). However, consumers significantly differentiate MO-coated from noncoated eggs for overall surface (R-index = 56.40); this was due to the significant bipolar R-indices for surface smoothness and glossiness (64.44 and 61.43% for R-index$_{more}$ values, respectively; critical R-index value = 57.53%, a two-tailed test, $\alpha = 0.05$). A large sensory discrimination study is being developed to compare MO- and emulsion-coated eggs against noncoated eggs in our laboratory. Further long-term storage studies are also needed with different initial egg qualities (initial Haugh unit of 75.62, yolk index of 0.43 in this study) since the quality and shelf-life of eggs may vary depending on this factor under room and refrigerated temperatures.

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CHAPTER 5. EFFECTS OF INITIAL ALBUMEN QUALITY AND MINERAL OIL-CHITOSAN EMULSION COATING ON INTERNAL QUALITY AND SHELF-LIFE OF EGGS DURING ROOM TEMPERATURE STORAGE

5.1 Introduction

Eggs are widely consumed throughout the world; therefore eggs represent an important segment of the world food industry and an important commodity of international trades (Stadelman, 1995b). According to the Food and Agriculture Organization (FAO), the production of egg worldwide in 2009 totaled 67.4 million metric tons, which represented an increase of 1.97% in the production compared with that of 2008 (FAO, 2010). However, interior quality deterioration and microbial contamination during storage cause a serious economic loss to the poultry industry (Stadelman, 1995b; Wong *et al*., 1996). During storage, the loss of moisture and carbon dioxide via the shell pores causes negative quality changes in albumen and yolk as well as weight loss of eggs (Stadelman, 1995b). Altough, low temperature refrigeration is considered the single most important preservation treatment for eggs (in the United States, eggs are required to be refrigerated at 7 °C or below), in some developing regions of the world, refrigeration of eggs is seldom practiced, and coating of eggs is an alternative and effective method to preserve the internal quality.

Coating materials including mineral oil (Obanu & Mpieri, 1984; Waimaleongora-Ek *et al*., 2009; Jirangrat *et al*., 2010) and chitosan (No *et al*., 2005; Kim *et al*., 2006, 2007, 2008) have been applied to the surface of egg shells for preserving the internal quality of eggs. Despite the fact that chitosan films are efficient barriers against permeation of oxygen, these films act as low water barriers due to their strong hydrophilic properties (Butler *et al*., 1996). Mineral oil is another coating material currently used to preserve the internal quality of eggs. Nonetheless, a problem associated with mineral oil coating is that oil dries very slowly compared with chitosan

solution when applied on the surface of the eggshell without wiping it dry (Torrico *et al*., 2010a). Thus, coating of eggs with emulsion of mineral oil (MO) and chitosan solution (CH) may considerably reduce the drying time. More recently, Torrico *et al*. (2010a) found that three emulsions of MO and CH (MO:CH = 75:25, 50:50 and 25:75 ratios) were as equally effective as MO but were more effective than CH as coating materials in preserving the internal quality of eggs at room temperature storage (25 °C). Among these emulsions, only MO:CH = 25:75 emulsion-coated eggs were not sensorially glossier than noncoated eggs.

Other important factors that affect internal quality of eggs during storage are initial egg quality, egg size and storage conditions (Muller, 1958; Goodwin *et al*., 1962; Silversides & Scott, 2001). Sabrani & Payne (1978) reported a significant interaction ($P < 0.05$) between age of hens (young and old hens with eggs having different initial internal qualities) and coating material (linseed oil) during 24 days of storage at 28 °C, in which oiling significantly decreases the rate of internal quality deterioration. However, shell color and visual appearance of eggs may be altered by the coating material used. Wong *et al*. (1996) reported that egg shells coated with mineral oil possessed a higher L^* value (lightness) than noncoated eggs (87.05 vs. 83.90), possibly due to a glossier surface. Caner & Cansiz (2008) observed changes in L^* (ranged from 92.89 to 93.73), a^* (−0.40 to −0.57, indicates greenness), and b^* (1.85 to 2.83, indicates yellowness) values of noncoated and chitosan coated eggs after 4 weeks of storage at 25 °C. To date, there is no information available on the combined effects of MO:CH emulsion as a coating material and different initial albumen qualities (expressed as Haugh unit) on the internal quality and shelf life of eggs during room temperature storage.

The objective of this study was to evaluate the effects of MO and MO:CH emulsion (MO:CH = 25:75 ratio) as coating materials in preserving the internal quality (weight loss,

Haugh unit, yolk index, albumen pH) of coated eggs having three different initial albumen qualities at coating, during 5 weeks of storage at 25 °C. Consumer perception and purchase intent of freshly coated eggs were also evaluated by a sensory discrimination test at week 0 of storage.

5.2 Materials and Methods

5.2.1 Materials

Mineral oil (viscosity = 34 mPa s; transparent, odorless and food-grade) was obtained from Ste Oil Company® Inc. (San Marcos, TX, USA). Chitosan (molecular weight = 223 kDa), acid soluble and white-colored powder prepared from crab leg shell, was purchased from Biotech (Mokpo, Korea). Emulsifier Eficacia XE (Acacia gum purified and instantised) was obtained from Colloides Naturels International (Rouen Cedex, France). This emulsifier type was selected among others for its ability to form a stable emulsion between mineral oil (MO) and chitosan (CH) at a ratio of MO:CH = 25:75 (Torrico *et al*., 2010b).

Unwashed, feces-free, white-shell eggs were obtained from three different batches of hens (52-weeks-old hens for 'High', 48-weeks-old hens for 'Medium' and 54-weeks-old hens for 'Low' initial Haugh unit before coating). All eggs in this study were from Hyline W-36 hens with a weight range of 50-70 g, and were obtained from Cal-Maine Foods (Jackson, MS, USA). Immediately after collected from the farm, all eggs were screened for defects and desirable weight range. Eggs from batches of 52-weeks-old and 48-weeks-old hens (for 'High' and 'Medium' initial Haugh unit before coating) were stored in the cold room (approximately 7 °C) overnight. Before coating, eggs were kept at room temperature (approximately 25 °C) for 2 hours to avoid water condensation on the egg surface that could interfere with coating. On the other hand, eggs from a batch of 54-weeks-old hens (for 'Low' initial Haugh unit before coating)

were stored at room temperature (approximately 25 °C) for 4 days to quickly decrease the initial albumen quality (expressed as Haugh unit) before coating. Data from 'Medium' initial Haugh unit before coating were adopted from Torrico *et al*. (2010b).

5.2.2 Preparation of Mineral Oil/Chitosan Solution Emulsion

Chitosan solution was prepared by dissolving chitosan in 1% (v/v) acetic acid at 2% (w/v) concentration (Kim *et al*., 2009). Emulsion of mineral oil (MO) and chitosan solution (CH) was prepared at a fixed ratio of 25:75 MO:CH (Torrico *et al*., 2010a) by adding 1% of emulsifier Eficacia XE as described in following procedure: Emulsifier was added to CH and mixed by using a hand blender (Model # 59780R, Hamilton Beach® Brands Canada, Inc., Picton, Ontario, Canada) at a low speed for 2 min at 25 °C. The mixture stood for 30 min at room temperature, and subsequently MO was added and mixed by using the hand blender at a high speed for 6 min at 25 °C. The CH and MO:CH emulsion were prepared on the day of the coating experiment.

5.2.3 Coating Treatment and Storage of Eggs

Eggs with three different intial albumen qualities (expressed as Haugh unit) before coating were evaluated: (1) 'High' = egg with an initial Haugh unit of 87.76, (2) 'Medium' = egg with an initial Haugh unit of 75.62, and (3) 'Low' = egg with an initial Haugh unit of 70.88 before coating. Eggs were individually weighed with a balance (TS400, Ohaus Corp., Florham Park, NJ, USA), coated with MO or MO:CH emulsion by using a sponge brush, and dried overnight at room temperature (25 ± 2 °C). The noncoated eggs served as the control. Coating treatment with 100% CH was excluded in this study because CH was found to be less effective than MO and 25:75 MO:CH emulsion as a coating material in preserving the internal quality of eggs in our previous investigation (Torrico *et al*., 2010a). All eggs were placed in a small-end down position (Kim *et al*., 2009) in cardboard egg racks and stored at room temperature (25 ± 2

°C) (50 eggs/treatment) and at 60-65% of RH. For determination of weight loss, Haugh unit, yolk index, and albumen pH, ten eggs per each treatment were taken weekly for 5 weeks at 25 °C. After 3 weeks of storage at room temperature (25 °C), all noncoated eggs were disregarded due to their extremely low albumen quality (Haugh units were below 25, C grade).

5.2.4 Determination of Weight Loss

Weight loss (%) of the coated whole egg during storage was calculated as {[initial whole egg weight (g) after coating at day 0 – whole egg weight (g) after storage]/initial whole egg weight (g) after coating at day 0} × 100. Weight loss (%) of the control noncoated whole egg was calculated as {[initial whole egg weight (g) at day 0 – whole egg weight (g) after storage]/initial whole egg weight (g) at day 0} × 100. The weight of whole eggs was measured with a balance (TS400S, Ohaus Corp., Florham Park, NJ, USA). Ten measurements per treatment were taken.

5.2.5 Determination of Haugh Unit and Yolk Index

The height of albumen and yolk was measured with a tripod micrometer (Model S-6428, B.C. Ames Inc., Melrose, MA, USA). The yolk width was measured with a digital caliper (General Tools & Instruments, New York, NY, USA). The Haugh unit was calculated as 100 log (H – 1.7 $W^{0.37}$+ 7.57), where H is the albumen height (mm) and W is the weight (g) of egg (Haugh, 1937). The yolk index was calculated as yolk height/yolk width (Stadelman, 1995a; Lee *et al*., 1996). Ten measurements per treatment were taken.

5.2.6 Measurement of Albumen pH

After measurement of Haugh unit and yolk index, the albumen was separated from the yolk. The thin and thick albumen were mixed thoroughly prior to measuring pH with a pH meter

(IQ150, IQ Scientific Instruments, San Diego, CA, USA). Ten measurements per treatment were taken.

5.2.7 Color Measurement of Egg Shells

Color of egg shells was measured with a Minolta hand-held spectrophotometer model CM-508d (Minolta Co., Ltd., Osaka, Japan) with the operation conditions of illuminant D65 and 2° observer, obtaining the color parameters L^* (lightness), a^* (+ for redness and – for greenness) and b^* (+ for yellowness and – for blueness). Color measurement was conducted with eggs with the 'High' initial Haugh unit before coating. For color measurements, eggs were horizontally placed on a cardboard rack, and the lens of the Minolta hand-held spectrophotometer was placed flat against the surface of the shell pointing at the longitudinal middle of the egg. Three measurements were made at different locations around the surface of the egg shell and were averaged. Five eggs (replicates) per each treatment were measured weekly for 5 weeks at 25 °C. Whiteness index (WI) of egg shells was calculated as $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$. The size of the color difference (ΔE^*) of egg shells by using noncoated egg shells at week 0 as a reference (L^*_o, a^*_o and b^*_o) was calculated as $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ where $\Delta L^* = L^* - L^*_o$, $\Delta a^* = a^* - a^*_o$ and $\Delta b^* = b^* - b^*_o$.

