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Effects of Assorted Coating Materials and Room Temperature Storage on Internal Quality and Oxidative Stability of Shell Eggs

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EFFECTS OF ASSORTED COATING MATERIALS AND ROOM
TEMPERATURE STORAGE ON INTERNAL QUALITY AND OXIDATIVE
STABILITY OF SHELL EGGS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Food Science

by
Jinjuta Jirawatjunya
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ABSTRACT

Surface coating is an effective/inexpensive method to preserve internal quality and minimize weight loss of shell eggs during storage without refrigeration. No research has been done to evaluate effects of coating on oxidative stability of cooked eggs. In the first study, soybean oil (SO) as a coating material was evaluated for its effects on internal quality and flavor volatile compounds of eggs throughout 5-weeks of storage at 25°C. Haugh unit (HU) and yolk index (YI) values decreased whereas weight loss increased during storage. After 5-weeks, SO-coated eggs had consistently higher HU (67.72 vs. 48.45) and YI (0.29 vs. 0.40) than the control. SO-coating significantly minimized weigh loss (0.95%) of eggs after 5-weeks storage (vs. 6.47% for the control). The major flavor volatiles in cooked eggs were aldehydes, ketones, alcohols, and sulfides. Soybean oil coted boiled eggs yielded the highest hexanal contents. In the second study, effectiveness of surface coatings using proteins, polysaccharides, or lipids in preserving quality and lipid oxidation of shell eggs was evaluated. After 5-weeks, lipid-based coatings had consistently higher HU (69.25 vs. 41.01-57.75) and YI (0.37 vs. 0.22-0.32) than control and other treatments. Lipid-based coating significantly minimized weigh loss (0.35%) of eggs after 5-weeks storage (vs. 4.84-6.84% for control and other treatments). After 5-weeks storage, compared with the control (8.37 μ g/100 g), hexanal of yolk of eggs coated with SO was higher (14.09 μ g), but that of eggs coated with α - and β -chitosans, WPC, and MO was lower (3.24, 3.89, 4.34, and 6.13 μ g). SO-coated eggs had highest TBARs values after 5-weeks storage. Palmitic, oleic, and linoleic acids were most abundant fatty acids in the yolk. The proportion of MUFAs and saturated fatty acids increased while the proportion of PUFAs decreased after 5 weeks of storage. This study demonstrated that surface coating can extend shelf-life of eggs, but

potentially affect chemical compounds related to lipid oxidation in egg yolk during 5-weeks storage at 25°C.

CHAPTER 1. INTRODUCTION

Eggs are typically inexpensive, yet being a good source of low calorie nutrients, such as minerals, proteins, and unsaturated fatty acids (Djousse & Gaziano, 2008; Plagemann *et al.*, 2010). In addition, eggs offer a variety of functional properties, including foaming, whipping, gelling, and emulsifying (Chang & Chen, 2000). Those functional properties are influenced by egg quality which is easily deteriorated by improper storage handling (Wardy *et al.*, 2010). Egg quality begins to deteriorate immediately after laying due to the loss of moisture and CO₂ through the pores of eggshell (Obanu & Mpieri, 1984; Stadelman, 1995) resulting in higher pH and thin albumen. In the United States, there were 92.9 billion eggs produced in 2012 (USDA, 2012); however, there were 179 million dozens of shell eggs broken (USDA, 2013). Because eggshell is a fragile material, shell broken has become the critical problem causing to economic loss.

To overcome these problems, surface coatings have been applied on eggs for quality deterioration protection. Edible films and coatings play an important role in food industries due to their versatile properties. Surface coating on eggs could increase shell strength and their shelf-life leading to reduced number of cracked eggs and the growth of sale exports (Biladeau & Keener, 2009). In addition, refrigeration might not be available in some developing areas of the world due to its high cost; thus, surface coatings could be an alternative method to extend egg shelf-life and minimize economic loss simultaneously.

Chitosan is a natural biopolymer of carbohydrate derived by deacetylation of chitin (poly β -(1-4)-N-acetyl-D-glucosamine) found in the shell of crustacean skeletons such as crab, shrimp, and crawfish (Bhale *et al.*, 2003). Recent studies (Caner, 2005; Kim *et al.*, 2009; Torrico *et al.*, 20) revealed that chitosan coatings could preserve and extend egg quality longer than that

of noncoated eggs at 25 °C. Whey protein is a byproduct of dairy industry. Whey protein films possess stronger oxygen and aroma barriers than synthetic polymer films under dry environment (Li & Chen, 2002). Both polysaccharide and protein films are effective gas barriers; however, they are poor moisture barriers due to hydrophilic structure (Yoshida & Antunes, 2004). In contrast, lipid films are hydrophobic; thus, they are excellent for moisture partition. With their properties and low cost, oil coatings have been found to be the most effective coatings for extending egg shelf life (Wardy *et al.*, 2010). Although there are a number of publications about the effects of edible oil coatings on egg quality, very few mentioned about their effects on flavor volatile changes in eggs.

Flavor is another factor indicating egg quality. Undesirable flavors lead to unacceptable products. Changes of desirable and undesirable flavors in chicken eggs depend on diet feedings, storage handling, and egg processing (Yang & Baldwin, 1995). According to Macleod & Cave (1975), the off-flavors of eggs are more readily detected due to their high intensity which would be prominent during or after cooking. There is a lack of updated information about egg volatiles since the available information was published over a decade ago. This provides a justification for the development of this thesis work.

This thesis is divided into five chapters. Chapter one provides a summarized introduction and the justification of this research. Chapter two gives a literature review with concepts associated with this work. Chapter three presents an investigation of effects of soybean oil as a coating material on changes in internal quality and oxidative stability of cooked whole eggs with three different cooking methods. Chapter four represents the effects of assorted coating materials on changes in internal quality of raw eggs and oxidative stability of boiled yolk during storage at 25 °C. Chapter five consists of a brief summary of composite findings of this work. Appendices

contain the proposal and other figures. Finally, the VITA of the author of this research is included on the last page of the thesis.

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CHAPTER 2. LITERATURE REVIEW

2.1. Hen Eggs

2.1.1 Egg Production

Eggs are inexpensive, but serve as an essential source of nutrients, including proteins, unsaturated fatty acids, vitamins, and minerals. In addition, they are one of the products that are widely consumed in many countries throughout the world (Stadelman, 1995a); hence, they have become one of the most important products in food industries for both domestic and international trade. The world production of hen eggs has increased for the last 10 years (2001-2010) (Figure 2.1). The current production of global eggs is around 1,182 billion eggs per year (Watt, 2011).

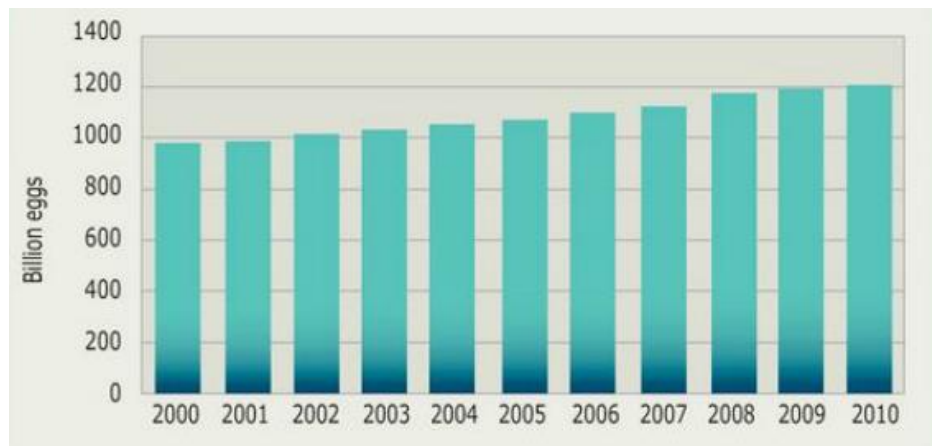


Figure 2.1 World Egg Production (Watt, 2011)

In 2011, the top 9 countries that are the largest egg producers are shown in Figure 2.2. These countries produce almost 65% of the world's egg supplies. The largest section of egg producers is in Asia, especially China. The United States of America (USA) is the second largest egg producer worldwide. The averaged egg production was 76 billion eggs, but expanded to 79 billion eggs in 2011, leading to the value of \$7.4 billion (Green & Cowan, 2012). The five

largest states of egg production are Iowa (highest), Ohio, Pennsylvania, Indiana, and California, respectively.

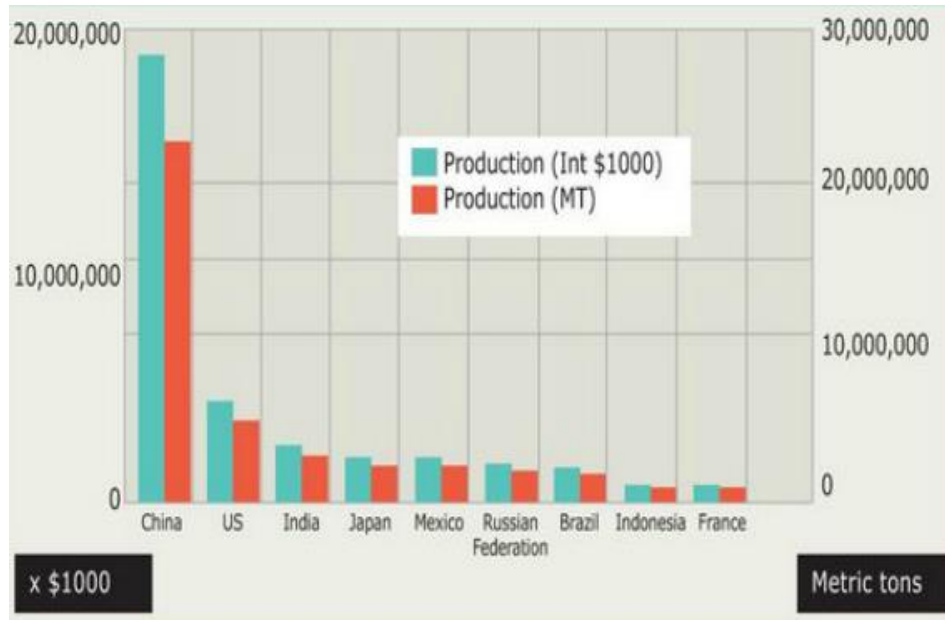


Figure 2.2 Top Egg Producer Countries (Watt, 2011)

Although eggs are surplus for consumption in some countries, egg production is not sufficient in some areas, particularly some countries in Africa. According to Food Agriculture organization (FAO), the annual consumption of eggs in some countries in Africa (e.g., Rwanda, Burundi, Chad, and Niger) is 300 g per person comparing to 19.1 kg per person in Japan based on the wealthy level in those countries (FAO, 2012). Only 9 of 43 countries in sub-Saharan Africa have an average consumption of egg higher than 2 kg per person per year, while Asian and American people consume at least 4 kg eggs per person per year. With these problems, the family farms and commercial productions of poultry products in the developing countries need to be urgently improved. More production areas, farm management, technical staffs, and consumer demands are required to increase the egg production since eggs are important for a healthy diet (FAO, 2012).

2.1.2 Structure of Hen Eggs and Composition

The structure of hen's egg is shown in Figure 2.3. A hen egg is composed of shell, egg white or albumen, and yolk. An egg weight and egg's composition depends on breed, hen's age, and feeding. The weight distribution of shell, albumen, and yolk of a white leghorn is in the range of 9-11%, 60-63%, and 28-29%, respectively (Okubo *et al.*, 1997).

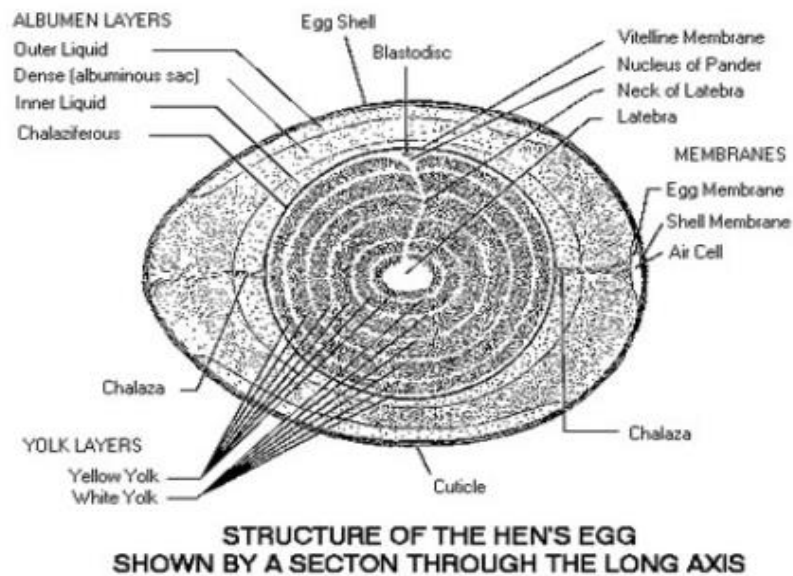


Figure 2.3 Structure of Hen Egg (Anonymous, 2012)

An eggshell is composed of a thin film of cuticle, shell stratum (calcium carbonate layer), and two shell membranes (inner and outer membranes). The main function of an eggshell is to prevent egg from deterioration by external environments. The cuticle is the most external part that covers pore canals. An egg has about 10,000 pore canals on the shell surface which allow the movement of air and moisture between inside and outside of an eggshell (Stadelman, 1995b). The main function of cuticle is to protect an egg from moisture movement and invasion of microorganisms, but it can be easily removed by washing eggs with water or soaking them in some solutions (Okubo *et al.*, 1997). Shell membrane, another important component of an

eggshell, can impede the microbial invasion by catching them in the meshwork before they reach the inside. A whole egg is composed of 12% lipids, 12% proteins, and the rest is water and small amounts of carbohydrates and minerals (Sugino et al., 1997).

The egg albumen consists of thin and thick albumen, a chalaziferous layer, and a chalazae code. Thick albumen directly contacts to the shell membrane and has a high viscosity because of the high content of protein. In addition, it covers the inner thin albumen and the chalaziferous layer by holding the egg yolk in the center of the egg. Major proteins presenting in egg albumen are ovoalbumin (54% of the total albumen solids), conalbumin (13%), ovomucoid (11%), lysozyme (3.5%) and ovomucin (1.5%) which is found in a much greater concentration in the thick layer than in the thin layer.

Egg yolk is usually in the center of the albumen. It is circled by the membrane called vitelline and surrounded by an albumen layer. The important contents of yolk are yellow and white yolk. The yellow consists of the layers of alternate light and deep yellow yolks. These two materials are the alteration of the deep components inside which are formed during the day or at night. Lipids, including fats, phosphorous, nitrogen and/or sugar-containing lipids, and sterols, are mostly contained in egg yolk (two-thirds of egg weight).

2.1.3 Shell Egg Quality

The characteristics of shell quality that need to be considered are cleanliness, soundness, smoothness, and shapes, and those characteristics could affect the acceptability of consumers (Stadelman, 1995c). For marketing, the quality control is very critical because it can be defined as the maintenance of the characteristics of a product level and tolerances acceptable to final users. The United States Department of Agriculture establishes 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 which is widely used for measurement of the albumen quality is derived from the relation of egg weight and height of the thick albumen. Besides, the Haugh unit is used as an important instrument for measuring the internal quality of eggs which is related to the USDA egg grades as follows: AA (above 72 units), A (72-60 units), B (59-31 units), and C (below 30 units, inedible or loss) (Lee *et al.*, 1996). The next factor used to determine egg quality is weight loss. The weight loss of eggs is caused by the respiration resulting in the loss of moisture and CO₂ (Caner, 2005) leading to the changes of weight loss, albumen, and yolk quality. As the storage time increases, eggs lose CO₂ and moisture through the shell pores resulting in the larger air cell and the egg's pH becomes more alkaline; eventually, the structural albumen changes (Akyurek & Okur, 2009).

Eggs are a sensitive agricultural product which can be affected by undesirable environments such as temperature, humidity, air circulation, handling, and storage time. Egg quality starts deteriorate immediately when eggs are laid by losing moisture and CO₂ through the pores of eggshell (Obanu & Mpieri, 1984). Not only eggs are perishable products but also eggshell is a fragile material. About 10% of eggs would be cracked or broken during transportation through retail market, and this represents a financial loss for egg industry (Zeidler, 2002). In addition, broken eggs will induce deterioration of internal egg quality and microbial invasion despite eggs being stored under refrigerated conditions.

Although refrigeration is the basic place to store food in order to retard deterioration in food quality, it may not be used in some countries or areas, especially rural areas, due to the high costs of refrigeration or insufficient electric power. Obanu & Mpieri (1984) reported that not only lack of electric power and refrigeration costs but also small line of egg productions can inhibit refrigerated storage in tropical areas.

Table 2.1 U.S. Standards for Quality of Individual Shell Eggs

| Quality Factor | AA Quality | A Quality | B Quality |
|---|--|---|--|
| Shell | Clean. Unbroken. Practically normal. | Clean. Unbroken. Practically normal. | Clean to slightly stained.* Unbroken. Abnormal. |
| Air Cell | 1/8 inch or less in depth. Unlimited movement and free or bubbly. | 3/16 inch or less in depth. Unlimited movement and free or bubbly. | Over 3/16 inch in depth. Unlimited movement and free or bubbly. |
| White | Clear. Firm. | Clear. Reasonably firm. | Weak and watery. Small blood and meat spots present.** |
| 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 not blood. Other serious defects. |
| For eggs with dirty or broken shells, the standards of quality provide two additional qualities. They are: | | | |
| Dirty | | Check | |
| Unbroken. Adhering dirt or foreign material, prominent stains, moderate stained areas in excess of B quality. | | Broken or cracked shell but membranes intact, not leaking.*** | |

* Moderately stained areas permitted (1/32 of surface if localized, or 1/16 if scattered).

** If they are small (aggregating not more than 1/8 inch in diameter).

*** Leaker has broken or cracked shell membranes, and contents leaking or free to leak.

Source: USDA (2000)

2.2 Coating Materials

2.2.1 Importance of Coatings

Edible films and coatings have been applied in food industries for many years. Many factors related to the application of edible films and coatings are consumer demand, new application, nontoxic food packaging materials, and new products (Gennadios *et al.*, 1997). Edible films and coatings can provide various benefits for meat and poultry products in different purposes. They can prevent moisture loss and drip loss leading to undesirable texture, flavor, and color. Volatilization of aromatic components can be reduced (Alleoni & Antunes, 2004). They can enhance the food quality by keeping water inside. They can also decrease gas diffusion (O₂

and CO₂) by maintaining low O₂ permeability which may reduce lipid and myoglobin oxidation resulting in brown color in raw meat. Some edible films and coatings are inherently antimicrobial, such as chitosan; however coating materials can be the carriers of additional antioxidants, antimicrobials, nutrients, or color in order to improve the quality of food and extend its shelf life, particularly meat rancidity and discoloration.

