

Efficacy of various protein-based coating on enhancing the shelf life of fresh eggs during storage

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ABSTRACT The effectiveness of various coatings (whey protein isolate [WPI], whey protein concentrate [WPC], zein, and shellac) on functional properties, interior quality, and eggshell breaking strength of fresh eggs were evaluated during storage at 24°C for 6 weeks. Coatings and storage time had significant effects on Haugh unit, yolk index, albumen pH, dry matter (DMA), relative whipping capacity (RWC), and albumen viscosity. Uncoated eggs had higher albumen pH (9.56) and weight loss, and lower albumen viscosity (5.73), Haugh unit (HU), and yolk index (YI) during storage. Among the coated eggs, the shellac and zein coated eggs had the highest value of albumen viscosity (27.26 to 26.90), HU (74.10 to 73.61), and YI (44.84 to 44.63) after storage. Shellac (1.44%) was more effective in preventing weight loss than WPC (4.59%), WPI (4.60%), and zein (2.13%) coatings. Uncoated eggs had the highest value (6.71%) of weight lost. All coatings increased shell strength (5.18 to 5.73 for top and 3.58 to 4.71 for bottom) significantly ($P < 0.05$) compared to

the uncoated eggs (4.70 for top and 3.15 for bottom). The functional properties such as albumen DMA (14.50 to 16.66 and 18.97 for uncoated) and albumen RWC (841 to 891 and 475 for uncoated) of fresh eggs can be preserved during storage when they are coated. The shellac and zein coatings were more effective for maintaining the internal quality of fresh eggs during storage. Fourier transform near infrared (FT-NIR) in the 800 to 2500 nm reflection spectra were used to quantify the contents of the fresh eggs at the end of storage. Eggs coated with shellac or zein displayed a higher absorbance at 970 and 1,197 nm respectively (OH vibration of water) compared with those coated with WPI or WPC and the uncoated group at the end of storage. The coatings improved functional properties and also shell strength and could be a viable alternative technology for maintaining the internal quality of eggs during long-term storage. This study highlights the promising use of various coatings to both enhance the functional properties and to reduce the breakage of eggs.

Key words: protein coatings, shell eggs, egg quality, shelf life, functional properties

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INTRODUCTION

Eggs are a versatile food which constitute high quality complete proteins containing all 9 essential amino acids, including the vitamins and minerals essential for optimal health. Eggs are widely used in the food industry due to their multifunctional properties (e.g., foaming, gelling, coagulation, and emulsifying) (Hernandez-Ledesma and Chia-Chien, 2013). Between 2000 and 2012, global egg output expanded by more than 2% a year from 51 million tonnes to around 65 million tonnes (Anonymous, 2014). In addition, 69.43 million egg shells are broken annually in the United States (Gupta, 2008).

It is estimated that more than 10% of eggs produced in the hen house are uncollectable or break before their intended use. The first 2 to 5% are unsuitable for collection due to a cracked or broken shell. Another 3 to 8% are lost during collection, whilst moving through the belts, or during cleaning, packing, and transportation to the end user (Gupta, 2008). Improving shell quality and reducing egg breakage are thus important factors and improving shell strength will potentially decrease the number of cracked eggs and result in significant savings to the industry.

Eggs also undergo a sequence of interior (functional) quality changes and microbial contamination during storage (Jones et al., 2004). The eggshell, with its porous structure, allows carbon dioxide and moisture to escape and contaminants such as bacteria and odours to enter the egg (Berrang et al., 1999; De Reu et al., 2006; Leleu et al., 2011). As such, eggs are highly perishable and can rapidly lose their internal qualities (Caner and Cansız, 2007, 2008) and it is therefore important to protect the shells from mass transfer. A process that

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seals the pores of the shell should not only reduce mass transfer but also improve the strength of the shell, extending shelf-life and reducing breakage.

Extending shelf-life and maintaining the quality of fresh foods is challenging and novel methods to achieve this must be developed. Several technologies, including cold storage, UV, modified atmosphere packaging, and ozonation have been used for reducing deterioration in the quality of fresh products and to prolong shelf life (Allende et al., 2006; Debabandya et al., 2013). There has been increasing interest in using coatings as a food preservation method, and as a tool to enhance quality, safety, and stability. Such coatings are used as a thin layer to protect perishable foods by controlling the internal gaseous atmosphere (Allende et al., 2006; Olivas and Barbosa-Canovas, 2005). Using coatings on eggshells may increase their strength and potentially decrease the number of cracked eggs. Even a small improvement would result in significant savings for the egg industry. Potentially, a thin protective coating layer could provide a barrier against mass transfer and may preserve viscosity, whipping, and foam stability (Foegeding et al., 2006; Lomakina and Mikova, 2006).

Various raw edible materials are suitable for coating: proteins (e.g., whey, corn, and soy), polysaccharides (e.g., cellulose derivatives or starches), lipids (e.g., waxes, shellac), and even some synthetic polymers (e.g., polyvinyl acetate) (Attila and Orts, 2009). Selecting a suitable coating for fresh eggshell quality is important to minimize mass transfer, oxidation processes, or microbial growth. Barrier and mechanical properties of coatings or films depend on their molecular structure and it is therefore important to use appropriate coatings that will provide the best protection for the internal quality (weight loss, pH, HU, and YI) of fresh eggs.

Protein coatings show potential as value-added applications that might receive little resistance from regulators for food use. Protein based biopolymers such as whey proteins and corn zein have desirable barrier properties (Kirsten et al., 2009). Whey is one of the most promising proteins due to its gas barrier properties and glossy appearance (Hossein, 2011). Whey protein (WPC, protein concentration 65 to 80% in dry matter, or WPI, protein concentration over 90% in dry matter), a byproduct of the cheese industry, has excellent nutritional and functional properties and has the potential to be used in edible films. Other proteins such as corn zein, obtained from the corn gluten that is a by product of the corn industry, also has great potential for food packaging applications (Padua and Wang, 2002). Use of corn zein for eggshell coating is very attractive because it has better barrier characteristics to moisture and oxygen when compared to other proteins.

Numerous other food-grade coating materials (mineral oils, waxes, whey protein, soy protein, gluten, chitosan, and cellulose-based materials) have also proven to be effective in reducing mass transfer by sealing pores and have been researched extensively (Anonymous,

2012; Caner, 2005b; Hernandez-Ledesma and Chia-Chien, 2013; Rhim et al., 2004; Waimaleongora-Ek et al., 2009; Wong et al., 1996; Xie et al., 2002). Little research has however been conducted into the preservation of internal quality and functional properties of fresh eggs using food proteins such as WPC, WPI, and corn zein as coatings. The goal of this research then was to compare the impacts different types of food protein coatings namely; (WPI, WPC, corn zein, and food grade shellac (Musa et al., 2011).