5.2.8 Sensory Discrimination and Consumer Purchase Intent

Consumers (n = 110) were recruited from Baton Rouge, Louisiana, USA to participate in the sensory discrimination of coated eggs (with MO and 25:75 MO:CH emulsion) compared with control noncoated eggs at week 0. Eggs for the sensory discrimination analysis were retrieved from the 'High' initial Haugh unit before coating batch. Consumers were first presented with the labeled control egg, followed by two unlabeled coated eggs and one unlabeled control (to ascertain the "noise" level). Unlabeled eggs were individually compared to the labeled

control for specified attributes. For surface glossiness and smoothness, consumers were asked to indicate whether the unlabeled coated and unlabeled control eggs were perceived as "more," "the same," or "less" in the specified attribute compared with that of the labeled control, and whether they were "sure" or "unsure" about their decision; in this case, as the direction of a given attribute was of interest, the bipolar R-index was used. For surface odor and color, and overall surface appearance, consumers were asked if the unlabeled coated and unlabeled control eggs were "different from" or "the same as" the labeled control, and whether their decision was "sure" or "unsure"; in this case, as the direction of a given attribute was not measured, the unipolar R-index was used. Consumers self-paced their evaluation. Afterward, these consumers evaluated purchase intent for all eggs on a yes/no scale, and it was reported as % positive purchase intent.

5.2.9 Statistical Analysis

For internal quality parameters (Haugh unit, weight loss, yolk index and albumen pH) of eggs, mean ± standard deviation values were reported based on ten measurements (eggs) per treatment. Conversely, for color parameters [whiteness index (WI) and color difference (ΔE^*)] of egg shells, mean ± standard deviation values were reported based on five measurements per treatment. Data generated from the experiment was carried out in a Complete Randomized Design (CRD) [6×3×3 factorial (6 storage time periods, 3 coating treatments and 3 initial albumen qualities before coating) for internal quality parameters and 6×3 factorial (6 storage time periods and 3 coating treatments) for color parameters]. Analysis of Variance (ANOVA) was used to determine differences among main effects and all their interactions at a significance level (α) of 0.05. When main effects were significant, the Tukey's studentized range test at α=0.05 was performed for post-hoc multiple comparisons. All analyses were done with the SAS software (SAS, 2003).

Data obtained from the sensory discrimination test were converted into frequency counts, and then the R-index was calculated for each attribute and expressed as a percentage of sensory discrimination (% R-index). The bipolar R-index for surface glossiness and smoothness, and the unipolar R-index for odor, color and overall surface appearance were computed from the equations as in Bhale *et al*. (2003).

The significance of the R-index was determined using the table provided by Bi and O'Mahony (2007). At the significance of 5%, the observed R-index value was significant if it exceeded the critical R-index of 56.35% for the unipolar R-index test. For the bipolar R-index, the result was significant if it exceeded the critical R-index of 57.53%. For the purchase intent (%), the Cochran's Q test and simultaneous confidence interval testing were used for multiple comparisons.

5.3 Results and Discussion

5.3.1 Effects of Mineral Oil and 25:75 MO:CH Emulsion as Coating Materials on Haugh Unit

The Haugh unit (HU), calculated by taking the logarithmic function of albumen height corrected by the egg weight, is an expression that measures the albumen quality of eggs. The higher the HU, the better the albumen quality of eggs (Zeidler, 2001; Stadelman, 1995a). During storage of eggs, the HU decreases principally due to a progressive deterioration of the thick albumen that changes into thin albumen (thinning). Recent studies associate this quality decline with chemical alterations of albumen proteins, primarily ovomucin. (Stevens, 1996). Table 5.1 and 5.2 show the changes in the HU of noncoated, MO and 25:75 MO:CH emulsion coated eggs (using eggs with different initial HU before coating) during 5 weeks of storage at 25 °C (interaction between coating treatments * storage periods * initial qualities, $P = 0.002$, Table 5.1).

Table 5.1 Anova Table for Haugh Unit and Weight Loss by Using Proc GLM for a CRD Design with a 6x3x3 Factorial Treatment Arrangement*

		Haugh unit			Weight loss		
Source*	**DF****	**Mean square****	**Pr > F****	**Rank**	**Mean square****	**Pr > F****	**Rank**
Storage periods	5	20957.43	<.0001	1***	78.26	<.0001	2***
Coating treatments	2	13374.79	<.0001	2	527.71	<.0001	1
Initial qualities	2	9701.09	<.0001	3	16.76	<.0001	3
Initial qualities*Coating treatments	4	1177.72	<.0001	1****	7.10	<.0001	2****
Coating treatments*Storage periods	10	819.28	<.0001	2	41.35	<.0001	1
Initial qualities*Storage periods	10	277.23	<.0001	3	1.69	<.0001	3
Interaction of all three factors	18	139.49	0.002	4	1.37	<.0001	4

*Anova=Analysis of Variance. GLM=Generalized Linear Model. CRD=Complete Randomized Design. 6x3x3=6 storage periods (0, 1, 2, 3, 4 and 5 weeks), 3 initial albumen qualities of eggs before coating based on the Haugh unit (HU) (High=87.76 HU, Medium=75.62 HU and Low=70.88) and 3 coating treatments (Control, MO and 25:75 MO:CH emulsion). Control = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75 by using Eficacia XE emulsifier. (CH) = chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid. Data for Medium initial HU before coating were adopted from Torrico et al. (2010b).

**DF= Degrees of Freedom. Mean square=Sums squares of each source of variation/DF. F value=Mean square/Mean square error. Mean square error of Haugh unit=61.76. Mean square error for weight loss=0.15.

***Ranking of the main effects (source of variation) according to their mean squares (The lower rank value indicates the higher impact on the weight loss).

****Ranking of the interactions (source of variation) according to their mean squares (The lower rank value indicates the higher impact on the Haugh unit).

Table 5.2 Haugh Unit* (HU) and Grade** of Noncoated and Coated Eggs during 5 Weeks of Storage at 25 °C at Different Initial Albumen Qualities Before Coating

Initial HU***	Coating****	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
High	Control	87.8±5.0	51.8±5.4[c]	43.1±9.7[b]	29.9±7.5[b]	–*****	–*****
		AA	B	B	C	C	C
	MO	87.8±5.0	75.5±6.7[a]	70.8±5.9[a]	64.6±7.8[a]	60.7±4.3[a]	55.9±10.5[a]
		AA	AA	A	A	A	B
	25:75 MO:CH	87.8±5.0	66.7±8.0[b]	65.5±4.0[a]	64.3±3.6[a]	63.9±7.1[a]	53.2±8.4[a]
		AA	A	A	A	A	B
Medium	Control	75.6±3.4	57.0±8.3[b]	39.3±7.4[b]	23.8±9.1[b]	–*****	–*****
		AA	B	B	C	C	C
	MO	75.6±3.4	66.7±6.7[a]	58.6±6.5[a]	43.6±7.8[a]	44.4±10.4[a]	38.1±9.5[a]
		AA	A	B	B	B	B
	25:75 MO:CH	75.6±3.4	69.7±6.6[a]	55.3±6.5[a]	49.9±5.4[a]	43.2±8.9[a]	42.7±8.2[a]
		AA	A	B	B	B	B
Low	Control	70.9±8.0	56.0±7.3[a]	35.9±13.8[b]	28.3±8.5[b]	–*****	–*****
		A	B	B	C	C	C
	MO	70.9±8.0	62.4±7.7[a]	46.4±9.2[ab]	44.9±10.0[a]	33.5±10.8[a]	38.8±10.4[a]
		A	A	B	B	B	B
	25:75 MO:CH	70.9±8.0	57.1±11.1[a]	48.8±6.9[a]	33.9±10.3[b]	37.7±9.4[a]	39.1±10.3[a]
		A	B	B	B	B	B

*Means ± standard deviations of 10 measurements. [a-c]Means with different superscripts within a column and within an initial HU before coating indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD).

**Based on the Haugh unit values (HU); AA, above 72; A, 71 to 60; B, 59 to 31; C, below 30.

***Initial albumen qualities of eggs before coating: High=87.76 HU, Medium=75.62 HU and Low=70.88 HU. Data for Medium initial HU before coating were adopted from Torrico et al. (2010b).

****Control = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75 by using Eficacia XE emulsifier. (CH) = chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.

*****Not determined as the HU of noncoated eggs was below 25 after 3 weeks.

ANOVA of HU means (Table 5.1) illustrates that differences of three main factors, (1) storage periods, (2) coating treatments, (3) initial albumen qualities, and all their interactions, (1) initial qualities by coating treatments, (2) coating treatments by storage periods, (3) initial qualities by storage periods and (4) interaction of all three factors, were significant ($P < 0.05$) in that order, ranked by their mean square (MS). The overall mean square error (MSE) of the HU was 61.76 that represents a standard error of 7.86. Considering these three main factors, storage period was the most influential factor affecting the HU. The HU significantly decreased with increased storage periods; however, this decrease significantly progressed at a much slower rate for eggs coated with MO and/or 25:75 MO:CH emulsion than for noncoated eggs, and likewise for eggs with 'High' than with 'Medium' and 'Low' initial HU before coating (Table 5.1 and 5.2). Compared with noncoated eggs, eggs coated with MO and/or 25:75 MO:CH emulsion had significantly higher HU throughout the 5 weeks ($P < 0.05$), irrespective of their initial HU before coating, except for eggs with 'Low' initial HU at 1 week of storage, in which HU of eggs coated with MO and/or 25:75 MO:CH emulsion was not significantly different ($P > 0.05$) from that of noncoated eggs (Table 5.2). Irrespective of their initial HU before coating, no significant differences ($P > 0.05$) in HU were observed between MO and 25:75 MO:CH emulsion coated eggs throughout the 5 weeks of storage, except for 'High' initial HU at 1 week and 'Low' initial HU at 3 weeks, in which HU of eggs coated with MO was significantly higher ($P < 0.05$) than that of egg coated with 25:75 MO:CH emulsion (75.5 vs. 66.7 and 44.9 vs. 33.9, respectively).

The HU of noncoated eggs decreased from 87.8, 75.6 and 70.9 (eggs with 'High', 'Medium' and 'Low' initial HU before coating, respectively) to 51.8, 57.0 and 56.0 after 1 week and to 43.1, 39.3 and 35.9 after 2 weeks of storage (Table 5.2). By using eggs with 'High' initial HU before coating, the HU of eggs coated with MO (53.2) and/or 25:75 MO:CH emulsion (55.9)

after 5 weeks were higher than that (51.8) of noncoated eggs after 1 weeks of storage; this implies that coating with MO and/or 25:75 MO:CH could preserve the albumen quality for at least 4 more weeks compared with that of noncoated eggs at 25 °C (Table 5.2). On the other hand, the HU of MO and/or 25:75 MO:CH coated eggs after 5 weeks was comparable to that of noncoated eggs after 2 weeks of storage by using eggs with 'Medium' and 'Low' initial HU before coating; this implies that MO and/or 25:75 MO:CH coating were also effective in preserving the albumen quality of eggs for at least 3 more weeks compared with that of noncoated eggs at 25 °C for those initial HU before coating (MO-25:75 MO:CH vs. control, 38.1-42.7 vs. 39.3 for 'Medium' and 38.8-39.1 vs. 35.9 for 'Low', respectively).

These results were substantiated by previous observations for MO and/or MO:CH emulsion coated eggs (Kamel *et al*., 1980; Waimaleongora-Ek *et al*., 2009; Jirangrat *et al*., 2010; Torrico *et al*., 2010a; Torrico *et al*., 2010b). It is important to mention that noncoated eggs with 'High' initial HU before coating had a elevated HU decline (36 units) after 1 week of storage (from 87.8 to 51.8, Table 5.2). This was also observed by Sabrani & Payne (1978) in which noncoated eggs from young hens (28-week-old hens, initial HU before coating of 89.0) had a HU decline of 29.2 units after 1 week of storage at 28 °C (from 89.0 to 59.8).

