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Formulation and Application of Cinnamon Oil-Chitosan Emulsion Coating to Increase the Internal Quality and Shelf Life of Shelled Eggs

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By

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Formulation and Application of Cinnamon Oil-Chitosan Emulsion Coating

to Increase the Internal Quality and Shelf life

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To my father, Bahman Vandyousefi, and

to the memory of my mother, Farideh Shams-Azaran.

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ABSTRACT

Eggs are one of the most versatile and nutritious foods and a good source of high- quality protein, vitamins, and minerals. However, as time passes, eggs' internal quality deteriorates due to evaporation of carbon dioxide and moisture through the eggshell pores. To increase the internal quality and shelf life of eggs, different types of coating materials such as oil, chitosan, and combination of variety of oils with chitosan have been applied to the surface of eggs. However, there is no information available on the combined effect of cinnamon oil (CO)-chitosan (CH) emulsion coating on chicken eggs. In this study, cinnamon oil- chitosan emulsions were prepared by dispersing cinnamon oil in Tween-80 emulsifier and water. The coating materials were applied to the entire surface of each egg with a sponge brush. CO-CH emulsions (CO: CH 3:97, 6:94, 9:91, 12:88 ratios), one 100% chitosan emulsion, and one uncoated control group were evaluated in this research study. Sixty eggs (10 eggs/treatment), of the total of 96 eggs, were evaluated weekly during five weeks of storage at room temperature (25±2°C). The remaining 36 eggs were evaluated every three weeks throughout nine weeks of storage at 4°C. Quality measurements including the Haugh unit (HU), weight loss percentage, yolk index, air cell height, and pH were done for all six treatments. Noncoated eggs' grade declined from AA to C after four weeks of storage at 25°C while CO: CH 9:91 and 12:88 coated eggs' grade was turned to B after five weeks of storage at 25±2°C. CO:CH 9:91 and 12: 88 emulsions coating significantly ($p<0.05$) retarded the yolk index and Haugh unit decline rate as well as the increase rate of weight loss percentage, pH value, and air cell height of eggs regardless of temperature. This study demonstrated that CO:CH coatings with ratios of 9:91 and 12:88 could preserve the internal quality of eggs and prolong their shelf life compared to the other treatments.

Keywords: Cinnamon oil, chitosan, egg, emulsion coating, internal quality, shelf life

CHAPTER ONE: INTRODUCTION

1.1 Introduction and Statement of the Problem

Eggs are one of the most versatile and nutritious foods and a good source of high- quality protein, vitamins, and minerals (Food and Agriculture Organization of the United Nations, 2013). Eggs are also one of the most beneficial products in both food and non-food industries. According to the American Egg Board (2013), eggs have approximately 93% item penetration, which means that eggs can be found in almost all homes. However, eggs are very perishable and their poor internal quality and shelf life may cause a decline in the efficacy of the egg industry as well as consumers' loss of confidence in purchasing the product. Hence, preserving the internal quality and shelf life of eggs has become one of the major concerns of food industries and marketing agencies.

As time passes, eggs' internal quality deteriorates due to evaporation of carbon dioxide and moisture throughout the eggshell pores. Thinner egg white, flatter yolk, and larger air cell characterize the poor internal quality of an egg (Jeffrey A. Coutts, Graham C. Wilson, Sergio Fernández, 2007). Since quality determines a product's acceptability and suitability to its consumers, increasing the quality of eggs at all levels of marketing and storage is essential for both the consumer and the industry (FAO, 2003).

It has been suggested that coating the egg's shell and covering its pores help decrease carbon dioxide and water loss through pores and consequently maintaining internal quality and shelf life of eggs (FAO, 2003). Application of a variety of oil emulsions such as mineral oil and soybean oil to the surface of eggs have been suggested for preserving chicken egg's quality and shelf life during storage (Jirangrat, Torrico, No, No, & Prinyawiwatkul, 2010; Nongtaodum et al., 2013). Different oils have been reported as good coating materials for egg preservation because of their hydrophobic nature, good sealing characteristics, and long-term stability during storage (Nongtaodum et al., 2013). However, it takes longer time for oils to dry compared to the other coating emulsions particularly chitosan emulsion (Torrico et al., 2010).

Kim, Youn, No, Choi, and Prinyawiwatkul (2009) suggested chitosan emulsion as an effective coating material for maintaining the internal quality of eggs during storage because of the fact that chitosan dries faster (less than 15 minutes) than oil and its film can modify the internal atmosphere of egg (Torrico et al., 2010). However, these films are hydrophilic in nature and lack resistance to water transmission (Butler, Vergano, Testin, Bunn, & Wiles, 1996). Recent advances emphasize the combination of chitosan and oil as a coating material, which incorporates both lipid and hydrocolloid component (Wardy et al., 2013). It has been reported that the combination of oil and chitosan profoundly affect the internal quality and shelf life of eggs more than 100% oil or chitosan emulsion (Torrico et al., 2010; Torrico et al., 2014; Wardy et al., 2011; Wardy et al., 2013).

Application of essential oil-chitosan emulsions as coating materials to preserve the internal quality and shelf life of eggs has not been reported. Cinnamon oil is one of the robust essential oils that have a strong antibacterial, antiviral, and antifungal characteristic, which makes them unique from all other oils. Because of its antibacterial characteristic, low cost, and high accessibility, cinnamon oil was selected as an alternative to the other oil coatings in this study. Since cinnamon oil has an aromatic scent, only small quantities of oil were applied in this research study. Therefore, the purpose of this research study is to evaluate the efficacy of cinnamon oil-chitosan emulsion coating on the internal quality and shelf life of shelled egg.

1.2 Hypothesis and Objectives of the Study

1.2.1 Hypothesis: Cinnamon oil-chitosan emulsion coating can be applied to increase the internal quality and shelf life of chicken eggs.

1.2.2 Objectives and Specific Aims: In order to evaluate the efficacy of cinnamon oil-chitosan emulsion as a coating material, the specific objectives of the study were to:

1. Formulate cinnamon oil-chitosan emulsion and apply the emulsion at fixed ratios of 3:97, 6:94,9:91, and 12:88 to the surface of shelled eggs
2. Evaluate egg quality parameters including weight loss percentage, yolk index, Haugh unit, albumen pH, and air cell height of chicken eggs after coating during storage at 25±2°C and 4°C

CHAPTER TWO: REVIEW OF THE LITERATURE

2.1 Benefits and applications of Chicken Eggs

Chicken eggs have several health and nutraceutical benefits related to their high-quality protein, significant levels of essential vitamins, minerals, antioxidants (Phosvitin; egg yolk protein), as well as antibacterial components. For instance, Lysozyme extracted from egg white has been used in eye drops and various cold remedies. It has also been used as a cheese preservative, which prevents cheese from late blowing, preserves flavor and texture, and prolongs its shelf life. Lutein and Choline from egg yolk are also substances effective in cataracts prevention and memory development, respectively (American Egg Board (AEB), 2014).

In food industries, eggs provide very important functions such as emulsification, coagulation, adhesion, and binding in many food products. Eggs are also beneficial in non-food industries. For instance, cosmetic manufacturers apply egg white in their products. Egg yolk has been used in various shampoos and hair conditioners. Eggs are also applicable in science and research. Egg Yolk Agar (EYA) is an enriched non-selective and differential medium that contains a suspension of egg yolk (AEB, 2014).

2.2 Nutrient Facts of Chicken Egg

As it can be seen in the figure 1, one large egg contains approximately 70 calories, six grams of high quality protein, and 4.5 grams of fat. Figure.1 also displays that eggs contain vitamins including vitamin D, vitamin A, riboflavin, B12, B6, and essential minerals, including phosphorus, iron, zinc, and calcium. All of these minerals and vitamins play essential and functional roles in the human's body. Eggs perform multiple functions in making and processing food.

Nutrition Facts
Serving Size 1 egg (50g)
Servings per Container 12

Amount Per Serving

Calories 70	Calories from Fat 45
	% Daily Value*
Total Fat 5g	**8%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 185mg	**62%**
Sodium 70mg	**3%**
Potassium 70mg	**2%**
Total Carbohydrate 0g	**0%**
Protein 6g	**12%**

Vitamin A 6%	• Vitamin C 0%
Calcium 2%	• Iron 4%
Vitamin D 10%	• Thiamin 0%
Riboflavin 15%	• Vitamin B6 4%
Folate 6%	• Vitamin B12 8%
Phosphorus 10%	• Zinc 4%

Not a significant source of dietary fiber and sugars.

* Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Potassium		3,500mg	3,500mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g
Protein		50g	65g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Figure 1 Egg nutrition facts panel for one large egg

Source: American Egg Board, www.aeg.org

2.3 Egg Composition

As it is shown in the Figure 2, an egg consists of four main layers including shell, membrane, albumen, and yolk.

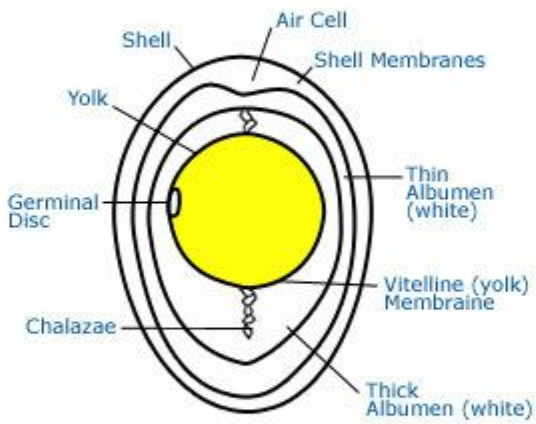

Figure 2 Egg Composition

Source: American Egg Board, www.aeg.org

2.3.1 Eggshell: The outer layer of egg with white or brown color depending on breed of chicken, eggshell, consists almost entirely of calcium carbonate (94%) (AEB, 2014). Eggshell is covered with 7000-17000 pores that are passages for air and moisture transmission (Waimaleongora-Ek, Garcia, No, Prinyawiwatkul, & Ingram, 2009).