The research was performed by: (1) observing changes in the interior quality of eggs during storage with attention focused on changes in quality indicators such as HU, YI, albumen, and yolk pH, as well as functional properties such as albumen and whole egg RWC and (2) measuring eggshell strength during storage at 24°C for 6 weeks.

MATERIAL AND METHODS

Clean, white shell (Lohmann White laying hen breed), unfertile, large, freshly laid (1-day-old) unwashed eggs supplied by A.B Foods Inc. (Bandirma, Turkey) were used in the present study according to (Caner, 2005b; Caner and Cansız, 2008). Treatments consisted of coating with WPI (DaviCo Foods International Inc, Eden Prairie, MN), WPC (DaviCo Foods International Inc, Eden Prairie, MN), zein, and shellac (Mantrose-Haeuser Co, Westport CT) and uncoated eggs were used as a control.

Preparation of Coating Solutions and Coating of Shell Eggs

Whey protein films were prepared at 10% (w/w protein) using WPI and WPC in 100 ml water. Glycerol was then added to give a protein:plasticizer ratio of 2.5:1 w/w while the solution was stirred continuously in a magnetic stirrer at 80°C for 30 min (Caner, 2005b). Shellac and zein were mixed with ethyl alcohol (10:90 v/v). Glycerol was then added to give a plasticizer:protein ratio of 2.5:1 w/w while the solution was stirred continuously (Caner, 2005a).

After washing with water at 24°C for 1 min followed by 5 min drying time, the clean eggs were immersed individually by hand in the coating solutions at 24°C for 1 min, left to dry for 5 min, then immersed again for 1 min (second layer) so that the coating visibly covered the entire surface. They were then left to dry at ambient temperature (Caner and Cansız, 2008). The uncoated washed eggs served as a control. The eggs were subsequently placed in open moulded plastic egg trays in storage at 24°C until testing. Ten eggs each per treatment were taken at each storage interval for the evaluation of quality parameters each of which was analysed three times.

Weight Loss

Weight loss (%) of the eggs during storage was calculated by subtracting the final weight of the egg from the initial weight and then dividing by the initial weight and multiplying by 100 as described in Caner and Cansız (2008). Ten eggs for each treatment type were taken at weekly intervals for determination of weight loss. Each egg was weighed to within ± 0.001 g using a sensitive laboratory electronic balance.

Haugh Unit and Yolk Index

Haugh units were calculated for 3×10 eggs for each treatment using digital callipers (CD-15CP, Mitutoyo Ltd, Andover, Hampshire, UK) based on following equation (Yuceer and Caner, 2014).

$$\text{Haugh unit (HU)} = 100 \times \log_{10} \\ \times (h - 1.7G0.37 + 7.6)$$

where h is the thickness of albumen (mm) and G is the mass of the entire egg (g). The parameter h was estimated by averaging 3 measurements carried out at different points at a distance 10 mm from the yolk using digital callipers (CD-15CP, Mitutoyo Ltd, Andover, Hampshire, UK). The eggs were graded as follows (Yuceer and Caner, 2014):

$$\text{AA, HU} > 72; \text{A, HU} = 71 - 60; \\ \text{B, HU} = 59 - 31; \text{C, HU} < 30.$$

The yolk index was calculated as yolk height divided by yolk width. Yolk height and width were measured without removing the albumen with digital callipers (CD-15CP, Mitutoyo Ltd, UK). Three measurements were taken for each of the 10 eggs per treatment type at 2-week intervals (week 0, 1, 3, and 5) for determination of the HU and YI.

pH Measurements

The albumen and yolk were separated and then small volumes of firm and thin albumen were homogenized for 20 s in a blender (Model 32BL80, Waring, Torrington, CT) and each individual egg albumen and yolk measured using a pH 210 meter (Hanna Instruments, Woonsocket, RI) (Caner and Cansız, 2007). The eggs (3×10 eggs) for each treatment were measured at each week intervals for determination of albumen and yolk pH during storage.

Albumen Viscosity

For each treatment type, 10 eggs were broken, chalazae were separated, homogenized as described in Lucisano et al. (1996), and the albumen was collected in

a vessel for measuring viscosity for each sample. Albumen viscosity (mPa s) measurements were carried out at $20 \pm 0.5^\circ\text{C}$ using a Brookfield viscometer (Model DV II+Pro D 220, TC-502 temperature controller unit and Rheocalc software; Brookfield Engineering Laboratories, Inc., Middleboro, MA). The spindle (UL adapter, 30 rpm) was selected based on the torque measurement suggested by the manufacturer of between 10 and 100%. The results were recorded after a 20 s rotation of the spindle. Silicone oil standard solution (Brookfield Engineering Laboratories, Inc., Middleboro, MA) was used to calibrate the viscometer (Yuceer and Caner, 2014).

Total Solids (Dry Matter) of Albumen and Yolk

Total solids (dry matter) (% w/w) in 5 g of egg albumen and yolk were determined using an Abbe refractometer with a Peltier system (DR-A1, Atago Co. Ltd, Tokyo, Japan) at $20 \pm 1^\circ\text{C}$ (Yuceer and Caner, 2014). Four samples were obtained from each specimen. 3×10 eggs for each treatment were measured each week intervals for determination of albumen and yolk pH during storage.

Foaming Properties

Relative whipping capacity and foam stability of the egg albumen and whole egg samples were measured at 20°C . Foam was obtained by whipping 75 mL of egg albumen /whole egg in a Hobart mixer (N50CE, Hobart Foster Scandinavia A/S, Aalborg, Denmark) at speed 2 for 90 s and then speed 3 for 90 s at room temperature.

Foam stability was measured with a graduated cylinder after the foam had been allowed to rest for 1 h, as described by Lomakina and Mikova (2006) using a small volume. Foam stability was measured in the same vessel as the volume of released fluid at the bottom 1 h after whipping. Egg relative foaming capacity calculated at the following formula:

$$\text{Volume (\%)} = (\text{volume of prepared foam} \\ - \text{volume of liquid drainage}) / \\ \text{original volume of liquid} \times 100$$

The experiment was repeated three times and mean values were calculated. The eggs (3×10 eggs) for each treatment were measured at each week intervals for determination of albumen and whole egg RWC.

Eggshell Breaking Strength

Eggshell breaking strength (puncture strength) was determined using a texture analyzer (TA.XT2, Texture Technologies Corp., Scarsdale, NY). Each egg was mounted on a texture analyzer platform and the eggshell was punctured at the top (small end) and

bottom (large end) using a 3 mm die probe at 5 mm s⁻¹ constant speed and with a 30 kg load cell in compression mode. Three replicates were made for each sample. Ten eggs for each treatment were measured 3× each after treatment and at the end of the 4 week storage period. The force required to puncture the shell was recorded as the eggshell breaking strength (N) (Caner and Cansız, 2008; Yuceer and Caner, 2014).