Based on the HU, eggs can be classified into four grades: AA (above 72), A (72-60), B (59-31), and C (below 30) (Lee *et al*., 1996). Changes in classified egg grade during 5 weeks of storage at 25 °C as affected by different initial HU before coating are shown in Table 5.2. Irrespective of their initial HU before coating, grades of noncoated eggs decreased rapidly from AA to B and C after 1 and 3 weeks, respectively (Table 5.2). However, eggs with 'High' initial HU before coating coated with MO and/or 25:75 MO:CH emulsion changed from AA to B grade after 5 weeks, thus preserving A grade quality up to 4 weeks.

On the other hand, MO and/or 25:75 MO:CH emulsion coated eggs with 'Medium' and 'Low' initial HU changed from AA to B grade after 3 weeks, preserving this B-grade up to 5 weeks of storage. Hence, this demonstrated that whether the eggs with 'Medium' or 'Low' initial HU, all eggs will end up with B-grade after 5 weeks of storage. Therefore, coating treatments (MO and/or 25:75 MO:CH emulsion) had more impact on the final HU than the initial HU before coating (Rank 2 vs. 3, Table 5.1).

5.3.2 Effects of Mineral Oil and 25:75 MO:CH Emulsion as Coating Materials on Weight Loss

ANOVA of weight loss percentage (WL) means (Table 5.1) illustrates that differences of three main factors, (1) coating treatments, (2) storage periods, (3) initial qualities and all their interactions, (1) coating treatments by storage periods, (2) initial qualities by coating treatments, (3) initial qualities by storage periods and (4) interaction of all three factors, were significant ($P < 0.05$) in that order, ranked by their mean square (MS). The overall mean square error (MSE) of the WL was 0.15 that represents a standard error of 0.39. Considering these three main factors, WL progressively and significantly increased with increased storage periods; however, the extent was significantly lesser in coated (MO and/or 25:75 MO:CH emulsion) than in noncoated eggs (Table 5.1 and 5.3). Without exception, all eggs coated with MO and/or 25:75 MO:CH emulsion, irrespective of their initial HU before coating, had significantly ($P < 0.05$) lesser WL than noncoated eggs throughout 5 weeks of storage at 25 °C (interaction between coating treatments * storage periods, $P < 0.0001$, Table 5.1). Furthermore, WL of eggs coated with MO and/or 25:75 MO:CH emulsion remained below 1.0% during the entire storage period of 5 weeks. Eggs coated with MO and/or 25:75 MO:CH emulsion, irrespective of their initial HU before coating, after 5 weeks had significantly lesser WL than that of noncoated eggs after 1 week of storage (0.28-0.74% for MO and 0.85-0.92% for 25:75 MO:CH emulsion vs. 1.11-2.25% for noncoated, Table 5.3).

Table 5.3 Weight Loss (%)* Of Noncoated and Coated Eggs during 5 Weeks of Storage at 25 °C at Different Initial Albumen Qualities (Haugh Unit=HU) Before Coating**

Initial HU**	Coating***	1 week	2 weeks	3 weeks	4 weeks	5 weeks
High	Control	2.25±0.3^{a}	3.23±0.3^{a}	4.66±0.4^{a}	–****	–****
	MO	0.15±0.1^{c}	0.49±0.4^{b}	0.70±0.5^{b}	0.57±0.2^{a}	0.74±0.4^{a}
	25:75 MO:CH	0.43±0.3^{b}	0.48±0.1^{b}	0.58±0.1^{b}	0.75±0.5^{a}	0.92±0.4^{a}
Medium	Control	2.18±0.3^{a}	4.64±1.5^{a}	6.73±1.0^{a}	–****	–****
	MO	0.27±0.1^{b}	0.37±0.1^{b}	0.45±0.2^{b}	0.62±0.3^{a}	0.72±0.3^{a}
	25:75 MO:CH	0.24±0.1^{b}	0.49±0.2^{b}	0.87±0.5^{b}	0.80±0.4^{a}	0.86±0.3^{a}
Low	Control	1.11±0.2^{a}	2.44±0.3^{a}	3.32±0.7^{a}	–****	–****
	MO	0.04±0.0^{c}	0.19±0.2^{b}	0.27±0.1^{b}	0.26±0.1^{a}	0.28±0.2^{b}
	25:75 MO:CH	0.27±0.2^{b}	0.29±0.2^{b}	0.38±0.2^{b}	0.36±0.2^{a}	0.85±0.4^{a}

*Means ± standard deviations of 10 measurements. $^{a\text{-}c}$Means with different superscripts within a column and within an initial HU before coating indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD).

**Initial albumen qualities of eggs before coating: High=87.76 HU, Medium=75.62 HU and Low=70.88 HU. Data for Medium initial HU before coating were adopted from Torrico et al. (2010b).

***Control = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75 by using Eficacia XE emulsifier. (CH) = chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.

****Not determined as the HU of noncoated eggs was below 25 after 3 weeks.

Temperature, humidity, air movement and storage time can cause loss of water through the porous shell, resulting in a loss of weight in eggs (FAO, 2003). This can be explained by a significant decrease in the weight percentage of albumen (Wardy *et al*., 2010). No significant differences ($P > 0.05$) in WL were observed between MO and 25:75 MO:CH emulsion coated eggs after 5 weeks of storage, except in eggs with 'Low' initial HU, in which WL of eggs coated with 25:75 MO:CH emulsion was significantly higher ($P < 0.05$) than that of eggs coated with MO (0.85 vs. 0.28, Table 5.3). Waimaleongora-Ek *et al.* (2009) stated that MO (viscosity = 26 mPa s) coating significantly reduced WL (0.85%) of coated eggs, compared to that (8.78%) of noncoated eggs, after 5 weeks of storage at 25 °C by using eggs with an initial HU before coating of 84.12. Besides, Torrico *et al.* (2010a) reported that WL of eggs coated with MO (0.69-0.70%) and 25:75 emulsion (1.03%) after 5 weeks of storage was lower than that (1.43%) of noncoated eggs after 1 week of storage at 25 °C by using eggs with an initial HU before coating of 83.79. Slight differences in WL among these studies may be due to different coating materials used, storage period, temperature, egg size, shell porosity, relative humidity and hens' age (Muller, 1958; Williams, 1992).

According to FAO (2003), a WL of 2-3% is common in marketing eggs and is hardly noticeable to consumers. This study demonstrated that MO and 25:75 MO:CH emulsion coatings (irrespective of the initial HU before coating of eggs) can similarly offer protective barriers against the loss of moisture through the eggshell during 5 weeks of storage at 25 °C, thus minimizing WL of eggs (< 0.92%, Table 5.3).

5.3.3 Effects of Mineral Oil and 25:75 MO:CH Emulsion as Coating Materials on Yolk Index

The yolk index (YI), an indication of yolk freshness by measuring the ratio between yolk height and width, decreases during storage as a result of a yolk flattening and its reduced

resistance to breaking. This internal quality deterioration is largely attributed to a progressive weakening of the vitelline membranes and liquefaction of the yolk due to diffusion of water from the white into the yolk (Stadelman, 1995a; Obanu & Mpieri, 1984). In our present study, the YI decreased with increased storage periods (Table 5.4). This decrease was affected by coating treatments, storage period at 25 °C, and, in a lesser extent, by the initial HU of eggs before coating (interaction between coating treatments * storage periods * initial qualities, $P = 0.0096$; complete data not shown).

For eggs with 'High' initial HU before coating, YI of eggs coated with MO (0.36) and/or 25:75 MO:CH emulsion (0.36) after 5 weeks were comparable (not significant different, $P > 0.05$) to that (0.36) of noncoated eggs after 1 weeks of storage (Table 5.4). On the other hand, for eggs with 'Medium' and 'Low' initial HU before coating, YI of eggs coated with MO (0.29-0.31) and/or 25:75 MO:CH emulsion (0.32) after 5 weeks were comparable (not significant different, $P > 0.05$) to that (0.27-0.31) of noncoated eggs after 2 weeks of storage (Table 5.4). By using eggs with an initial HU before coating of 84.12, Waimaleongora-Ek *et al*. (2009) reported that YI (0.37) of MO (26 mPa s) coated eggs after 5 weeks was comparable with that (0.35) of noncoated eggs after 1 week of storage at 25 °C. Torrico *et al*. (2010a) observed that 25:75 MO:CH emulsion coated eggs after 5 weeks had similar (not significant different, $P < 0.05$) YI to that of noncoated eggs after 2 weeks of storage at 25 °C (0.33 vs. 0.30) by using eggs with an initial HU before coating of 83.79. Data from Tables 5.2 (HU), 5.3 (WL) and 5.4 (YI) collectively imply that coating with MO and/or 25:75 MO:CH emulsion can preserve both albumen and yolk quality for at least 4 more weeks by using eggs with 'High' initial HU before coating and at least 3 more weeks by using eggs with 'Medium' or 'Low' initial HU before coating compared with that observed for noncoated eggs at 25 °C.

Table 5.4 Yolk Index* of Noncoated and Coated Eggs during 5 Weeks of Storage at 25 °C at Different Initial Albumen Qualities (Haugh Unit=HU) Before Coating**

Initial HU**	Coating***	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
High	Control	0.48	0.36^{c}	0.32^{b}	0.28^{b}	–****	–****
	MO	0.48	0.42^{a}	0.40^{a}	0.40^{a}	0.37^{a}	0.36^{a}
	25:75 MO:CH	0.48	0.39^{b}	0.38^{a}	0.40^{a}	0.36^{a}	0.36^{a}
Medium	Control	0.43	0.36^{b}	0.27^{b}	0.23^{b}	–****	–****
	MO	0.43	0.39^{a}	0.35^{a}	0.33^{a}	0.32^{a}	0.29^{a}
	25:75 MO:CH	0.43	0.40^{a}	0.35^{a}	0.32^{a}	0.31^{a}	0.32^{a}
Low	Control	0.40	0.35^{a}	0.31^{b}	0.29^{b}	–****	–****
	MO	0.40	0.35^{a}	0.33^{a}	0.32^{a}	0.30^{a}	0.31^{a}
	25:75 MO:CH	0.40	0.37^{a}	0.33^{a}	0.31^{ab}	0.31^{a}	0.32^{a}

*Means ± standard deviations of 10 measurements. Standard deviations for all yolk-index values ranged from 0.01 to 0.04. $^{a\text{-}c}$Means with different superscripts within a column and within an initial HU before coating indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD).

**Initial albumen qualities of eggs before coating: High=87.76 HU, Medium=75.62 HU and Low=70.88 HU. Data for Medium initial HU before coating were adopted from Torrico et al. (2010b).

***Control = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75 by using Eficacia XE emulsifier. (CH) = chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.

****Not determined as the HU of noncoated eggs was below 25 after 3 weeks.

5.3.4 Effects of Mineral Oil and 25:75 MO:CH Emulsion as Coating Materials on Albumen pH

Freshly laid eggs contain 1.44-2.05 mg CO_2/g of albumen (Keener *et al*., 2001; Biladeau & Keener, 2009) and have an albumen pH value of 7.6-8.7 (Goodwin *et al*., 1962; Rhim *et al*., 2004; Waimaleongora-Ek *et al*., 2009). During storage, carbon dioxide escapes via eggshell pores, resulting in thinning of the thick albumen and an increased albumen pH value up to 9.6-9.7 (Li-Chan & Nakao, 1989; Kemps *et al*., 2007). In this study, the albumen pH values of all noncoated and coated eggs ranged from 8.38 to 9.34 (Figure 5.1).