2.3.2 Shell Membrane: Two membranes inside the shell, inner and outer membranes, are referred to as shell membranes. They surround albumen and act as a barrier against bacterial penetration (AEB, 2014).

2.3.3 Air Cell: The air sac or air pocket formed at the large end of egg. After egg is laid, inner contents shrink due to cooling and an air cell forms between the outer and inner membranes of eggshell. As egg ages, the air cell becomes larger due to loss of water and carbon dioxide through eggshell pores.

2.3.4 Albumen (Egg white):

2.3.4.1 Thin Albumen: The thin liquid spread around the thick albumen, thin albumen, is the nearest layer to the eggshell.

2.3.4.2 Thick Albumen: The thick and dense layer of albumen, thick albumen, spreads less in high-grade eggs. Thick albumen is the main source of protein and riboflavin in egg. In low quality eggs, thick albumen becomes thinner and indistinguishable from thin albumen (Vaclavik & Christian, 2008).

2.3.5 Chalazae: The twisted, cordlike fibrous filaments of egg white that keep egg yolk in the center of egg. Chalazae in fresh eggs are more prominent (AEB, 2014).

2.3.6 Vitelline Membrane: The outer layer of egg yolk, Vitelline, holds yolk contents and prevents egg yolk from mottling.

2.3.7 Egg Yolk: The yellow portion of egg, egg yolk, is the main source of egg fat, vitamins, and minerals.

2.4 Egg Quality and Grading

According to the United States Department of Agriculture egg grading manual (USDA, 2000), there are three main classifications for eggs including AA, A, and B that determine the quality of eggs by the grade. Grading eggs is based on the eggs' Haugh unit, air cell height, break out, and the quality of the albumen, yolk, and eggshell. Figure 3 shows a summary of the USDA and American Egg Board grading standards.

	 **Grade AA**	 **Grade A**	 **Grade B**
Break Out Appearance	Covers a moderate area	Covers a moderate area	Covers a wide area
Air Cell Height	1/8 inch or 3/16 inch or less Over 3/16 inch	1/8 inch or 3/16 inch or less Over 3/16 inch	1/8 inch or 3/16 inch or less Over 3/16 inch
Albumen Quality	-White is reasonably thick and stands high -Prominent chalazae		-Small amount of thick white -Chalazae small or absent -Appears weak and watery
Yolk Quality	Yolk is firm, round, and high		Yolk is somewhat flattened and enlarged.
Shell Appearance	Approximates usual shape; generally clean, unbroken; ridges/rough spots that do not affect the shell strength are permitted.		Abnormal shape; some slight stained areas permitted; unbroken; pronounced ridges/thin spots permitted.
Haugh Unit	72≤ HU	60≤HU<72	HU<60
Usage	Ideal for any use, but are especially desirable for poaching, frying and cooking in shell		Good for scrambling, baking, and use as an ingredient in other foods

Figure 3 Grades of eggs

(*Sources:* American Egg Board, www.aeg.org ; USDA Egg Grading Manual (2000), www.ams.usda.gov)

2.5 Application of Edible Emulsion Coating on Eggs

Application of a variety of emulsions such as oil, carbohydrate, and protein-based emulsions on eggs has been suggested previously as effective coating materials (Biladeau & Keener, 2009; Jirangrat et al., 2010; Nongtaodum et al., 2013). Coating eggs seals the eggshell pores and decreases microbial permeability. Likewise, coating helps eggs to hold more moisture, retain carbon dioxide, and delay pH rise during storage (FAO, 2003).

2.5.1 Coating Eggs with Oil

Oils serve as good coating materials for egg preservation due to their hydrophobic nature, good sealing characteristics and long-term stability throughout storage (Nongtaodum et al., 2013; Wardy et al., 2011). Nongtaodum et al. (2013) suggested that coating eggs with edible oils increase the quality of shelled eggs compared to the noncoated eggs. They applied different types of oils including coconut, rice bran, soybean, palm, and glycerol to the surface of eggs for five weeks at room temperature. They evaluated the Haugh unit, weight loss percentage, yolk index, albumen pH and viscosity, air cell height, gloss value, sensory discrimination, and consumer acceptance of noncoated and coated eggs. The results of their study clearly indicated that coated eggs had a better quality than noncoated eggs.

2.5.2 Coating Eggs with Chitosan

Chitosan is deacetylated form of chitin that is obtained from shellfish waste and provides excellent oxygen barrier and antimicrobial properties (Badawy & Rabea, 2011). Kim, Youn, No, Choi, and Prinyawiwatkul (2009) suggested chitosan as an effective coating material on the internal quality and shelf life of eggs. They measured weight loss, Haugh unit, yolk index, albumen pH, albumen amino acid and fatty acid content of chitosan coated eggs for five weeks at 25±2°C. They also evaluated the effect of eggs' storage position on the internal quality and shelf life of eggs. They stated that quality of small-end down stored eggs was significantly better than eggs placed small end up during storage period.

2.5.3 Coating Eggs with the Combination of Chitosan and Oil

Recent advances emphasize the combination of chitosan and oil as coating emulsion, which offers a combined effect of both lipophilic (oil) and hydrophilic (chitosan) components (Wardy et al., 2013). The effectiveness of oil and chitosan combinations as coating materials on the internal quality and shelf life of eggs has been suggested by numerous studies (Torrico et al., 2010; Torrico et al., 2014; Wardy et al., 2011; Wardy et al., 2013). Wardy et al. (2013) evaluated the internal quality of eggs coated with soybean oil, chitosan solution, and soybean oil- chitosan emulsions at ratios of 60:40, 50:50, and 40:60 during seven and 20 weeks of storage at 25±2°C and 4 °C, respectively. They reported that soybean oil increased the efficacy of chitosan as eggs' coating material. All eggs coated with soybean oil-chitosan emulsions had less weight loss compared to the non-coated eggs and maintained A grade after seven weeks and 20 weeks of storage at 25±2°C and 4 °C, respectively.

Similarly, Torrico et al. (2011) evaluated the efficacy of mineral oil–chitosan emulsions at the fixed ratio of 25:75 as a coating material on the internal quality and shelf life of eggs during five weeks and 20 weeks of storage at 25±2°C and at 4 °C, respectively. They stated that compared to the noncoated samples, mineral oil–chitosan emulsion coated eggs had a better internal quality and longer shelf life irrespective of their storage temperature.

2.6 Characteristics of Essential Oils

As stated previously, numerous researchers have been evaluating the effects of variety of edible oils such as mineral oil and soybean oil as coating materials on enhancing the internal quality and shelf life of eggs (Torrico et al., 2011; Wardy et al., 2013). However, the effect of essential oils such as cinnamon oil and rosemary oil as coating emulsions on the quality and shelf life of eggs has not yet been studied. Essential oils are aromatic oils extracted from plant resources such as flowers and herbs. They can be obtained by fermentation, extraction, or steam distillation, which is the most common method used for commercial production. Terpenes and terpenoids are the primary constituents of the essential oils' chemical structure. Beside their aroma that is important for fragrance companies, essential oils' antibacterial, antiviral, antifungal, and antioxidant properties make them specially appropriate for medical usages (Prabuseenivasan, Jayakumar, & Ignacimuthu, 2006).

Prabuseenivasan et al. (2006) evaluated 21 different essential oils to find an essential oil that has the strongest antibacterial efficacy. Out of 21 essential oils, 19 oils displayed antibacterial activity against one or more bacteria. Cinnamon, clove, geranium, lemon, lime, orange, and rosemary oils showed the maximum inhibitory effects against all the bacterial species tested. Cinnamon, clove, and lime oils inhibited both gram-negative and gram-positive microorganisms and cinnamon oil itself had the strongest antibacterial efficacy even at low concentrations. The effects of essential oils as antimicrobial additives on several food models such as apple films have been studied (Du et al., 2009); however, the current research study will be the first study that explores essential oils' efficacy as a coating material on the internal quality and shelf life of shelled eggs.

CHAPTER THREE: METHODOLOGY

3.1 Eggs and Preparation of Coating Emulsions

Ninety-six white-shelled large fresh eggs, all AA grade, were obtained from the Crestview Inc. Farm (Arcadia, OK, USA). Eggs were screened for defects (breakage, crack, and cleanliness) and the desirable weight range of 50-70 grams (Torrico et al., 2011) upon arrival from the farm. Chitosan with the molecular weight of 223-kDa as suggested by Wardy, Torrico, No, Prinyawiwatkul, and Saalia (2010) and pure cinnamon oil (Olive Nation LLC. MA, USA) were used as eggs' coating materials.

Primarily, chitosan solution was prepared by dissolving chitosan in 1% (v/v) acetic acid at 2g/100 mL (w/v) concentration (Kim et al., 2009). Emulsions of cinnamon oil and chitosan (CO: CH 3:97, 6:94, 9:91, 12:88 ratios) were prepared by dispersing cinnamon oil and chitosan solutions in Tween-80 emulsifier. The resulting mixture was homogenized for five minutes at low speed and another five minutes at high speed using a hand blender (Hamilton Beach, Model 59765, USA) at 25±2°C.

3.2 Application of Chitosan and Cinnamon Oil-Chitosan Emulsion Coating on Eggs

Of the total of 96 eggs, 60 eggs (10 eggs/treatment) were evaluated weekly for five weeks of storage at room temperature (25±2°C). The remaining 36 eggs were evaluated every three weeks during nine weeks of storage at 4°C. Each egg was weighed with a scientific balance (model Mettler PJ400) to measure the initial weight in gram. Eggs were stored at 4°C temperature for about 24 hours. To prevent water concentration on the eggs' shell that could adversely affect the efficacy of coatings, eggs were kept at room temperature (approximately 25°C) for two hours before coating.