2.2.2 Whey Protein Concentrate

Whey protein is the collection of globular proteins isolated from whey, a by-product of cheese manufactured from cow's milk. Whey protein is typically a mixture of beta-lactoglobulin (~65%), alpha-lactalbumin (~25%), and serum albumin (~8%), which are soluble in their native forms, and each has independent pI. The protein fraction in whey (approximately 10% of the total dry solids within whey) comprises four major protein fractions and six minor protein fractions. The major protein fractions in whey are beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, and immunoglobulins. Whey proteins typically are available in three forms: concentrate (WPC), isolate (WPI), and hydrolysate (WPH).

WPC typically has a low, but still significant level of fat and cholesterol. In general, it has higher levels of bioactive compounds, and carbohydrates in the form of lactose. It is composed of 29%–89% of protein by weight.

WPI is processed to remove fat and lactose, but it is usually lower in bioactivated compounds. It is composed of 90% or higher of protein by weight. Like WPC, WPI has mild to slightly milky in taste.

WPH is whey protein that has been predigested and partially hydrolyzed for the purpose of easier metabolizing, but their cost is generally higher. Highly-hydrolysed whey may be less allergenic than other forms of whey.

Whey proteins have been widely used to form edible films because they are biodegradable and possess stronger oxygen and aroma barriers than synthetic polymer films under dry environment (Lee & Chen, 2001). Besides, they are abundant with nutritive value, their flavor is bland, and they can carry flavorings and functional ingredients. Edible protein films can improve food texture; however, their original film structures are breakable at low relative humidity; thus plasticizers, such as glycerol, are required to increase flexibility (Yoshida & Antunes, 2004). Although whey protein films are good for gas barrier, they are poor for moisture barrier because they are hydrophilic. The new promising development in food science is using milk proteins as the natural antioxidants. The properties of proteins help protect cut fruits and vegetables from enzymatic browning (Letien *et al.*, 2001). Milk proteins could prevent the formation of hydroperoxides and lipid peroxidation. In order to indicate the egg's quality, Wong *et al.* (1996) studied the properties of protein coatings on shell egg, and they observe that at the storage period during the fourth week, the greatest weight losses for both coated and non-coated eggs shall be occurred, by having values of 4.8% and 6.0%, respectively. Over four weeks of coated eggs' storage, albumen pH shall range from 8.01 to 8.33. The fine layer of albumen could be a primary barrier for gas diffusion, which helps prevent the free diffusion of CO₂ during long storage periods.

2.2.3 Chitosan

Chitosan is a polycationic and hydrophilic biopolymer of carbohydrate. It is derived from N-deacetylation of chitin (poly β -(1-4)-N-acetyl-D-glucosamine), which can be found in the shell of crustacean skeletons such as crab, shrimp, and crawfish (Figure 2.4). The three polymorphisms of chitin have been found in nature, α , β , and γ . Alpha-chitin (α) has α -crystallographic structure having the main chains arranged in an anti-parallel structure with strong

intermolecular hydrogen bonding. This chitin is mostly found in shrimp and crab shells (Wang *et al.*, 2006). Beta-chitin (β) possesses β -cristallographic structure obtained from squid pens. This chain arranges in a parallel structure with relatively weak intermolecular forces resulting in higher solubility, reactivity, and swelling than α -chitin (Kurita *et al.*, 1994). Gamma-chitin (γ) is, however, rarely identified. Chitin is largely acetylated and insoluble in water and acid while chitosan is largely deacetylated (Koide, 1998). Chitosan is insoluble in water but soluble in weak acid such as acetic acid, formic acid, lactic acid, and malic acid due to the positive charge on the second C of the glucosamine monomer at pH below 6 (Lopez-Caballero, 2005; Rabea *et al.*, 2003). The characteristics of chitosan depend on the degree of deacetylation and molecular weight (Lopez-Caballero *et al.*, 2005; No *et al.*, 2007). The viscosity of chitosan solutions is influenced by the degree of deacetylation, ionic strength, pH, and temperature.

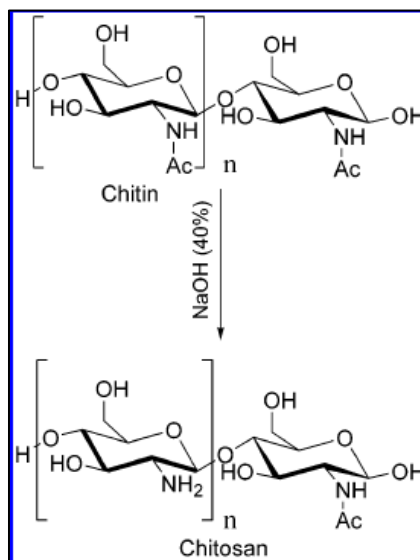


Figure 2.4 Preparation of Chitosan from Chitin (Rabea *et al.*, 2003).

Chitosan is widely used as edible films and coating materials due to its film forming properties of being tough, long lasting, flexible, and difficult to tear (Nadarajah *et al.*, 2006; Rabea *et al.*, 2003). Regardless of cellulose, Chitin is naturally the most abundant polysaccharide

(Aider, 2010; Wang *et al.*, 2006) after cellulose. Chitosan has many beneficial properties: gas impermeability, and being non-toxic and biodegradable. Although chitosan films cannot prevent moisture transmission, they are excellent for oxygen barriers. However, the most well-known property of chitosan is its antimicrobial activity. Several factors associate with the antimicrobial activity of chitosan: type of chitosan used (deacetylation degree, molecular weight), pH of matrix and temperature, and food components. The mechanism of antimicrobial activity is from the interactions between cationic chitosan and the electronegative charges on cell surfaces related to the permeable changes in cell. The higher pH, the more power to reduce microbial activity (Devlieghere *et al.*, 2004).

Many applications of chitosan as the edible films and coatings have been published. Chitosan coatings have been documented that they could extend shelf life of eggs by preventing changes of internal quality of eggs and had no effect on consumer acceptance (Lee *et al.*, 1996; Bhale *et al.*, 2003; No *et al.*, 2005; Liu *et al.*, 2009) In addition, chitosan coating could inhibit browning by protect the surface of mushroom from O₂ and delay the activity of PPO in fresh cut mushroom compared to uncoated samples at 4 °C (Eissa, 2008). According to Devlieghere *et al.* (2004), chitosan coating as an antimicrobial preservative cannot be used in every fruit and vegetable. It is applicable for food products containing low protein and NaCl; it imparts bitter taste in lettuce. Moreover, chitosan coating could be another alternative to decrease microorganisms, inhibit yellowing florets, and improve sensory quality of fresh cut broccoli (Moreira *et al.* 2011)

2.2.4 Oil Coatings

Most of lipid films and coatings are in the form of waxes (e.g., paraffin wax, beeswax), oils (e.g. mineral oil and vegetable oil), and glycerides. Wax is an ester of a long chain acid and

alcohol. It is generally found on the surface of plant or animal tissues to prevent water loss. Waxes can be named based on their original sources, such as beeswax (animal), Carnauba wax (plant), and petroleum waxes (mineral). Waxes have been widely applied on the surface on fruits and vegetables to protect moisture loss during storage. Ustunol (2009) stated that the effectiveness of lipid films depends on types of lipid, chemical structure and arrangement, polarity or hydrophobicity, physical appearance (i.e., liquid or solid), and the interactions between lipid and other components, such as proteins and polysaccharides. Lipid films are effective in preventing moisture and gas migration because of the hydrophobic structure. However, fats or oils have specific different flavor profiles from their natural sources, and their fatty acid profiles can be the onset of lipid oxidation leading to undesirable or off-flavor.

White mineral oil is a petroleum-based product, being a mixture of liquid paraffinic and naphthenic hydrocarbons. The basic structures of mineral oil are composed of n-alkanes which are chemically inert; thus, mineral oil is not involved in any chemical reactions. In addition, it has been approved for use as a food-release agent and a protective coating for fresh foods (Baldwin, 1999). Mineral oil to be used for egg coating must be odorless, colorless, and free of fluorescent materials (Stadelman 1995b).

Most vegetable oils contain a highly level of unsaturated fatty acids, primarily C 18 series. Olive and canola oils have higher proportion of oleic acid, soybean and corn oils are high in linoleic acid, and linseed oil is high in linoleic acid (Julian & Decker, 2008). Obanu & Mpieri (1984) reported suitable oil for egg coating should be light and no color, odor, and taste. According to their study, three vegetable oils (groundnut oil, cottonseed oil, and coconut oil) as coatings are efficient in preserving egg quality for 36 days under storage at 25-32 °C compared to noncoated eggs. Tropical oil coating can be another option used to decline quality losses in eggs. Comparing with proteins and polysaccharide based coatings, oil coatings have been

documented as the most effective coatings among others in preserving egg internal quality (Wardy *et al*, 2010; Biladeau & Keener, 2009).

2.3. Egg Flavors

Flavor is another consideration related to egg quality. Eggs can absorb other strong flavors from different sources, such as fruits and vegetables, organic solvents, or oil in the same environment, especially in the storage area (Yang & Balwin, 1995). Afterwards, eggs will progressively develop off-flavors as a result of deterioration of egg quality. The analysis of volatile compounds of eggs is another possibility to determine egg quality or freshness (Adamiec, *et al*, 2002). The accumulation of volatile compounds could be used as the indicators of deterioration in raw eggs (Brown *et al.*, 1986). Surprisingly, flavor components of hen eggs have not been extensively studied.

2.3.1 Flavor Changes of Egg

The flavor and odor of fresh eggs are weaker than cooked eggs because the development of characteristic of egg flavor is mostly generated by heating (Yang & Baldwin, 1995; Umamo *et al.*, 1990). When eggs are heated with different cooking methods, both desirable and undesirable flavors occur. In addition, the favorable egg volatile compounds decreases with increased storage time, resulting in higher undesirable flavors in stored eggs. The off-flavor of eggs is bland (Maga, 1982). The reduction of some volatile compounds may be caused by the loss of some compounds through the pores of shell egg or the participation in any reactions associated with other constituents of eggs. Comparing between raw and cooked eggs, heating could develop new compounds that may not originally be in raw eggs (Adamiec *et al.*, 2002). Warren *et al.* (1995) described sulfur compounds, such as dimethyl sulfide, was readily decomposed by heating.

Due to the abundance of lipids, especially in yolk, it is likely that the autoxidation plays an important role in producing most flavors or odors in eggs. When heat is accompanied with lipid in foods, hydroperoxides, the primary products of lipid oxidation process, are formed. They are unstable and, eventually, change to the secondary products, such as aldehydes, ketones, alcohols, alkanes, and acids compounds (Shahidi & Pegg, 1994). The major volatile compounds found in whole cooked eggs are aldehyde compounds (Umano *et al.*, 1990), and among those compounds, hexanal has been documented as the indicator of lipid oxidation due to its increased rate that is faster than other aldehydes (Shahidi & Pegg, 1994). According to Chitsamphandhvej *et al.* (2008), the hexanal content was used to determine the quality of fried food samples. Aldehyde and ketone compounds yield painty, fatty, metallic, and candle-like flavors (Lindsey, 2008) and the aroma quality of aldehydes is varied by the structural hydrocarbon chains. For example, C₆ and C₉ primarily are aldehydes and alcohol. C₆ compounds give green plant-like and fresh cut grass-like aromas, and C₉ compounds smell like melon and cucumbers. C₈ compounds, secondary alcohols and ketones, yield mushroom-like or violet and geranium leaves.

Off-flavors or undesirable volatiles in raw eggs are developed as the storage period increases and when the storage handling is improper. However, cooking and preparation methods with thermal processing will also create other flavors. Flavors generated during cooking are associated with browning reaction. Maillard nonenzymatic browning reaction is the chemical reaction between carbonyl compounds in reducing sugars and amino compounds associated with heat induction. It is the major of chemical reaction involving the development of flavors in foods. While some flavor compounds are acyclic, many of them are heterocyclic with substituent molecules of nitrogen, oxygen, or sulfur (Figure 2.5) contributing roasted, nutty, toasted, burnt, or caramel odors depending on temperature and time during processing.

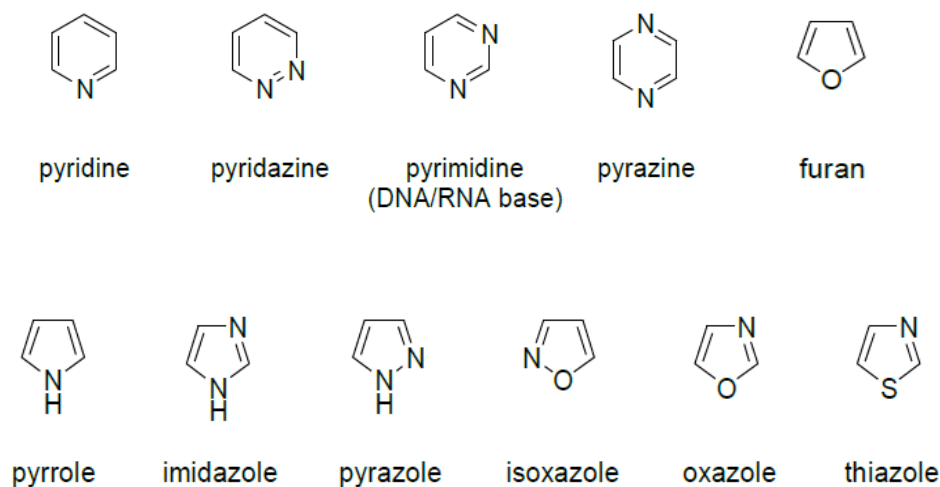


Figure 2.5 Some Common Heterocyclic Structures in Flavor Compounds associated with Thermally Induced or Browning Flavors (Gilchrist, 1992).

More volatiles were found in whole egg compared to either egg yolk or egg white alone. Because lipids and proteins are the major components in egg yolk, aldehydes and pyrazines are the major volatiles found in egg yolk. 2-methylpropanal, the richest aldehydes in egg yolk (MacLeod & Cave, 1976), may be produced from lipid oxidation of amino acid of valine upon Strecker degradation reaction (Lundberg, 1962). Sulfurous flavor in cooked egg is contributed from amino acid of methionine. Cooked egg white is a primary source that produces H_2S and NH_3 odors (Yang & Baldwin, 1995). When egg white is heated, the proteins become loosen. $-SH$ groups, in cysteine and methionine, are oxidized to $-S-S-$; hence, a subsequent release of H_2S . H_2S has a positive effect on flavor at a low concentration, while a high concentration can make eggs unacceptable.

2.3.2 Factoring Influence Egg Flavor

Different volatiles were found among cooked egg yolk, white, and whole egg (Umano *et al.*, 1990). Undesirable flavors or off- flavors in eggs are varied by feed ingredients, handling and storage of eggs before and after cooking, and processing (Yang and Baldwin, 1995). Different diets of hens influenced volatile flavors in raw egg yolk (Plagemann, 2011). In

addition, the type of cooking and container used can be influential to flavor changes. Chen & Hsu (1981) found that scrambled eggs in a double boiler had less H₂S and NH₃ than those in pouches and cooked eggs either in boiling water or by microwaves. In addition, the uses of ingredients during cooking methods will provide more attractive aromas, in, for instance, fried or scrambled eggs. It is highly possible that using oil during cooking can influence flavor components (Miller *et al.*, 1960).

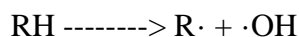
2.4 Lipid Oxidation

Lipid oxidation is the reaction of chemical changes from double bonds of unsaturated fatty acid in food that interact with atmospheric oxygen. It can be referred to the primary cause of deterioration in food products. When the oxidation of double bond takes place, intermediate peroxides or primary oxidation products are formed. They, however, are not stable; consequently, they decompose into many stable compounds, including aldehydes, ketones, alcohols, hydrocarbons and other products. The generated compounds from lipid oxidation are both desirable and undesirable or off-flavor compounds. Among secondary products from lipid oxidation, aldehydes are most important because they have a direct impact on off-flavor development, known as oxidative rancidity or rancid smell. Rancid flavor which is developed during storage from oxidative rancidity is an important problem in meat and meat products (Khaksar *et al.*, 2010). Boyd *et al.* (1992) stated that secondary products from lipid oxidation were low molecular weight volatile compounds generating off-flavors and odors. It has been documented that the contents of linoleic and linolenic acids influence to the oxidative stability of fats in food matrix (Frankel & Huang, 1994).

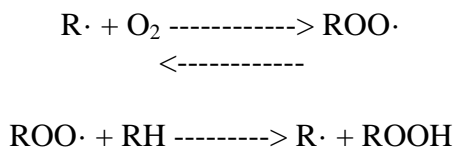
2.4.1 Mechanisms of Lipid Oxidation

Autoxidation occurs when a free radical in food lipids reacts with oxygen molecule. The reaction automatically progresses into a chain of mechanism (free radical chain reaction). This autoxidation consists of three main steps:

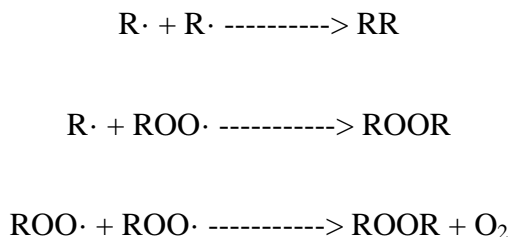
(1) Initiation step: a fatty acid radical is formed ($R\cdot$).



(2) Propagation step: a fatty acid radical is not stable, so it continuously reacts with oxygen molecule and forms peroxy radicals ($ROO\cdot$). The peroxy radicals are also not stable, so they immediately combine with other unsaturated fatty acids, and hydroperoxides ($ROOH$) are formed.



(3) Termination step: non radical species are produced from the interaction of two radicals, and the interaction between radical ($R\cdot$) and peroxy radicals ($ROO\cdot$) automatically occurs under the presence of oxygen.



The hydroperoxides ($ROOH$) formed during propagation step are the primary products of lipid oxidation. They are non-volatile, odorless, and unstable. Eventually, they decompose to

volatile aromatic compounds, especially in the forms of aldehydes and ketones. These compounds, such as hexanal, heptanal, and others, are indicator for undesirable flavors or aromas (off-flavors) in food products.

Lipid oxidation in foods begins with an induction period followed by an exponentially increased oxidation rate (Figure 2.6; Medina *et al.*, 2012).

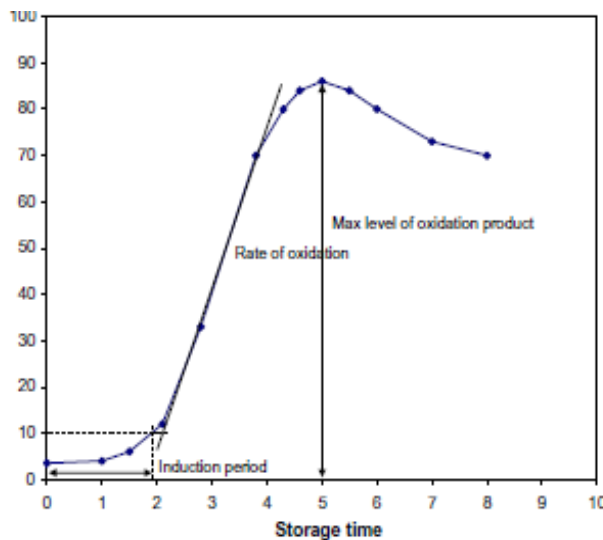


Figure 2.6 The Formation of Oxidation Products (such as peroxides, TBARS or volatiles) in a Fish Lipid Matrix (Medina *et al.*, 2012).