Albumen pH values of eggs coated with MO and/or 25:75 MO:CH emulsion, irrespective of their initial HU before coating, were significantly ($P < 0.05$) lower than those of noncoated eggs throughout 5 weeks of storage at 25 °C (Figure 5.1). This implies that MO and/or 25:75 MO:CH emulsion as coating materials could retard loss of carbon dioxide through eggshell pores by acting as gas barriers (Obanu & Mpieri, 1984; Stadelman, 1995b). The pattern for changes in albumen pH during the storage periods differed between noncoated and coated eggs (interactions between coating treatments * storage periods, $P < 0.0001$), but it was similar among eggs with different initial HU before coating ('High', Medium' and 'Low') (Figure 5.1). Irrespective of their initial HU before coating, the albumen pH of noncoated eggs slightly increased from values of 8.81 for 'High', 9.20 for 'Medium' and 8.71 for 'Low' initial HU before coating to 9.33, 9.28 and 9.29, respectively, after 3 weeks of storage (Figure 5.1). The albumen pH of noncoated eggs after 3 weeks of storage was not measured due to their low HU, with a C-grade quality.

Conversely, albumen pH of eggs coated with MO and/or 25:75 MO:CH emulsion gradually decreased from 8.71-9.20 to 8.46-8.38 for 'High' and to 8.58-8.61 for 'Medium' before coating. For 'Low' initial HU eggs, albumen pH of coated eggs slightly increased during the 3 first weeks and then decreased to values of 8.73-8.59 after 5 weeks of storage (Figure 5.1).

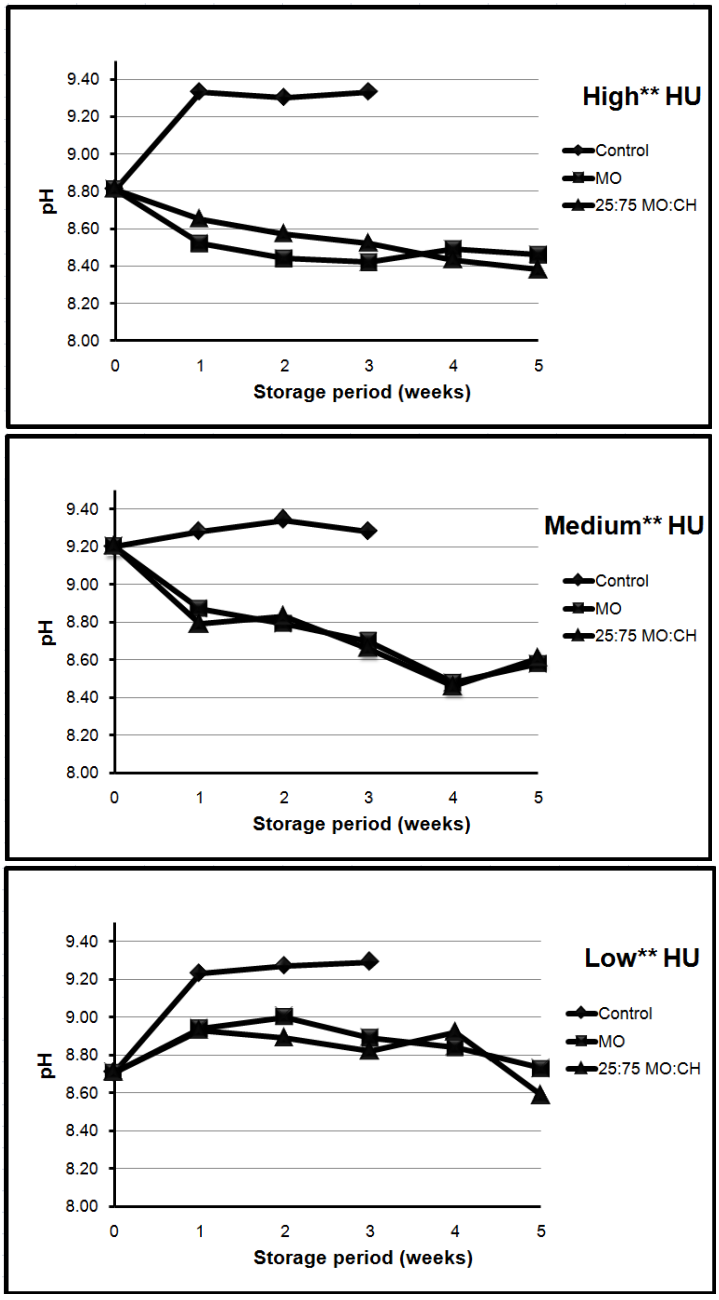

Figure 5.1 Variations in Albumen pH* of Noncoated and Coated Eggs during 5 Weeks of Storage at 25 °C at Different Initial Albumen Qualities (Haugh Unit=HU) Before Coating**

*Each point represents an average value of 10 measurements. For all data, standard deviation values ranged from 0.03 to 0.34. **Control = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75 by using Eficacia XE emulsifier. (CH) = chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid. Initial albumen qualities of eggs before coating: High=87.76 HU, Medium=75.62 HU and Low=70.88 HU. Data for Medium initial HU before coating were adopted from Torrico et al. (2010b).

The decrease in albumen pH during storage may be due to the continuing breakdown of the constituents in egg white and/or a change in the bicarbonate buffer system (Obanu & Mpieri, 1984; Biladeau & Keener, 2009). Kamel *et al*. (1980) reported the increased albumen pH of noncoated eggs from an initial value of 8.64 to 9.51 after 5 weeks of storage at 25 °C. Sabrani & Payne (1978) reported that albumen pH of noncoated eggs from young hens (28-weeks-old hens, initial HU before coating of 89.0) and old hens (60-weeks-old hens, initial HU before coating of 76.3) increased from 7.62-7.57 to 9.50-9.59 after 24 days at 28 °C, whilst eggs coated with linseed oil had a markedly slowed increase from 7.62-7.57 to 8.29-7.98 for young and old hen eggs. Differences in egg size and storage conditions (temperature, humidity, and period) may affect albumen pH before and after storage (Muller, 1958; Goodwin *et al*., 1962; Silversides & Scott, 2001).

5.3.5 Color Measurements of Noncoated and Coated Egg Shells

Visual appraisal is the first sense that consumers use in making a decision to purchase products, and one of its main components is color (Caner & Cansiz, 2008). Table 5.5 shows the whiteness index (WI) and color difference (ΔE^*) of egg shells for noncoated and coated eggs during 5 weeks of storage at 25 °C. Overall, WI decreased and ΔE^* increased as storage time increased. WI of noncoated egg shells decreased from a value of 94.5 to 91.1 after 3 weeks. On the other hand, WI of egg shells coated with MO and/or 25:75 MO:CH decreased from 93.5-92.7 to 90.8-90.9, respectively, after 5 weeks of storage. Color changes of egg shells are results of changes in the L^*, a^* and b^* values (complete data not shown). The decrease of the WI in noncoated and coated egg shells is largely explained by the decrease of L^* (lightness) values during the 5 weeks of storage (L^* decreased from initial 93.11-94.94 to 91.89-91.98) indicating a possible darkening of the natural cuticle or coating material.

Table 5.5 Whiteness Index (WI)* and Color Difference (ΔE^*)* Values of Egg Shells* for Noncoated and Coated Eggs during 5 Weeks of Storage at 25 °C

Color parameter**	Coating***	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
WI	Control	94.5±0.7[a]	90.8±1.1[a]	92.2±0.5[a]	91.1±0.8[a]	–****	–****
	MO	93.5±1.6[a]	91.3±0.4[a]	91.0±0.8[b]	90.9±0.7[a]	91.2±0.7[a]	90.8±0.3[a]
	25:75 MO:CH	92.7±1.3[a]	90.9±0.8[a]	90.2±0.6[b]	90.2±0.6[a]	89.8±0.5[b]	90.9±0.7[a]
ΔE^*	Control	0.0±0.0	3.9±1.2[a]	2.6±0.9[b]	3.6±0.8[a]	–****	–****
	MO	2.0±1.2[a]	3.4±0.6[a]	3.8±1.1[ab]	3.9±0.8[a]	3.5±0.9[b]	3.9±0.9[a]
	25:75 MO:CH	2.2±1.2[a]	3.9±1.0[a]	4.6±1.2[a]	4.7±0.5[a]	5.1±0.8[a]	3.9±1.3[a]

Means ± standard deviations of 5 measurements from eggs with High initial Haugh unit (HU=87.76) before coating. [a-b]Means with different superscripts within a column and within a color parameter (WI or ΔE) indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD).

**Whiteness index of the egg shell was calculated as $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$. Color difference ($\Delta E^*$) of between egg shells and initial noncoated egg shells at week 0 (L^*_o, a^*_o and b^*_o) was calculated as $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ where $\Delta L^* = L^* - L^*_o$, $\Delta a^* = a^* - a^*_o$ and $\Delta b^* = b^* - b^*_o$.

***Control = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75 by using Eficacia XE emulsifier. (CH) = chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid.

****Not determined as the Haugh unit of noncoated eggs was below 25 after 3 weeks.

Table 5.5 also shows the size of the ΔE^* of egg shells using noncoated egg shells at week 0 as the reference. The ΔE^* of noncoated egg shells increased from 0.0 to 3.9 and to 3.6 after 1 and 3 weeks of storage, respectively. In contrast, ΔE^* of egg shells coated with MO and/or 25:75 MO:CH emulsion increased from 2.0-2.2 to 3.4-3.9 and to 3.9-4.7 after 1 and 3 weeks of storage, respectively. After 5 weeks of storage, there were not significant differences ($P > 0.05$) between ΔE^* (3.9 for both) of egg shells coated with MO and 25:75 MO:CH emulsion. At week 0, MO and 25:75 MO:CH coated egg shells had ΔE^* of 2.0 and 2.2, respectively, mainly due to a lesser L^* value, imparted by these coating materials, compared with that of noncoated egg shells (93.11-93.77 vs. 94.94). For the noncoated and coated egg shells during storage, decreased L^* values were also observed with increases of $-a^*$ (greenness) and $+b^*$ (yellowness) values ($-a^*$ ranged from –0.36 to –2.20 and $+b^*$ ranged from +1.89 to +4.36). According to Caner & Cansiz (2008), ΔE^* values less than 3.0 could not be easily detected by the naked human eye. Therefore, ΔE^* values of egg shells coated with MO or 25:75 MO:CH at week 0 may not be detected by naked human eye. However after 1 week of storage, noncoated and coated egg shells had ΔE^* values greater than 3.0 except for noncoated egg shells at week 2 (ΔE^* of 2.6). Sensory discrimination was further performed to see if color differences were detected.

5.3.6 Sensory Discrimination and Purchase Intent of Noncoated and Coated Eggs

The R-index (%) was used to measure the degree of difference between the noncoated eggs (control) and freshly (week 0) coated eggs (Table 5.6). A value of 100% indicates perfect discrimination, whereas a chance value of 50% indicates that the two samples cannot be differentiated (Bhale *et al*., 2003). As shown in Table 5.6, more consumers indicated that the coated eggs (MO and/or 25:75 MO:CH) were perceived to be significantly ($P < 0.05$) glossier than the noncoated eggs.