The coating materials were applied to the entire surface of each egg with a sponge brush and were left to dry on racks in the horizontal position at room temperature (Wardy et al., 2010). Chitosan, cinnamon oil (CO)-chitosan (CH) emulsions (CO: CH 3:97, 6:94, 9:91, 12:88 ratios), and one uncoated control group were evaluated in this research study. The coated eggs were placed small end down on egg racks upon drying (Kim et al., 2009) and were stored for five and nine weeks at 25±2°C and 4°C temperatures, respectively. Quality measurements were done on each replicate (two eggs per replicate) per each treatment every week for five weeks of storage at 25±2°C and for nine weeks of storage (intervals of three weeks) at 4°C.

Total number of Eggs = 96 → 60 (at 25±2°C) + 36 (at 4°C)

- **60 eggs for five weeks at 25±2°C** (10 eggs/ treatment →Evaluated 2 eggs/week): 10 eggs for each CO: CH 3:97, 6:94, 9:91, and 12:88 ratios + 10 noncoated eggs + 10 chitosan coated eggs.

- **36 eggs at intervals of three weeks for nine weeks** (intervals of three weeks) at 4°C:

  6 eggs/ treatment →Evaluated 2 eggs/ every three weeks

Figure 4 Labeling and coding eggs: (a) chitosan coated eggs; (b) all treatments (mixed)

(C: control group, O: chitosan emulsion, T: treatment (1, 2, 3, & 4), W: week, a: sample a, b:sample b)

Figure 5 Cinnamon oil-chitosan coated eggs stored at 25±2°C

3.3 Evaluation of the Internal Quality of Eggs and their Shelf Life during Storage

3.3.1 Weight Measurement

Eggs' weights in gram at week zero (W_0) were measured using a scientific balance (model Mettler PJ400). Weight measurement was done every week during the eggs' storage period.

Figure 6 Scientific balance (model Mettler PJ400)

3.3.2 Height and Width Measurement:

A vernier caliper micrometer gauge (AGPtek iT4-2 Stainless Steel 150 mm LCD Digital) was used to measure the yolk height, albumen height, and yolk width in millimeter.

Figure 7 Measuring yolk width by a digital vernier caliper micrometer gauge

3.3.3 Yolk Index

Yolk index represents the physical quality and freshness of eggs. Its measure depends on the quality of the vitelline membrane. The yolk index formula takes into account the yolk height and width in millimeters.

- **Yolk Index = [yolk height (mm)/yolk width (mm)]**

3.3.4 Haugh Unit

The Haugh Unit (HU) was calculated based on the Raymond Haugh formula. Dr. Raymond Haugh introduced the Haugh unit formula in 1937 and since then it has been used as a method of egg quality and freshness measurement all over the world. This formula takes into account albumen height in millimeters and egg weight in grams. A range of HU varies from single numbers in very poor quality eggs to over 100 in high quality fresh eggs (Haugh, 1937).

- **Haugh unit = 100 log (H - $1.7\ W^{0.37}$ + 7.57)**

3.3.5 Air Cell Height:

The air cell height increases over time. In this research, the air cell height in millimeter was measured for each egg using a vernier caliper micrometer gauge.

3.3.6 Weight Loss Percentage:

Weight loss percentage of the coated and noncoated eggs for the duration of storage was calculated as [(Initial egg weight (g) after coating at day 0 - egg weight (g) after storage) ÷ initial egg weight (g) after coating at day 0] - 100.

$$\textbf{Weight Loss (\%)} = \frac{(\textbf{initial egg weight (g) after coating at day 0} - \textbf{egg weight (g) after storage})}{\textbf{initial egg weight (g) after coating at day 0}} \times \mathbf{100}$$

3.3.7 Albumen pH Measurement:

Albumen pH is one of the indicators of eggs' internal quality and freshness. In this study, each egg's albumen (thick and thin mixture) was poured into a beaker by a pipette. Then, the albumen pH was measured by a digital pH meter (pH/ ORP Meter model HI 9125).

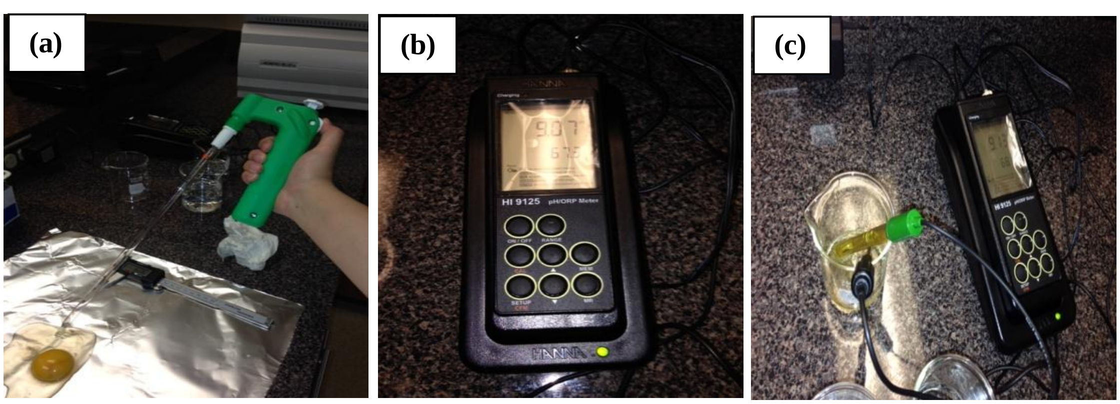

Figure 8 Albumen pH measurement: (a) pouring the albumen into a beaker by a pipette; (b) digital pH meter (pH/ ORP Meter model HI 9125); (c) measuring the albumen pH

3.4 Statistical Analysis

Means and standard deviations of all replicates were computed for the Haugh unit, weight loss percentage, yolk index, albumen pH, and air cell height. Overall differences among non-coated, CO-CH coated and CH-coated eggs were determined using Microsoft Excel 2010, considering all variables (yolk index, Haugh unit, weight loss percentage, albumen pH, and air cell height) concurrently. T-test was used to determine the significant differences of mean values. *P*-value of less than 0.05 was considered statistically significant.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Effects of Chitosan Solution and Cinnamon Oil- Chitosan Emulsion Coating on Weight Loss

Throughout storage period, eggs' weight decreases due to evaporation of carbon dioxide and water through the eggshell pores. In this study, differences in the weight loss among the control group (noncoated eggs) and those coated with chitosan solution (CH) and four cinnamon oil-chitosan emulsions (CO: CH 3:97, 6:94, 9:91, and 12:88) were evaluated during five weeks of storage at 25 ± 2°C (Table 1) and nine weeks of storage at 4°C (Table 2). The chitosan coated and noncoated eggs exhibited a significant weight loss ($P < 0.05$) compared to the cinnamon oil-chitosan (CO: CH) emulsion coated eggs. However, no significant differences ($P > 0.1$) were observed between the weight loss percentage of noncoated (12.89%) and chitosan coated (9.83%) eggs after five weeks of storage at 25 ± 2°C and nine weeks of storage at 4°C (Figures 9 and 10).

The weight loss percentage of eggs coated with cinnamon oil-chitosan (CO: CH) emulsion at ratios of 9:91 CO: CH (5.64%) and 12:88 CO: CH (5.08%) was significantly ($P<0.05$) lower than those of noncoated (12.89%) and chitosan coated (9.83%) eggs after five weeks of storage at 25 ± 2°C (Table 1). Among all cinnamon oil-chitosan coated eggs, the weight loss percentages of treatments one (3:97 CO: CH) and two (6:94 CO: CH) were more than treatments three (9:91 CO: CH) and four (12:88 CO: CH) irrespective of their storage temperature. In addition, no significant difference ($P>0.1$) was observed between the weight loss percentages control group and treatment one (Tables 1 and 2).

Table 1 Weight loss (%) of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 5 weeks of storage at 25±2 °C

Coating*	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Control Group**	2.85 ± 0.49	4.48 ± 0.57	8.65 ± 0.16	9.59 ± 0.16	12.89 ± 0.77
Chitosan (CH)	2.64 ± 0.14	4.15 ± 0.57	7.63 ± 0.20	8.59 ± 0.36	9.83 ± 0.62
Treatment.1 (3:97 CO:CH)	1.65 ± 0.04	3.75 ± 0.43	5.71 ± 0.25	6.25 ± 0.14	8.29 ± 0.55
Treatment.2 (6:94 CO:CH)	1.51 ± 0.10	3.37 ± 0.42	4.75 ± 031	5.74 ± 0.01	6.3 ± 0.05
Treatment3 (9:91 CO:CH)	1.18 ± 0.14	2.96 ± 0.19	4.06 ± 0.23	5.17 ± 0.18	5.64 ± 0.60
Treatment.4 (12:88 CO:CH)	1.06 ± 0.12	2.78 ± 0.02	3.91 ± 0.54	4.39 ± 0.26	5.08 ± 0.52

*Means ± standard deviations of two measurements
**Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid; Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2 = coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3 = coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4 = coating with a ratio of 12 ml cinnamon oil: 88 ml chitosan

Besides, there was an insignificant difference ($0.05<P<0.1$) between treatment two and noncoated eggs during storage at room temperature (25 ± 2°C).

As it can be seen in Figure 10, the weight loss percentage rate of refrigerated eggs was significantly slower than those eggs that were stored at room temperature (Figure 9). For instance, treatment four group (12:88 CO:CH) had the weight loss percentage of 1.86% after nine weeks of storage at 4°C, which is noticeably lower than their weight loss percentage (2.78%) at the second week of storage at 25 ± 2°C (Tables 1 and 2). Accordingly, cinnamon oil-chitosan emulsion coating had a greater effect on the weight loss percentage of eggs throughout the storage at 25 ± 2°C than at 4 °C. The weight loss percentages of treatments three (9:91 CO: CH) and four (12:88 CO: CH) were significantly ($P < 0.05$) less than that of noncoated eggs stored at both room and refrigerator temperatures. Conversely, there were no significant differences (0.1<*P*) among the treatments one and two, CH group, and control group.