The length of the induction period reflects the quality of food is still high, and the rancidity has not been detected. When the exponential period is reached, lipid oxidation takes place, quickly accelerates, and rapidly develops off-aroma and flavor. The rate of lipid oxidation depends on several factors, such as oxygen content, light, metal, heat, surface area, and moisture. After the decomposition of hydroperoxides, many reactions occur, and many oxidation products are produced. Products from those reactions will depend on the type of fatty acid and the location of hydroperoxide on fatty acid. Simultaneously, the decomposition from lipid oxidation products will generate a number of different aromas based on fatty acid type which can have strong effect on sensory acceptance. Lipid oxidation produces a variety form of compounds in both low and

high molecular weight. For instance, lipid oxidation of vegetable oils with ω -6 fatty acids will produce “beany” and “grassy” odors, whereas the oxidation of long chain ω -3 fatty acids in marine oils will produce “fishy” aromas.

2.4.2 Egg Yolk Lipids

Most of egg lipids are in egg yolk, including triglycerides, phospholipids, and sterols. An egg yolk contains more unsaturated fatty acids than saturated fatty acids which mostly are found in animal lipids (Table 2.2). The difference between unsaturated and saturated fatty acids is the linked position of α and β . The fatty acids linked at the β -position are mostly unsaturated fatty acids, while saturated fatty acids are linked at α -position (Sugino et al., 1997). In 100 g of raw yolk, it consists of 26.54 g of lipid, of which 9.551 g is from total saturated fatty acids, 11.738 g from total monounsaturated fatty acids, 4.204 g total poly unsaturated fatty acids, and 1085 mg from cholesterol (USDA National Nutrient Database, 2012).

Table 2.2 Fatty Acids Composition of Egg Yolk (Gutierrez *et al.*, 1997)

| Saturated | Monounsaturated | Polyunsaturated |
|-----------|-----------------|-----------------|
| 14:0 | 14:1 | 18:2 |
| 16:0 | 16:1 | 18:3 |
| 18:0 | 18:1 | 20:4 |

Oleic (18:1), palmitic (16:0), linoleic (18:2), and stearic acid (18:0) are the major fatty acids in the form of triglycerides, whereas polyunsaturated fatty acids, such as arachidonic acid (20:4), are concentrated in the phospholipid lipid. Egg yolk contains many essential fatty acids for human nutrition, such as α -linolenic (ω -3) and linoleic (ω -6) acids. In addition, the composition of fatty acid in eggs can be affected by the feeding diet of hens. Feeding hens with fish oil or flax seeds would increase the long chain omega-3 polyunsaturated fatty acids (ω -3

PUFA) in yolk phospholipids. ω -3 PUFAs not only are essential for brain function and visual system for adults but also help develop neural system in newborn children because they can obtain those from breast milk. Beynen (2004) studied the effects of different feeding diets of hens on the composition of fatty acids in eggs, and found that groundnut feeding raised the content of oleic acid, linseed feeding produced more α -linolenic acid, and soya bean feeding provided high level of linoleic acid. Dietary of 1.5 and 3% of menhaden oil lower arachidonic acid (20:4n-6) content in raw egg yolk by 40 and 50%, respectively (Van Elswyk, 1995).

2.4.3 Measurements of Lipid Oxidation

Because of many different unsaturated fatty acids in food lipids, a number of oxidation products can be formed. In addition, lipid oxidation can occur in different ways. Each pathway can produce either primary or secondary lipid oxidation products or both. The methods for monitoring lipid oxidation in foods are complicated. There are many techniques that are used to determine the lipid oxidation; however, the analytical methods for the secondary lipid oxidation products will be focused at this time.

Secondary lipid oxidation products are the compounds derived from the decomposition of hydroperoxides at the last step of lipid oxidation. Numerous compounds, both volatile and nonvolatile, are generated. However, it is impossible to measure all the compounds simultaneously; consequently, the analysis of lipid oxidation will be focused on a single compounds or class of compounds. Nevertheless, it can be difficult to measure the oxidation since the concentrations of secondary products can be very low. Because of volatility, secondary lipid oxidation products can easily evaporate to the atmosphere. In addition, the compositions in food, such as carbohydrate, protein, and others, can interact with secondary oxidation products, and make them difficult to measure. The measurements of secondary lipid oxidation products

will measure the products from fatty acid decomposition related to the rancidity or off-flavors and off-aromas in oils which are directly related to sensory perception.

2.4.3.1 Volatile Analysis by Gas Chromatography. GC is the typical method used to analyze volatile compounds in the headspace of sample. Headspace techniques consist of static, dynamic, and solid-phase micro extraction (SPME) methods. Lipid oxidation can be measured by this method using its specific products. For example, hexanal is used for lipids with high ω -6 fatty acids, while propanal is used for lipids with high ω -3 fatty acids. In addition, a group of volatile compounds can be identified (e.g., aldehydes, hydrocarbons, or carbonyls). Hexanal is the most popular compound from lipid oxidation because it is mostly found in food lipids (Shahidi & Pegg, 1994). Consequently, it is selected to be the indicator for lipid oxidation in food, especially in meats. The advantages of using gas chromatography are that the volatile lipid oxidation product can give a high correlation with sensory analysis, and it is a reliable technique for measuring oxidation. However, this instrument is expensive and time consuming. The amount of samples can be limited. The GC requires a high temperature treatment of sample to concentrate volatile in the headspace; however, heat can increase the oxidation rates in some foods, especially meats; therefore, loss of volatile compounds during processes is another additional problem.

2.4.2.2 Thiobarbituric Acid Reactive Substances (TBARS). The TBA measurement is used to determine the decomposition of peroxides resulting in the development of off-flavors in food product (Amani & Manal, 2011). The TBARS is the assay based on the reaction between TBA and carbonyls to form pink chromophores which can be absorbed at 532 nm. The compound that acts like the primary lipid oxidation product by TBA is malondialdehyde (MDA). The advantages of this method are simple and inexpensive. However, there are several

disadvantages of using the TBA method. Non-lipid components (e.g., protein or carbohydrate) in food matrix can interfere during reaction. In addition, many variations, such as reagents or heating, can affect the condition of activity.

2.4.2.3 Fatty Acids Profile. Fatty acids are the main components of lipid, excluding mono-, di-, and triglycerides, and phospholipids. The fatty acid compounds consist of a chain of aliphatic hydrocarbon combined to carboxylic acid group at the end. Fatty acids can be classified as saturated or unsaturated containing double bonds. In addition, unsaturated fatty acids can be described as either monounsaturated (MUFAs, with only 1 double bond) or polyunsaturated (PUFAs, more than 1 double bond). Most fatty acids naturally are composed of an even number of carbon atoms in a straight chain. Naturally, the structure of fatty acids are formed in C₄ to C₂₂, but the most common form is in C₁₈ (Scrimgeour, 2005). Short chain of fatty acids, < 14 carbons, may be found but not common (Table 2.3).

Table 2.3 Names and Sources of Common Fatty Acids

| Common Name | Abbreviation | Sources |
|--------------------|----------------------|--|
| <i>Saturated</i> | | |
| Capric | C10:0 | Dairy fat, coconut, and palm kernel oil |
| Lauric | C12:0 | Coconut, and palm kernel oil |
| Myristic | C14:0 | Dairy fat, coconut, and palm kernel oil |
| Palmitic | C16:0 | Most fats and oils |
| Stearic | C18:0 | Most fats and oils |
| Arachidic | C20:0 | Peanut oil |
| <i>Unsaturated</i> | | |
| <i>(cis-MUFAs)</i> | | |
| Palmitoleic | 16:1Δ ⁹ c | Marine oils, macadamia oil, most animal and vegetable oils |

(Table 2.3 continued)

| Common Name | Abbreviation | Sources |
|---|-----------------------|--|
| Oleic <i>Unsaturated</i> (<i>cis</i> -PUFAs) | 18:1 Δ 9c (OA) | All fats and oils, especially olive oil, canola oil and high-oleic sunflower and safflower oil |
| linoleic acid | 18:2n-6 (LA) | Most vegetable oils |
| γ - linolenic acid | 18:3n-6 (GLA) | Evening primrose, borage and blackcurrant seed oils |
| arachidonic acid | 20:4n-6 (AA) | animal fats, liver, egg lipids, fish |
| α -linolenic | 18:3n-3 (ALA) | flaxseed oil, perilla oil, canola oil, soybean oil |
| EPA | 20:5n-3 | } fish, especially oily fish (salmon, herring, anchovy, smelt and macker |
| DPA | 22:5n-3 | |
| DHA | 22:6n-3 | |

Reference: FAO (2010)

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CHAPTER 3. THE EFFECTS OF SOYBEAN OIL AS A COATING MATERIAL ON CHANGES IN INTERNAL QUALITY AND FLAVOR VOLATILE COMPOUNDS OF COATED EGGS COOKED WITH THREE DIFFERENT METHODS

3.1 Summary

Effects of soybean oil (SO) as a coating material on changes in internal quality and flavor volatile compounds of eggs were evaluated during 5 weeks at $25 \pm 2^\circ\text{C}$. For the control and SO-coated eggs, as storage time increased, weight loss increased while Haugh unit and yolk index decreased. SO-coating significantly minimized weight loss (0.95%) of eggs after 5-weeks storage (vs. 6.47% for the control). Albumen pH was higher for the control compared with SO-coated eggs. Forty one volatile compounds, including aldehydes, ketones, alcohols, and sulfides, were primarily found in cooked whole eggs. Off-flavors presented in aldehyde compound, particularly hexanal due to lipid oxidation, significantly increased ($P \leq 0.05$) at the early storage and decreased near the end of storage time. The boiled eggs with SO coated produced more off-flavors than control noncoated eggs and microwaved and scrambled eggs. This demonstrated that SO coating induces higher off-flavors in cooked eggs.

3.2 Introduction

Eggs are typically inexpensive, yet offering a good source of minerals, proteins, and unsaturated fatty acids (Djousse & Gaziano, 2008; Plagemann *et al.*, 2010). In addition, eggs have a variety of functional properties including foaming, whipping, gelling, and emulsifying (Chang & Chen, 2000). Those functional properties are dependent on egg quality which is easily deteriorated by improper storage handlings (Wardy *et al.*, 2010). Egg quality begins deteriorating immediately after laying due to the loss of moisture and CO_2 through the pores of eggshell (Obanu & Mpiéri, 1984; Stadelman, 1995), resulting in higher pH and thin albumen. In the United States, there were 92.9 billion eggs produced in 2012 (USDA, 2012) of which 79 million

dozens were broken (USDA, 2013). Because eggshell is made of a fragile material, shell broken has become a big problem causing economic loss.

To surmount these problems, surface coatings have been applied on eggs for quality deterioration protection. With their versatile properties, edible films and coatings have played an important role in food industries. Surface coatings on eggs could increase shell strength and their shelf-life leading to reduced number of cracked eggs and the growth of sale exports (Biladeau & Keener, 2009). In addition, refrigeration might not be available in some developing areas of the world due to its high cost; surface coating could be the possible method to extend egg shelf-life and minimize economic loss simultaneously.

There are a number of publications about egg coatings with various types of coating materials: lipid, protein, or polysaccharide based coatings. Biladeau & Keener (2009) investigated the effects of food- grade coatings (paraffin wax, mineral oil, soy protein isolate and whey protein isolate) on egg's shelf-life under refrigerated storage. Torrico *et al.* (2010) evaluated the effects of chitosan-mineral oil emulsion as a coating material on internal quality and shelf life of eggs during room temperature storage. The effects of shellac as a coating material on internal quality and sensory evaluation during 30 days at 40 °C was studied by Musa *et al.* (2011).

Lipid based coatings, such as waxing and oiling, have been proved to be the most effective coatings among others due to their excellent sealing properties and their hydrophobicity to prevent gas and moisture loss (Biladeau & Keener, 2009; Wardy *et al.*, 2010; Jirangrat *et al.*, 2010). Among oil coatings, soybean oil has been documented as the best alternative option due to its low cost and availability (Ryu *et al.*, 2011). These publications only focused on internal

quality changes (i.e., physical attributes); however, they have not mentioned about the effects of oil coatings on flavor volatile changes in eggs.

Aroma is another important index that could affect consumer acceptance (Tananuwong & Lertsiri, 2010); thus, egg flavor can represent its quality. Generally, the flavor of fresh egg is bland and mild (Maga, 1982). The concentration of volatile compounds in fresh eggs is very low, but it simultaneously accumulates with the increased storage time (Adamiec *et al.* 2002). However, it is difficult to identify the volatile components since their original sources are more complicated to explain, and both desirable and undesirable volatiles could form at the same time. The changes of desirable and undesirable flavors in chicken eggs depend on feeding diets, storage handling, and egg processing (Yang & Baldwin, 1995). There is, however, still a lack of updated information about egg volatiles since the available information was published decades ago.

The development and distribution of egg flavor mostly occur during cooking due to changes of some chemical compounds. Plagemann *et al.* (2011) found that raw yolk contained low contents of volatiles, and its primary odor was expressed as onion-like. Since egg yolk contains more amino acids and lipids, it is more likely that the lipid oxidation and Strecker Degradation under Maillard reaction, accompanied with heating, may play an important role in forming egg volatiles resulting in the flavor compounds of pyrazines and aldehyde compounds (Macleod and Cave, 1976; Plagemann *et al.*, 2011). Kato *et al.* (1978) reported that heterocyclic compounds containing nitrogen, such as pyridines, pyrazines, and thiazoles in cooked egg white were important for cooked egg flavor.

Only a few methods have been applied for volatile extractions. Adamiec *et al.*, 2002 compared three methods of volatile extraction using a dynamic headspace, a Solid Phase

Microextraction (SPME), and a modified Likens and Nickerson extraction apparatus of simultaneous distillation-extraction (SDE). Macleod and Cave (1975) modified the Likens and Nickerson extraction apparatus of SDE to investigate egg volatiles, and they found that off-flavors had stronger flavors than desirable flavors; off-flavors would be easily detected in order to determine food quality. Umamo *et al.* (1990) used the headspace technique to determine the forms of volatile compounds in cooked whole egg, egg yolk, and egg white. However, these studies only focused on egg volatiles, no association with coatings. Although it is well known that coatings on egg shell can extend egg shelf-life and increase shell strength, there is no report whether coating treatments could affect volatile compound leading to the alteration of chemical changes that may lead to lipid oxidation.

The objectives of this study were to determine (1) the volatile compounds in cooked whole eggs, (2) the effects of soybean oil as a coating material on changes in internal quality and oxidative stability of cooked whole eggs with three different cooking methods.

3.3 Materials and Methods

3.3.1 Materials

Washed white-shelled eggs (Hyline, from 30-weeks old; a weight range of 50-70 g) were obtained from Cal-Maine Foods (Jackson, MS, USA). Great Value[®] soybean oil (SO) was purchased from Walmart (AR, USA).

3.3.2 Coating Treatment and Storage of Raw Eggs

Eggs were immediately screened based on desirable weight range of 50-70 g and defects (crack, breakage, and surface cleanliness) after they were collected from the farm and stored in the cold room (approximately 7 °C). For the next day, eggs were placed at room temperature (approximately 25 °C) for 2 h to avoid water condensation on the egg surface that could interfere

during coating. Eggs were individually weighed using a balance (TS400; Ohaus Corp., Florham Park, NJ, USA), coated with SO using a sponge brush to the entire surface of eggs, and dried overnight at room temperature (25 ± 2 °C) on racks. After eggs were dried, all treatments were placed in a small-end down position (Kim *et al.*, 2009). Two coating treatments consisting of control (noncoated) and SO coating were evaluated throughout 5 weeks of storage at room temperature (25 ± 2 °C) and averaged 60-65% RH. In 1 week intervals, twelve eggs per each treatment were taken for measuring weight loss, Haugh unit, yolk index, and albumen pH, and fifteen eggs per each treatment were taken for determination of flavor volatile compounds.

3.3.3 Physical Measurements of Raw Eggs

3.3.3.1 Measurement of Weight Loss. Weight loss (%) of the coated whole egg during storage was calculated as $\{[\text{initial whole egg weight (g) after coating at day 0} - \text{whole egg weight (g) after storage}] / \text{initial whole egg weight (g) after coating at day 0}\} \times 100$. Weight loss (%) of the control (noncoated) whole egg was calculated as $\{[\text{initial whole egg weight (g) at day 0} - \text{whole egg weight (g) after storage}] / \text{initial whole egg weight (g) at day 0}\} \times 100$. Twelve measurements per treatment were taken, and the egg weights were measured using a balance (TS400; Ohaus Corp., Florham Park, NJ, USA).

3.3.3.2 Measurement of Haugh Unit and Yolk Index. A Tripod micrometer (Model S-6428; B.C. Ames Inc., Melrose, MA, USA) was used to measure the height of yolk (mm) and albumen (mm). A digital caliper (General Tools & Instruments, New York, NY, USA) was used to measure the yolk width. 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 the ratio of yolk height and width (Stadelman, 1995a; Lee *et al.*, 1996). Twelve measurements per treatment were taken.

3.3.3.3 Measurement of Albumen pH. After the height of yolk (mm) and albumen (mm) were measured, the albumen was separated from the yolk. The thin and thick albumen were thoroughly mixed, and the pH was measured using a pH meter (Accumet[®] AP61; Fisher Scientific, Pittsburgh, PA, USA). Twelve measurements per treatment were taken.

3.3.3.4 Microbial Analysis. The control (noncoated) eggs and coated eggs with all treatments were analyzed for Aerobic Plate Count (APC) and *Salmonella* at day 0 and after 5 weeks of storage. Yolk and albumen of egg samples were homogenized using a stomacher-Easy mix (AES-Chemunex, Hamilton, NJ, USA) in a dilution of 1:10 phosphate buffered saline (PBS) 0.02 M: sodium chloride, sodium phosphate monobasis, and sodium phosphate dibasis. For APC, viable cells (CFU/g of egg) were enumerated on 3M[™] Petrifilm[™] Aerobic Count Plates (St. Paul, MN, USA) followed by incubation at 37 °C for 48 h (incubator VWR, model no 2015, Cornelius, OR, USA).

For *Salmonella spp.* detection, homogenates of egg samples were enriched by using TT Broth base Hajna dehydrated (BD Difco[™], Sparks, MD, USA) and incubated at 37 °C for 24 h. After enrichment, subcultures were plated onto XLD agar (BD Difco[™], Sparks, MD, USA) and incubated at 37 °C for 24 h prior to detection. All microbial assays were done in duplicate for each coating treatment.

3.3.4 Preparation of Cooked Eggs

Fifteen eggs per treatments were taken. Eggs were randomly separated to three groups (five eggs per groups). Each group was cooked in three different cooking methods: boiled, microwaved, and scrambled eggs.

For boiled eggs, five eggs were put in a Teflon pot with water. The stove was preheated at medium-high energy (approximately 6.5) for 5 minutes, and a pot of eggs and water was

placed on the stove. After the water reached 100 °C, the timer was set to 10 minutes. Later, eggs were cool down in cold water for 10 minutes and peeled.