Table 5.6 R-Index (% Sensory Discrimination)[1] Comparing Noncoated Eggs with Freshly Coated Eggs and Their Purchase Intent

Coating[2]	Surface glossiness		Surface smoothness		Surface odor	Surface color	Overall surface appearance	Purchase intent (%)[4]
	R-index more[3]	R-index less[3]	R-index more[3]	R-index less[3]	R- index	R-index	R-index	
Control								69[a]
MO	***61.43****	38.41	***64.44****	68.83	49.52	48.96	56.40*	75[a]
25:75 MO:CH	***59.54****	43.90	***55.35***	62.30	47.91	53.90	53.46	72[a]

[1]Based on 110 consumers. The R-index with * indicates significant difference at $P < 0.05$. The critical R-index value for a bipolar test is 57.53%, and the critical R-index value for a unipolar test is 56.35% ($\alpha = 0.05$).

[2] Control = noncoated eggs; MO = unwiped after coating with 100% mineral oil (MO); 25:75 MO:CH = coating with MO:CH emulsion at a ratio of 25:75 by using Eficacia XE emulsifier. (CH) = chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid. Eggs with High initial Haugh unit (HU=87.76) before coating were used for the sensory discrimination.

[3]Bold italicized values indicate that more responses were selected from R index more or R index less by consumers.

[4]Percentages of purchase intent (%) with different letter within the column indicate significant differences (Cochran's Q (df =5) = 2.18, $P > 0.05$).

Besides, only eggs coated with MO were perceived to be significantly ($P < 0.05$) smoother than the noncoated eggs. For surface odor and color, the unipolar R-index values for all coated eggs fell between 47.91 and 53.90, indicating that consumers could not significantly ($P > 0.05$) differentiate the coated eggs from noncoated eggs (critical R-index value = 56.35%, one tailed test, $\alpha = 0.05$). For overall surface appearance, only eggs coated with 25:75 MO:CH emulsion were perceived as not significant different ($P > 0.05$) from the control. Conversely, consumers significantly differentiate MO coated eggs from noncoated eggs for overall surface (R-index = 56.40, Table 5.6); this could be due to MO coated eggs had significant ($P < 0.05$) bipolar R-indices for surface smoothness and glossiness (64.44 and 61.43% for $\text{R-index}_{\text{more}}$ values, respectively; critical R-index value = 57.53%, a two-tailed test, $\alpha = 0.05$). Table 5.6 also shows that the purchase intents of MO and/or 25:75 MO:CH coated eggs were above 70% compared with 69% for the noncoated eggs (not significant different, $P > 0.05$, Table 5.6).

5.4 Conclusions

Based on data from HU, WL, YI and albumen pH, it is implied that coating with MO and/or MO:CH emulsion can preserve both albumen and yolk quality for at least 4 more weeks by using eggs with 'High' initial HU before coating and at least 3 more weeks by using eggs with 'Medium' or 'Low' initial HU before coating compared with that observed for noncoated eggs at 25 °C during 5 weeks of storage. Color difference (ΔE*) of egg shells coated with MO and/or 25:75 MO:CH at week 0 cannot be detected by naked human eye ($\Delta E^* < 3.0$) using noncoated egg shells as the reference. However, after 1 week of storage, all coated egg shells become detectable ($\Delta E^* > 3.0$). According to the sensory discrimination, surface glossiness of eggs coated with MO and/or 25:75 MO:CH emulsion was significantly different ($P < 0.05$) from the control (noncoated) whereas only eggs coated MO exhibited greater surface smoothness ($P <$

0.05) than the control. Consumers significantly ($P < 0.05$) differentiate MO coated from noncoated eggs for overall surface appearance possible due to their greater surface glossiness and smoothness. Eggs coated with MO and/or 25:75 MO:CH emulsion had at least 70% positive purchase intent that was not significant different ($P > 0.05$) from the purchase intent of noncoated eggs (69%). Ideally, eggs with 'High' initial HU before coating (HU=87.8) coated with emulsion of 25:75 MO:CH would be most effective in view of preservation of the internal egg quality. Nonetheless, 25:75 MO:CH emulsion coating was also capable to maintain the internal quality of 'Medium' and 'Low' initial HU before coating (HU=75.6 and 70.9, respectively) eggs with a B-grade after 5 weeks of storage.

5.5 References

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CHAPTER 6. SUMMARY AND CONCLUSIONS

Eggs are highly susceptible to internal quality deterioration. The most profound factor that affects quality deterioration rate of eggs is storage temperature. In some developing countries of the world where refrigeration of eggs is seldom practiced, surface coating is an alternative and effective method to preserve the internal quality of eggs and to prevent microbial contamination. Based on the internal quality (weight loss, Haugh unit, yolk index and albumen pH), mineral oil (MO) and emulsions of mineral oil and chitosan solution (MO:CH=75:25, 50:50 and 25:75) were more effective than chitosan solution (CH) as coating materials in preserving the internal quality of eggs at room temperature (25 °C). MO and MO:CH emulsion coatings reduced weight loss and preserved the internal quality of eggs for at least 3 weeks longer than observed for the noncoated eggs during 5 weeks of storage at 25 °C. MO:CH emulsions, especially at the ratio of 25:75, and CH solution require much less drying time than MO when applied on the surface of the eggshell. According to the sensory discrimination, surface glossiness of eggs coated with emulsion of MO:CH = 25:75 was not significantly different from the noncoated eggs whereas eggs coated with MO and two other emulsions (MO:CH = 75:25 and 50:50) exhibited greater surface glossiness than the noncoated eggs. All emulsion-coated eggs had at least 80% of positive purchase intent and were negative for *Salmonella* spp.

A synergistic effect between refrigeration and coating material (MO and/or MO:CH, irrespective of the emulsifier types) was evidenced in maintaining lower weight losses (<2%) and preserving albumen and yolk qualities during 5 weeks storage at 25 °C and 20 weeks at 4°C. At 4 °C, the grade of noncoated eggs decreased from AA to A and B after 5 and 10 weeks, respectively. Nonetheless, eggs coated with MO and/or four emulsions changed from AA to A grade after 5 weeks and maintained this grade up to 10 weeks (5 weeks longer than observed for

nonocoated eggs). Compared with 4 °C, the increasing weight loss showed stronger negative correlation (P<0.01) with the decreasing Haugh unit (-0.46 to -0.89) and yolk index (-0.36 to -0.89) at 25 °C. The emulsifier type used did not insert significant effect on the internal quality of eggs.

Coating with MO and/or MO:CH emulsion can preserve both albumen and yolk quality for at least 4 more weeks by using eggs with 'High' initial Haugh unit before coating and at least 3 more weeks by using eggs with 'Medium' or 'Low' initial Haugh unit before coating compared with that observed for noncoated eggs at 25 °C during 5 weeks of storage. Color difference (ΔE^*) of egg shells coated with MO and/or 25:75 MO:CH at week 0 could not be detected by naked human eye ($\Delta E^* < 3.0$) using noncoated egg shell as the reference. According to the sensory discrimination, surface glossiness of eggs coated with MO and/or 25:75 MO:CH emulsion was significantly different ($P < 0.05$) from the control (noncoated) whereas only eggs coated MO exhibited greater surface smoothness ($P < 0.05$) than the control. Consumers significantly ($P < 0.05$) differentiated MO coated from noncoated eggs for overall surface appearance possible due to their greater surface glossiness and smoothness. Coating eggs with 'High' initial HU before coating (HU=87.8) with emulsion of 25:75 MO:CH would be most effective in view of preservation of the internal egg quality, sensory perception, purchase intent, and drying time.

Under the conditions of this project, there was a synergistic effect between the MO:CH emulsion coating and the refrigeration storage. MO:CH emulsion coating mainly aided in minimizing the overall weight loss of eggs in order to meet an acceptable range (less than 3%, FAO 2003); whilst, refrigeration storage preserved the albumen and yolk quality of coated eggs and extend their shelf life.

APPENDIX A: STUDY 1

a. Research Consent Form

I, _____, agree to participate in the research entitled "Sensory Discrimination of Surface Properties of Eggs," which is being conducted by Witoon Prinyawiwatkul, Professor of the Department of Food Science at Louisiana State University Agricultural Center, (225)578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. 100 consumers will participate in this research. For this particular research, about 10-15 minutes participation will be required for each consumer.

The following points have been explained to me:
1. In any case, it is my responsibility to report prior participation to the investigator any food allergies I may have. **In this study, however, taste testing is not required.**

2. The reason for the research is to gather information on **sensory perception of the surface properties of eggs**. The benefit that I may expect from it is a satisfaction that I have contributed to solution and evaluation of problems relating to such examinations.

3. The procedures are as follows: Eight coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.

4. Participation entails minimal risk: No taste testing is required in this study. The only but unlikely risk which can be envisioned is that of an allergic reaction via touching of mineral oil, emulsifier, and biopolymer from crustacean shell.

5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.

6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University AgCenter that involves human participation is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. David Morrison, Assistant Vice Chancellor of LSU AgCenter. I agree with the terms above.

_____ _____

Signature of Investigator Signature of Participant

Witness: _____ Date: _____

b. Questionnaire Form

1. Examine **Sample A**. Are the attributes of samples B, C, D, E, F, G and H listed below **MORE**, **SAME** or **LESS** compared to sample A? Are you **SURE** or **UNSURE** of your selection? Please check one box.

ATTRIBUTE	SAMPLE	MORE		SAME		LESS	
		SURE	UNSURE	SURE	UNSURE	SURE	UNSURE
Surface Smoothness	B						
	C						
	D						
	E						
	F						
	G						
	H						
Surface Glossiness or Shininess	B						
	C						
	D						
	E						
	F						
	G						
	H						

2. Examine **Sample A**. Are the attributes of samples B, C, D, E, F and G listed below **SAME** or **DIFFERENT** compared to sample A? Are you **SURE** or **UNSURE** of your selection? Check one box.