Figures 9 and 10 illustrate the differences among the weight loss percentages of all coated and noncoated eggs. Among all treatments, treatments three (9:91 CO: CH) and four (12:88 CO:CH) had the minimum weight loss percentages regardless of their storage temperature. There were no significant differences (0.1<*P*) among the weight loss percentages of noncoated, chitosan coated, and 3:97 CO: CH coated eggs. Additionally, there was a slight difference (0.05<*P*<0.1) between the weight loss percentages of the treatment two (6:94 CO: CH) and the control group. Overall, coating with oil can decrease the weight loss percentage of eggs during storage (Nongtaodum et al., 2013).

Table 2 Weight loss (%)* of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 9weeks of storage at 4°C

Coating**	**Week 3**	**Week 6**	**Week 9**
Control Group	2.05 ± 0.41	2.63 ± 0.28	3.27 ± 0.32
Chitosan (CH)	1.82 ± 0.40	2.39 ± 0.23	3.18 ± 0.66
Treatment.1 (3:97 CO:CH)	1.02 ± 0.23	1.93 ± 0.07	2.9 ± 0.01
Treatment.2 (6:94 CO:CH)	0.88 ± 48	1.84 ± 0.19	2.86 ± 0.37
Treatment3 (9:91 CO:CH)	0.69 ± 0.23	1.56 ± 0.27	1.95 ± 0.81
Treatment.4 (12:88 CO:CH)	0.53 ± 0.12	1.49 ± 0.05	1.86 ± 0.42

*Means ± standard deviations of two measurements
**Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid;Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2 = coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3 = coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4 = coating with a ratio of 12 ml cinnamon oil: 88 ml chitosan

Coating eggs with chitosan has been suggested to slow down weight loss and maintain the internal quality of eggs during storage (Kim et al., 2009). However, the combination of chitosan and oil has been reported as a more successful coating material for retarding the weight loss of eggs (Torrico et al., 2011; Wardy et al., 2011). In this study, the weight loss rate of all coated eggs was less when compared to the control group. The results also showed that eggs coated with the combination of cinnamon oil and chitosan had a slower weight loss rate than chitosan-coated eggs. Similarly, Wardy et al. (2013) suggested that the combination of soybean oil and chitosan can act as a stronger coating material than chitosan emulsion in delaying eggs' weight loss. They also stated that eggs coated with emulsions that contained a higher amount of soybean oil than chitosan, had less weight loss when compared to the other treatments.

According to the results of this study, treatments three (9:91 CO: CH) and four (12:88 CO: CH) were the most successful coating materials compared to the other treatments that contain less cinnamon oil. Therefore, increasing the ratio of cinnamon oil to chitosan can decrease the rate of weight loss irrespective of storage temperature.

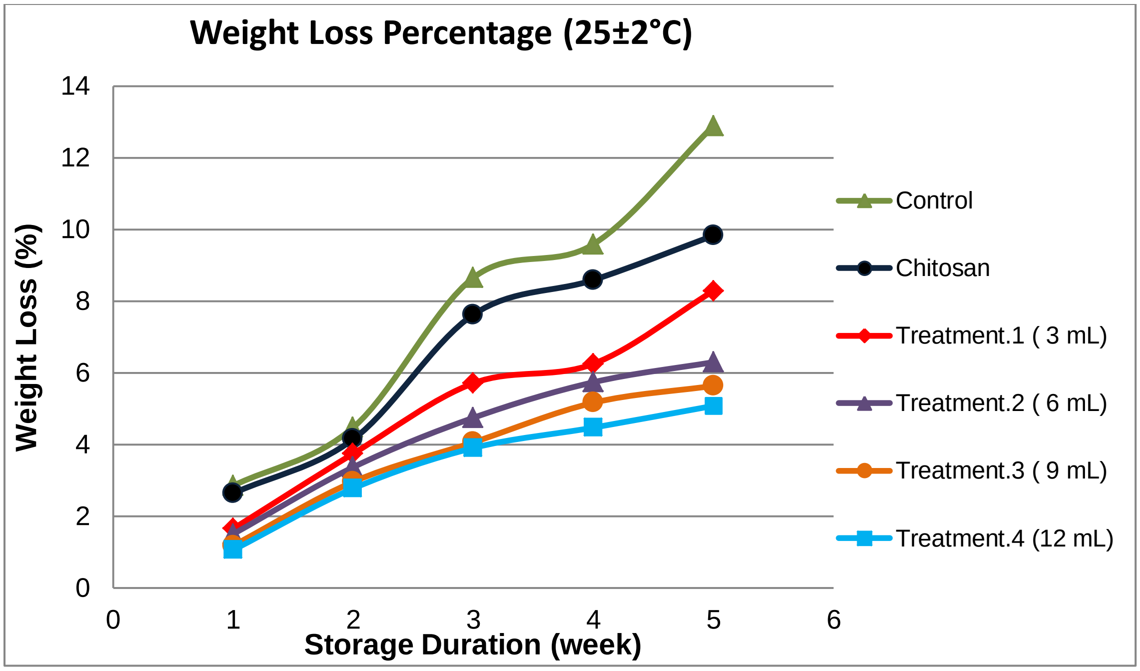

Figure 9 Average weight loss (%) of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 5 weeks of storage at 25±2°C

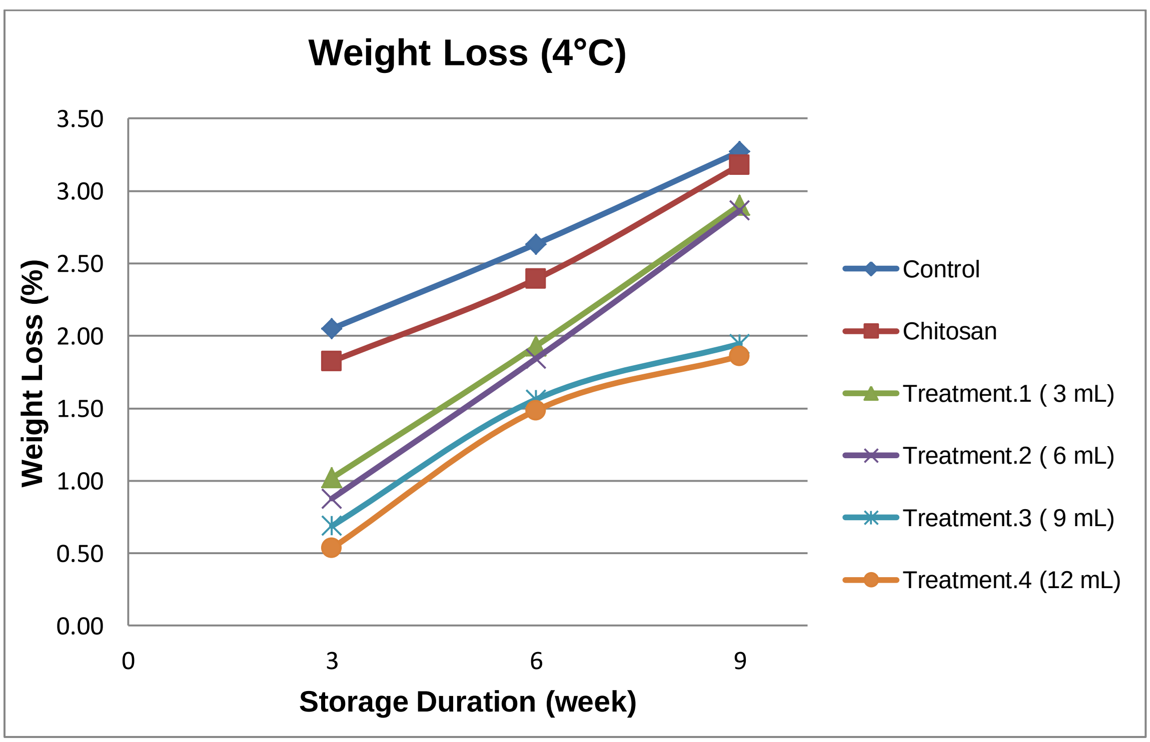

Figure 10 Average weight loss (%) of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 9weeks of storage at 4°C

4.2 Effects of Chitosan Solution and Cinnamon Oil-Chitosan Emulsion Coating on the Haugh Unit

The Haugh unit is a measurement of the weight and height of the thick albumen that represents the quality, freshness, and grade of eggs. According to the United States Department of Agriculture (USDA, 2014), the greater the Haugh unit value, the higher the grade and quality of eggs. In this study, the Haugh unit of all eggs, coated and noncoated, decreased during the storage time irrespective of their storage temperature. The quality of all coated eggs, chitosan and cinnamon oil- chitosan coated eggs decreased with a slower rate than noncoated eggs during storage at room temperature. As a case in point, noncoated eggs' grade declined to the grade of B after two weeks of storage at 25±2°C while the other coated eggs' grade remained the grades of AA (9:81 and 12:88 CO:CH) and A (3:87 and 6:94 CO:CH, and CH).

In addition, the initial Haugh unit of noncoated eggs stored at 25±2°C significantly decreased from 84.60 (Grade AA) to 9.11 (Grade C) after five weeks of storage. This Haugh unit decline rate was considerably slower for eggs coated with 9:91 and 12:88 CH: CO emulsions than for noncoated and CH-coated eggs. For instance, the initial Haugh unit of the treatment four (12:88 CO: CH) was decreased from 84.60 (Grade AA) to 59.93(Grade B) after five weeks of storage at 25±2°C, while the Haugh unit of noncoated and CH-coated eggs harshly decreased to 9.11 (Grade C) and 20.44 (Grade C), respectively (Table 3). Similarly, Damir D. Torrico et al. (2010) reported that the initial Haugh unit (83.79) and grade (AA) of the control group in their study decreased to 24.06 and grade C , while mineral oil-chitosan (25:75 MO: CH) emulsion coated eggs' grade turned to grade B after five weeks of storage at 25°C.