For microwave cooking, each egg was cracked, stirred, and put in a Nordic ware 2 egg microwave poacher (Walmart,USA) (Figure 3.1).



Figure 3.1 Nordic Ware 2 Egg Microwave Poacher

For scrambled eggs, an empty Teflon pot was preheated on the stove for five min using a medium-high power. Five eggs were cracked, stirred, and scrambled in a hot pot for 1-2 min until cooked. No oil was used for cooking. The samples were kept in the close container to protect loss of volatiles.

Egg samples from each method were separately blended using a hand blender Hamilton Beach 59780R (Walmart, USA). Then, the samples were put in the Ziploc bag for sampling during extraction methods.

3.3.5 Analysis of Cooked Egg Volatiles

Twenty five grams of whole cooked egg samples was well mixed with 250 µl of internal standard (4-methyl-2 pentanone) in a round bottom flask. Then, the flask was incubated in water bath at 60 °C. The headspace volatiles of the sample were extracted using a SPME fiber (75 µm, Carboxen-PDMS, Supelco, Bellefonte, PA, USA) for 30 min. After the extraction, the SPME

fiber was injected and desorbed in a GC-MS to perform volatile analysis. The SPME fiber was cleaned each time before reused using a flow of helium gas for 5 minutes at 200 °C.

Gas chromatography-Mass spectrometry: The gas chromatography (Varian CP-3800 GC, Walnut Creek, CA, USA) with DB-5 column (L 60 m x i.d. 0.25 mm and d_f 0.25 μm thin coating film, Supelco, Bellefonte, PA, USA) and Variance Saturn 2200 mass spectrometer (MS) were used to identify volatile compounds in eggs. The flow rate of helium carrier gas was 1 ml/min. The oven temperature was maintained at 40 °C for 5 min and then changed to 50 °C at 2.0 °C/min. Then, the temperature was increased to 200 °C at 10 °C/min. The total analysis time was 30 min. The injection temperature was 250 °C. The identification of volatiles was obtained by the comparison of the targeted compound mass spectra to that found in NIST (National Institute of Standards and Technology) library database.

To measure the selective level of each volatile, 4-methyl-2 pentanone was used as an internal or reference standard. The relative abundance of a volatile compound was calculated as the following equation (modified from Macleod & Cave, 1976):

$$\text{Peak height ratio} = \frac{\text{peak height of interested compound}}{\text{peak height of internal standard}}$$

3.4 Statistical Analysis

Data were reported as mean ± standard deviations based on twelve measurements (eggs) per treatment for weight loss, Haugh unit, yolk index, and albumen pH while three replicates for volatile detection. The statistical analysis software (SAS, 2003) was used to analyze the data using Analysis of Variance followed by the Tukey's studentized range test ($\alpha = 0.05$).

3.5 Results and Discussion

3.5.1 The Effects of Soybean Oil Coating on the Internal Quality of Raw Eggs

Significant changes in all parameters of internal quality between the control noncoated eggs and soybean oil coated eggs were observed (interaction between coating treatments * storage periods, $P < 0.0001$) (Table 3.1). It was found that eggs coated with soybean oil had higher quality than noncoated eggs for all parameters.

3.5.1.1 Effects of Soybean Oil Coating on Weight Loss. Weight loss of egg has been found to decrease significantly with increased storage periods due to the movement of moisture and CO₂ from the albumen through the pores of eggshell (Obanu & Mpiéri, 1984). Throughout storage time, % weight loss of control eggs increased faster than SO-coated eggs (1.88-6.47% vs. 0.20-0.95%) (Table 3.1). At the end of storage, weight loss of noncoated eggs was approximately 7 times higher than SO coated eggs, which implied that SO was more effective in preventing the loss of moisture and gas due to its hydrophobicity. Similarly, soybean oil coating could reduce weight loss of eggs about 9.8 times (0.77%) compared control eggs (7.55%) after 5 weeks of storage at 25 °C (Wardy *et al.*, 2011). Moreover, Ryu *et al.* (2011) reported that the weight loss of oil coated eggs was up to 14.2 times less than that of controls, and soy bean oil itself reduced weight loss 9.1 times compared to noncated eggs after 5 weeks of storage at 25 °C. Differences in weight loss among these studies may be influenced by hen ages used, egg size, shell porosity, and environmental storage condition. Since soybean oil has hydrophobic structure, it has excellent sealing properties for moisture barrier that could inhibit the loss of egg weight (Wardy *et al.*, 2011). According to FAO (2003), the acceptable weight loss of eggs during marketing is 2-3%. In this study, SO coating was able to maintain the loss of egg weight within the acceptable range after 5 weeks at 25 °C.

Table 3.1 The Internal Quality* of Control compared to SO Coated Eggs during 5 Weeks of Storage at 25 °C.

| Parameters** | Coating*** | W0 | W1 | W2 | W3 | W4 | W5 |
|--------------|------------|----------------------------------|----------------------------------|---------------------------------|----------------------------------|----------------------------------|---------------------------------|
| WL (%) | C | - | 1.88 ± 0.32 ^{Ea} | 2.97 ± 0.19 ^{Da} | 3.95 ± 0.65 ^{Ca} | 5.04 ± 0.49 ^{Ba} | 6.47 ± 0.54 ^{Aa} |
| | SO | - | 0.20±0.05 ^{Bb} | 0.25 ± 0.12 ^{Bb} | 0.40 ± 0.12 ^{Bb} | 0.43 ± 0.28 ^{Bb} | 0.95 ± 0.55 ^{Ab} |
| HU | C | 83.46 ± 6.31 ^{Aa} AA | 67.66 ± 9.73 ^{Bb} A | 58.49 ± 6.20 ^{Cb} B | 54.66 ± 4.41 ^{CDb} B | 53.65 ± 4.40 ^{CDb} B | 48.45 ± 4.37 ^{Db} B |
| | SO | 83.46 ± 6.31 ^{Aa} AA | 77.4 ± 4.81 ^{Aba} AA | 71.64 ± 3.05 ^{Ba} A | 70.34 ± 5.28 ^{Ba} A | 69.96 ± 4.41 ^{BCa} A | 62.72 ± 5.71 ^{Ca} A |
| ApH | C | 9.20 ± 0.03 ^{Da} | 9.39 ± 0.05 ^{BCa} | 9.34 ± 0.06 ^{Ca} | 9.40 ± 0.03 ^{Ba} | 9.44 ± 0.02 ^{ABa} | 9.46 ± 0.02 ^{Aa} |
| | SO | 9.20 ± 0.03 ^{Da} | 8.57 ± 0.07 ^{Bb} | 8.45 ± 0.08 ^{Bb} | 8.46 ± 0.15 ^{Bb} | 8.46 ± 0.10 ^{Bb} | 8.45 ± 0.06 ^{Bb} |
| YI | C | 0.46 ± 0.02 ^{Aa} | 0.41 ± 0.01 ^{Bb} | 0.38 ± 0.03 ^{Cb} | 0.34 ± 0.02 ^{Cb} | 0.33 ± 0.02 ^{Db} | 0.29 ± 0.01 ^{Eb} |
| | SO | 0.46 ± 0.02 ^{Aa} | 0.43 ± 0.01 ^{ABa} | 0.46 ± 0.01 ^{Aa} | 0.43 ± 0.02 ^{ABa} | 0.42 ± 0.02 ^{BCa} | 0.40 ± 0.02 ^{Ca} |

* Means ± SD of 10 measurements. ^{A-E} For all parameter, means with different superscripts within a row indicate significant differences (P<0.05). ^{a-b} For all parameter, means with different superscripts within a column indicate significant differences (P<0.05). ** WL = % weight loss, HU = Haugh unit, ApH = Albumen pH, YI = Yolk Index; *** C = noncoated eggs, SO = soybean oil coating. Quality of individual shell eggs; AA (firm) = HU above 72; A (reasonably firm) = 71-60; B (weak and watery) = lower than 60 (USDA, 2000).

3.5.1.2 Effects of Soybean Oil Coating on Haugh Unit. The Haugh unit is calculated based on the relation between the thickness of albumen and egg weight, and it generally decreases with increasing storage time. The higher the Haugh unit values, the better the albumen quality (Stadelman, 1995; Adamiec *et al.*, 2002). Overall, the Haugh unit values significantly decreased with increased storage periods; however, this decrease slowly progressed in SO coated eggs. As seen from Table 3.1, the Haugh unit values of SO coated eggs were significantly higher than those of control eggs throughout 5 weeks of storage. At the end of storage, the averaged decline rate of the Haugh unit values of noncoated eggs was higher than SO coated eggs (41.95% vs. 18.86%). This implied that SO coating could preserve the albumen quality for at least 5 weeks at room temperature. Once the Haugh unit values are determined, egg grading could be classified. It was found that control eggs changed from AA to B grade within 2 weeks, whereas SO coated eggs were able to maintain A grade up to week 5. This implied that SO coating was effective in preserving egg quality. The Haugh unit value is directly related to the thickness of albumen. The albumen height inherently degrades to thin albumen immediately after laying eggs due to water loss (Leeson & Caston, 1997). With oil coatings, the weight loss can be reduced, and the albumen quality can be maintained for a longer time. Similar to previous studies, Wardy *et al.* (2011) and Ryu *et al.* (2011) reported that soybean oil coated eggs could maintain A grade up to 5 weeks at 25 °C, while noncoated control egg grades changed to C and B grade, respectively.

3.5.1.3 Effects of Soybean Oil Coating on Albumen pH. Albumen pH is another indicator of egg freshness (Obanu & Mpieri, 1984). Newly laid eggs should have the albumen pH around 7.6-8.5 (Waimaleongora-Ek *et al.*, 2009). In this study, the initial albumen pH value was quite high (9.20; Table 3.1). This may be due to lack of good care during transportation because eggs begin to deteriorate right after the moment of laying (Obanu & Mpieri, 1984). Eggs generally

lose moisture and CO₂ over time through the pores of eggshell. During the movement of CO₂, the carbonic acid formation in albumen starts breaking down resulting in migration of more CO₂ and a change of pH from neutral to more basic (Stadelman, 1995; Akyurek & Okur, 2009). As seen from Table 3.1, the albumen pH of control noncoated eggs gradually increased from 9.20 to 9.46, implying more loss of CO₂. However, the albumen pH was not significantly different in SO coated eggs during storage, and it was significantly lower than that of the control throughout storage periods. This implied that SO coating could lower the albumen pH closed to the fresh egg. The possible explanation is that oil may possibly oxidize to the form of free fatty acids and migrated to the albumen resulting in lower pH (Biladeau & Keener, 2009). Ryu *et al.* (2011) reported that no significant differences were observed in albumen pH among oil coated eggs during 5 weeks of storage at 25 °C, while the albumen pH of control eggs gradually increased from 8.81 to 9.37 after storage. Similarly, Wisdom *et al.* (2010) reported the increasing trend of albumen pH was observed in control eggs, whereas the decreasing trend was observed in oil coated eggs.

3.5.1.4 Effects of Soybean Oil Coating on Yolk Index. A yolk index value is used to express the spherical nature of egg yolk according to the ratio of yolk height and width (Stadelman, 1995a). The higher the yolk index value, the better the yolk quality. Significant changes in the yolk index values of noncoated and SO coated eggs during 5 weeks of storage at 25 °C were observed (interaction between coating treatments * storage periods, $P < 0.0001$) (Table 3.1). Overall, the yolk index values of all treatment decreased with increased storage periods at 25 °C. A decrease in a yolk index value is caused by the weakening of the vitelline membrane. Vitelline membrane is an important part between egg white and egg yolk, and its strength decreased with increased of storage time (Obanu & Mpieri, 1984). In addition, it is a

barrier that prevents the movement of bacteria from the albumen to the yolk (Gast, 2005). When vitelline membrane becomes weak, the water moves from albumen to the yolk, causing the yolk to collapse. Eventually, the yolk becomes susceptible to microorganisms, especially *Salmonella*, that can penetrate to the yolk due to its abundant nutrients (Keneer *et al.*, 2005). According to this study, the yolk index values in control eggs during storage significantly decreased from the initial value, while the yolk index values of SO coated eggs remained significantly unchanged. However, SO coated eggs had significantly higher yolk index values than noncoated eggs which implied that soybean oil coating could preserve the yolk quality at 25 °C. Similar results were observed, i.e., oil coated eggs, regardless of oil sources, had significantly higher yolk index value than control eggs (0.33-0.39 vs. 0.22) after 5 weeks of storage at 25 °C (Ryu *et al.*, 2011). Wardy *et al.* (2010) reported that the yolk index values of soybean oil and mineral oil coated eggs were significantly higher than control eggs (0.36-0.37 vs. 0.23) at the end of storage at 25 °C.

3.5.2 Microbial Analysis of Raw Eggs

Aerobic plate count (APC) is a quality indicator of populations of bacteria providing the information related to a food processor with raw materials, processing conditions, storage conditions and handling of product (Morton, 2001). Bacteria, particularly *Salmonella*, may be present at an intact of hatching egg and penetrate to the shell membrane (Messens *et al.*, 2005). According to the USDA (2012), raw eggs are not allowed to be consumed by people if they are contaminated with *Salmonella*. According to the results (data not shown), all treatments, before and after 5 weeks of storage at 25 °C, had non-detectable levels of bacterial counts. No *Salmonella* colonies were detected in all treatments, following the requirement of FDA.

3.5.3 The Effects of Soybean Oil Coating on the Flavor Volatile Profiles of Eggs Cooked with Three Different Methods

3.5.3.1 The Flavor Components in Cooked Whole Eggs. The profiles of volatile compounds in the cooked whole eggs with three different cooking methods were compared. Typical chromatograms of major volatile compounds are shown in Figure 3.2. Overall, it was found that the three cooking methods, regardless of coating treatments, did provide some differences in aroma compound profiles. Those compounds were identified by comparing their mass spectra to the mass spectra of the standard from the mass spectrometry library. The peak height ratio of each compound and the internal standard represented the relative abundance of that compound in each extraction. Approximately, 41 volatile compounds were found in cooked whole eggs. The volatile chemical names are described in Table 3.2.

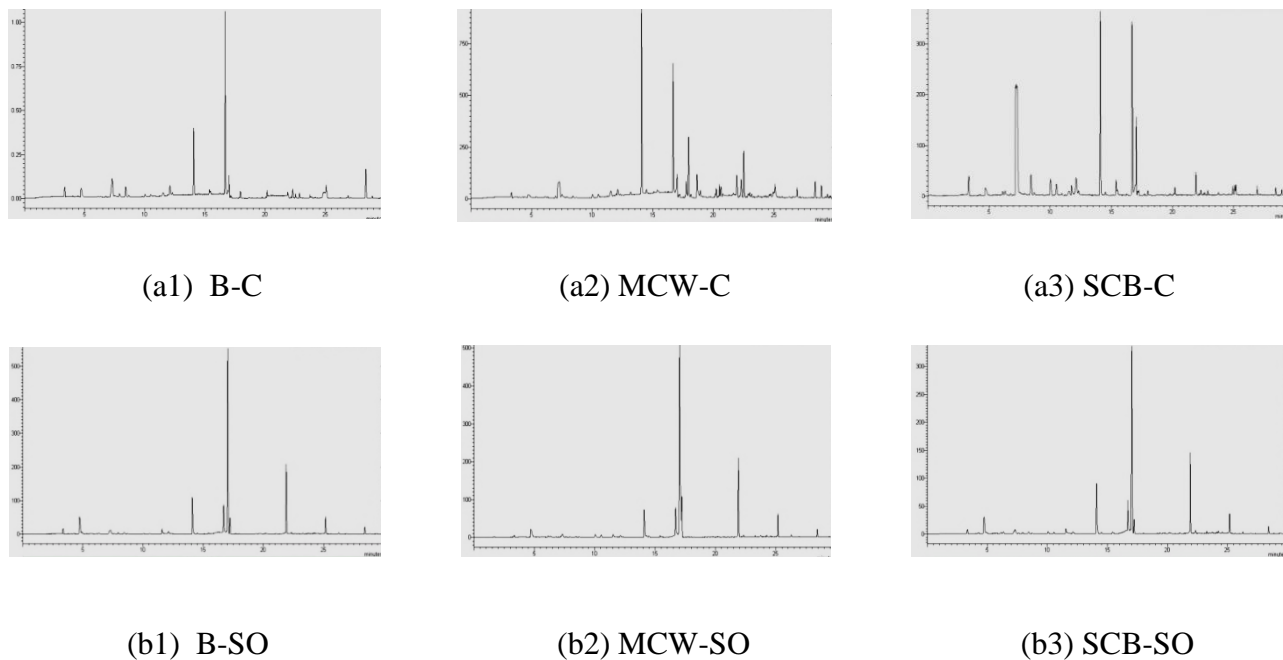


Figure 3.2 The Sample Chromatograms of Volatile Compounds in Cooked Whole Eggs (week 1): a1 = boiled, noncoated eggs, a2 = microwaved noncoated eggs, a3 = scrambled, noncoated eggs, b1 = boiled, soybean oil coated eggs, b2 = microwaved, soybean oil coated eggs, b3 = scrambled, soybean oil coated eggs.

Table 3.2 Summary Volatile Groups in Cooked Whole Wggs

| Volatile named by Classes | Compounds |
|--------------------------------------|--|
| Aldehyde | hexanal, heptanal, octanal, nonanal, 2-methylbutanal, 3-methylbutanal, 2-methylpropanal, acetaldehyde, 2,4-decadienal, t-2 nonenal |
| Alcohol | 2-methyl-2 pentanol, 2-Furanmethanol, pentane-3-ol |
| Alkanes | hexane, decane, methylcyclopentane, 2-methyl butane |
| Aromatics | toluene, benzaldehyde, 2-acetylthiazole, 1,2-dimethylbenzene, trimethylbenzene |
| Chlorine | Chloroform |
| Ester | ethyl acetate |
| Fatty acids | acetic acid, heptanoic acid, oleic acid |
| Furan | furfural, 2-pentylfuran, 3-Furaldehyde , 3-Furanmethanol |
| Ketone | acetone, 2-heptanone, 4-methyl-2-pentanone, 2,3- octanedione |
| Pyrazine | 2,5-dimethyl pyrazine |
| Sulfur | dimethyl disulfide, dimethyl sulfide |
| Others | 2-methyl-2-propen-1-ol, 4-pyridinamine, nitriles, indoles |

According to Figure 3.2, all treatments had the same profiles of flavor volatile compounds of aldehydes, ketones, furans, alcohols, alkanes, aromatic derivatives, and others. However, pyrroles and pyrazines were found in the scrambled eggs rather than in the other two cooked eggs. These compounds may be produced from the Mailard reactions between amino acids and carbonyl compounds at low moisture. They provide toasted or burnt aromas in heated foods. The results were supported by previous studies. Macleod & Cave (1975) found 65 volatile compounds in cooked eggs using a modified Likens and Nickerson extraction apparatus. Umamo *et al.* (1990) identified 87 out of 141 compounds in cooked whole eggs consisting of nitriles,

alkylbenzenes, ketones, aldehydes, pyrazines, pyrroles, and pyridines using the same method with Macleod & Cave (1975). Matiella & Hsieh (1991) determined the volatile compounds in scrambled eggs using the dynamic headspace sampling method and found 38 compounds, including aldehydes, ketones, alcohols, furans, esters, benzene derivatives, alkanes, sulfur-containing compounds, and a terpene.