ATTRIBUTE	SAMPLE	SAME		DIFFERENT	
		SURE	UNSURE	SURE	UNSURE
COLOR	B				
	C				
	D				
	E				
	F				
	G				
	H				
ODOR	B				
	C				
	D				
	E				
	F				
	G				
	H				
OVERALL SURFACE DIFFERENCE	B				
	C				
	D				
	E				
	F				
	G				
	H				

3. Base on **external appearance,** would you BUY the following sample?

Sample B ____ YES ____ NO Sample F ____ YES ____ NO

Sample C ____ YES ____ NO Sample G ____ YES ____ NO

Sample D ____ YES ____ NO Sample H ____ YES ____ NO

Sample E ____ YES ____ NO

APPENDIX B: STUDY 2

a. Haugh Unit* of Noncoated and Coated Eggs during 5 wk of Storage at 25° C

Coating**	0wk	1wk	2wk	3wk	4wk	5wk
CONTROL	75.62±3.41^{A}	57.01±8.25^{B,c}	39.34±7.41^{C,c}	23.78±9.10^{D,b}	-***	-***
MO	75.62±3.41^{A}	66.71±6.73^{AB,ab}	58.63±6.49^{B,ab}	43.62±7.83^{C,a}	44.42±10.38^{C,a}	38.08±9.47^{C,a}
TANDEM	75.62±3.41^{A}	67.06±6.49^{A,ab}	49.89±10.02^{B,b}	45.96±7.13^{BC,a}	40.42±13.55^{BC,a}	37.02±5.99^{C,a}
TIC	75.62±3.41^{A}	72.79±5.19^{A,a}	66.68±7.07^{A,a}	51.28±4.89^{B,a}	35.60±12.28^{C,a}	38.89±7.27^{C,a}
TWEEN	75.62±3.41^{A}	62.42±6.17^{B,bc}	57.89±3.91^{BC,ab}	50.41±9.49^{CD,a}	46.48±7.72^{DE,a}	37.52±13.16^{E,a}
EFICACIA	75.62±3.41^{A}	69.69±6.60^{A,ab}	55.28±6.50^{B,b}	49.87±5.44^{BC,a}	43.23±8.89^{C,a}	42.70±8.15^{C,a}

*Means ± standard deviations of 10 measurements. Chitosan solution (CH) at 2% (w/v) was dissolved in 1% of acetic acid (v/v).
**CONTROL = noncoated eggs; MO = unwiped after coating with 100% of mineral oil (MO); TANDEM = coating with MO:CH emulsion at a ratio of 25:75 by using emulsifier Tandem® 552K; TIC = coating with MO:CH emulsion at a ratio of 25:75 by using emulsifier Tic Pretested®Ticaloid®210 S Powder; TWEEN = coating with MO:CH emulsion at a ratio of 25:75 by using emulsifier Tween 80; and EFICACIA = coating with MO:CH emulsion at a ratio of 25:75 by using emulsifier Eficacia XE.
***Not determined as the Haugh unit of noncoated eggs was below 25 after 3 wk.
$^{A-E}$Means with different superscripts within a row indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD)
$^{a-c}$Means with different superscripts within a column indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD)

b. Haugh Unit* of Noncoated and Coated Eggs during 20 wk of Storage at 4° C

Coating**	0wk	5wk	10wk	15wk	20wk
CONTROL	75.62±3.41^{A}	60.22±6.28^{B,b}	54.64±7.47^{BC,c}	54.31±6.34^{BC,a}	50.84±7.42^{C,a}
MO	75.62±3.41^{A}	69.61±6.47^{A,a}	60.31±4.41^{B,bc}	56.42±6.02^{B,a}	56.79±6.79^{B,a}
TANDEM	75.62±3.41^{A}	64.35±6.21^{B,ab}	60.84±3.93^{BC,bc}	56.04±5.46^{C,a}	58.43±7.30^{BC,a}
TIC	75.62±3.41^{A}	70.44±3.59^{A,a}	71.73±3.49^{A,a}	59.71±4.87^{B,a}	55.34±10.11^{B,a}
TWEEN	75.62±3.41^{A}	70.16±4.96^{AB,a}	63.37±6.40^{BC,b}	57.53±9.50^{C,a}	59.44±6.97^{C,a}
EFICACIA	75.62±3.41^{A}	67.25±5.61^{B,ab}	65.44±5.15^{B,ab}	57.54±4.36^{C,a}	58.86±3.80^{C,a}

*Means ± standard deviations of 10 measurements. Chitosan solution (CH) at 2% (w/v) was dissolved in 1% of acetic acid (v/v).
**CONTROL = noncoated eggs; MO = unwiped after coating with 100% of mineral oil (MO); TANDEM = coating with MO:CH emulsion at a ratio of 25:75 by using emulsifier Tandem® 552K; TIC = coating with MO:CH emulsion at a ratio of 25:75 by using emulsifier Tic Pretested®Ticaloid®210 S Powder; TWEEN = coating with MO:CH emulsion at a ratio of 25:75 by using emulsifier Tween 80; and EFICACIA = coating with MO:CH emulsion at a ratio of 25:75 by using emulsifier Eficacia XE.
$^{A-C}$Means with different superscripts within a row indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD)
$^{a-c}$Means with different superscripts within a column indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD)

APPENDIX C: STUDY 3

a. Haugh Unit (HU)* of Noncoated and Coated Eggs during 5 Weeks of Storage at 25 °C at Different Initial Albumen Qualities**

Coating^	Initial HU**	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
CONTROL	High	87.76±5.01^{a}	51.81±5.36^{a}	43.05±9.65^{a}	29.90±7.54^{a}	26.52±9.09^{a}	24.15±5.36^{a}
	Medium	75.62±3.41^{b}	57.01±8.25^{a}	39.34±7.41^{a}	23.78±9.10^{a}	-***	-***
	Low	70.88±8.00^{b}	56.00±7.25^{a}	35.92±13.80^{a}	28.28±8.48^{a}	23.64±6.45^{a}	21.99±5.34^{a}
MO	High	87.76±5.01^{a}	75.45±6.74^{a}	70.75±5.87^{a}	64.60±7.77^{a}	60.67±4.29^{a}	55.93±10.48^{a}
	Medium	75.62±3.41^{b}	66.71±6.73^{b}	58.63±6.49^{b}	43.62±7.83^{b}	44.42±10.38^{b}	38.08±9.47^{b}
	Low	70.88±8.00^{b}	62.37±7.68^{b}	46.43±9.19^{c}	44.93±10.00^{b}	33.52±10.83^{c}	38.84±10.42^{b}
TANDEM	High	87.76±5.01^{a}	72.03±4.60^{a}	72.94±5.72^{a}	61.12±6.93^{a}	60.13±6.29^{a}	57.26±5.18^{a}
	Medium	75.62±3.41^{b}	67.06±6.49^{ab}	49.89±10.02^{b}	45.96±7.13^{b}	40.42±13.55^{b}	37.02±5.99^{b}
	Low	70.88±8.00^{b}	58.53±11.72^{b}	54.34±8.76^{b}	38.02±10.17^{b}	36.64±7.14^{b}	39.42±12.49^{b}
TIC	High	87.76±5.01^{a}	73.62±3.86^{a}	68.06±4.71^{a}	65.14±6.49^{a}	58.77±4.61^{a}	54.41±4.13^{a}
	Medium	75.62±3.41^{b}	72.79±5.19^{a}	66.68±7.07^{a}	51.28±4.89^{b}	35.60±12.28^{b}	38.89±7.27^{b}
	Low	70.88±8.00^{b}	59.96±6.89^{b}	49.04±9.66^{b}	45.19±13.25^{b}	33.73±8.45^{b}	34.27±9.09^{b}
TWEEN	High	87.76±5.01^{a}	71.44±4.60^{a}	65.31±6.43^{a}	67.65±2.97^{a}	60.09±3.91^{a}	56.25±6.43^{a}
	Medium	75.62±3.41^{b}	62.42±6.17^{b}	57.89±3.91^{b}	50.41±9.49^{b}	46.48±7.72^{b}	37.52±13.16^{b}
	Low	70.88±8.00^{b}	60.61±5.84^{b}	46.91±8.52^{c}	43.84±12.60^{b}	33.70±12.42^{c}	38.47±3.83^{b}
EFICACIA	High	87.76±5.01^{a}	66.74±8.00^{ab}	65.48±4.01^{a}	64.32±3.57^{a}	63.88±7.06^{a}	53.22±8.39^{a}
	Medium	75.62±3.41^{b}	69.69±6.60^{a}	55.28±6.50^{b}	49.87±5.44^{b}	43.23±8.89^{b}	42.70±8.15^{b}
	Low	70.88±8.00^{b}	57.07±11.08^{b}	48.78±6.89^{b}	33.91±10.33^{c}	37.72±9.44^{b}	39.14±10.33^{b}

*Means ± standard deviations of 10 measurements.

**Initial qualities: High=87.76 HU, Medium=75.62 HU and Low=70.88 HU.

***Not determined as the HU of noncoated eggs was below 20 after 3 weeks.

^ See Appendix B for coating nomenclature.

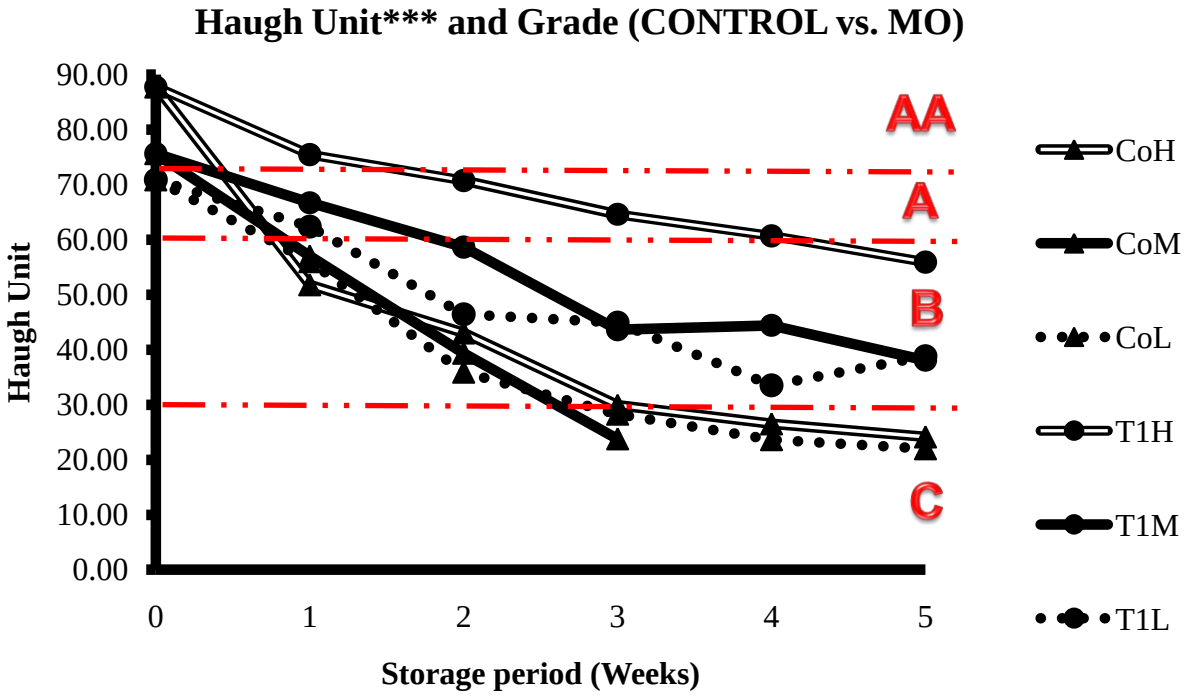
$^{a-c}$Means with different superscripts within a column and within a treatment indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD).