Table 3 Haugh unit* and grade** of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 5 weeks of storage at 25±2 °C

Coating***	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Control Group	84.60 ± 2.01 AA	61.89 ± 0.45 A	55.17 ± 0.97 B	44.09 ± 6.16 B	21.74 ± 1.67 C	9.11 ± 0.86 C
Chitosan (CH)	84.60 ± 2.01 AA	64.40 ± 2.53 A	60.87 ± 1.23 A	51.47 ±8.15 B	40.27 ±3.15 B	20.44 ± 0.31 C
Treatment.1 (3:97CO:CH)	84.60 ± 2.01 AA	69.19 ± 2.35 A	65.58 ± 1.24 A	55.40 ± 0.85 B	50.41 ± 0.31 B	26.56 ± 0.76 C
Treatment.2 (6:94 CO:CH)	84.60 ± 2.01 AA	71.28 ±0.21 A	66.60 ±0.37 A	57.06 ±3.41 B	52.60 ±6.43 B	28.61 ±1.40 C
Treatment.3 (9:91 CO:CH)	84.60 ± 2.01 AA	76.47 ± 0.89 AA	74.90 ± 0.39 AA	65.33 ± 1.68 A	56.56 ± 0.94 B	52.37 ± 0.47 B
Treatment.4 (12:88 CO:CH)	84.60 ± 2.01 AA	79.71 ± 6.12 AA	77.78 ± 6.19 AA	70.87 ±1.79 A	65.18 ±4.48 A	59.93 ± 0.08 B

*Means ± standard deviations of 2 measurements
**Based on the USDA Haugh unit values; AA, above 72; A, 71 to 60; B, 59 to 31; C, below 30
***Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid; Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2= coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3= coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4= coating with a ratio of 12 ml cinnamon oil: 88 ml chitosan

Overall, the treatments three and four (9:91 and 12:88 CH: CO) emulsions had significantly ($P < 0.05$) the higher Haugh unit throughout five weeks of storage when compared to the other treatments. Additionally, no significant differences in the Haugh unit were observed among the treatment one (3:97 CO: CH), treatment two (6:94 CO: CH), chitosan, and noncoated eggs (Table 3). Compared to the eggs stored at 25±2°C, it was noticeable that eggs' Haugh unit declining rate at 4 °C was significantly less.

In addition, the Haugh units of all treatments, coated and noncoated, after nine weeks were higher or about the same as the Haugh units of eggs stored at 25±2°C in the first week of storage. For instance, the Haugh unit of treatment three at week nine (76.3) was about the same as the Haugh unit of the same treatment in the first week of storage (76.47) at 25°C (Table 4). As it can be seen in Table 4, there were slight differences (0.05<*P*<0.1) among the treatments three, four, chitosan, and control group. Nevertheless, there were no significant differences (0.1<*P*) among treatments two, three, chitosan, and control group.

Table 4 Haugh unit* and grade** of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 9 weeks of storage at 4°C

Coating* **	**Week 0**	**Week 3**	**Week 6**	**Week 9**
Control Group	84.60 ± 2.01 AA	77.08 ± 0.87 AA	74.57 ± 2.40 AA	66.58 ± 1.20 A
Chitosan (CH)	84.60 ± 2.01 AA	79.65 ± 2.22 AA	76.51 ± 4.04 AA	69.94 ± 1.21 A
Treatment.1 (3:97 CO:CH)	84.60 ± 2.01 AA	86.58 ± 0.85 AA	78.27 ± 1.90 AA	69.91 ± 2.61 A
Treatment.2 (6:94 CO:CH)	84.60 ± 2.01 AA	88.45 ± 1.05 AA	80.71 ± 0.24 AA	71.56 ± 0.42 A
Treatment3 (9:91 CO:CH)	84.60 ± 2.01 AA	85.09 ± 0.57 AA	83.03 ± 1.46 AA	76.3 ± 0.59 AA
Treatment.4 (12:88 CO:CH)	84.60 ± 2.01 AA	89.32 ± 3.46 AA	84.44 ± 2.57 AA	78.82 ± 2.80 AA

*Means ± standard deviations of two measurements
**Based on the USDA Haugh unit values; AA, above 72; A, 71 to 60; B, 59 to 31; C, below 30
***Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid; Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2= coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3= coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4= coating with a ratio of 12 ml cinnamon oil: 88 ml chitosan

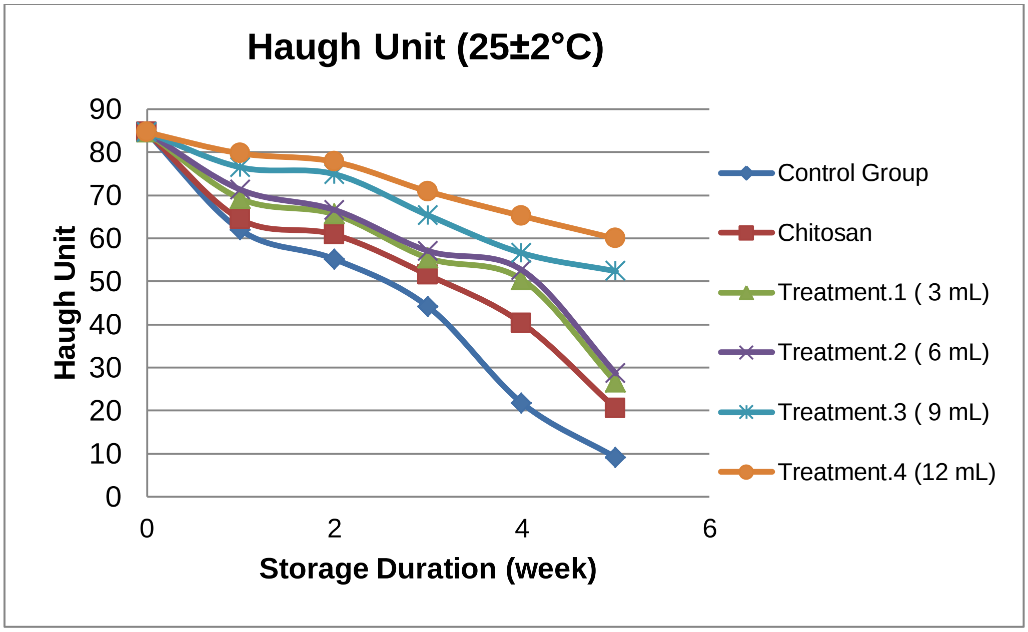

Figure 11 Haugh unit changes of eggs coated with chitosan and four cinnamon oil-chitosan emulsions during 5 weeks of storage at 25±2°C

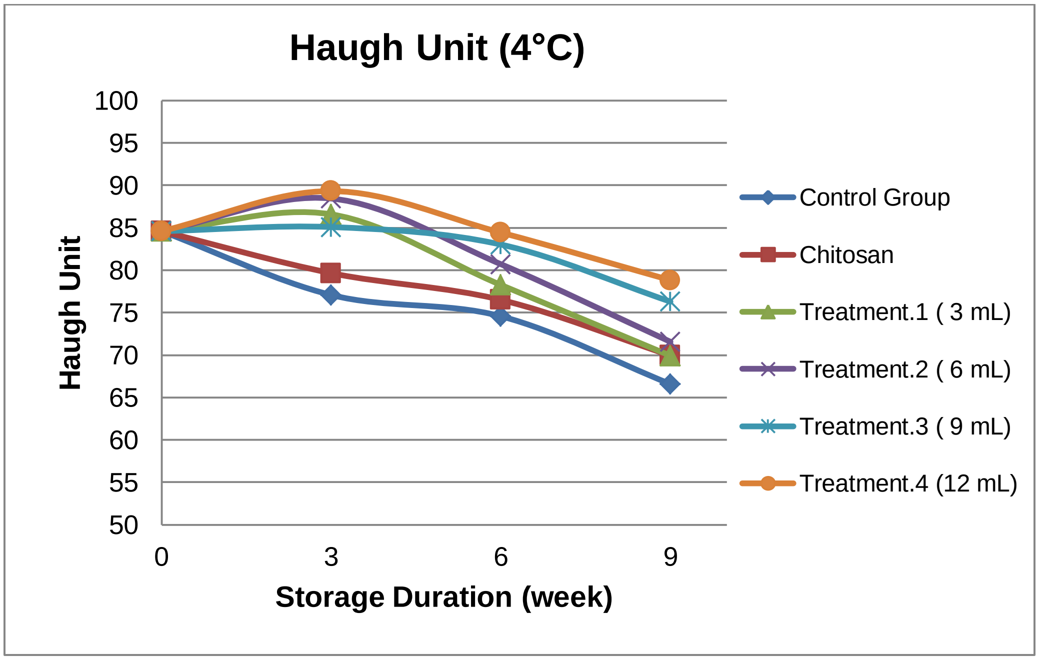

Figure 12 Haugh unit changes of eggs coated with chitosan and four cinnamon oil-chitosan emulsions during 9 weeks of storage at 4°C

Figures 11 and 12 show the Haugh unit changes of eggs stored at both room and refrigerator temperatures. The Haugh unit of all treatments decreased during storage; however, the rate of decrease at 4°C was significantly (P<0.05) lower when compared to the decrease rate at 25±2°C. Likewise, Wardy et al. (2010) compared the effects of different oil emulsions as coating materials with chitosan emulsion on the Haugh unit of eggs stored in the refrigerator and at room temperature and reported that refrigeration slows down the Haugh unit deterioration of eggs particularly oil coated eggs.