The number of volatile compounds found in this study was less than that of previous research. This may be due to the different methods used during volatile extraction. In addition, inherent compounds in eggs could be affected by hen feedings (Yang & Baldwin, 1995; Plagemann, 2011).

Aldehyde compounds, including hexanal, heptanal, octanal, 3-methylbutanal, 2-methylpropanal, were found to be the major aromas in cooked egg. Warren *et al.* (1995) stated that aldehydes ranked the highest concentration of volatile compounds in cooked whole eggs, particularly acetaldehyde, 3-methylbutanal, hexanal, and 2-methylbutanal. Matiella & Hsieh (1991) reported that saturated aldehyde of C₅ to C₉ were the most abundant volatile compounds in scrambled eggs. Since eggs contain a high amount of lipids, those aldehyde compounds could be originally produced from lipid oxidation during cooking, Amino acids in eggs could play a role in forming some aldehyde compounds as well (Lungberg, 1962).

In this study, 2,5-dimethylpyrazine was found in the scrambled egg. Pyrazine is a heterocyclic compound, along with pyridines, pyrroles, and thiazoles. Typically, pyrazines are produced in the small amounts, but they are well known as the producer of strong flavor in heated food (Macleod & Cave, 1975). According to Umano *et al.* (1990), those compounds are mainly produced by the Mailard reaction and contribute to roasted, burnt, or toasted flavors in cooked foods.

Few ketones were presented in this study. Ketones are another group of volatile compounds produced from lipid oxidation. Those ketone compounds were also found in previous studies (Macleod & Cave, 1975, 1976; Matiella & Hsieh, 1991; Warren *et al.*, 1995). Acetone which has low molecular weight was found in a high amount in cooked whole egg and egg white (Umano *et al.*, 1990).

Some alcohol compounds were found in cooked whole eggs, regardless of the cooking methods. Matiella & Hsieh (1991) identified 4 saturated alcohols in scrambled egg. Octenol was the only alcohol found at very low concentrations in cooked egg yolk, white, and combinations (Warren *et al.*, 1995). Few alcohol compounds found in cooked whole egg were related to lipid oxidation as well (Umano *et al.*, 1990). Aromatic hydrocarbons, such as benzene and toluene, were found in chicken and egg volatiles. Those compounds may be due to the thermal degradation of carotenoids in egg yolk, and degradation of amino acids of phenylalanine and tyrosine to benzene and toluene, respectively (Macleod & Cave, 1975).

Regardless of the cooking methods, some furan compounds were found in cooked eggs. Particularly, 2-pentyl furan was also found in the previous research for overheated eggs (Umano *et al.* 1990; Warren *et al.*, 1995; Matiella & Hsieh, 1991). It was described as beany or grassy characteristics of off-flavors.

Ethyl acetate was the only ester found in this study. This compound was also found in the study of Warren *et al.* (1995). Methyl butyrate and butyl acetate were found in scrambled eggs (Matiella & Hsieh, 1991). Indole compounds may be derived from tryptophan (Macleod & Cave, 1975). However, the original sources of these compounds are still unclear (Matiella & Hsieh, 1991). Four n-alkanes were observed in this study in all cooking methods. Similar to the studies of Macleod & Cave (1975) and Umano *et al.* (1990), some n-alkanes were also found in their

study. However, they have no influence to flavor in foods. Dimethyl disulfide compound was originally formed by Strecker degradation of the sulfur component in amino acids, methionine and cysteine. Yang & Baldwin (1995) stated that sulfur flavor was mostly found in cooked egg white due to the release of H₂S gas when protein becomes degraded during heating. According to Umamo *et al.* (1990) and Warren *et al.*, (1995), dimethyl disulfide was usually found in cooked egg samples.

3.5.3.2 The Alterations of Major Volatile Compounds during Storage. Among 41 volatile compounds found in cooked eggs with three cooking methods associated with coating treatments, not every compound appeared consistently. It was difficult to compare every compound due to the loss of some compounds during cooking. In addition, the inherent flavor compounds in egg depend on egg quality. Many uncontrollable variations could interfere during the experiment. Therefore, the interested volatile compounds, i.e., the aldehyde group, were chosen and compared based on their importance. However, not every high peak would be the target volatile compounds because some compounds were from the SPME fiber.

Significant changes in each compound and cooking methods between the control noncoated eggs and soybean oil coated eggs were observed (interaction between coating treatments * storage periods, $P < 0.0001$) (Table 3.3). The alteration of major volatile compounds of cooked eggs with three different cooking methods were compared based on their peak height ratios. The higher the peak height ratio, the more relative abundance of the compound. The major compounds found in this study were the aldehyde group which is mainly produced from lipid oxidation of oxidized oleate, linoleate, and linolenate contributing to off-flavors (Tananuwong & Lertsiri, 2010).

Table 3.3 The changes of peak height ratios* of major volatiles compounds in cooked whole eggs during 5 weeks of storage at 25 °C.

| Volatile Compounds | Coatings | Cooking Methods** | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
|---------------------------|-----------------|--------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 3-methylbutanal | Control | B | 0.072 ^{Da} | 0.046 ^{Fd} | 0.064 ^{Ed} | 0.118 ^{Cb} | 0.542 ^{Aa} | 0.316 ^{Ba} |
| | | MCW | 0.049 ^{Eb} | 0.070 ^{Cc} | 0.057 ^{De} | 0.047 ^{Fd} | 0.082 ^{Ac} | 0.079 ^{Bd} |
| | | SCB | 0.033 ^{Ec} | 0.040 ^{De} | 0.053 ^{Cf} | 0.040 ^{Df} | 0.074 ^{Ad} | 0.069 ^{Be} |
| | SO-coating | B | 0.072 ^{Fa} | 0.682 ^{Ba} | 1.492 ^{Aa} | 0.205 ^{Da} | 0.103 ^{Eb} | 0.222 ^{Cb} |
| | | MCW | 0.049 ^{Eb} | 0.095 ^{Bb} | 0.085 ^{Cc} | 0.109 ^{Ac} | 0.082 ^{Dc} | 0.085 ^{Cc} |
| | | SCB | 0.033 ^{Dc} | 0.02 ^{Ef} | 0.088 ^{Ab} | 0.041 ^{Ce} | 0.06 ^{Be} | NP |
| 2-methylbutanal | Control | B | NP | 0.037 ^{Ed} | 0.059 ^{Dd} | 0.111 ^{Cc} | 0.465 ^{Aa} | 0.297 ^{Ba} |
| | | MCW | NP | 0.055 ^{Cc} | 0.053 ^{De} | 0.049 ^{Ee} | 0.065 ^{Bf} | 0.081 ^{Ad} |
| | | SCB | NP | 0.026 ^{Ee} | 0.040 ^{Cf} | 0.031 ^{Df} | 0.074 ^{Ad} | 0.064 ^{Bf} |
| | SO-coating | B | NP | 0.500 ^{Ba} | 0.941 ^{Aa} | 0.167 ^{Da} | 0.197 ^{Cb} | 0.089 ^{Ec} |
| | | MCW | NP | 0.080 ^{Eb} | 0.098 ^{Cb} | 0.136 ^{Bb} | 0.137 ^{Ac} | 0.093 ^{Bb} |
| | | SCB | NP | 0.019 ^{Ef} | 0.093 ^{Ac} | 0.06 ^{Dd} | 0.073 ^{Ce} | 0.079 ^{Be} |

(Table 3.3 continued)

| Volatile Compounds | Coatings | Cooking Methods** | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
|--------------------|------------|-------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Hexanal | Control | B | 0.596 ^{Fa} | 0.922 ^{Dc} | 1.525 ^{Bb} | 1.982 ^{Ac} | 1.358 ^{Cd} | 0.839 ^{Ed} |
| | | MCW | 0.228 ^{Fb} | 1.165 ^{Bb} | 1.235 ^{Ae} | 1.11 ^{Ce} | 0.805 ^{De} | 0.554 ^{Ee} |
| | | SCB | 0.065 ^{Fc} | 0.468 ^{Df} | 0.637 ^{Bf} | 0.716 ^{Af} | 0.561 ^{Cf} | 0.426 ^{Ee} |
| | SO-coating | B | 0.596 ^{Fa} | 0.820 ^{Ed} | 1.851 ^{Da} | 8.016 ^{Ba} | 8.165 ^{Aa} | 5.515 ^{Ca} |
| | | MCW | 0.228 ^{Fb} | 1.242 ^{Ea} | 1.408 ^{Dc} | 5.474 ^{Ab} | 3.633 ^{Bb} | 2.058 ^{Cb} |
| | | SCB | 0.065 ^{Fc} | 0.660 ^{Ee} | 1.370 ^{Dd} | 1.972 ^{Cd} | 2.616 ^{Ac} | 1.993 ^{Bc} |
| Heptanal | Control | B | NP | 0.016 ^{Ee} | 0.187 ^{Aa} | 0.080 ^{Bc} | 0.070 ^{Db} | 0.074 ^{Cb} |
| | | MCW | NP | 0.034 ^{Da} | 0.184 ^{Ab} | 0.044 ^{Ce} | 0.023 ^{Ef} | 0.074 ^{Bb} |
| | | SCB | NP | 0.034 ^{Ea} | 0.048 ^{Bf} | 0.039 ^{Df} | 0.045 ^{Ce} | 0.070 ^{Ac} |
| | SO-coating | B | NP | 0.023 ^{Ec} | 0.154 ^{Bc} | 0.183 ^{Aa} | 0.088 ^{Ca} | 0.076 ^{Da} |
| | | MCW | NP | 0.029 ^{Eb} | 0.066 ^{Be} | 0.081 ^{Ab} | 0.054 ^{Cd} | 0.051 ^{De} |
| | | SCB | NP | 0.019 ^{Ed} | 0.090 ^{Ad} | 0.053 ^{Dd} | 0.063 ^{Bc} | 0.058 ^{Cd} |

(Table 3.3 continued)

| Volatile Compounds | Coatings | Cooking Methods** | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
|--------------------|------------|-------------------|--------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Octanal | Control | B | NP | 0.023 ^{Da} | 0.019 ^{Ed} | 0.083 ^{Ab} | 0.062 ^{Bb} | 0.055 ^{Ca} |
| | | MCW | NP | 0.020 ^{Db} | 0.027 ^{Cc} | 0.045 ^{Be} | 0.019 ^{Ee} | 0.055 ^{Aa} |
| | | SCB | NP | 0.009 ^{Ae} | NP | 0.045 ^{Be} | NP | NP |
| | SO-coating | B | NP | 0.012 ^{Dd} | 0.191 ^{Aa} | 0.099 ^{Ba} | 0.074 ^{Ca} | NP |
| | | MCW | NP | 0.019 ^{Ec} | 0.141 ^{Ab} | 0.073 ^{Bc} | 0.048 ^{Cc} | 0.045 ^{Db} |
| | | SCB | NP | 0.012 ^{Dd} | 0.018 ^{Ce} | 0.047 ^{Ad} | 0.041 ^{Bd} | NP |
| Nonanal | Control | B | NP | 0.041 ^{Cb} | NP | 0.223 ^{Ab} | 0.102 ^{Bc} | NP |
| | | MCW | NP | 0.033 ^{Bc} | 0.575 ^{Aa} | NP | NP | NP |
| | | SCB | NP | NP | 0.484 ^{Ab} | 0.144 ^{Bd} | 0.112 ^{Db} | 0.136 ^{Ca} |
| | SO-coating | B | NP | 0.023 ^{Ce} | 0.023 ^{Ce} | 0.469 ^{Aa} | 0.120 ^{Ba} | NP |
| | | MCW | NP | 0.049 ^{Ea} | 0.085 ^{Dc} | 0.177 ^{Ac} | 0.120 ^{Ba} | 0.117 ^{Cb} |
| | | SCB | NP | 0.028 ^{Cd} | 0.030 ^{Bd} | NP | 0.047 ^{Ad} | NP |

* Means of 3 measurements, NP = No Peak. ** B = Boiled; MCW = Microwaved, SCB = scrambled

^{A-E} For hexanal parameter, means with different superscripts within a row indicate significant differences (P<0.05).

^{a-b} For hexanal parameter, means with different superscripts within a column indicate significant differences (P<0.05).

Overall, some volatile compounds tended to progressively increase with the increased storage time, while some volatile compounds tended to increase during storage time until they reached the maximum point, then they tended to decrease until the end of storage, with a plateau pattern and some fluctuations. No peak was observed in some compounds at each storage time. This may be caused by the structural degradation and the loss of some volatile compounds during cooking methods and environmental such as temperature. Regardless of coating treatment, it was found that the off-flavors of boiled eggs were significantly higher than those of the other two cooking methods which implied that cooking methods affected the distribution of off-flavors. The higher temperature and longer time, the more volatile compounds (Parliament *et al.*, 1989). For scrambled and microwaved eggs, these methods required less cooking time (2 min) than boiling (~ 20 min). For scrambled eggs, the egg samples were highly exposed to the air during cooking. Although microwaved eggs were cooked within a closed container, the container would be automatically opened due to the pressure of heat; thus this may lead to the loss of some volatile compounds. This demonstrated that heating is the major factor accelerating lipid oxidation. Chen & Hsu (1981) also confirmed that the cooking methods affected the major volatile contents of cooked egg products. Dried egg products were easy to produce the off-flavors during storage (Macleod & Cave, 1976).

For all cooking methods, it was found that SO coated eggs yielded higher off-flavors contents which implied that oil coating associated with heating could induce more lipid oxidation in eggs. This may be because the fatty acids in soybean oil got oxidized, and oxidative product migrated inside the egg resulting in higher off-flavor contents which were developed over time. Surprisingly, there is little information on the effects of oil coatings on egg volatile changes. Stadelman (1995) described that egg flavors (e.g., desirable and undesirable flavors) have

inherently been developed over time inside a shell. After eggs are coated with oil prior to storing, the oil seal could inhibit the movement of flavors from inside to outside. When storage period increases, favorable aromas are replaced with off-flavors leading to the higher intense of off-flavors. Consequently, off-flavors in eggs coated with oil are higher than those in noncoated eggs. Stadelman (1995) also claimed that oil dipping would create more off-flavors than oil spraying because dipping could completely seal the pores leading to the faster development of off-flavors than noncoated shell. However, there is still a lack of information to explain how oil coating material on eggshell influences the development of off-flavors.

Among these off-flavor compounds, the hexanal compound was found to appear mostly and consistently throughout storage periods. Hexanal is very important because it is widely used as the indicator of lipid oxidation in food lipids (Elisia & Kitts, 2011) because it is the dominant product produced from lipid oxidation of linoleic acid (Panseri *et al.*, 2011). It is simple to detect hexanal due to its low odor threshold at 5 ppb (Buttery *et al.*, 1988) and the faster rate of increasing compared to other aldehydes (Shahidi & Pegg, 1994). Its odor is described as “beany” or “grassy” which contributed to off-flavors. For all treatments, the hexanal contents tended to increase over time with the maximum values were observed at week 3. Then, the hexanal contents tended to decrease after week 3 as the plateau pattern. This implied that the hexanal compound mostly took place and influenced the flavor changes at the beginning of storage and declined due to the reduction of the substrate from lipid oxidation process. Similar result was observed by Tulyathan *et al.* (2008). They investigated the effect of coating of brown rice with flour gel on the amount of 2-acetyl-1-pyrroline (2AP) and *n*-hexanal, and found that the *n*-hexanal content in rice significantly increased from month 1 to 4 and then significantly decreased during months 5-6 which was the end of storage time. Panseri (2011) quantified the hexanal in

fresh butter during 6 months of storage at 4 °C using the headspace solid-phase microextraction (HS-SPME), and found that the hexanal constantly increased until it reached the maximum value at 6 months which referred to the commercial shelf-life. Lam & Proctor (2003) evaluated the development of hexanal and other volatile compounds in milled rice, and found that the hexanal increased significantly during the first 3 days of storage until it reached a plateau and stabilized; then, it continuously increased until 50 days of storage.

3.6 Conclusion

Aldehyde compounds were found as the major compounds in cooked whole eggs which were produced from lipid oxidation during cooking processes. Although soybean oil coating was more effective in preserving the internal quality of eggs throughout 5 weeks of storage at 25 °C, it caused higher off-flavors in cooked eggs, and the combination of soybean oil coating and boiling as a cooking method showed the highest hexanal content.

3.7 References

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CHAPTER 4. THE EFFECTS OF ASSORTED COATING MATERIALS AND ROOM-TEMPERATURE STORAGE ON INTERNAL QUALITY OF RAW EGGS AND OXIDATIVE STABILITY OF COOKED EGGS

4.1 Summary

Effects of surface coating using polysaccharides, proteins, and lipids in preserving internal quality and minimizing lipid oxidation of shell eggs were evaluated during 5 weeks at $25\pm 2^{\circ}\text{C}$. After 5-weeks, lipid-based coatings had consistently higher Haugh unit (HU) and yolk index (YI) than control and other coating treatments. Lipid-based coating significantly ($P \leq 0.05$) minimized weight loss (0.35%) of eggs after 5-weeks storage (vs. 4.84-6.84% for control and other coating treatments). For the oxidative stability measurement, SO-coated eggs had the highest hexanal content and TBARs value of boiled yolk after 5-weeks storage. The proportion of monounsaturated fatty acids (MUFAs) and saturated fatty acids increased while the proportion of polyunsaturated fatty acids (PUFAs) decreased after 5 weeks of storage. Palmitic, oleic, and linoleic acids were most abundant fatty acids in boiled yolk. This study demonstrated that SO coating can affect chemical compounds related to lipid oxidation in egg yolk although it was the most effective coating that can preserve the internal quality of eggs during storage time.

4.2 Introduction

Eggs are typically inexpensive, yet offering a good source of minerals, proteins, and unsaturated fatty acids (Djousse & Gaziano, 2008; Plagemann *et al.*, 2010). In addition, eggs have a variety of functional properties, including foaming, whipping, gelling, and emulsifying (Chang & Chen, 2000). Those functional properties depend on egg quality which is easily deteriorated by improper storage handlings (Wardy *et al.*, 2010). Egg quality begins deteriorating immediately after laying due to the loss of moisture and CO_2 through the pores of eggshell (Obanu & Mpiერი, 1984; Stadelman, 1995), resulting in higher pH and thin albumen. Shell

broken is another problem due to its fragile material causing economic loss. In the United States 2012, there are 2,114 million dozens of shell egg broken out of 92.9 billion eggs in the annual egg production (USDA, 2013).

To solve the problems, surface coatings have been applied on eggs for quality deterioration protection. Surface coating on eggs could increase shell strength and the shelf-life of eggs leading to the reduction of the number of cracked eggs and the growth of sale exports (Biladeau & Keener, 2009). In addition, refrigeration might not be available in some developing areas of the world due to its high cost; surface coating could be the possible method to extend egg shelf-life and increase shell strength. Various types of edible films and coatings have been widely used in food industry, including whey protein, soy protein, chitosan, shellac, paraffin waxes, mineral oil, and soybean oil (Obanu & Mpieri 1984; Caner, 2005, Bhale *et al.*, 2003; Kim *et al.*, 2008; Torrico *et al.* 2010 and 2011; Wardy *et al.*, 2010 and 2011). These coatings are biodegradable due to their natural sources.