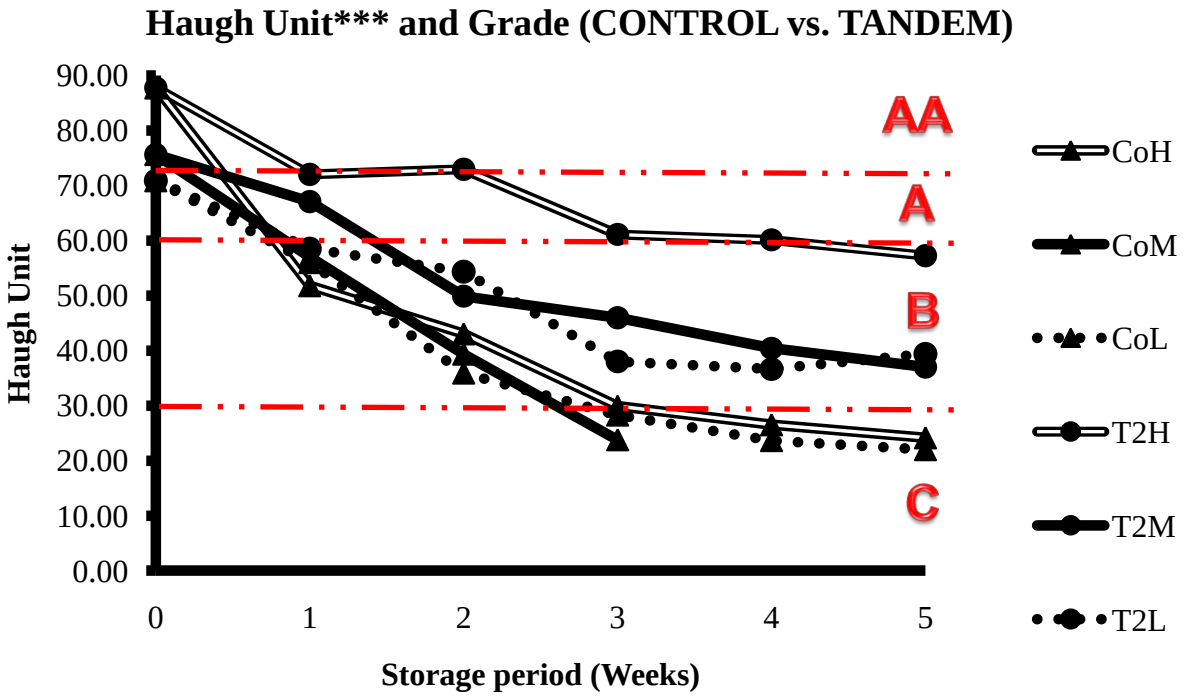
b. Grades* of Noncoated and Coated Eggs during 5 Weeks of Storage at 25 °C at Different Initial Albumen Qualities**

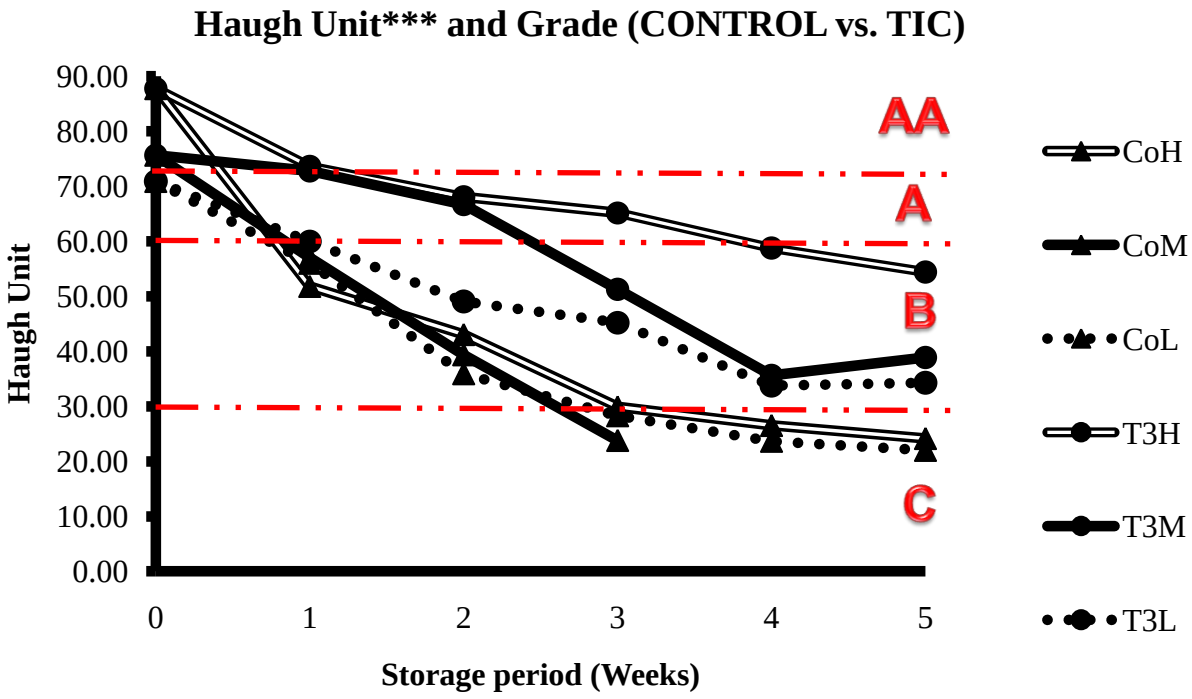
Coating^	Initial HU**	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
CONTROL	High	AA	B	B	C	C	C
	Medium	AA	B	B	C	C	C
	Low	A	B	B	C	C	C
MO	High	AA	AA	A	A	A	B
	Medium	AA	A	B	B	B	B
	Low	A	A	B	B	B	B
TANDEM	High	AA	AA	AA	A	A	B
	Medium	AA	A	B	B	B	B
	Low	A	B	B	B	B	B
TIC	High	AA	AA	A	A	B	B
	Medium	AA	AA	A	B	B	B
	Low	A	B	B	B	B	B
TWEEN	High	AA	A	A	A	A	B
	Medium	AA	A	B	B	B	B
	Low	A	A	B	B	B	B
EFICACIA	High	AA	A	A	A	A	B
	Medium	AA	A	B	B	B	B
	Low	A	B	B	B	B	B

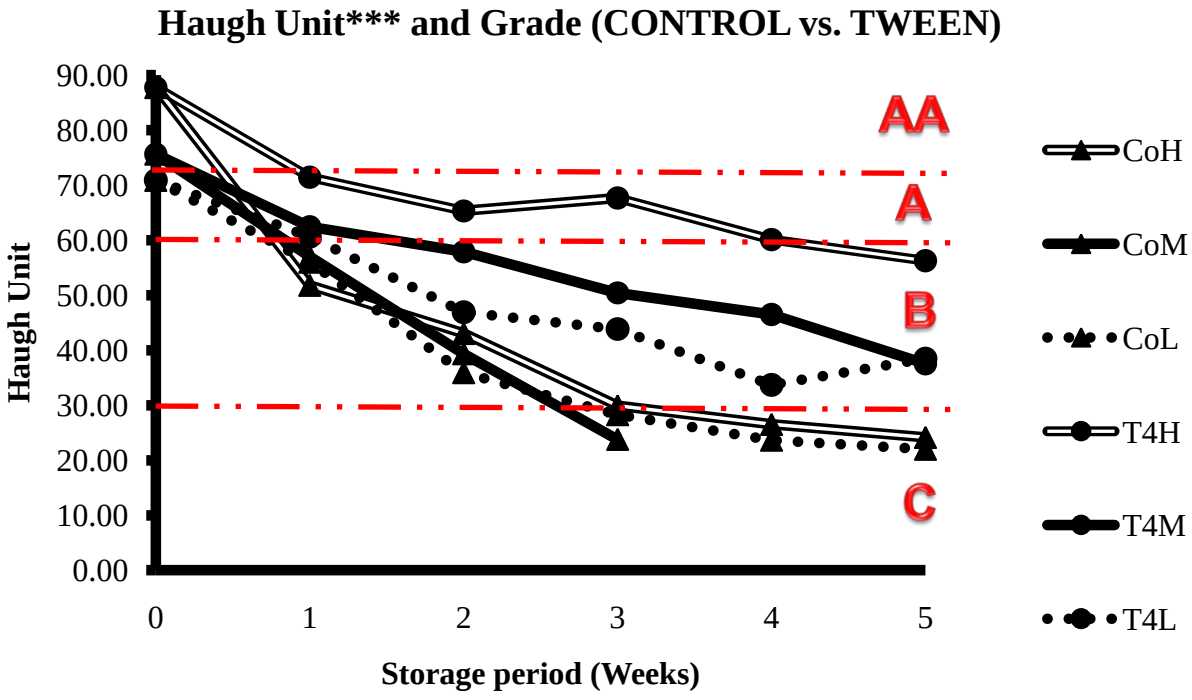
*Quality grades of eggs based on the Haugh unit values where AA is above 72; A, 71 to 60; B, 59 to 31 and C is below 30.
**Initial qualities: High=87.76 HU, Medium=75.62 HU and Low=70.88 HU.
^ See Appendix B for coating nomenclature.

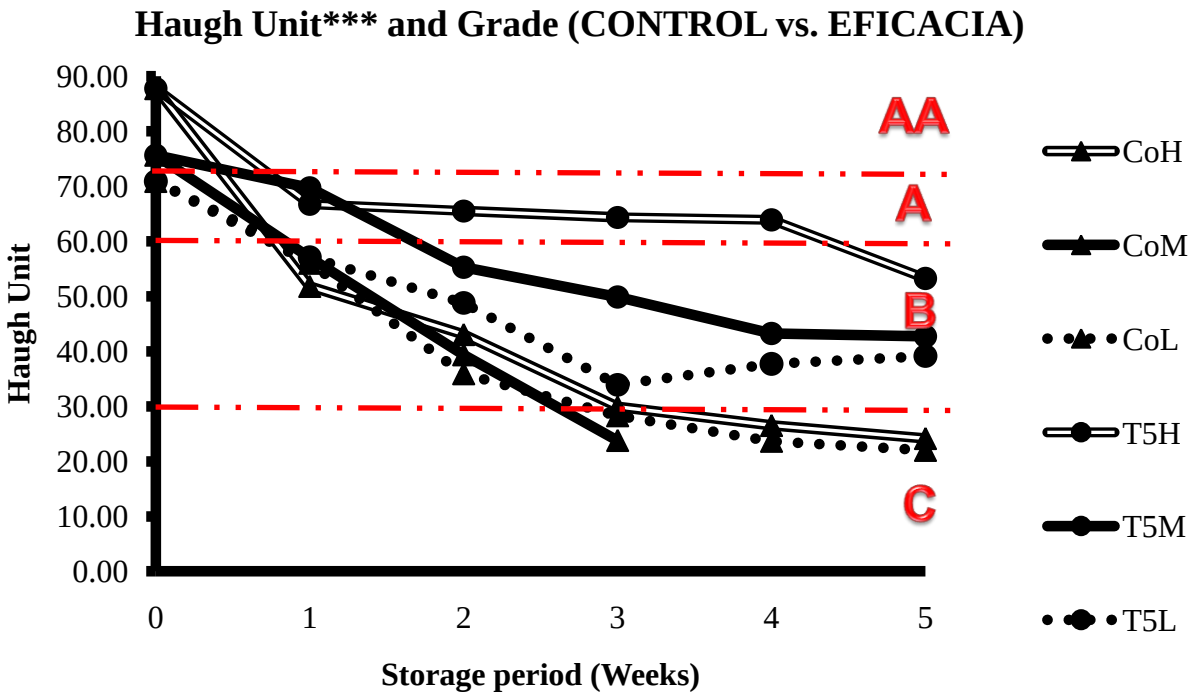
c. Haugh Unit (HU)* of Noncoated and All Emulsion-Coated^ Eggs during 5 Weeks of Storage at 25 °C at Different Initial Albumen Qualities**






Haugh Unit*** and Grade (CONTROL vs. TIC)
Haugh Unit
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
1
2
3
4
5
Storage period (Weeks)
AA
A
B
C
CoH
CoM
CoL
T3H
T3M
T3L


Haugh Unit*** and Grade (CONTROL vs. TWEEN)
Haugh Unit
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
1
2
3
4
5
Storage period (Weeks)
AA
A
B
C
CoH
CoM
CoL
T4H
T4M
T4L