Those eggs coated with a combination of CO-CH had lesser Haugh unit decline when compared to the noncoated and CH-coated eggs. Torrico et al. (2011) reported that the Haugh unit of CH coated eggs was significantly (P<0.05) lower than mineral oil (MO) and MO-CH coated eggs during storage. In this study, treatments three and four (9:91 and 12:88 CH: CO) had the minimum Haugh unit changes and the highest grades compared to the treatments one and two at both 25°C and 4°C. This shows that increasing the amount of cinnamon oil ratio to chitosan can be effective in preventing the grade and quality loss of eggs.

4.3 Effects of Chitosan Solution and Cinnamon Oil- Chitosan Emulsion Coating on the Yolk Index

The yolk index represents the physical quality of eggs. The yolk index is one of the indicators of eggs' freshness that takes into account the yolk height and width in millimeters. Its measure depends on the quality of the vitelline membrane. Normally, the yolk index values decrease during storage periods (Wardy et al., 2011). In this research, the yolk index of all eggs decreased after storage time at both 25±2°C and 4 °C; however, the rate of decline was significantly (P<0.05) slower for eggs stored at 4°C when compared to those stored at 25±2°C. As a case in point, the yolk index of the eggs coated with 12:88 CO:CH was decreased to 0.42 at the second week of storage at 25±2°C while the yolk index of the same group was decreased to 0.43 after nine weeks of storage at 4°C (Tables 5 and 6).

Table 5 Yolk index* of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 5 weeks of storage at 25±2°C

Coating**	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Control Group	0.46	0.37	0.31	0.28	0.24	0.22
Chitosan (CH)	0.46	0.38	0.32	0.29	0.26	0.24
Treatment.1 (3:97 CO:CH)	0.46	0.38	0.33	0.31	0.29	0.25
Treatment.2 (6:94 CO:CH)	0.46	0.39	0.33	0.31	0.29	0.26
Treatment3 (9:91 CO:CH)	0.46	0.42	0.41	0.38	0.35	0.33
Treatment.4 (12:88 CO:CH)	0.46	0.44	0.42	0.39	0.37	0.34

*Means of 2 measurements ± standard deviations range of 0.01-0.04.
**Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid; Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2= coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3= coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4= coating with a ratio of 12 ml cinnamon oil: 88 ml chitosan.

These differences in the yolk index deterioration show that refrigeration retards the rate of the yolk index and egg quality degeneration. Likewise, Jirangrat et al. (2010) reported that the initial yolk index (0.47) of the mineral oil coated eggs was decreased to 0.21 after five weeks of storage at 25±2°C while there were no alterations in the yolk index of eggs after five weeks of storage at 4°C. The yolk index of all room temperature stored eggs decreased significantly (P <0.01) after five weeks of storage. There were also significant differences (P<0.05) among the yolk indices of treatments three (9:91 CO: CH) and four (12:88 CO: CH) and the other groups irrespective of their storage temperature. In addition, there was no significant difference (P>0.1) between the treatments three (9:91 CO: CH) and four (12:88 CO: CH) during storage at either 25±2°C or 4°C.

Table 6 Weight loss (%)* of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 9 weeks of storage at 4°C

Coating**	**Week 0**	**Week 3**	**Week 6**	**Week 9**
Control Group	0.46	0.41	0.39	0.37
Chitosan (CH)	0.46	0.42	0.40	0.38
Treatment.1 (3:97 CO:CH)	0.46	0.42	0.40	0.39
Treatment.2 (6:94 CO:CH)	0.46	0.43	0.41	0.39
Treatment3 (9:91 CO:CH)	0.46	0.46	0.44	0.42
Treatment.4 (12:88 CO:CH)	0.46	0.46	0.45	0.43

*Means of 2 measurements ± standard deviations range of 0.01-0.04.
**Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid; Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2= coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3= coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4= coating with a ratio of 12 ml cinnamon oil: 88 ml chitosan.

No significant differences ($0.1 < P$) were observed among the treatment one, treatment two, CH, and control group during storage and regardless of temperature (Table 5). The yolk index of noncoated and CH-coated eggs were decreased significantly ($P < 0.05$) at 4°C. Similarly, Wardy et al. (2013) reported that there was a significant decrease in the yolk indices of control and CH groups in their study after five weeks of storage at both 25±2°C and 4°C. In contrast, there were no significant differences ($0.1 < P$) between the yolk indices of the treatments three (0.42) and four (0.43) and the initial yolk index (0.46) after nine weeks of storage at 4°C, in the present study (Table 6).

Overall, treatments three and four had the minimum rate of yolk index reduction when compared to the other coating materials at both room and refrigerator temperatures. According to the data from Tables 5 and 6, treatments three and four can be applied as effective coating materials to delay the yolk index deterioration rate of eggs throughout storage.

Figures 13 and 14 show the yolk index fluctuations during five weeks of storage at 25±2°C and nine weeks of storage at 4°C, respectively.

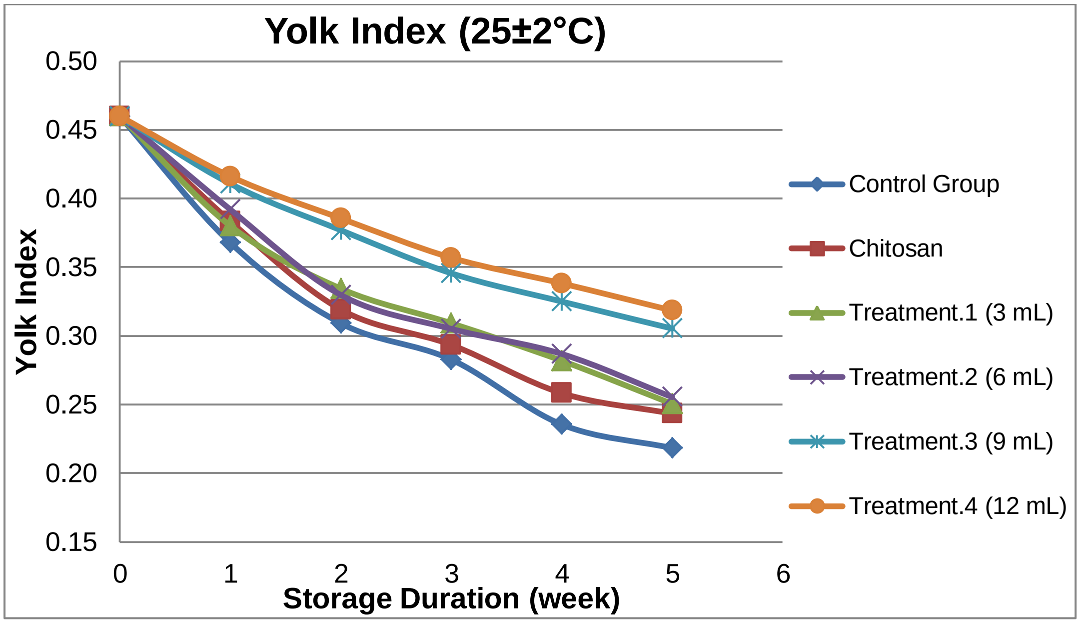

Figure 13 The yolk index changes of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 5 weeks of storage at 25±2°C

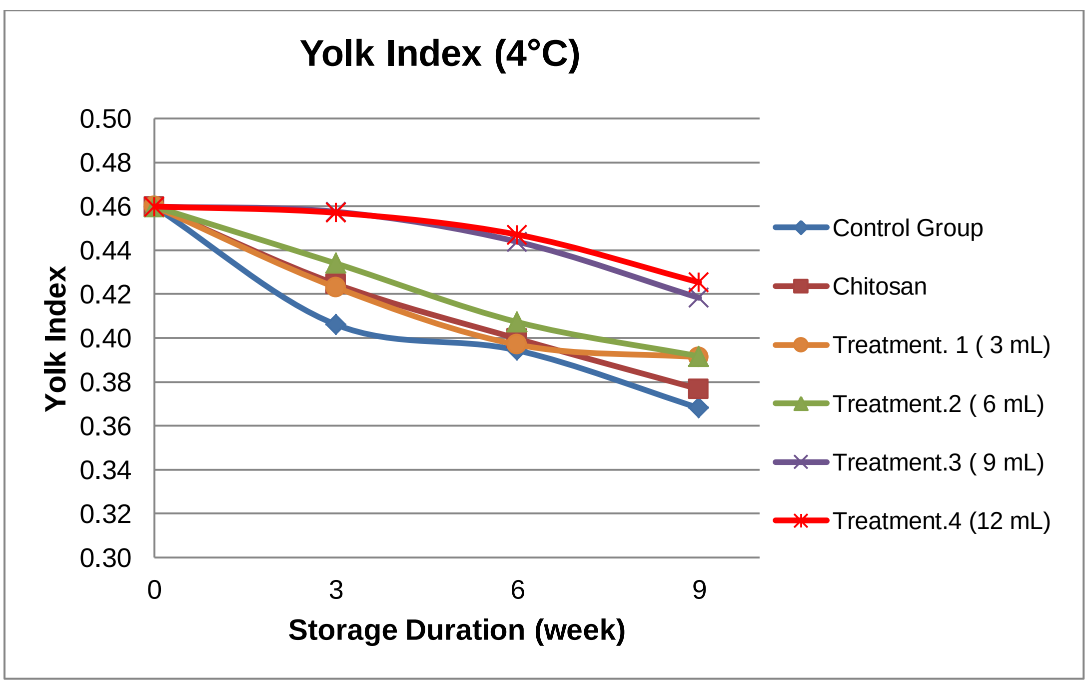

Figure 14 The yolk index changes of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 9 weeks of storage at 4°C

4.4 Effects of Chitosan Solution and Cinnamon Oil- Chitosan Emulsion Coating on the Albumen pH

Albumen pH measures the internal quality and freshness of eggs (Waimaleongora-Ek et al., 2009). Normally, the albumen pH value of freshly laid egg is between 7.6 and 8.7 (Keener, LaCrosse, & Babson, 2001). Because of carbon dioxide outflow through eggshell pores during storage, the thick albumen becomes thinner and albumen pH value increases up to 9.6-9.7 (Damir Dennis Torrico et al., 2011). In this study, the albumen pH values of all treatments ranged from 8.01 to 9.40 (Figures 15 and 16). The albumen pH values of all CO: CH coated eggs were significantly (P<0.05) lower than noncoated eggs stored at 25±2°C. Therefore, cinnamon oil-chitosan emulsion coating delays carbon dioxide loss and its subsequent pH value rise.