FT-NIR Measurements

Spectral measurements were taken on the eggs yolk and albumen in reflectance and transmittance modes using an FT-NIR spectrometer according to Aday and Caner (2010). Spectral measurements were performed using a Bruker multi-purpose analyser (MPA) FT-NIR spectrometer (Bruker Optik, GmbH, Ettlingen, Germany) equipped with InGaAs detectors (TE-InGaAs internal for reflectance and RT-InGaAs external for transmittance) and a 20 W high-intensity tungsten-halogen NIR light source. Reflectance measurements obtained with a fibre optic probe (type IN 261) covered the wavelength range 780 to 2,500 nm. During transmittance measurements, the albumen and yolks were placed on the transmittance probe so that light beam from the light source entered at the centre. Collection of the transmitted light from the samples was performed via an RT-InGaAs detector. Thirty-two scans were performed for each reflectance spectrum in about 15.32 s and 128 scans for transmittance in about 62 s. Resolution was 8 cm⁻¹ for both spectral measurement modes. To locate the OH band, wave number (cm⁻¹) was transformed to wavelength (nm) using the OPUS software (Bruker Optik GmbH, Germany) (Aday and Caner, 2010).

Data Analysis

This study aimed to evaluate the effects of the protein based coatings and storage time (6 weeks at 24°C) on the quality characteristics of eggs. Analysis of the variance was carried out on all measured parameters among the control (uncoated) and coated eggs to determine

any significant differences during or after storage. Statistical procedures were performed using LSM-PROG GLM of the SAS program (SAS Institute, Cary, NC). Statistical significance was defined from the means at $P < 0.05$ with a Tukey multiple comparison test.

RESULTS AND DISCUSSION

Weight Loss

The weight loss of control (uncoated) and coated eggs (WPC, WPI, zein, and shellac) is shown in Table 1 during 5 weeks storage at 24°C. Egg weight decreased significantly during storage. The weight loss of the eggs increased with storage period and was higher for the control group followed by the weight loss of the WPC (4.59%), WPI (4.60%), zein (2.13%), and shellac (1.44%) coated eggs respectively (Table 1). The control group showed an increasing level of weight loss, reaching a value of 6.71% at the end of the storage period. All coatings showed a significant reduction ($P < 0.05$) in weight loss during the storage period. Differences in weight loss between WPC and WPI coatings were not significant until 3 weeks had passed. The shellac coating rendered excellent sealing properties of the pores of the eggs, preventing the evaporation of moisture and gases. Differences in weight loss based on coating type were strongly influenced by the coating's ability to block pores on the surface of the eggs.

In this study, both zein and shellac egg coating materials kept the weight loss within the acceptable range of 2 to 3% of food and agriculture organization (FAO) at the end of the 5 week storage period at 24°C (FAO, 2003). Shellac coating renders excellent sealing properties and extends storage time. Eggs coated with WPC and WPI showed a significant reduction ($P < 0.05$) in weight loss compared with the control eggs. Eggs coated with zein showed a significant reduction ($P < 0.05$) in weight loss compared with WPC and WPI coatings and the control eggs. Various studies have shown the enhancement effects of WPC coatings Alleoni and Antunes (2004b), WPI coatings Caner (2005b), zein coatings Wong, et al. (1996), and shellac coatings (Musa et al., 2011) associated to lower water loss from the eggs

Table 1. Effect of different coatings on egg weight loss during 5 weeks of storage at 24°C.¹

Coatings ^a	Egg weight loss (%)					
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	0.00 ± 0.00 ^{A,a}	1.06 ± 0.07 ^{B,a}	1.82 ± 0.06 ^{C,a}	3.84 ± 0.51 ^{D,a}	5.28 ± 0.52 ^{E,a}	6.71 ± 0.73 ^{F,a}
WPI	0.00 ± 0.00 ^{A,a}	0.89 ± 0.07 ^{B,a,b}	1.47 ± 0.13 ^{C,b}	2.28 ± 0.22 ^{D,b}	3.44 ± 0.34 ^{E,b}	4.60 ± 0.41 ^{F,b}
WPC	0.00 ± 0.00 ^{A,a}	0.83 ± 0.11 ^{B,a}	1.52 ± 0.09 ^{C,a}	2.19 ± 0.14 ^{D,b}	3.61 ± 0.14 ^{E,b}	4.59 ± 0.18 ^{F,b}
Zein	0.00 ± 0.00 ^{A,a}	0.56 ± 0.08 ^{B,c}	0.84 ± 0.14 ^{C,b}	1.21 ± 0.21 ^{C,c}	1.71 ± 0.31 ^{D,c}	2.13 ± 0.39 ^{D,c}
Shellac	0.00 ± 0.00 ^{A,a}	0.34 ± 0.04 ^{B,c}	0.59 ± 0.06 ^{B,C,c}	0.83 ± 0.06 ^{B,C,c}	1.22 ± 0.12 ^{C,D,d}	1.44 ± 0.10 ^{D,d}

Data are expressed as Means ± standard deviations.

^{a-d}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-F}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

¹n = 30 eggs per mean.

during storage. The present results are in agreement with those references reporting significant ($P < 0.05$) egg weight reductions.

Haugh Unit and Egg Grade

The HU is a measure of egg quality based on the height of albumen and the egg's weight. A fresh, good quality egg has a HU index of around 80 and an older egg would have a lower HU (Caner, 2005a; Caner and Cansız, 2008; Yuceer and Caner, 2014). Changes in HU of the various coated and control (uncoated) eggs are shown in Table 2. Haugh units significantly decreased during storage time in all groups. The control eggs exhibited a significantly lower ($P < 0.05$) HU than that of the coated eggs during storage. Haugh unit value rapidly decreased for the control eggs during storage, in agreement with previous investigations (Caner, 2005a; Jones and Musgrove, 2005; Yuceer and Caner, 2014). The decrease in HU is due to weakening of the albumen (Yuceer and Caner, 2014). The current main hypotheses for this phenomenon concerns the break down of the ovomucin-lysozyme complex, decreasing carbohydrate content of ovomucin and increasing pH and water loss (Chen et al., 2005; Yuceer and Caner, 2014). During storage of eggs, the gelatinous structure of the thick albumen gradually deteriorates, changing into thin albumen, which is associated with either ovomucin-lysozyme interactions, disulfide bonds or carbohydrate moieties of ovomucin, or interrelations between α and β ovomucins (Hernandez-Ledesma and Chia-Chien, 2013; Li-Chan and Nakai, 1989).