*Means of 10 measurements.
**Initial qualities: High(H)=87.76 HU, Medium(M)=75.62 HU and Low(L)=70.88 HU.
*** CoH=CONTROL with high initial HU. CoM=CONTROL with medium initial HU. CoL=CONTROL with low initial HU. HU of control was not determined after 3 weeks since the HU was below 20. T1H=MO with high initial HU. T1M=MO with medium initial HU. T1L=MO with low initial HU. T2H=TANDEM with high initial HU. T2M=TANDEM with medium initial HU. T2L=TANDEM with low initial HU. T3H=TIC with high initial HU. T3M=TIC with medium initial HU. T3L=TIC with low initial HU. T4H=TWEEN with high initial HU. T4M=TWEEN with medium initial HU. T4L=TWEEN with low initial HU. T5H=EFICACIA with high initial HU. T5M=EFICACIA with medium initial HU. T5L=EFICACIA with low initial HU.
^See Appendix B for coating nomenclature.

d. Color Parameters* of Noncoated and Coated Eggs during 5 Weeks of Storage at 25 °C

Parameter**	Coating^	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
L*	CONTROL	94.94±0.49^{a}	91.53±1.08^{a}	93.12±0.60^{a}	92.17±0.55^{a}	93.14±0.60^{a}	92.29±0.92^{a}
	MO	93.77±1.59^{a}	92.00±0.31^{a}	91.84±0.65^{b}	91.86±0.47^{ab}	92.07±0.53^{abc}	91.90±0.30^{a}
	TANDEM	94.07±0.75^{a}	92.17±0.57^{a}	91.88±0.50^{b}	91.92±0.51^{ab}	92.16±0.42^{ab}	91.98±0.20^{a}
	TIC	92.54±1.45^{a}	91.44±0.59^{a}	90.97±0.84^{b}	91.10±0.56^{b}	91.60±0.87^{bc}	91.85±0.42^{a}
	TWEEN	93.28±1.13^{a}	91.19±0.58^{a}	90.88±0.39^{b}	91.27±0.27^{ab}	91.00±0.52^{c}	91.67±0.37^{a}
	EFICACIA	93.11±1.28^{a}	91.70±0.60^{a}	91.46±0.55^{b}	91.18±0.49^{b}	91.00±0.29^{c}	91.89±0.51^{a}
a*	CONTROL	0.07±0.31^{ab}	-1.15±0.12^{a}	-1.08±0.10^{a}	-1.28±0.24^{a}	-1.01±0.14^{a}	-1.29±0.30^{a}
	MO	0.10±0.24^{a}	-1.11±0.10^{a}	-1.32±0.20^{ab}	-1.35±0.20^{ab}	-1.23±0.15^{a}	-1.27±0.53^{a}
	TANDEM	0.00±0.19^{abc}	-1.23±0.17^{a}	-1.71±0.22^{bc}	-1.50±0.24^{abc}	-1.30±0.17^{ab}	-1.53±0.07^{a}
	TIC	-0.34±0.22^{bc}	-1.34±0.18^{a}	-1.76±0.21^{c}	-1.70±0.19^{bcd}	-1.65±0.23^{b}	-1.55±0.15^{a}
	TWEEN	-0.37±0.11^{c}	-1.31±0.65^{a}	-2.00±0.18^{c}	-1.80±0.06^{cd}	-2.03±0.19^{c}	-1.65±0.10^{a}
	EFICACIA	-0.36±0.15^{c}	-1.32±0.20^{a}	-1.89±0.28^{c}	-1.96±0.25^{d}	-2.20±0.18^{c}	-1.43±0.12^{a}
b*	CONTROL	2.10±0.88^{a}	3.41±0.50^{a}	3.46±0.45^{a}	3.95±0.68^{a}	3.46±0.62^{a}	4.21±0.91^{a}
	MO	1.89±0.52^{a}	3.08±0.51^{a}	3.59±0.43^{a}	3.79±0.54^{a}	3.54±0.47^{a}	4.05±0.20^{a}
	TANDEM	1.90±0.64^{a}	3.02±0.39^{a}	3.79±0.40^{a}	3.54±0.48^{a}	3.51±0.57^{a}	3.79±0.51^{a}
	TIC	3.26±1.18^{a}	3.41±0.20^{a}	4.60±0.94^{a}	4.54±0.88^{a}	4.52±0.89^{a}	4.54±0.64^{a}
	TWEEN	2.43±0.59^{a}	3.74±0.69^{a}	4.02±0.75^{a}	3.68±0.46^{a}	4.18±0.68^{a}	4.19±0.56^{a}
	EFICACIA	2.40±0.79^{a}	3.53±0.77^{a}	4.36±0.37^{a}	3.87±0.53^{a}	4.32±0.56^{a}	3.90±0.67^{a}

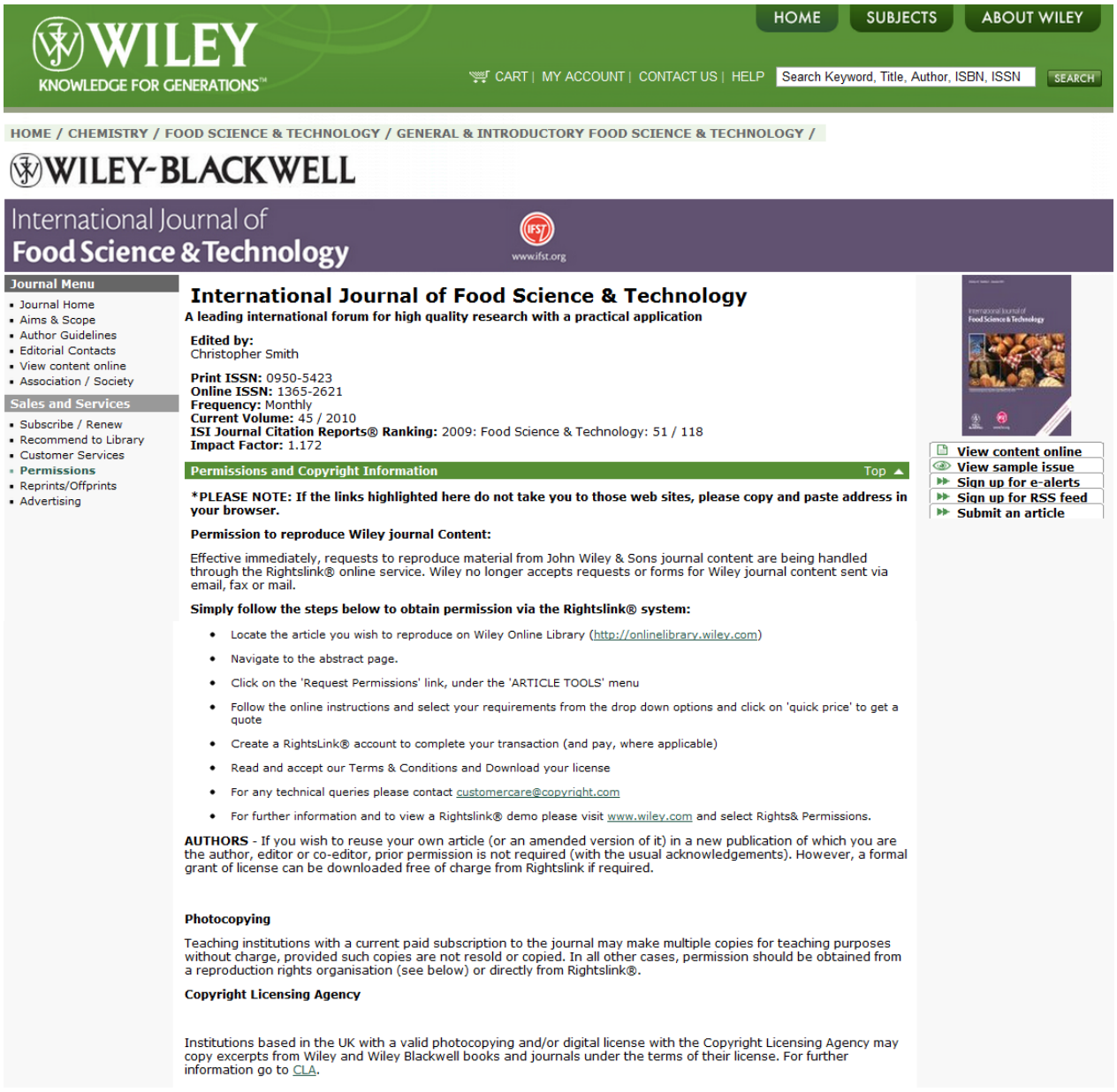
*Means ± standard deviations of 5 measurements.
**Lightness (L*), redness or greenness (a*), yellowness or blueness (b*).
$^{a-c}$Means with different superscripts within a column and within a treatment indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD).
^See Appendix B for coating nomenclature.

e. Color Parameters* of Noncoated and Coated Eggs during 5 Weeks of Storage at 25 °C

Parameter	Coating^	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
c*	CONTROL	1.96±0.93^{ab}	3.60±0.52^{a}	3.63±0.44^{b}	4.16±0.70^{a}	3.60±0.63^{bc}	4.41±0.95^{a}
	MO	2.15±0.38^{ab}	3.28±0.51^{a}	3.82±0.45^{ab}	4.02±0.57^{a}	3.75±0.47^{abc}	4.31±0.19^{a}
	TANDEM	1.08±0.99^{b}	3.26±0.38^{a}	4.16±0.43^{ab}	3.85±0.50^{a}	3.51±0.57^{c}	4.10±0.49^{a}
	TIC	3.29±1.18^{a}	3.67±0.25^{a}	4.94±0.92^{a}	4.85±0.86^{a}	4.82±0.88^{ab}	4.80±0.61^{a}
	TWEEN	2.46±0.60^{ab}	4.05±0.68^{a}	4.49±0.74^{ab}	4.17±0.47^{a}	4.65±0.68^{abc}	4.50±0.54^{a}
	EFICACIA	2.44±0.78^{ab}	3.79±0.75^{a}	4.77±0.26^{a}	4.43±0.48^{a}	4.86±0.47^{a}	4.17±0.63^{a}
H°	CONTROL	83.12±10.85^{c}	108.84±1.05^{a}	107.49±2.07^{c}	108.15±2.31^{c}	106.49±1.22^{d}	107.13±2.08^{b}
	MO	87.45±5.47^{bc}	110.02±1.91^{a}	110.29±2.36^{bc}	109.67±1.01^{c}	109.37±2.27^{cd}	109.82±2.06^{ab}
	TANDEM	92.09±6.68^{abc}	112.34±3.44^{a}	114.25±2.34^{ab}	112.54±1.89^{abc}	112.02±2.60^{bc}	112.21±2.29^{a}
	TIC	95.93±3.99^{ab}	111.43±1.87^{a}	111.12±2.84^{abc}	110.87±3.10^{bc}	110.31±2.87^{cd}	109.14±2.95^{ab}
	TWEEN	98.66±2.06^{ab}	112.71±3.57^{a}	116.90±3.52^{a}	116.14±2.88^{ab}	116.16±2.34^{ab}	111.65±1.96^{ab}
	EFICACIA	99.51±3.68^{a}	111.06±3.94^{a}	113.81±4.23^{ab}	117.23±4.48^{a}	117.40±4.14^{a}	110.51±3.61^{ab}

*Means ± standard deviations of 5 measurements.
**Chroma value (c*), Hue value (H°).
$^{a-c}$Means with different superscripts within a column and within a treatment indicate significant differences ($P < 0.05$). Tukey's Studentized Range (HSD).
^See Appendix B for coating nomenclature.

APPENDIX D: PERMISSION OF THE "INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY"



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