Table 7 pH* of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 5 weeks of storage at 25±2°C

Coating**	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Control Group	8.44 ± 0.04	8.91 ± 0.16	9.15 ± 0.05	9.22 ± 0.01	9.32 ± 0.05	9.40 ± 0.11
Chitosan (CH)	8.44 ± 0.04	8.73 ± 0.64	8.69 ± 0.01	8.89 ± 0.14	9.11 ± 0.08	9.14 ± 0.16
Treatment.1 (3:97 CO:CH)	8.44 ± 0.04	8.51 ± 0.04	8.37 ± 0.17	8.43 ± 0.13	8.77 ± 0.40	9.10 ± 0.05
Treatment.2 (6:94 CO:CH)	8.44 ± 0.04	8.50 ± 0.03	8.53 ± 0.08	8.40 ± 0.04	8.68 ± 0.01	8.96 ± 0.02
Treatment3 (9:91 CO:CH)	8.44 ± 0.04	8.22 ± 0.08	8.21 ± 0.06	8.09 ± 0.01	8.34 ± 0.03	8.31 ± 0.01
Treatment.4(12:88 CO:CH)	8.44 ± 0.04	8.19 ± 0.08	8.19 ± 0.04	7.96 ± 0.08	8.33 ± 0.04	8.13 ± 0.05

*Means ± standard deviations of 2 measurements.
**Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid; Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2 = coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3 = coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4 = coating with a ratio of 12 ml cinnamon oil : 88 ml chitosan.

There were significant differences (P < 0.05) between pH values of noncoated eggs and both treatment three and treatment four throughout their storage period at 25±2°C and 4°C; however, no significant difference was observed between treatments three and four (Tables 7 and 8). This denotes that 9:91 and 12:88 CO: CH emulsions as coating materials could considerably slow down carbon dioxide loss through eggshell pores by acting as gas barriers (Torrico et al., 2011). In addition, there were no significant differences (P >0.1) among the pH values of the treatment one, treatment two, and CH group regardless of temperature.

Table 8 pH* of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 9 weeks of storage at 4°C

Coating**	Week 0	Week 3	Week 6	Week 9
Control Group	8.44 ± 0.04	8.75 ± 0.01	9.12 ± 0.01	9.20 ± 0.05
Chitosan (CH)	8.44 ± 0.04	8.68 ± 0.01	9.06 ± 0.04	9.11 ±0.02
Treatment.1 (3:97 CO:CH)	8.44 ± 0.04	8.61 ± 0.04	8.78 ± 0.08	8.82 ± 0.01
Treatment.2 (6:94 CO:CH)	8.44 ± 0.04	8.56 ± 0.03	8.62 ± 0.01	8.68 ± 0.05
Treatment.3 (9:91 CO:CH)	8.44 ± 0.04	8.29 ± 0.05	8.19 ± 0.04	8.10 ± 0.01
Treatment.4 (12:88 CO:CH)	8.44 ± 0.04	8.23 ± 0.01	8.12 ± 0.04	8.01 ± 0.02

*Means ± standard deviations of 2 measurements.
**Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid; Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2= coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3= coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4= coating with a ratio of 12 ml cinnamon oil: 88 ml chitosan.

Among all CO: CH coated eggs, the pH value of treatments three (9:91 CO: CH) and four (12:88 CO: CH) were gradually decreased through storage period and regardless of their storage temperature (Figures 15 and 16).

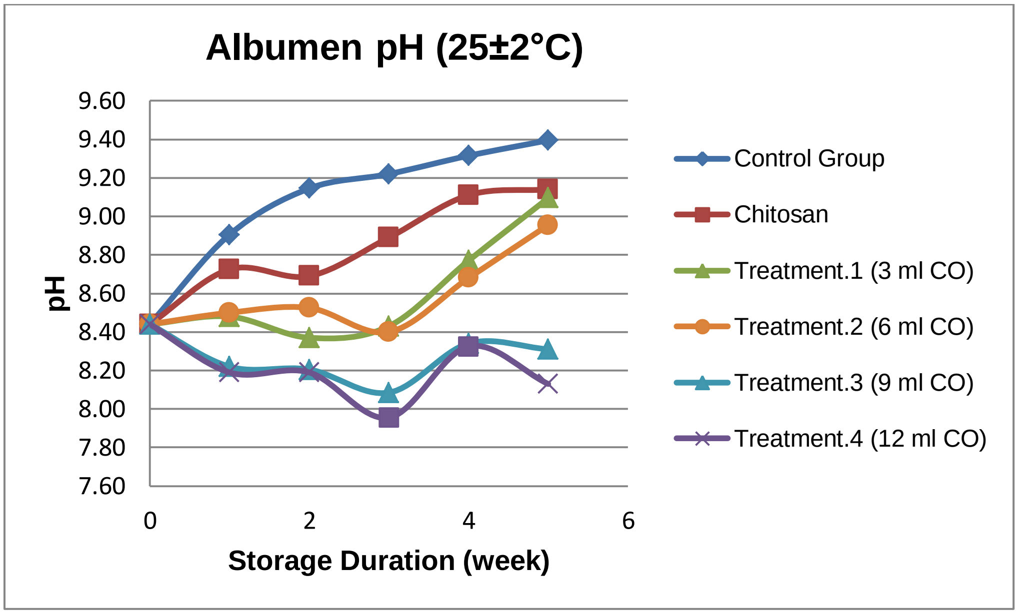

Figure 15 pH values of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 5 weeks of storage at 25±2°C

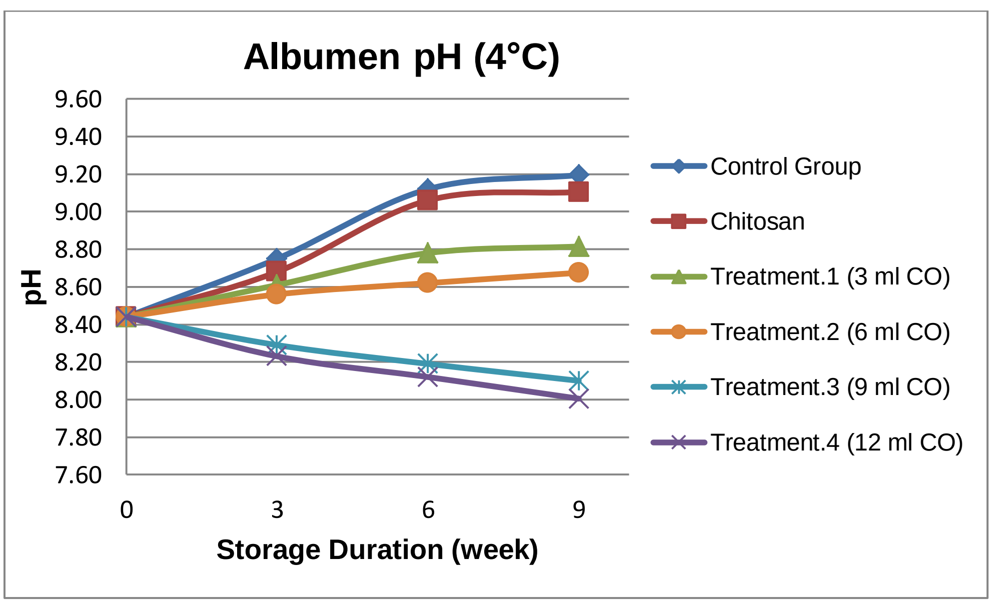

Figure 16 pH values of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 9 weeks of storage at 4°C

During storage at 25±2°C the initial pH value (8.44) of the control group and chitosan coated eggs was increased to 9.40 and 9.14, respectively (Table 7). The increase in the pH value of the same groups was slower during their storage at 4°C than storage at 25±2°C. For instance, the pH value of the refrigerated noncoated eggs at week nine (9.20) was less than the pH value of the room temperature stored noncoated eggs (9.22) at the third week of storage (Table 8).

Overall, the results of this study clearly show that coating materials can decrease the pH value of eggs during storage regardless of temperature. In a similar study by Wardy et al. (2013), they reported that the pH values of noncoated and CH-coated eggs was increased significantly ($P<0.05$) irrespective of storage temperature. They also reported that pH values of soybean oil-chitosan coated eggs were decreased throughout the storage time at both 25°C and 4°C.

4.5 Effects of Chitosan Solution and Cinnamon Oil- Chitosan Emulsion Coating on the Air Cell

During eggs' storage period, air cell height increases because of the two shell membranes separation due to moisture and carbon dioxide loss (Nongtaodum et al., 2013) . Storage time and temperature are two major factors that affect the air cell height. As time and temperature of storage increase, the air cell height increases as well (Samli, Agma, & Senkoylu, 2005). In this study, the air cell height of all eggs were increased throughout five weeks of storage at 25±2°C (Table 9) as well as nine weeks of storage at 4°C (Table 10).