Eggs coated with shellac and zein had significantly higher values than the control eggs and those coated with WPI or WPC (Table 2). No significant differences were observed in HU of eggs coated with WPI and WPC after 1 week of storage. The shellac and zein coatings were more effective than the WPI and WPC coatings in preserving albumen quality during storage. Eggs coated with shellac and zein retained a value of HU at 74.10 (Shellac) and 73.61 (Al-Bachir and Zeinou,

2006) respectively after 5 weeks of storage, compared with 67.99 (WPC) and 69.07 (WPI), and 58.93 for the control eggs (Table 2). This indicated that the coatings, especially shellac and zein, helped seal pores on the eggshell and slow down the escape of carbon dioxide and water during long term storage, maintaining albumen quality.

While eggs coated with zein or shellac stored at 24°C maintained a grade "AA" (HU > 72) through the entire 5 week period, eggs coated with WPI or WPC maintained grade "AA" only for 3 weeks. The uncoated eggs dropped from grade "AA" to "A" (HU from 81.23 to 70.86) after 1 week of storage and then to grade "B" after 5 weeks (Table 2). Thus the shelf life of the eggs was prolonged by at least 3 weeks by WPI or WPC coating, and by at least 5 weeks by zein or shellac coating. Caner (2005a) reported that among eggs coated and stored at 24°C, the shellac coated eggs had the highest value of HU during storage. Chitosan and shellac effectively maintained the eggs at grade "A" over 3 weeks and WPI over 2 weeks. Wong, et al. (1996) reported that eggs coated with soy and corn remained at grade "B" after 28 days of storage, while uncoated eggs changed from grade "A" to grade "B" after 1 week of storage. Wong, et al. (1996) reported that the HU value for eggs coated with corn zein, wheat gluten, soy protein isolate, and mineral oil were 52.6, 52.3, 51.1 and 34.6 respectively on the 28th day at room temperature. The zein coating showed a higher HU value when compared with the other coatings over the same storage period (Wong et al., 1996). Eggs coated with WPI 6% remained at grade "A" over 2 weeks, and eggs coated with WPI 12% and 18% remained at grade "A" over 3 weeks of storage at room temperature. Eggs coated with wheat gluten solutions resulted in the maintenance of quality grade "A" for 30 days at room temperature (Caner, 2005b).

These studies demonstrated that various coatings can preserve albumen quality during long-term storage at 24°C. These results are in agreement with Yuceer and Caner (2014), Caner (2005a) and Bhale, et al. (2003).

Table 2. Effect of the coatings on Haugh unit (HU) and egg grade during 5 weeks of storage at 24°C.¹

Coating ^a	Haugh unit (Egg grade) ^b			
	0 weeks	1 week	3 weeks	5 weeks
Control	81.23(AA) ± 0.94 ^{A,a}	74.73(AA) ± 1.81 ^{B,a}	70.86(A) ± 1.09 ^{C,a}	58.93(B) ± 0.96 ^{D,a}
WPI	81.23(AA) ± 0.94 ^{A,a}	78.03(AA) ± 0.80 ^{B,b}	75.65(AA) ± 0.98 ^{B,b}	69.07(A) ± 1.40 ^{C,b}
WPC	81.23(AA) ± 0.94 ^{A,a}	79.06(AA) ± 0.76 ^{A,b,c}	75.93(AA) ± 1.53 ^{B,b}	67.99(A) ± 1.17 ^{C,b}
Zein	81.23(AA) ± 0.94 ^{A,B,a}	81.48(AA) ± 1.20 ^{A,c}	78.77(AA) ± 1.81 ^{B,c}	74.10(AA) ± 1.29 ^{C,c}
Shellac	81.23(AA) ± 0.94 ^{A,a}	80.26(AA) ± 0.88 ^{A,b,c}	78.92(AA) ± 1.00 ^{A,c}	73.61(AA) ± 1.39 ^{B,c}

Data are expressed as Means ± standard deviations.

^{a-c}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-D}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

^bEgg grades: AA, HU > 72; A, HU = 71–60; B, HU = 59–31; C, HU < 30.

¹n = 30 eggs per mean.

Table 3. Effect of the coatings on yolk index during 5 weeks of storage at 24°C.¹

Coating ^a	Yolk index			
	0 weeks	1 week	3 weeks	5 weeks
Control	0.49 ± 0.05 ^{A,a}	0.41 ± 0.01 ^{B,a}	0.37 ± 0.01 ^{B,a}	0.33 ± 0.02 ^{C,a}
WPI	0.49 ± 0.05 ^{A,a}	0.44 ± 0.01 ^{B,a,b}	0.41 ± 0.01 ^{B,a,b}	0.38 ± 0.02 ^{C,b}
WPC	0.49 ± 0.05 ^{A,a}	0.44 ± 0.01 ^{B,a,b}	0.42 ± 0.01 ^{B,C,a,b}	0.40 ± 0.02 ^{C,b,c}
Zein	0.49 ± 0.05 ^{A,a}	0.45 ± 0.01 ^{B,b,c}	0.44 ± 0.01 ^{B,b,c}	0.42 ± 0.02 ^{B,c}
Shellac	0.49 ± 0.05 ^{A,a}	0.46 ± 0.01 ^{A,b,c}	0.45 ± 0.01 ^{A,B,c}	0.42 ± 0.01 ^{B,c}

Data are expressed as Means ± standard deviations.

^{a-c}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-C}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

¹n = 30 eggs per mean.

Yolk Index

The yolk index is a measure of egg freshness and is based on the ratio of yolk height to yolk width (Yuceer and Caner, 2014). During storage at room temperature, the YI value decreased as a result of a progressive weakening of the vitelline membranes and liquefaction of the yolk caused mainly by diffusion of water from the albumen to yolk part (Hernandez-Ledesma and Chia-Chien, 2013; Stadelman, 1995; Torricco et al., 2011; Wardy et al., 2011). The YI of the uncoated eggs was significantly lower than all the coated eggs during storage. Storage time had a significant effect on YI. After 5 weeks of storage, the YI of the uncoated eggs decreased from 0.49 to 0.33, while eggs coated with shellac, zein, WPI, or WPC displayed YI values of 0.42, 0.42, 0.40 and 0.38 respectively. Yolk index values of the coated eggs at 5 weeks were similar to the YI values of the control eggs at 1 week (Table 3). Eggs coated with shellac or zein had significantly higher YI values than those of WPC or WPI coated eggs after 3 weeks of storage.