Chitosan is a natural biopolymer of carbohydrate derived from deacetylated chitin (poly β -(1-4)-N-acetyl-D-glucosamine) found in the shell of crustacean skeletons such as crab, shrimp, and crawfish (Bhale *et al.*, 2003). Recent studies (Caner, 2005; Kim *et al.*, 2009; Torrico *et al.*, 20) revealed that chitosan coatings could preserve and extend egg quality longer than that of noncoated eggs at 25 °C. Whey protein, a byproduct of dairy industry, possesses stronger oxygen and aroma barriers than synthetic polymer films under dry environment (Li & Chen, 2002). Both polysaccharide and protein films are good for gas barrier; however, they are poor for moisture resistance due to hydrophilic structure (Yoshida & Antunes, 2004). Lipid films, in contrast, are hydrophobic; thus, they are good for moisture barrier. However, oil coatings have been found to be the most effective coatings for extending egg shelf life due to low cost (Wardy *et al.*, 2010).

Although surface coatings have been documented to improve egg quality during storage time, their effects on chemical changes in eggs have been neglected. Flavor is another consideration related to egg quality. Eggs can absorb other strong flavors from different sources, such as fruits and vegetables, organic solvents, or oil in the same environment, especially in the storage area (Yang & Balwin, 1995). Afterwards, eggs will progressively develop off-flavors as a result of deterioration during storage. The accumulation of volatile compounds could be used as the indicators of deterioration in raw eggs (Brown *et al.*, 1986). Because the main component in egg is lipid, lipid oxidation may play a role resulting in chemical changes during storage.

Hexanal is widely used as the important indicator of lipid oxidation because it is a predominant lipid oxidation product directly related to off-flavors even at a low odor threshold at 5 ppb (Shahidi & Pegg, 1994; Buttery *et al.*, 1988). The precursor of hexanal is linoleic acid, and this fatty acid is one of the most abundant fatty acids in egg yolk. In addition, hexanal is the most abundant compounds in many food lipids, including, meats, milk, or cooked whole eggs (Umano *et al.*, 1990; Shahidi & Pegg, 1994; Elisia & Kitts, 2011). The TBA measurement is another method used to determine the decomposition of peroxides resulting in the development of off-flavors in food product (Amani & Manal, 2011). It measures the reaction between TBA and malonaldehyde produced from lipid oxidation to form a pink chromophore detected under the absorbance 532 nm. However, it is still lack of specificity and is interfered by non-lipid components in food matrix.

Surprisingly, no study has been done on changes in oxidative stability of cooked eggs accompanied with coatings of raw egg. The objective of this research was to investigate (1) the effects of assorted coating materials on changes in internal quality of raw eggs and (2) oxidative stability of boiled yolk during storage at 25 °C.

4.3 Materials and Methods

4.3.1 Materials

Washed white-shelled eggs (Hyline, from 58-weeks old; a weight range of 50-70 g) were obtained from Cal-Maine Foods (Jackson, MS, USA). α -Chitosan (molecular weight of 1017 of kDa, acid soluble, and white-colored flaky powder prepared from crab leg shell) was purchased from Keumbo Chem (Seoul, South Korea). β -Chitosan (molecular weight of 927 kDa, acid soluble, and white-colored powder prepared from crab leg shell) was purchased from Recell Tech G&B (Seoul, South Korea). Whey protein concentrate (80% WPC; Arla Foods, Basking Ridge, NJ, USA), food grade mineral oil (MO; viscosity = 26 mPa s; transparent, odourless) obtained from Penreco[®] (Karns City, PA, USA), and Great Value[®] soybean oil (SO; Walmart, AR, USA) were also used as coating materials for eggs.

4.3.2 Preparation of Coating Solutions

Chitosan coating solution was prepared by dissolving chitosan in 1% (v/v) acetic acid at 1% (w/v) concentration (modified from Kim *et al.*, 2009). WPC coating solution was prepared by following the method of Gennadios *et al.* (1993). 3.5 g of glycerol was added to 10.78 g of WPC in order to yield approximately a protein to a plasticizer ratio of 3:1 and made up to 100 g using distilled water. The solution was homogenized until it completely dissolved and placed in a water bath at 90 °C for 30 min with stirring. The solution was adjusted to a neutral pH using 1.0 N NaOH after it was cooled until 25 °C. Chitosan and WPC coating solutions were prepared a day before the coating experiment and stored in a cold room (approximately 7 °C). MO and SO were used without modification to apply on eggs.

4.3.3 Coating Treatments and Storage of Raw Eggs

Eggs were screened based on desirable weight range of 50-70 g and defects (i.e., crack,

breakage, and surface cleanliness) immediately after they were collected from the farm and stored in the cold room (approximately 7 °C). For the next day, eggs were left at room temperature (approximately 25 °C) for 2 h to avoid water condensation on the egg surface that could interfere during coating. Eggs were individually weighed using a balance (TS400; Ohaus Corp., Florham Park, NJ, USA) and randomly coated with MO, SO, WPC, and chitosan (α and β) with a sponge brush to the entire surface of eggs. Eggs were dried overnight at room temperature (25 ± 2 °C) on racks. After they were dried, all coated eggs were placed in a small-end down position (Kim *et al.*, 2009). Six coating treatments consisting of control (noncoated), WPC, α -Ch, β -Ch, MO, and SO were evaluated throughout 5 weeks of storage at room temperature (25 ± 2 °C) and averaged 60-65% RH. Quality measurements were measured on ten eggs per each treatment at 1-week intervals.

4.3.4 Physical Measurements of Raw Eggs

4.3.4.1 Measurement of Weight Loss. Weight loss (%) of the coated whole egg during storage was calculated as $\{[\text{initial whole egg weight (g) after coating at day 0} - \text{whole egg weight (g) after storage}] / \text{initial whole egg weight (g) after coating at day 0}\} \times 100$. Weight loss (%) of the control (noncoated) whole egg was calculated as $\{[\text{initial whole egg weight (g) at day 0} - \text{whole egg weight (g) after storage}] / \text{initial whole egg weight (g) at day 0}\} \times 100$. Ten measurements per treatment were taken. The weight of the egg was measured using a balance (TS400; Ohaus Corp., Florham Park, NJ, USA)

4.3.4.2 Measurement of Haugh Unit and Yolk Index. A Tripod micrometer (Model S-6428; B.C. Ames Inc., Melrose, MA, USA) was used to measure the height of yolk (mm) and albumen (mm); while, a digital caliper (General Tools & Instruments, New York, NY, USA) was used to measure the yolk width. 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 (mm)/yolk width (mm) (Stadelman, 1995; Lee *et al.*, 1996). Ten measurements per treatment were taken.

4.3.4.3 Measurement of Albumen pH. After the height of yolk (mm) and albumen (mm) were measured, the albumen was separated from the yolk. The thin and thick albumen were thoroughly mixed and the pH was measured using a pH meter (Accumet[®] AP61; Fisher Scientific, Pittsburgh, PA, USA). Ten measurements per treatment were taken.

4.3.4.4 Microbial Analysis. The control (noncoated) eggs and coated eggs with all treatments were analyzed for Aerobic Plate Count (APC) and *Salmonella* at day 0 and after 5 weeks of storage. Yolk and albumen of egg sample were homogenized using a stomacher-Easy mix (AES-Chemunex, Hamilton, NJ, USA) in a dilution of 1:10 phosphate buffered saline (PBS) 0.02 M: sodium chloride, sodium phosphate monobasis, and sodium phosphate dibasis. For APC, viable cells (CFU/g of egg) were enumerated on 3M[™] Petrifilm[™] Aerobic Count Plates (St. Paul, MN, USA) followed by incubation at 37 °C for 48 h (incubator VWR, model no 2015, Cornelius, OR, USA).

For *Salmonella spp.* detection, homogenates of egg samples were enriched by using TT Broth base Hajna dehydrated (BD Difco[™], Sparks, MD, USA) and incubated at 37 °C for 24 h. After enrichment, subcultures were plated onto XLD agar (BD Difco[™], Sparks, MD, USA) and incubated at 37 °C for 24 h prior to detection. All microbial assays were done in duplicate for each coating treatment.

4.3.5 Preparation of Boiled Eggs

Nine eggs were placed in a Teflon pot with approximately 18 cm. diameter. 700 ml water was added into a pot to cover all eggs. The stove was preheated at medium-high energy (~6.5)

for 5 minutes; a pot with eggs and water was placed on the stove. After the water reached 100 °C, the time was set to 10 minutes. Then, eggs were cooled in cold water for 10 minutes and peeled. Each egg was cut in a half and only the yolk was taken out. Yolks were homogeneously blended using a hand blender (Hamilton Beach, 59780R). The sample was put in 4*6 inch ziploc bag to be ready for the extraction.

4.3.6 Chemical Measurements of Cooked Yolk

4.3.6.1 Determination of Hexanal Contents. Twenty five grams of yolk samples was well mixed with 1 ml of internal standard (4-methyl-2 pentanone) in a round bottom flask. Then, the flask was incubated in a water bath at 60 °C. The headspace volatiles of the sample were extracted using a SPME fiber (75 µm, Carboxen-PDMS, Supelco, Bellefonte, PA, USA) for 30 min. After the extraction, the SPME fiber was injected and desorbed in a GC-MS to perform volatile analysis. The SPME fiber was cleaned each time before reuse using a flow of helium gas for 5 minutes at 200 °C.

Gas chromatography-Mass spectrometry: The gas chromatography (Varian CP-3800 GC, Walnut Creek, CA, USA) with DB-5 column (L 60 m x i.d. 0.25 mm and d_f 0.25 µm thin coating film, Supelco, Bellefonte, PA, USA) and Varian Saturn 2200 mass spectrometer (MS) were used to identify volatile compounds in eggs. The flow rate of helium carrier gas was 1 ml/min. The oven temperature was maintained at 40 °C for 5 min and then changed to 50 °C at 2.0 °C/min. Then, the temperature was increased to 200 °C at 10 °C/min. The total analysis time was 30 min. The injection temperature was 250 °C. The identification of volatiles was obtained by the comparison of the targeted compound mass spectra to that found in NIST (National Institute of Standards and Technology) library database. To quantify hexanal in yolk samples, external standard curves were constructed (Figure 4.1)

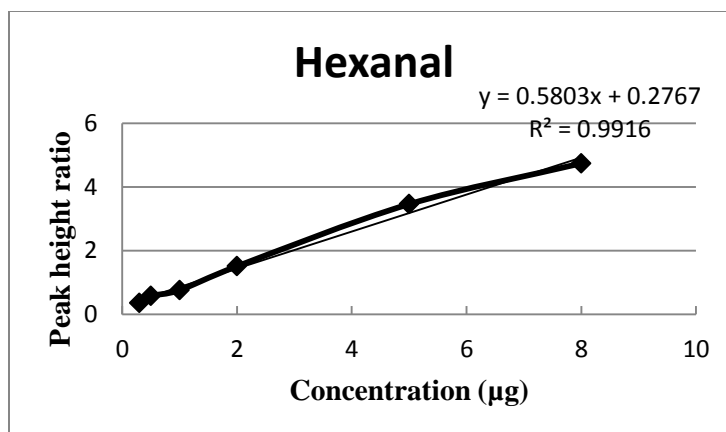


Figure 4.1 External Standard Curve of Hexanal

4.3.6.2 Determination of Thiobarbituric Acid Value (TBARS). The concentration of malondialdehyde (MDA) in cooked yolk was determined using a modified method of the Current Protocols in Food Analytical Chemistry (2001). 2.5 g of yolk samples were put in the centrifuge tubes. 5 ml of 1:1TCA reagent; water, 5 ml of water, and 0.25 of ml antioxidant solution (propyl gallate and ethylenediaminetetraacetic acid or EDTA) were added into the tubes. The solution was mixed well and centrifuged for 10 minutes. 5 ml aliquot was pipetted into a glass tube, and 5 ml of 0.02 M TBA was added into the tube. The tubes were capped, vortexed, and heated for 35 minutes at 95 °C in water. The tubes were cooled down under running tap water for 5 minutes. The MDA was quantified using a spectrophotometer at 532 nm based on the external standard curve of 1,1,3,3-Tetramethoxypropane (TMP). The TBARS value was reported as milligrams of malondialdehyde per kilogram of sample.

4.3.6.3 Analysis of Fatty Acid Compositions by Gas Chromatography. The fatty acid in cooked yolk was determined using a modified method from Yue *et al.* (2008). The extraction of yolk oil was done by mixing 2 g of cooked yolk with 2 ml of hexane and 2 ml of heptadecanoic acid (C17:0) (0.1 mg/mL in hexane), as an internal standard. The solutions were extracted using sonication for 10 minutes in cold water and centrifuged at 5000 g for 10 minutes. The hexane

and aqueous layers were transferred to the clean tube. Hexane was evaporated by using nitrogen flow. The extracted oil was derivatized by adding 2 ml of BCl₃-methanol. All test tubes were capped and incubated in a 60 °C water bath for 30 minutes to perform the delivatization of fatty acid esters. Then, the tubes were cooled in ice water for 10 minutes to stop the reaction. 2 ml of hexane and 1 ml of distilled water were added to test tubes and vortexed. The upper hexane layer was transferred to another tube, dried with anhydrous sodium sulfate, and transferred to a GC injection vial.

Gas chromatography: The gas chromatography Hewlett Packard 580 Series II plus flame ionization detector (FID) and column SP2380, 30 m x 0.25 mm i.d. x 0.25 µm (Supelco, Bellefonte, PA, USA) were used. The helium carrier gas flow rate was 1.2 ml/min. The injection volume was 5µL. The oven temperature was set at 50 °C for the first 3 min. Later, the temperature was programmed to 250 °C at 4°C/min.

4.4 Statistical Analysis

The data were reported as mean ± standard deviations based on ten measurements (eggs) per treatment for weight loss, Haugh unit, yolk index, and albumen pH while three replicates for hexanal, TBARS, and fatty acid analysis. The statistical software (SAS, 2003) was used to analyze the data using Analysis of Variance followed by the Tukey's test ($\alpha = 0.05$).

4.5 Results and Discussion

4.5.1 The Effects of Assorted Coating Materials on the Internal Quality of Raw Eggs

4.5.1.1 The Effects of Assorted Coating Materials on Haugh units. Haugh unit is the most widely tool for measuring the albumen quality. It is the primary indicator of egg freshness because it progressively decreases with increased storage time (Silversides & Budgell 2004). The higher the Haugh unit value (i.e., thick albumen), the better the albumen quality of eggs

(Stadelman, 1995; Adamiec *et al.*, 2002). Haugh unit is derived from adjusting the height of inner thick albumen to egg weight (Haugh, 1937). 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 4.1). Overall, the Haugh unit significantly decreased when storage periods increased (Table 4.1). Obviously, all coated eggs had significantly higher Haugh unit values than that of noncoated eggs which implied that coating material could maintain the albumen quality during 5 weeks of storage at 25 °C. The albumen height inherently degrades to thin albumen immediately after laying eggs due to water loss (Leeson & Caston, 1997). Loss of albumen quality is associated with egg ages, time, temperature, humidity, and storage handling. Temperature and carbon dioxide movement directly influence the rate of change in the albumen (Stadelman, 1995). Ovomucin is the major component in albumen that affects the albumen height. The alterations of albumen height may be related to several properties: proteolysis of ovomucin, cleavage of disulfide bonds, interactions with lysozyme, and changes in the interaction between α and β ovomucins (Stevens, 1996).

As seen from Table 4.1, the Haugh unit values of eggs decreased from 93.09 to 41.01 (noncoated), 57.75 (WPC), 48.43 (α -cH), 48.56 (β -cH), 68.51 (MO), and 69.25 (SO) after 5 weeks of storage. Similar results were observed by Wardy *et al.* (2010) who compared the effects of α -chitosan, WPC, mineral oil, and soybean oil on Haugh units of unwashed eggs. It was found that the Haugh units of eggs decreased from 86.50 to 27.04 (noncoated), 39.07 (α -chitosan), 33.50 (WPC), 58.43 (mineral oil), and 58.72 (soybean oil) after 5 weeks of storage at 25 °C.

Table 4.1 Haugh unit (HU)* and Grades** of Noncoated and Coated Eggs during Storage at 25 °C

| Coating*** | 0 week | 1 week | 2 week | 3 week | 4 week | 5 week |
|------------|----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|----------------------------------|----------------------------------|
| C | 93.09 ± 2.30 ^{Aa} AA | 71.37 ± 2.27 ^{Bd} A | 55.55 ± 4.26 ^{Cc} B | 50.48 ± 3.69 ^{CDc} B | 45.06 ± 6.76 ^{DEc} B | 41.01 ± 9.06 ^{Ec} B |
| WPC | 93.09 ± 2.30 ^{Aa} AA | 86.19 ± 3.49 ^{Abc} AA | 78.25 ± 8.20 ^{Bab} AA | 71.78 ± 2.51 ^{BCb} A | 68.92 ± 1.66 ^{Cab} A | 57.75 ± 5.74 ^{Db} B |
| α-cH | 93.09 ± 2.30 ^{Aa} AA | 84.21 ± 1.76 ^{Bbc} AA | 73.91 ± 1.22 ^{Cb} AA | 70.12 ± 2.21 ^{CDb} A | 65.37 ± 4.29 ^{Db} A | 48.43 ± 5.15 ^{Ebc} B |
| β-cH | 93.09 ± 2.30 ^{Aa} AA | 83.86 ± 3.00 ^{Bc} AA | 73.60 ± 4.69 ^{Cb} AA | 70.88 ± 4.57 ^{Cb} A | 61.93 ± 5.61 ^{Db} A | 48.56 ± 8.58 ^{Ebc} B |
| MO | 93.09 ± 2.30 ^{Aa} AA | 88.61 ± 3.36 ^{Bab} AA | 79.92 ± 2.94 ^{Cab} AA | 77.20 ± 1.11 ^{CDa} AA | 75.30 ± 3.40 ^{Da} AA | 68.51 ± 0.55 ^{Ea} A |
| SO | 93.09 ± 2.30 ^{Aa} AA | 91.58 ± 3.22 ^{Aa} AA | 82.36 ± 4.75 ^{Ba} AA | 80.23 ± 2.87 ^{Ca} AA | 76.22 ± 4.69 ^{Ca} AA | 69.25 ± 3.41 ^{Da} A |

* Means ± SD of 10 measurements.

^{A-E} For HU parameter, means with different superscripts within a row indicate significant differences (P<0.05). ^{a-b} For HU parameter, means with different superscripts within a column indicate significant differences (P<0.05). Tukey's Studentized Range (HSD) was used for mean separation.

**Quality of individual Shell Eggs; AA (firm) = HU above 72; A (reasonably firm) = 71-60; B (weak and watery) = 59-31 and C is below 30 (USDA, 2000).