Table 9 Air cell height* of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 5 weeks of storage at 25±2°C

Coating**	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Control Group	1.62 ± 0.04	2.30 ± 0.04	3.16 ± 0.87	4.12 ± 0.44	5.48 ± 0.52	7.30 ± 0.33
Chitosan (CH)	1.62 ± 0.04	2.18 ± 0.33	3.08 ± 0.08	3.43 ± 0.28	4.48 ± 0.08	6.27 ± 0.96
Treatment.1 (3:97 CO:CH)	1.62 ± 0.04	2.14 ± 0.13	2.58 ± 0.11	3.33 ± 0.06	3.78 ± 0.11	5.12 ± 0.59
Treatment.2 (6:94 CO:CH)	1.62 ± 0.04	1.91 ± 0.06	2.25 ± 0.26	3.07 ± 0.65	3.60 ± 0.08	4.70± 0.23
Treatment3 (9:91 CO:CH)	1.62 ± 0.04	1.75 ± 0.08	2.06 ± 0.47	2.36 ± 0.47	2.80 ± 0.36	3.15 ± 0.08
Treatment.4(12:88 CO:CH)	1.62 ± 0.04	1.63 ± 0.13	1.99 ± 0.73	2.27 ± 0.49	2.56 ± 0.13	2.65 ± 0.22

*Means ± standard deviations of 2 measurements.
**Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid; Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2 = coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3 = coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4 = coating with a ratio of 12 ml cinnamon oil : 88 ml chitosan.

Table 10 Air cell height* of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 9 weeks of storage at 4°C

Coating**	Week 0	Week 3	Week 6	Week 9
Control Group	1.62 ± 0.04	2.87 ± 0.34	3.65 ± 0.01	4.78 ± 0.11
Chitosan (CH)	1.62 ± 0.04	2.73 ± 0.01	3.49 ± 0.24	4.44 ± 0.46
Treatment.1 (3:97 CO:CH)	1.62 ± 0.04	2.55 ± 0.17	2.79 ± 0.08	3.64 ± 0.40
Treatment.2 (6:94 CO:CH)	1.62 ± 0.04	2.31 ± 0.24	2.65 ± 0.03	3.35 ± 0.36
Treatment3 (9:91 CO:CH)	1.62 ± 0.04	1.83 ± 0.05	2.07 ± 0.08	2.36 ± 0.05
Treatment.4 (12:88 CO:CH)	1.62 ± 0.04	1.74 ± 0.11	1.96 ± 0.04	2.21 ± 0.06

*Means ± standard deviations of 2 measurements
**Control = noncoated eggs; CH = coating with 100% chitosan solution (CH). Chitosan solution at 2% (w/v) was prepared in 1% (v/v) acetic acid; Treatment.1 = coating with a ratio of 3 ml cinnamon oil: 97 ml chitosan; Treatment.2= coating with a ratio of 6 ml cinnamon oil: 94 ml chitosan; Treatment.3= coating with a ratio of 9 ml cinnamon oil: 91 ml chitosan; Treatment.4= coating with a ratio of 12 ml cinnamon oil: 88 ml chitosan.

There were significant differences (P <0.05) among the noncoated eggs, treatment three (CO: CH 9:91), and treatment four (CO: CH 12:88). On the contrary, no significant differences were observed among the control group, CH group, treatment one, and treatment two during storage at 25±2°C (Figure 17). The increase rate of air cell height of all treatments kept at refrigerator was slower than room temperature stored eggs. As a case in point, the initial air cell height of noncoated eggs (1.62 mm) was increased to 4.78 after nine weeks of storage in the refrigerator and 7.30 mm after five weeks of storage at 25±2°C (Tables 9 and 10).

There were significant differences ($P < 0.05$) among the air cell heights of noncoated and CH coated eggs stored in the refrigerator and treatments three and four. In addition, there was a slight difference ($0.05 < P < 0.1$) between the air cell heights of treatments one and two stored at 4°C and treatments three and four, separately. In contrast there was no significant difference ($0.1 < P$) between the air cell heights of treatments three and four during storage at both room and refrigerator temperature.

Figures 17 and 18 clearly display that the combination of cinnamon oil and chitosan can be effective as a coating material on retarding the eggs’ air cell enlargement during storage. Increasing the amount of cinnamon oil could slow down the rate of eggs’ air cell expansion. Similarly, Nongtaodum et al. (2013) reported that coating eggs with a variety of edible oils such as coconut and soybean significantly decreased the eggs’ air cell height rise.

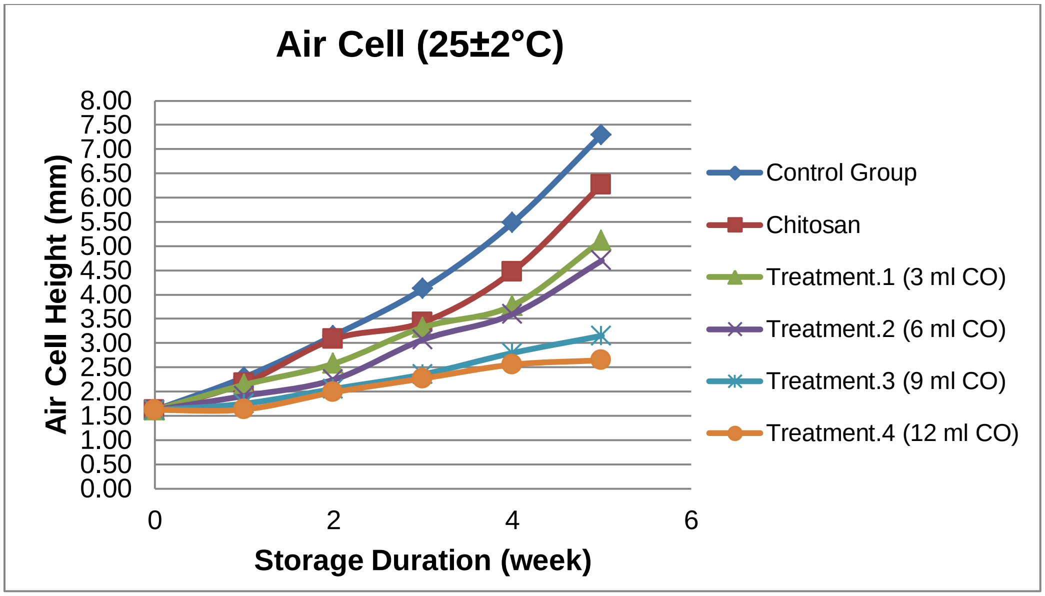

Figure 17 Air cell height of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 5 weeks of storage at 25±2°C

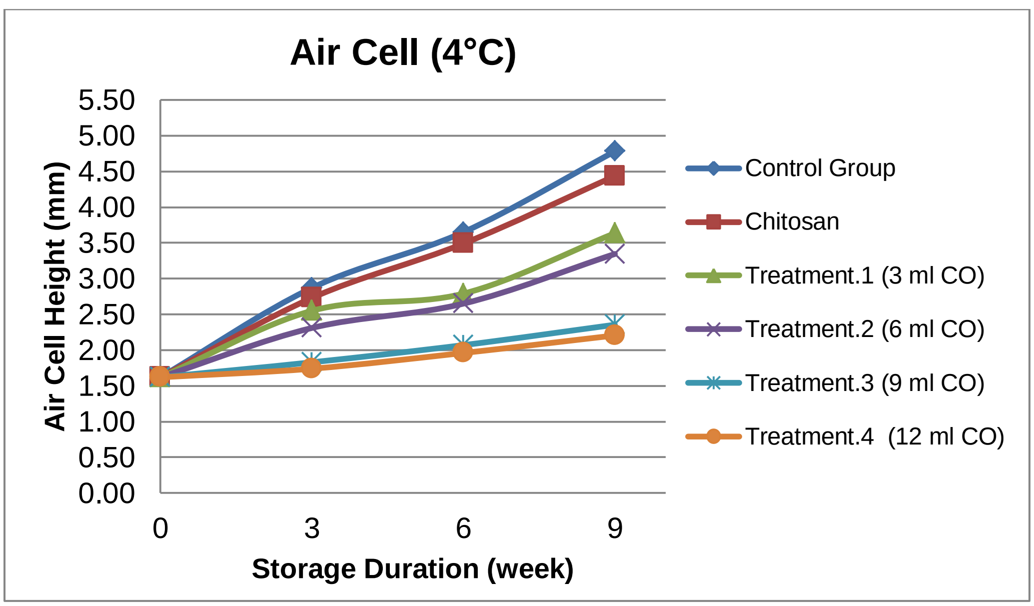

Figure 18 Air cell height of eggs coated with chitosan and four cinnamon oil- chitosan emulsions during 9 weeks of storage at 4°C

CHAPTER FIVE: CONCLUSION AND FUTURE DIRECTIONS

In this study, achieved data from the weight loss percentage, Haugh unit, pH, yolk index, and air cell height indicated that the combined emulsion of cinnamon oil and chitosan could preserve the internal quality of eggs more than chitosan itself during storage irrespective of storage temperature. Therefore, cinnamon oil can boost the effectiveness of chitosan as a coating material for eggs. Coating eggs with CO: CH emulsions at ratios of 9:91 and 12:88 maintained AA grade for two weeks and a grade of A for four weeks of storage at room temperature.

Moreover, CO: CH 9:91 and 12:88 coated eggs, maintained the grade of AA after nine weeks of storage at 4°C. Although treatments one (3:97 CO: CH) and two (6:94 CO: CH) had no significant differences ($0.1<P$) with CH and noncoated groups, they were more effective in preserving the eggs' internal quality than chitosan emulsion. Among all treatments, CO: CH emulsion at the ratio of 12:88 is recommended because of its capability in preserving the internal quality and grade of eggs during storage time and regardless of temperatures.

This study showed that even small amounts of the combined cinnamon oil and chitosan emulsion coating could maintain the quality of eggs and retard their internal quality deterioration. In this study, small quantities of cinnamon oil were used because of its aromatic characteristic. CO: CH coated eggs had no strong smell after two weeks of storage and their internal quality and odor was not affected by the eggshell coating. However, a larger sample size is required in order to get more valid and reliable results.

In addition, a customer acceptance test as well as sensory evaluation might be required. Further studies on the antimicrobial efficacy of these coating emulsions against *Salmonella Enteritidis*, a pathogen of concern to eggs, will provide an insight into the potential egg safety applications of these emulsions.

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