Shellac, zein, WPC, and WPI coatings all effectively reduced the mass transfer rate (water and CO₂ loss) from the albumen through the eggshell during long term storage. Consequently, this process inhibits albumen liquefaction and water uptake by the yolk and minimizes a reduction in yolk quality. The coatings with shellac, zein, WPC, and WPI were all able to preserve yolk quality for 3 to 4 weeks longer than the control eggs at room temperature. According to these results, various coatings potentially have significant preservative effects on the YI values, in agreement with Caner (2005a), Waimaleongora-Ek, et al. (2009), Bhale, et al. (2003) and Yuceer and Caner (2014).

pH Measurement

Besides the HU and YI, albumen pH can also be used as an indicator of egg freshness (Caner, 2005a; Yuceer and Caner, 2014). Freshly laid eggs contain 1.44 to 2.05 mg CO₂/g of albumen (Biladeau and Keener, 2009) and

have an albumen pH value of 7.5 to 8.5 (Scott and Silversides, 2000; Yuceer and Caner, 2014). Within a short time, the albumen pH increases to 9 owing to the release of CO₂ from the breakdown of carbonic acid in the albumen, resulting in changes to the bicarbonate buffer system (Biladeau and Keener, 2009; Scott and Silversides, 2000; Yuceer and Caner, 2014).

In this study, the pH of all the eggs albumen significantly increased with storage period. The albumen pH values were higher for uncoated eggs than for the coated eggs during storage (Tables 4 and 5). The albumen pH for uncoated eggs ranged from 7.50 initially to 9.50 at the end of storage (Table 4). For coated eggs, albumen pH values reached 9.33 (WPC), 9.31 (WPI), 8.90 (Zein) (Al-Bachir and Zeinou, 2006), and 8.83 (Shellac). Eggs coated with shellac and zein had significantly lower pH values than WPC or WPI coated eggs after 3 weeks (Table 4). There were no significant differences between WPC and WPI coated eggs. The albumen freshness of shellac and zein coated eggs after 5 weeks was comparable to that of the control eggs after 2 weeks and WPC and WPI chitosan-coated eggs after 3 weeks (Table 4). According to these result, eggshell coatings, especially shellac and zein, work as a barrier to CO₂, helping to maintain albumen quality by controlling the albumen pH. These results agree with those of Biladeau and Keener (2009), Yuceer and Caner (2014) and Caner (2005a).

Yolk pH weas also increased by storage time. The yolk pH in freshly laid eggs is generally about 6.0, but during storage of eggs, the pH gradually increases to 6.5. For coated eggs, yolk pH values reached 6.41 (WPI), 6.37 (WPC), 6.21 (Zein) (Al-Bachir and Zeinou, 2006), and 6.17 (Shellac). Eggs coated with shellac or zein had significantly lower yolk pH values than those coated with WPC or WPI after 2 weeks (Table 5).

Albumen Viscosity

The albumen that surrounds the yolk, which is called the thick albumen, progressively liquefies and thins

Table 4. Effect of the coatings on albumen pH during 5 weeks of storage at 24°C.¹

Coating ^a	Albumen pH					
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	7.50 ± 0.04 ^{A,a}	8.31 ± 0.02 ^{B,a}	8.82 ± 0.04 ^{C,a}	9.27 ± 0.02 ^{D,a}	9.38 ± 0.03 ^{E,a}	9.56 ± 0.02 ^{F,a}
WPI	7.51 ± 0.05 ^{A,a}	8.03 ± 0.09 ^{B,b}	8.35 ± 0.02 ^{C,b}	9.18 ± 0.03 ^{D,b}	9.24 ± 0.04 ^{E,b}	9.31 ± 0.02 ^{E,b}
WPC	7.52 ± 0.03 ^{A,a}	8.03 ± 0.04 ^{B,b}	8.47 ± 0.04 ^{C,c}	9.16 ± 0.05 ^{D,b}	9.27 ± 0.03 ^{E,b}	9.33 ± 0.01 ^{E,b}
Zein	7.52 ± 0.02 ^{A,a}	7.84 ± 0.04 ^{B,c}	8.09 ± 0.03 ^{C,d}	8.78 ± 0.04 ^{D,c}	8.78 ± 0.03 ^{D,c}	8.90 ± 0.06 ^{E,c}
Shellac	7.52 ± 0.03 ^{A,a}	7.92 ± 0.10 ^{B,c}	8.21 ± 0.08 ^{C,d}	8.80 ± 0.06 ^{D,c}	8.84 ± 0.03 ^{D,c}	8.83 ± 0.03 ^{E,c}

Data are expressed as Means ± standard deviations.

^{a-d}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-F}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

¹n = 30 eggs per mean.

Table 5. Effect of the coatings on egg yolk pH during 5 weeks of storage at 24°C.¹

Coating ^a	Egg Yolk pH					
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	5.86 ± 0.07 ^{A,a}	6.10 ± 0.01 ^{B,a}	6.22 ± 0.08 ^{C,a}	6.32 ± 0.04 ^{D,a}	6.47 ± 0.02 ^{E,a}	6.60 ± 0.01 ^{F,a}
WPI	5.85 ± 0.02 ^{A,a}	6.04 ± 0.05 ^{B,a}	6.11 ± 0.01 ^{C,b}	6.23 ± 0.02 ^{D,b}	6.28 ± 0.01 ^{D,b}	6.41 ± 0.01 ^{E,b}
WPC	5.84 ± 0.02 ^{A,a}	6.05 ± 0.05 ^{B,a,b}	6.13 ± 0.03 ^{C,b}	6.21 ± 0.01 ^{D,b}	6.24 ± 0.03 ^{D,b}	6.37 ± 0.01 ^{E,b}
Zein	5.84 ± 0.02 ^{A,a}	5.94 ± 0.03 ^{B,b}	6.05 ± 0.02 ^{C,c}	6.10 ± 0.01 ^{D,c}	6.15 ± 0.03 ^{D,E,c}	6.21 ± 0.01 ^{E,c}
Shellac	5.84 ± 0.02 ^{A,a}	5.88 ± 0.01 ^{A,b}	6.03 ± 0.06 ^{B,c}	6.06 ± 0.03 ^{B,c}	6.08 ± 0.04 ^{B,d}	6.17 ± 0.02 ^{C,c}

Data are expressed as Means ± standard deviations.

^{a-d}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-F}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

¹n = 30 eggs per mean.