*** C = Control; WPC = coating with whey protein concentrate solution; α- and β-cH = coating with α- and β-chitosan solutions; MO = coating with 100% mineral oil; SO = coating with 100 % soybean oil.

Without exception, the Haugh unit values of eggs coated with MO or SO were significantly higher than those of noncoated, α and β chitosan, and WPC coated eggs throughout 5 weeks of storage. This implied that lipid-based coatings were the most effective coatings in preserving the albumen quality for at least 5 weeks of storage at 25 °C. Oil coatings (e.g., MO or SO) are better barriers to moisture and gas permeability compared to polysaccharide-based (α , and β -cH) and protein-based (WPC) coatings due to their hydrophobic structures (Ustunol, 2009). Although, protein (WPC) and polysaccharide-based (α - and β -cH) coatings were less effective than lipid-based coatings, they could prolong the shelf life of eggs by at least 2 weeks longer than noncoated eggs at 25 °C. WPC and chitosan are poor moisture barriers due to their hydrophilicity (Yoshida & Antunes, 2004).

Egg quality grading can be classified based on the Haugh units: AA (>72), A (72-60), B (59-30), and C (\leq 30) (Lee *et al.*, 1996). As seen from table 4.1, control noncoated eggs changed from AA to B grade within 2 weeks and remained B grade throughout 5 weeks; whereas, eggs coated with WPC and α and β chitosan changed from AA to B grade at week 5. The AA grade could be maintained up to 4 week by MO and SO coatings but changed to A grade at week 5.

In brief, the deterioration of albumen quality of eggs could be minimized with surface coatings on eggshell. The oil coating material, particularly soybean oil, was the most effective coatings in preserving albumen quality during 5 weeks of storage at 25 °C and less expensive than other based coatings.

4.5.1.2 The Effects of Assorted Coating Materials on Weight Loss. The interaction ($P < 0.0001$) between time and coating treatments on weight loss of eggs was observed. Weight loss has been found to progressively increase when storage periods increased (Biladeau & Keener 2009; Torrico *et al.*, 2010; Wardy *et al.*, 2010) because of the evaporation of water and

CO₂ through the pores of eggshell, resulting in larger air cell (Akyurek & Okur, 2009). In this study, weight loss of all treatments significantly increased ($P < 0.0001$) during 5 weeks of storage at 25 °C (Table 4.2). However, the rate of weight loss (%) of noncoated eggs increased faster than coated eggs, i.e., from 1.31% after week 1 to 6.84% after 5 weeks vs. 0.35% to 6.46% after 5 weeks. At week 5, no significant differences ($P \geq 0.05$) were observed between α and β chitosan coatings. The weight loss (%) of control noncoated eggs was not significantly different from α chitosan, but it was significantly different from β chitosan and WPC. Nevertheless, oil coatings (i.e., mineral and soybean oil) showed the best results in reducing weight loss over storage time, and they were not significantly different ($P \geq 0.05$) from each other. Mineral and soybean oil had 17.54-19.54 times lesser in weight loss (%) than control eggs, while others had only 0.35-0.39 time lesser than control after 5 weeks of storage at 25 °C.

Similar to Wardy *et al.* (2010)'s study, weight loss (%) of mineral and soybean oil were 0.51 and 0.46, respectively, compared to 4.41 (whey protein concentrate), 4.91 (α - chitosan), and 4.80 (control) after 5 weeks of storage at 25 °C. Obanu & Mpieri (1984) found that vegetable oil coatings minimized weight loss of eggs (0.013-0.016 g) compared with that of noncoated eggs (0.186 g) for 36 days storage under 25-32 °C. Sealing pores of eggshell can prevent the movement of carbon dioxide and water from egg; as a result of, weight loss (%) decreases (Stadelman, 1995). Lipid-based film types are more resistant to moisture barriers because of their hydrophobic structure (Ustunol, 2009) while polysaccharides and protein based provide less ability against moisture loss (Banks *et al.*, 1997) due to the hydrophilic structure which could interact with the water molecules leading to higher moisture loss (Alleoni & Antunes, 2004; Wong *et al.*, 1996).

The 2-3 % weight loss of eggs during storage and distribution is considerably acceptable (FAO, 2003); therefore, this study demonstrated that mineral and soybean oil coatings were considered with the most suitable materials that could control the loss of egg weights to be in the desirable range throughout 5 weeks of storage at 25 °C.

Table 4.2 Weight loss (WL) (%)* of Noncoated and Coated Eggs during Storage at 25 °C.

| Coatings** | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
|------------|---------------------------|---------------------------------------|-----------------------------|----------------------------|---------------------------------------|
| C | 1.31 ± 0.21 ^{Da} | 2.74 ± 0.17 ^{Ca} | 5.02 ± 0.78 ^{Ba} | 6.42 ± 0.76 ^{Aa} | 6.84 ± 1.91 ^{Aa} |
| WPC | 0.71 ± 0.08 ^{Eb} | 1.96 ± 0.19 ^{Dc} | 3.08 ± 0.29 ^{Cc} | 4.06 ± 0.50 ^{Bc} | 4.84 ± 0.41 ^{Ac} |
| α-cH | 0.83 ± 0.11 ^{Eb} | 2.51 ± 0.30 ^{D^{ab}} | 4.00 ± 0.52 ^{Cb} | 5.37 ± 0.54 ^{Bb} | 6.46 ± 1.02 ^{A^{ab}} |
| β-cH | 0.77 ± 0.13 ^{Db} | 2.42 ± 0.20 ^{Cb} | 3.40 ± 0.20 ^{Bc} | 4.90 ± 1.07 ^{Ab} | 5.58 ± 0.48 ^{A^{bc}} |
| MO | 0.17 ± 0.12 ^{Bc} | 0.16 ± 0.07 ^{Bd} | 0.28 ± 0.11 ^{ABd} | 0.36 ± 0.10 ^{Ad} | 0.39 ± 0.14 ^{Ad} |
| SO | 0.10 ± 0.14 ^{Cc} | 0.18 ± 0.05 ^{BCd} | 0.24 ± 0.13 ^{ABCd} | 0.32 ± 0.18 ^{ABd} | 0.35 ± 0.10 ^{Ad} |

* Means ± SD of 10 measurements.

^{A-E} For WL parameter, means with different superscripts within a row indicate significant differences (P<0.05).

^{a-b} For WL parameter, means with different superscripts within a column indicate significant differences (P<0.05).

** See Table 4.1 for coating description.

4.5.1.3 The Effects of Assorted Coating Materials on Yolk Index. The yolk index, a spherical nature of egg yolk, represents the yolk quality (Stadelman, 1995). The higher the yolk index values, the higher the quality of the yolk. Overall, the yolk index decreased simultaneously with increased storage periods (Table 4.3). During storage, water in albumen diffuses to the yolk; thus, yolk becomes heavier (Stadelman, 1995), swelling, and collapsed (Obanu & Mpieri, 1984). Vitelline membrane is the important membrane which separates the yolk from the albumen. When vitelline membrane strength becomes weak, it is susceptible to microorganism to invade from albumen to the yolk due to an abundant source of nutrients (Akyurek & Okur, 2009). High

temperatures allow the faster rate of water movement (Stadelman, 1995). The application of coating treatments could reduce the rate of liquefiable yolk. In this study, the yolk index values of coated eggs were significantly higher than those of noncoated eggs during 5 weeks of storage at 25 °C.

The yolk index decreased from 0.47 to a range of 0.22 (noncoated) to 0.37 (mineral oil) (Table 4.3). With chitosan coatings at week 5, both α and β , had the lowest yolk indices compared to other coatings; however, their yolk index values were significantly higher than that of the control. At week 5, the yolk index of eggs coated with whey protein concentrate, mineral oil, and soybean oil were not significantly different ($P \geq 0.05$), but they had significantly ($P < 0.0001$) higher yolk index than chitosan coatings. According to the study by Wardy (2010), the yolk index of mineral and soybean oil coated eggs were significantly higher than those coating with whey protein (0.37 and 0.36 vs. 0.26). The initial quality of eggs is very important depending on hen's feeding, time for picking, handling, and others. Meanwhile, the quality of newly laid eggs is easily altered by time and environment, indicating that the initial egg quality is difficult to control. Eggs that were used in the experiment were randomly assigned to each coating treatments without knowing the quality inside. This might be the reason that why the yolk index of WPC was not significantly different from oil coatings. With oil coatings, the yolk index dropped only 0.10-0.11 unit after 5 weeks of storage. According to Obanu & Mpieri (1984), the yolk index values changed by 0.1-0.13 in eggs coated with vegetable oils (groundnut, cottonseed, and coconut) over 36 days of storage. Also, no significant differences ($P \geq 0.05$) were observed among oil coatings. Similar results were observed by Wardy *et al.* (2010), i.e., the yolk index of mineral and soybean oil coated eggs were 0.37 and 0.36, respectively after 5 weeks of storage at 25 °C. This study demonstrated that oil coatings were the most efficient in

maintaining the yolk quality than other coatings and uncoated eggs after 5 weeks of storage at 25 °C. Chitosan coatings, both α and β , slightly exhibited the preservative effects compared to control.

4.5.1.4 The Effects of Assorted Coating Materials on Albumen pH. Albumen pH is another important indicator for measuring egg freshness. Silversides and Scott (2001) claimed that measurement of albumen pH is better than measurement of albumen height in determining egg freshness. The pH range of albumen in newly laid eggs is 7.6-8.5 (Waimaleongora-Ek *et al.*, 2009). According to this study, the albumen pHs of noncoated and coated eggs were significantly different (interaction between coating treatments x storage time, $P < 0.0001$). The albumen pH of noncoated eggs gradually increased along with increased storage time while coated eggs could keep the pH down or delay the increased albumen pH throughout storage time (Table 4.4). The increased pH in noncoated eggs was caused by breaking down of carbonic acid into CO_2 which escapes through the pores. This changes neutral pH (7.6) to alkaline pH (9.7) (Obanu & Mpiერი, 1984), and the structure of albumen gel becomes watery. Both α and β chitosan delayed the alteration of albumen pH of fresh eggs until week 1, and the pH progressively increased after week 2 toward week 5. Whey protein concentrate could maintain the quality of fresh eggs longer than chitosan coatings by reducing the albumen pH from day 0 until week 3; however, the increased albumen pH was still lower than that of the control and chitosan coatings throughout week 5.

The albumen pH values of oil coated eggs (i.e, mineral and soybean oil) were not significantly different ($P \geq 0.05$) from each other but slightly decreased throughout week 5. However, oil coatings caused significantly ($p < 0.0001$) lower pH than other coatings and control over storage time, reflecting good albumen quality. This implied that oil coatings were the most

effective coating for preserving the changes in albumen pH due to their excellent sealing properties. Similar results were observed from previous studies. Wardy *et al.* (2010) demonstrated that the albumen pH increased from 9.04 to 9.33 (chitosan) and 9.24 (WPC) but decreased to 8.26 (mineral oil) and 8.34 (soybean oil). Keener & Biladeau (2009) revealed that the albumen pH of eggs coated with paraffin wax, mineral oil, soy protein isolate, and whey protein isolate was lower than control during 12 weeks of storage at 7 °C. Obanu & Mpieri (1984) reported that the albumen pH of coated eggs with groundnut, cottonseed, and coconut oil was in the range of 8.35-8.65 while control eggs had an albumen pH of 9.80 after 36 days storage under ambient conditions. The pH of all chitosan-coated eggs gradually increased with increased storage periods, from an initial value of 8.48 to 9.03–9.16 after 5 weeks of storage at 25 °C (Kim *et al.*, 2009).

4.5.1.5 Microbiological analysis of raw eggs. Aerobic plate count (APC) is a quality indicator of populations of bacteria providing the information related to a food processor with raw materials, processing conditions, storage conditions, and handling of product (Morton, 2001). Bacteria, particularly *Salmonella*, may be on the surface of intact hatching eggs and then penetrate the shell membrane (Messens *et al.*, 2005). According to USDA (2012), raw eggs are not allowed to be consumed by people if contaminated with *Salmonella*. All treatments, before and after 5 weeks of storage at 25 °C, had non-detectable levels of bacterial counts (results not shown). No *Salmonella* colonies were detected in all treatments. According to FDA, the presence of *Salmonella* should be negative (no colony).

Table 4.3 Yolk index (YI)* of Noncoated and Coated Eggs during Storage at 25 °C

| Coatings** | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
|------------|---------------------------|----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|
| C | 0.47 ± 0.01 ^{Aa} | 0.38 ± 0.01 ^{Bb} | 0.31 ± 0.00 ^{Cd} | 0.26 ± 0.01 ^{Dd} | 0.26 ± 0.02 ^{Dc} | 0.22 ± 0.01 ^{Ed} |
| WPC | 0.47 ± 0.01 ^{Aa} | 0.44 ± 0.02 ^{Aba} | 0.42 ± 0.10 ^{BCa} | 0.38 ± 0.02 ^{Dab} | 0.40 ± 0.02 ^{CDa} | 0.32 ± 0.04 ^{Ea} |
| α-cH | 0.47 ± 0.01 ^{Aa} | 0.44 ± 0.01 ^{Ba} | 0.39 ± 0.02 ^{Cbc} | 0.36 ± 0.02 ^{Db} | 0.34 ± 0.02 ^{Db} | 0.27 ± 0.02 ^{Ec} |
| β-cH | 0.47 ± 0.01 ^{Aa} | 0.44 ± 0.01 ^{Ba} | 0.37 ± 0.02 ^{Cc} | 0.34 ± 0.02 ^{Dc} | 0.34 ± 0.02 ^{Db} | 0.27 ± 0.02 ^{Ec} |
| MO | 0.47 ± 0.01 ^{Aa} | 0.44 ± 0.01 ^{Ba} | 0.41 ± 0.02 ^{Cab} | 0.39 ± 0.01 ^{DEa} | 0.40 ± 0.02 ^{CDa} | 0.37 ± 0.01 ^{Ea} |
| SO | 0.47 ± 0.01 ^{Aa} | 0.44 ± 0.01 ^{Ba} | 0.42 ± 0.10 ^{BCa} | 0.39 ± 0.01 ^{Ca} | 0.41 ± 0.03 ^{Ca} | 0.36 ± 0.01 ^{Da} |

* Means ± SD of 10 measurements.

^{A-E} For YI parameter, means with different superscripts within a row indicate significant differences (P<0.05).

^{a-b} For YI parameter, means with different superscripts within a column indicate significant differences (P<0.05).

** See Table 4.1 for coating description.

Table 4.4 Albumen pH* of Noncoated and Coated Eggs during Storage at 25 °C

| Coatings** | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
|------------|---------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|----------------------------|
| C | 8.62 ± 0.13 ^{Ca} | 9.30 ± 0.04 ^{Ba} | 9.41 ± 0.03 ^{Aa} | 9.43 ± 0.03 ^{Aa} | 9.27 ± 0.04 ^{Ba} | 9.38 ± 0.10 ^{Aa} |
| WPC | 8.62 ± 0.13 ^{Ba} | 8.15 ± 0.17 ^{Cc} | 8.23 ± 0.07 ^{Cd} | 8.56 ± 0.21 ^{Bd} | 8.81 ± 0.09 ^{Ac} | 8.87 ± 0.15 ^{Ab} |
| α-cH | 8.62 ± 0.13 ^{Da} | 8.56 ± 0.22 ^{Db} | 8.86 ± 0.18 ^{Cb} | 8.95 ± 0.21 ^{BCc} | 9.11 ± 0.06 ^{ABab} | 9.27 ± 0.09 ^{Aa} |
| β-cH | 8.62 ± 0.13 ^{Da} | 8.58 ± 0.23 ^{Db} | 8.90 ± 0.29 ^{Cb} | 9.20 ± 0.05 ^{ABb} | 9.03 ± 0.27 ^{BCb} | 9.30 ± 0.08 ^{Aa} |
| MO | 8.62 ± 0.13 ^{Aa} | 8.50 ± 0.11 ^{ABb} | 8.46 ± 0.09 ^{BCc} | 8.42 ± 0.07 ^{BCde} | 8.31 ± 0.12 ^{Cd} | 8.32 ± 0.14 ^{Cc} |
| SO | 8.62 ± 0.13 ^{Aa} | 8.42 ± 0.11 ^{Bb} | 8.41 ± 0.10 ^{BCd} | 8.33 ± 0.15 ^{BCe} | 8.24 ± 0.13 ^{Cd} | 8.25 ± 0.14 ^{BCc} |

* Means ± SD of 10 measurements.

^{A-E} For Albumen pH parameter, means with different superscripts within a row indicate significant differences (P<0.05).

^{a-b} For Albumen pH parameter, means with different superscripts within a column indicate significant differences (P<0.05).

** See Table 4.1 for coating description.

4.5.2 The Effects of Assorted Coating Materials on Fatty Acids Changes of Boiled Yolk during Storage at 25 °C

The effect of coating treatments and room temperature storage for 5 weeks on the fatty acid compositions of boiled egg yolk is shown in Table 4.5. These reported fatty acids were the major fatty acids in boiled yolk sample. Overall, slight differences were observed in changes of fatty acid compositions during storage time, and some changes were not statistically different.

Palmitic, oleic, linoleic, and arachidonic acids were not significantly different between coating treatments and storage periods. Comparing among coating treatments, soybean oil coated eggs yielded the highest portion of all fatty acid profiles. This may be due to the abundance of natural fatty acids from the original source in soybean oil. The portion of myristic acid (C14:0) of soybean oil coated egg yolk increased almost two times of that of other treatments.

Apparently, oleic (C18:1), palmitic (C16:0), and linoleic (C18:2 *n-6*) acids were the top three most abundant fatty acids in boiled egg yolk. These results supported the previous studies that those three fatty acids were accounted for 89% of the total fatty acid in yolk, and fatty acid compositions of yolk was not notably different after 5 weeks of storage at 25 °C (Kim *et al.*, 2009). Liu *et al.* (2005) also reported that oleic (C18:1) was the first major fatty acids in commercial egg products, with approximately 44-49%, followed by palmitic (C16:0) and linoleic (C18:2 *n-6*) about 24-28% and 10-15%, respectively.

Alteration of fatty acids in hen eggs depends on many factors: feeding diets, storage time, and thermal processing. Botsoglou *et al.* (2012) found that hen feedings with linseed and soybean oil affected the proportions of saturated fatty acids, MUFAs, and PUFAs of eggs throughout 60 days storage at refrigerated temperature. They also demonstrated that thermal processing by pasteurization, hard-boiling, and scrambling will decrease the proportions of PUFAs, especially *n-3* PUFAs. In addition, feedings with linseed oil diet had α -linolenic acid

(C18:3n-3) as the main portion, while feedings with sunflower oil did not affect to the amount of C18:3. Van Elswyk *et al.* (1995) demonstrated that feeding diets containing 1.5 and 3.0 % of menhaden oil resulted in a higher amount of total n-3 fatty acids in egg yolk.