Table 6. Effect of different coatings on egg albumen viscosity (20 s) during 6 weeks of storage at 24°C.¹

Coating ^a	Albumen viscosity (mPa s)						
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	60.46 ± 0.66 ^{A,a}	55.16 ± 0.95 ^{A,a}	41.83 ± 1.42 ^{B,a}	33.23 ± 1.61 ^{C,a}	24.70 ± 1.05 ^{D,a}	12.73 ± 1.33 ^{E,a}	5.73 ± 1.83 ^{F,a}
WPI	59.96 ± 1.5 ^{A,a}	55.30 ± 1.49 ^{A,a}	49.30 ± 0.62 ^{B,b}	45.26 ± 1.62 ^{B,b}	34.50 ± 1.21 ^{C,b}	29.36 ± 0.80 ^{D,b}	17.13 ± 1.18 ^{E,b}
WPC	60.06 ± 1.24 ^{A,a}	54.93 ± 1.34 ^{B,a}	48.36 ± 0.90 ^{C,b}	45.60 ± 0.60 ^{C,b}	35.60 ± 0.95 ^{D,b}	29.26 ± 1.59 ^{E,b}	17.23 ± 0.41 ^{F,b}
Zein	60.03 ± 1.88 ^{A,a}	58.78 ± 0.47 ^{A,a}	55.40 ± 1.01 ^{A,c}	50.23 ± 1.48 ^{B,c}	43.86 ± 1.71 ^{C,c}	36.86 ± 1.10 ^{D,c}	26.90 ± 1.53 ^{E,c}
Shellac	60.73 ± 1.59 ^{A,a}	59.20 ± 0.90 ^{A,a}	57.16 ± 0.80 ^{A,B,c}	53.03 ± 0.70 ^{B,c}	45.90 ± 1.57 ^{C,c}	37.36 ± 1.15 ^{D,c}	27.26 ± 0.64 ^{E,c}

Data are expressed as Means ± standard deviations.

^{a-c}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-F}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

¹n = 30 eggs per mean.

with time, transforming into thin albumen (deterioration of the gelatinous structure) (Silversides and Scott, 2001; Toussant and Latshaw, 1999). Viscosity of the egg albumen is one of the important characteristics that determine functional properties such as emulsification, whippability, and the gelling properties of the eggs (Kannan et al., 2013; Kemps et al., 2010). Any adverse effects on the viscosity of egg albumen will directly affect these properties and would make eggs unsuitable for food use. Also, a reduction in viscosity has been shown to have adverse effects on the shelf life of eggs. Viscosity is a critical parameter in determining the emulsifying properties of egg albumen (Kannan et al., 2013; Kemps et al., 2010). Egg albumen is a pseu-

doplastic fluid and its viscosity depends on the shear force. The albumen viscosity values measured during storage are given in Table 6. The less viscous the fluid, the greater its ease of movement (fluidity). The viscosity of the egg albumen decreased during storage. The gradual evaporation of water through the shell causes a decrease in density and the air cell enlarges. During storage, a decrease in viscosity was observed, confirming earlier results obtained by Kemps, et al. (2010) and Kannan, et al. (2013).

The albumen viscosity for uncoated eggs ranged from 60.46 initially to 5.73 at the end of storage (Table 6). For coated eggs, albumen viscosity values decreased to 27.26 (Shellac), 26.90

(Zein) (Al-Bachir and Zeinou, 2006), 17.23 (WPC), and 17.13 (WPI). Eggs coated with shellac or zein had significantly higher viscosity values than those coated with WPC or WPI after 2 weeks (Table 6).

The higher water content may explain the lower viscosity. The albumen viscosity depends on ovomucin-lysozyme complex, and ovomucin concentration is four times higher in thick albumen compared to liquefied albumen (Lucisano et al., 1996; Spada et al., 2012). The liquefaction of albumen is due to the increase in pH during storage and is influenced by the presence of ovomucin-lysozyme complex (Kannan et al., 2013; Spada et al., 2012). Thus the viscosity of the albumen changes during storage (Kannan et al., 2013; Spada et al., 2012).

Ovamucin is one of the major proteins in egg albumen and plays an important role in its gel-like structure. According to these result, the coatings minimize changes in the carbohydrate and protein moieties involved in the formation of ovomuc complex, resulting in a loss of gel-like structure (Lucisano et al., 1996). In addition, the coatings, especially shellac and zein, work as a barrier to CO₂ permeation through the pores of the shell during storage, minimizing changes in pH and maintaining albumen quality.

Total Solids (Dry Matter) of Albumen and Yolk

The total solid (dry matter) concentration of albumen (Heldman, 2011), has also been used as an indicator of egg freshness which is related to thinning or liquefaction of albumen. This liquefaction could occur due to protease enzymes, depolymerisation by hydroxyl ions at increasing pH values, and the interaction of ovomucin-lysozyme complex (Hidalgo et al., 1995; Lucisano et al., 1996). Water contained in the albumen permeates the yolk and some nutrients contained in the yolk can permeate the albumen. These osmotic tracks and changes in albumen and yolk concentrations can be measured by the refractometric method. During storage, the dry matter (DMA) increases due to

mixing of the yolk into the albumen. Albumen DMA values for uncoated eggs were higher than those for coated eggs (Table 7). The increase in DMA during storage has been attributed to liquefaction of the yolk and subsequent mixing into the albumen. Liquefaction is a result of an enhanced interaction between lysozyme and ovomucin as the pH increases during storage. The chemical cleavage effect of pH on the O-glycoside link between trisaccharides and β -ovomucin has been considered to be responsible for the collapse of the albumen structure. In general, liquefaction would result in an increase of fluidity in egg albumen and is associated with a deterioration in egg quality. Thick albumen is a gel and thin albumen is a fluid. During storage, the gelatinous structure of thick albumen changes its physical and chemical characteristics and gradually breaks down into a clear liquid, losing its consistency (Torrice et al., 2011). The DMA of the control (uncoated) egg albumen ranged from 11.47 initially to 18.97 at the end of storage (Table 7). For the coated eggs, albumen DMA values reached 16.66 (WPC), 16.53 (WPI), 14.72 (Zein) (Al-Bachir and Zeinou, 2006) and 14.50 (Shellac) at the end of storage. Significant differences in albumen DMA were observed between uncoated and coated samples ($P < 0.05$). Eggs coated with shellac or zein displayed similar values which were significantly lower than the DMA values of eggs coated with WPC or WPI after 2 weeks storage. There were no significant differences between WPC and WPI coated eggs. The albumen freshness of shellac or zein coated eggs after 5 weeks was comparable to that of the control eggs after 2 weeks, and that of the WPC or WPI chitosan-coated eggs after 3 weeks.

Yolk DMA values decreased significantly during storage. Water evaporation is the main reason for dry matter decrease (Lucisano et al., 1996). Yolk total solids decreased during storage from a maximum of 46.54% at 0 weeks to 40.53 to 44.84% at 5 weeks storage, in agreement with Jones (2007) and Yuceer and Caner (2014). Significant differences in DMA yolk values were observed between uncoated and coated samples ($P < 0.05$). The DMA of the uncoated egg yolks ranged from 46.54 initially to 40.53 at the end of storage (Table 8). Uncoated eggs had significantly lower yolk DMA values than the coated eggs (Table 8).