It is well known that the long chain PUFAs can enhance the susceptibility to lipid oxidation leading to off-flavors in food lipids. The enrichment of the level of PUFA in n-3 is susceptible to lipid oxidation in poultry meat leading to discoloration, drip loss, and development of off-flavors during storage Hugo *et al.* (2009). As egg lipid contains a high amount of unsaturated fatty acids, lipid oxidation may play an important role in egg quality deterioration by generating off-flavors. Linoleic and linolenic acids have been documented as a precursor of lipid oxidation (Koelsch *et al.*, 1991; Shahidi & Pegg, 1994; Panseri *et al.*, 2011). After it oxidizes, it alters to hexanal, the indicator of lipid oxidation. The oxidative rancidity of oils is varied by the level of unsaturated fatty acid compositions, particularly linoleic and linolenic acids (Frankel & Huang, 1994). Soybean oil, the most abundant source of vegetable oil in the world, is readily susceptible to lipid oxidation, resulting in “flavor reversion” (Frankel, 1980). Then, oxidative products can be analyzed from the intense of hexanal using a GC and TBA Test.

However, it is difficult to explain if the abundant fatty acids in soybean oil may get involved to some chemical changes. Many variations can occur during the experiment. The major variable found during the GC analysis was the shifting of some peaks with the changed retention time leading to miscalculations. In addition, the analysis of fatty acids using gas chromatography can measure all fatty acids that are not in free form. However, H₂O₂ from lipid oxidation will convert unsaturated fatty acids to saturated fatty acids (Khaksar *et al.*, 2010). This analysis only demonstrated some ideas about the changes of fatty acids in boiled egg yolk affected by coating treatments during storage and the fatty acids related to oxidative reaction.

Table 4.5 Effects of Coating Treatments on Fatty Acid Compositions (ppm) of Boiled yolk after 5 Weeks of Storage at 25 °C.

| Fatty acids | Storage Time | | | | | | |
|--|---------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|----------------------------|
| | Day 0 | Week 5 | | | | | |
| | Control** | Control** | WPC** | α -cH** | β -cH** | MO** | SO** |
| C14:0 myristic | 7.3 ± 0.1 ^b | 8.2 ± 1.0 ^b | 9.3 ± 0.1 ^b | 7.6 ± 0.2 ^b | 8.4 ± 0.6 ^b | 8.2 ± 0.0 ^b | 12.7 ± 0.7 ^a |
| C16:0 palmitic | 397.1 ± 31.6 ^a | 368.6 ± 31.6 ^a | 420.5 ± 5.0 ^a | 411.8 ± 4.2 ^a | 405.3 ± 25.6 ^a | 405.8 ± 6.6 ^a | 421.3 ± 13.0 ^a |
| C16:1 palmitoleic | 46.3 ± 6.6 ^a | 33.9 ± 0.0 ^b | 53.2 ± 3.2 ^a | 48.0 ± 0.8 ^a | 53.5 ± 0.0 ^a | 48.2 ± 0.0 ^a | 52.5 ± 0.0 ^a |
| C18:0 stearic | 196.7 ± 0.0 ^b | 177.9 ± 5.2 ^c | 172.4 ± 1.9 ^c | 172.8 ± 1.8 ^c | 177.8 ± 0.0 ^c | 195.5 ± 0.0 ^b | 207.0 ± 0.0 ^a |
| C18:1 oleic | 549.8 ± 46.3 ^a | 520.16 ± 38.3 ^a | 568.5 ± 7.0 ^a | 525.4 ± 38.3 ^a | 523.9 ± 52.5 ^a | 545.7 ± 19.2 ^a | 621.07 ± 10.4 ^a |
| C18:2 (<i>n</i> -6) linoleic | 232.2 ± 12.7 ^a | 199.4 ± 20.0 ^a | 226.3 ± 4.8 ^a | 220.6 ± 11.3 ^a | 203.6 ± 5.8 ^a | 225.5 ± 11.9 ^a | 234.2 ± 0.0 ^a |
| C18:3 (<i>n</i> -3) α -linolenic | 24.9 ± 1.3 ^{ab} | 17.6 ± 0.0 ^c | 21.8 ± 0.0 ^{abc} | 18.0 ± 3.8 ^c | 20.1 ± 0.0 ^{bc} | 19.7 ± 0.0 ^{bc} | 26.8 ± 0.0 ^a |
| C20:4 (<i>n</i> -6) arachidonic | 17.1 ± 0.5 ^a | 18.7 ± 0.0 ^a | 15.3 ± 3.2 ^a | 16.7 ± 1.0 ^a | 14.0 ± 0.6 ^a | 14.9 ± 2.4 ^a | 19.8 ± 1.7 ^a |
| Total SAT* | 601.1 | 554.7 | 602.2 | 599.8 | 591.5 | 609.5 | 641 |
| Total MUFAs* | 596.1 | 554.06 | 621.4 | 573.4 | 577.4 | 593.9 | 673.57 |
| Total PUFAs* | 274.4 | 235.7 | 263.4 | 255.3 | 237.7 | 260.1 | 280.8 |

Means ± SD of 3 measurements.

^{a-b}Means with different superscripts within a row indicate significant differences (P<0.05).

* SAT = Saturated fatty acids, MUFAs = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids

** See Table 4.1 for coating description.

4.5.3 The Effects of Assorted Coating Materials on Hexanal Changes in Boiled Yolk during storage at 25 °C

Hexanal is widely used as the indicator of lipid oxidation in foods (Elisia & Kitts, 2011) because it is the predominant product produced from lipid oxidation of linoleic acid (Koelsch *et al.*, 1991; Shahidi & Pegg, 1994; Panseri *et al.*, 2011). It is simple to detect hexanal due to its low odor threshold at 5 ppb (Buttery *et al.*, 1988) and the faster rate of increasing compared to other aldehydes (Shahidi & Pegg, 1994). Its odor is described as “beany” or “grassy” contributed to off-flavors. The changes of hexanal contents in boiled egg yolk were observed throughout 5 weeks of storage at 25 °C (the interaction between week x treatment; $P < 0.0001$) (Table 4.6).

The amount of hexanal on day 0 was obtained from the noncoated control eggs while the hexanal quantities in all coated eggs were measured on week 1. For all treatments, the hexanal contents tended to increase over time with the maximum values observed at week 3. Then, the hexanal contents tended to decrease as the plateau pattern. This implied that the hexanal compound mostly took place and influenced flavor changes at the beginning of storage and declined due to the reduction of the substrate from lipid oxidation process. Similar result was observed by Tulyathan *et al.* (2008). They investigated the effect of coating of brown rice with flour gel on the amount *n*-hexanal, and found that the *n*-hexanal content in rice significantly increased from month 1 to 4 and then significantly decreased during months 5-6 which was the end of storage time. Panseri (2011) quantified the hexanal in fresh butter during 6 months of storage at 4 °C using the headspace solid-phase microextraction (HS-SPME) and found that the hexanal constantly increased until it reached the maximum value at 6 months which referred to the commercial shelf-life. Lam & Proctor (2003) evaluated the development of hexanal and other volatile compounds in milled rice, and found that the hexanal increased significantly during the first 3 days of storage until it reached a plateau and stabilized; then, it continuously increased

until 50 days of storage. Chitsamphandhvej *et al.* (2008) analyzed the hexanal compounds in six food samples in Thailand: infant cereal, instant noodle, potato French fried, deep fried peanut, fried rice (Khawtan) and fried pork skin (Capmoo), and they found that the hexanal increased significantly with the increase of storage time in the range of 0.15-10.40 $\mu\text{g/g}$.

At the end of storage, the hexanal contents of eggs coating treatments, excluding SO coated eggs, were significantly lower than control (3.24 - 6.13 vs. 8.73) which implied that α and β chitosan, WPC, and MO coatings were more effective in preventing the lipid oxidation by prohibiting the pervasion of oxygen from the atmosphere to interact with the interior lipid in yolk, leading to higher hexanal. Based on coating properties, whey protein and chitosan films provide an excellent protection of oxygen barrier due to the tight structure of hydrogen bonds (Chen, 1995; Gallstedt & Hedenqvist, 2004), and they are naturally non-oxidized.

However, it was found that SO coated eggs yielded the highest hexanal content. Comparing to the control at week 5, the hexanal values increased 61.40% in SO coated, whereas the hexanal values decreased 29.78%, 50.28%, 55.44%, and 62.89% in MO, β chitosan, α chitosan, and WPC coated eggs, respectively. This may be possible that soybean oil got oxidized, and the oxidation products migrated to the yolk inside, resulting in high hexanal contents which were developed over storage time. Similar explanation was found by Stadelman (1995) that the development of off-flavors inside an egg generally increases with the increased storage periods. With soybean oil coatings prior to storage, the oil seal could inhibit the loss of off- flavors that are inherently occurred from an interior due to their excellent sealing properties and their natural unsaturated fatty acids composition. Mineral oil, in contrast, is from non-vegetable sources composed of n-alkanes which are chemically inert; therefore, it does not associate with any chemical reactions. In addition, oil coatings have drawbacks in some products. Wax coatings on

fruits and vegetables can develop off-flavors due to the inhibition of O₂ and CO₂ exchanges resulting in the fermentation of anaerobic system (Hagenmaier & Shaw, 1992).

4.5.4 The Effects of Assorted Coating Materials on TBARS Changes in Boiled Yolk during Storage at 25 °C

TBARS is another secondary method that is commonly used as the indicator of degree of rancidity in various products, particularly in meat and meat products (Shahidi & Pegg, 1994). The TBARS values of boiled yolks of non-coated raw eggs compared to those of coated raw eggs were observed during storage at 25 °C (Table 4.7).

Overall, TBARS values continuously increased until the maximum values were observed at week 5 ($p < 0.0001$). This may be because the ambient temperature accelerates the chain propagation reactions of rancidity in early stage of autoxidation and decomposition of peroxides leading to higher rate of lipid oxidation (Biswas *et al.*, 2011). Then, the TBARS values slightly decreased which may due to the loss of some low molecular weight volatile products from lipid oxidation (Artharn *et al.*, 2009).

The TBARS results supported the previous results of hexanal contents that SO coated egg yolk yielded the highest TBARS value, and the TBARS values of other coating treatments were significantly lower than control at the end of storage. Comparing to control, the TBARS values increased by 61.40% in SO coated yolk, whereas the TBARS values decreased from 29.78 to 62.89% in other coating treatments. This implied that both hexanal and TBARS methods could serve as the useful index of lipid oxidation in egg yolk. The explanations based on the effects of coating treatments to TBARS values were already given earlier. At the end of storage, TBARS values of all treatments ranged from 0.63-3.20 mg malonaldehyde/kg sample. The TBARS values of egg oils of commercial eggs in Taiwan, including tea egg, simmered egg, iron egg, salted egg, and pidan egg, were in the range of 0.17-2.73 mg malondialdehyde/kg oil (Liu *et al.*,

2005). Apparently, chitosan, protein based films, and mineral oil coatings could minimize lipid oxidation by inhibiting the penetration of oxygen inducing lipid oxidation in boiled egg yolk.

Similar results were observed from the previous studies Sathivel (2005) reported that chitosan and Soy Protein Concentrate coatings could retard lipid oxidation of pink salmon fillets after 3 months frozen storage; the reason was that the lipid oxidation in pink salmon fillets could be retarded due to antioxidant and oxygen barrier properties of chitosan (Sathivel *et al.*, 2007). Hasanzati. *et al.* (2010) demonstrated that coating fish with whey protein solutions could decrease lipid oxidation because whey protein prevented the lipid in fish muscle from interaction with oxygen. For lipid based coatings, soybean oil is biodegradable from a vegetable source, so it naturally contains unsaturated fatty acids in both MUFAs and PUFAs, which make the oil chemically unstable. However, mineral oil is less biodegradable, and mainly composed of hydrocarbon, so it is chemically stable. The higher the amount of unsaturated fatty acids, the higher the content of lipid oxidation products.

According to the objective of this study, the storage time of the experiment was set at 5 weeks. Since the TBARS values continued increasing at week 5, the TBARS measurement in week 7 was performed to find out the maximum point of TBARS values. The expected lipid oxidation curve should be like a dome or plateau pattern. Comparing between hexanal and TBARS methods, it was found that the hexanal method provided the specific method to determine rancidity because hexanal itself is the major off flavor directly produced from lipid oxidation. TBARS method, in contrast, provided the overall degree of lipid oxidation. Some limitations of using TBARS method were found. TBARS does not specifically indicate the off flavor compounds; as a result, it requires more time to reach the maximum value. Non-food lipids, such as carbohydrates and proteins, can interfere during TBARS extraction. In addition,

TBARS requires many reagents and heating under acidic condition; these could affect malonaldehyde formation and cause overestimation.

Table 4.6 The changes of hexanal contents ($\mu\text{g}/100$ g sample) in boiled yolk

| Coating** | Week 0 | Week 1 | Week 3 | Week 5 |
|--------------|-----------------------------|------------------------------|------------------------------|------------------------------|
| C | $6.72 \pm 0.09^{\text{Ba}}$ | $7.28 \pm 0.06^{\text{Ba}}$ | $9.60 \pm 0.29^{\text{Ab}}$ | $8.73 \pm 0.47^{\text{Ab}}$ |
| WPC | $6.72 \pm 0.09^{\text{Ba}}$ | $2.61 \pm 0.58^{\text{Bd}}$ | $7.32 \pm 0.02^{\text{Abc}}$ | $3.24 \pm 0.22^{\text{Bd}}$ |
| α -cH | $6.72 \pm 0.09^{\text{Ba}}$ | $2.28 \pm 0.10^{\text{Cd}}$ | $4.41 \pm 0.57^{\text{Bd}}$ | $3.89 \pm 0.14^{\text{Bd}}$ |
| β -cH | $6.72 \pm 0.09^{\text{Ba}}$ | $5.17 \pm 0.20^{\text{BCb}}$ | $7.10 \pm 0.78^{\text{Ac}}$ | $4.34 \pm 0.40^{\text{Cd}}$ |
| MO | $6.72 \pm 0.09^{\text{Ba}}$ | $3.04 \pm 0.50^{\text{Ccd}}$ | $8.78 \pm 0.18^{\text{Abc}}$ | $6.13 \pm 0.45^{\text{Bc}}$ |
| SO | $6.72 \pm 0.09^{\text{Ba}}$ | $4.34 \pm 0.61^{\text{Cbc}}$ | $17.01 \pm 1.02^{\text{Aa}}$ | $14.09 \pm 0.16^{\text{Ba}}$ |

Means \pm SD of 3 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 ($P < 0.05$).

** See Table 4.1 for coating description.

Table 4.7 The changes of TBARS value of boiled yolk during storage (mg malonaldehyde/kg sample)

| Coatings** | Week 0 | Week 1 | Week 3 | Week 5 | Week 7 |
|--------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| C | $0.82 \pm 0.05^{\text{Da}}$ | $1.02 \pm 0.07^{\text{Ca}}$ | $1.50 \pm 0.01^{\text{Ba}}$ | $3.34 \pm 0.01^{\text{Ab}}$ | $2.04 \pm 0.05^{\text{Cb}}$ |
| WPC | $0.82 \pm 0.05^{\text{Da}}$ | $0.17 \pm 0.02^{\text{Dd}}$ | $0.89 \pm 0.00^{\text{Bb}}$ | $1.10 \pm 0.02^{\text{Ad}}$ | $0.63 \pm 0.01^{\text{Ce}}$ |
| α -cH | $0.82 \pm 0.05^{\text{Da}}$ | $0.37 \pm 0.00^{\text{Dc}}$ | $0.95 \pm 0.01^{\text{Bb}}$ | $1.14 \pm 0.04^{\text{Ad}}$ | $0.75 \pm 0.01^{\text{Cd}}$ |
| β -cH | $0.82 \pm 0.05^{\text{Da}}$ | $0.17 \pm 0.01^{\text{Cd}}$ | $0.68 \pm 0.03^{\text{Bc}}$ | $0.88 \pm 0.02^{\text{Ae}}$ | $0.84 \pm 0.02^{\text{Ad}}$ |
| MO | $0.82 \pm 0.05^{\text{Da}}$ | $0.42 \pm 0.01^{\text{Ec}}$ | $0.58 \pm 0.02^{\text{Dd}}$ | $1.30 \pm 0.01^{\text{Ac}}$ | $0.99 \pm 0.02^{\text{Bc}}$ |
| SO | $0.82 \pm 0.05^{\text{Da}}$ | $0.61 \pm 0.00^{\text{Db}}$ | $0.70 \pm 0.02^{\text{Dc}}$ | $4.30 \pm 0.02^{\text{Aa}}$ | $3.20 \pm 0.01^{\text{Ba}}$ |

Means \pm SD of 3 measurements.

^{A-E}Means with different superscripts within a row indicate significant differences ($P < 0.05$).

^bMeans with different superscripts within a column indicate significant differences ($P < 0.05$).

** See Table 4.1 for coating description.

4.6 Conclusion

Lipid based films were the most effective in preserving the internal quality of eggs with A grade at room temperature for at least five weeks; however, soybean oil coating could affect some chemical compounds resulting in a high hexanal level accumulating in the cooked yolk. In this study, mineral oil coated eggs seems to be the most effective coating regarding to the internal quality and oxidative stability results. Palmitic, oleic, and linoleic acids were most abundant fatty acids in the yolk. The proportion of MUFAs and saturated fatty acids increased while the proportion of PUFAs decreased after 5 weeks of storage. Linoleic acid was abundant in boiled egg yolk, which was a precursor for lipid oxidation during cooking.

4.7 References

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CHAPTER 5 CONCLUSIONS

Egg quality declines during storage under room temperature. Control eggs (noncoated) deteriorated rapidly within two weeks, observing from the albumen quality changed from *AA* grade to *B* grade. It clearly confirmed that surface coatings were the alternative and effective method to prevent the deterioration of the internal quality of eggs, including the haugh unit, weight loss, yolk index, and albumen pH. In some developing countries of the world where egg production is very low, and the refrigeration is barely available; surface coatings can be a useful technique to preserve egg quality under ambient temperature. Among coating types, (i.e., chitosan, whey protein, mineral oil, and soybean oil), oil coatings were the most effective in preserving the internal quality of eggs at room temperature (25 °C). Soybean oil was found to be the most practical because it is not expensive and readily available.

However, soybean oil coating affected the oxidative stability by increasing the hexanal content in cooked whole egg. Cooking methods influenced the level of hexanal contents. Boiling produced more hexanal than scrambling and microwaving because it required higher cooking time and temperature. For egg flavors, aldehyde compounds were the primary compound in cooked eggs produced by lipid oxidation. Another involved reaction is the Mailard reaction undergone by Streaker degradation of amino acids and carbonyl compounds in eggs. For lipid oxidation measurement, both hexanal and TBARS could be used as the standard methods to determine the degree of rancidity in food lipids. Oleic (C18:1), palmitic (C16:0), and linoleic (C18:2n-6) acids were the top three most abundant fatty acids in boiled egg yolk. During storage, the proportion of monounsaturated fatty acids and saturated fatty acids increased while the proportion of polyunsaturated fatty acids decreased after storage; however, most of them were not statistically different.

Overall, this study demonstrated that soybean oil coating was effective in preserving egg quality but it increased the hexanal level in cooked eggs. Further studies should be focused on consumer perception to determine if consumers could detect the off-flavors caused by soybean oil coating. This is to determine if soybean oil coating is ultimately a practical presentation method for eggs.

THE VITA

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