Table 7. Effect of different coatings on total solids (dry matter) of albumen during 5 weeks of storage at 24°C.¹

Coating ^a	Albumen Total solids (Dry matter) (% w/w)					
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	11.47 ± 0.05 ^{A,a}	12.69 ± 0.17 ^{B,a}	15.00 ± 0.23 ^{C,a}	17.10 ± 0.11 ^{D,a}	17.94 ± 0.26 ^{E,a}	18.97 ± 0.25 ^{F,a}
WPI	11.47 ± 0.05 ^{A,a}	11.72 ± 0.11 ^{B,b}	12.18 ± 0.05 ^{C,b}	13.97 ± 0.06 ^{D,b}	15.53 ± 0.03 ^{E,b}	16.53 ± 0.05 ^{F,b}
WPC	11.48 ± 0.04 ^{A,a}	11.73 ± 0.05 ^{B,b}	12.27 ± 0.04 ^{C,b}	14.00 ± 0.07 ^{D,b}	14.80 ± 0.04 ^{E,c}	16.66 ± 0.04 ^{F,b}
Zein	11.47 ± 0.05 ^{A,a}	11.63 ± 0.06 ^{A,B,b}	11.85 ± 0.04 ^{B,c}	12.25 ± 0.07 ^{C,c}	13.54 ± 0.02 ^{D,d}	14.72 ± 0.03 ^{E,c}
Shellac	11.47 ± 0.04 ^{A,a}	11.54 ± 0.04 ^{A,B,b}	11.76 ± 0.05 ^{B,c}	11.99 ± 0.06 ^{C,d}	13.24 ± 0.02 ^{D,e}	14.50 ± 0.03 ^{E,c}

Data are expressed as Means ± standard deviations.

^{a-e}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-F}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

¹n = 30 eggs per mean.

Table 8. Effect of different coatings on total solids (dry matter) of yolk during 5 weeks of storage at 24°C.¹

Coating ^a	Egg Yolk Total solids (Dry matter) (% w/w)					
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	46.54 ± 0.04 ^{A,a}	45.86 ± 0.10 ^{B,a}	44.65 ± 0.12 ^{C,a}	44.10 ± 0.06 ^{D,a}	42.52 ± 0.10 ^{E,a}	40.53 ± 0.26 ^{F,a}
WPI	46.53 ± 0.05 ^{A,a}	46.08 ± 0.03 ^{B,a}	45.50 ± 0.05 ^{C,b}	44.92 ± 0.04 ^{D,b}	43.45 ± 0.04 ^{E,b}	42.53 ± 0.15 ^{F,b}
WPC	46.54 ± 0.02 ^{A,a}	46.20 ± 0.04 ^{B,b}	45.53 ± 0.03 ^{C,b}	45.04 ± 0.04 ^{D,b,c}	43.59 ± 0.04 ^{E,b}	43.08 ± 0.06 ^{F,c}
Zein	46.55 ± 0.02 ^{A,a}	46.42 ± 0.06 ^{A,c,d}	45.66 ± 0.03 ^{B,b}	45.25 ± 0.04 ^{C,c,d}	44.81 ± 0.04 ^{D,c}	44.63 ± 0.08 ^{D,d}
Shellac	46.54 ± 0.03 ^{A,a}	46.47 ± 0.03 ^{A,d}	45.96 ± 0.04 ^{B,c}	45.51 ± 0.05 ^{C,d}	45.13 ± 0.07 ^{D,d}	44.84 ± 0.04 ^{E,d}

Data are expressed as Means ± standard deviations.

^{a-d}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-F}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

¹n = 30 eggs per mean.

Relative Whipping Capacity (Foaming Properties)

The whippability of egg albumen can be assayed by measurement of foam volume and foam stability (amount of liquid released from the foam in a given time). The changes that occur in eggs during storage include: thinning of albumen, increase in pH, weakening and stretching of the vitelline membrane, and increase in water content of the yolk. Foam stability (FS) is determined by measuring the loss of liquid resulting from destabilization, i.e., leakage, measuring volume decrease, or density increase with time. In particular, ovomucin forms a film of insoluble material between the liquid lamella and air bubble, thereby stabilizing the foam. Foam stability reflects the water holding capacity of the foam and this is one way to determine the rate at which the liquid drains from it (Kemps et al., 2010). The liquid in foams is distributed between thin films and plateau borders. Because of the radius of curvature of a plateau border, the pressure inside is less than that in thin films due to capillary pressure. This phenomenon, known as plateau border suction, leads to drainage of liquid from thin films into the neighboring plateau border. All liquid in the plateau border of foams are subject to drainage between the bubbles caused by the action of gravity. The stability of foam is influenced by the thickness of the interface, foam size distribution, interface permeability, and surface tension. It is known that the egg albumen N-ovalbumin is transformed into S-ovalbumin (less hydrophobic than N-ovalbumin). This interferes with the formation of a cohesive film at the air/water interface, causing a decrease in FS (Kampf et al., 2003; Lomakina and Mikova, 2006).

A significant interaction between the coatings and storage time was observed via the albumen foaming properties. During storage, a decrease in RWC was observed ($P < 0.05$) over time, confirming earlier results Yuceer and Caner (2014). At all storage times, the volume of albumen foam (RWC) for the coated eggs was greater than that of the control eggs (Table 9). The pH in the aqueous phase determines the magnitude and nature of protein charges and therefore affects the foam stability (Alleoni and Antunes, 2004a; Kampf et al., 2003).

Higher foam expansion indicates that more air was trapped in the foam and egg albumen reducing surface and interfacial tension to levels sufficiently low to form an interfacial film that exceeds the critical thickness. Over time, these films became progressively thinner and ruptured (Kampf et al., 2003; Narsimhan, 1991). Throughout the storage period, fluid is lost by lamellar water drainage, resulting in foam collapse. There were significant differences in the RWC of albumen for control (475), and coated eggs WPI (841), WPC (841), zein (866), and shellac (891). The coatings stabilized the foam (Table 9). After 6 weeks of storage, the reductions in RWC were 49.52% (Control), 11.17% (WPI), 10.93% (WPC), 8.66% (Zein) (Al-Bachir and Zeinou, 2006) and 5.61% (Shellac). There were also significant differences in the RWC of whole eggs for the control (191), and coated eggs WPI (308), WPC (316), zein (380), and shellac (450). After 6 weeks of storage, the reductions in RWC of the whole eggs were 77.07% (control), 62.66% (WPI), 61.69% (WPC), 54.38% (Zein) (Al-Bachir and Zeinou, 2006) and 45.97% (Shellac) (Table 10). These results clearly demonstrate that the coatings maintained the RWC of both albumen and the whole eggs (foaming properties: whipping capacity) since they are an effective barrier against the loss of CO₂, avoiding changes in the pH of the egg albumen during storage.

Eggshell Breaking Strength

Egg shell quality is of considerable economic significance for commercial egg production. The shell protects the contents of the egg from mechanical impact to some extent, allows a controlled exchange of fluid and gas through the pores, and provides protection against microbial entry. Shell quality declines as the hens get older. The eggshell should be as strong as possible to maximize the number of eggs reaching to consumer (Caner and Cansız, 2008; Cordts et al., 2002). Cracked eggs are of major economic significance to those involved in the production and marketing of eggs. Improved shell strength would result in significant reductions in the number of eggs lost due to breakage or the occurrence of cracks during handling and storage. The

Table 9. Effect of different coatings on albumen relative whipping capacity during 5 weeks of storage at 24°C.¹

Coating ^a	Relative whipping capacity-Albumen					
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	941 ± 37 ^{A,a}	833 ± 25 ^{B,a}	802 ± 44 ^{C,a}	733 ± 25 ^{C,a}	632 ± 40 ^{D,a}	475 ± 27 ^{E,a}
WPI	935 ± 25 ^{A,a}	891 ± 37 ^{A,a,b}	875 ± 27 ^{A,a,b}	858 ± 37 ^{A,b}	850 ± 44 ^{A,b}	841 ± 37 ^{B,b}
WPC	933 ± 25 ^{A,a}	901 ± 31 ^{A,B,a,b}	883 ± 40 ^{A,B,b}	866 ± 40 ^{A,B,b}	858 ± 49 ^{A,B,b}	841 ± 49 ^{B,b}
Zein	941 ± 37 ^{A,a}	916 ± 25 ^{A,b}	900 ± 31 ^{A,b}	883 ± 51 ^{A,b}	875 ± 52 ^{A,b}	866 ± 40 ^{A,b}
Shellac	941 ± 20 ^{A,a}	933 ± 25 ^{A,b}	916 ± 40 ^{A,b}	908 ± 49 ^{A,b}	900 ± 31 ^{A,b}	891 ± 58 ^{A,b}

Data are expressed as Means ± standard deviations.

^{a-b}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-E}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI, Whey Protein Isolate; WPC, Whey Protein Concentrate.

¹n = 30 eggs per mean.

Table 10. Effect of different coatings on whole egg relative whipping capacity during 5 weeks of storage at 24°C.¹

Coating ^a	Relative whipping capacity – Whole egg					
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	833.3 ± 25.81 ^{A,a}	725.0 ± 27.38 ^{B,a}	680.0 ± 27.38 ^{B,a}	550.0 ± 44.72 ^{C,a}	341.6 ± 37.63 ^{D,a}	191.6 ± 37.63 ^{E,a}
WPI	825.0 ± 27.38 ^{A,a}	758.3 ± 37.63 ^{A,B,a,b}	725.0 ± 28.86 ^{B,a,b}	630.0 ± 27.38 ^{C,b}	450.0 ± 31.62 ^{D,b}	308.3 ± 37.63 ^{E,b}
WPC	825.0 ± 27.38 ^{A,a}	766.6 ± 25.81 ^{A,B,a,b}	730.0 ± 27.38 ^{B,a,b}	630.0 ± 44.72 ^{C,c}	441.6 ± 37.63 ^{D,b}	316.6 ± 40.08 ^{E,b}
Zein	833.3 ± 25.81 ^{A,a}	816.6 ± 25.81 ^{A,B,b,c}	758.3 ± 20.41 ^{B,b}	680.0 ± 27.38 ^{C,c,d}	530.0 ± 57.0 ^{D,c}	380.0 ± 57 ^{E,b,c}
Shellac	833.3 ± 25.81 ^{A,a}	833.3 ± 25.81 ^{A,c}	775.0 ± 27.38 ^{A,B,b}	730.0 ± 27.38 ^{B,d}	575.0 ± 27.38 ^{C,c}	450.0 ± 31.62 ^{D,c}

Data are expressed as Means ± standard deviations.

^{a-d}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-E}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

¹n = 30 eggs per mean.

top of the eggshell has a higher puncture strength than the bottom. Breaking strength of the eggs from the control group dropped from 3.34 to 3.15 kg after storage. For the coated eggs, the strength values increased these eggs displayed a higher puncture strength than the control eggs at both the top and bottom (Table 11) after 4 weeks of storage, however, no significant differences were found among the different coatings at this point with values of WPI (35.18), WPC (35.23), zein (46.25), and shellac (46.25). The higher puncture strength exhibited by the shellac coated eggs was probably a result of the mechanical properties of shellac.

These results are in agreement with Caner and Cansız (2008) where chitosan coatings improved shell strength with chitosan together with lactic acid being the best coating in this regard compared to acetic or propionic acids. Xie et al. (2002) reported that egg coatings based on soy protein, whey isolate, or wheat protein coated carboxy methyl cellulose also improved strength compared to uncoated eggs.

FT-NIR Analysis

The FT-NIR spectroscopy system acquired diffuse reflectance spectra in the range of 833 to 2500 nm. Using NIR spectroscopy, C-H, N-H and O-H bonds were induced to vibrate. The general profile of the absorption spectra for eggs are shown in (Figures 1 and 2). NIR

Table 11. Effect of different coatings on eggshell breaking strength (kgf) both top (11-a) and bottom (11-b) during 5 weeks of storage at 24°C.¹

a - Eggshell breaking strength (kgf) at top		
Coating ^a	0 weeks	4 weeks
Control	4.94 ± 0.87 ^{A,a}	4.70 ± 0.11 ^{A,a}
WPI	4.94 ± 0.87 ^{A,a}	5.18 ± 0.33 ^{A,b}
WPC	4.94 ± 0.87 ^{A,a}	5.50 ± 0.43 ^{A,b}
Zein	4.94 ± 0.87 ^{A,a}	5.56 ± 0.29 ^{B,c}
Shellac	4.94 ± 0.87 ^{A,a}	5.73 ± 0.27 ^{B,c}
b - Eggshell breaking strength (kgf) at bottom		
Coating ^a	0 weeks	4 weeks
Control	3.34 ± 0.25 ^{A,a}	3.15 ± 0.29 ^{A,a}
WPI	3.34 ± 0.25 ^{A,a}	3.58 ± 0.31 ^{A,b}
WPC	3.34 ± 0.25 ^{A,a}	3.59 ± 0.47 ^{A,b}
Zein	3.34 ± 0.25 ^{A,a}	4.38 ± 0.45 ^{B,c}
Shellac	3.34 ± 0.25 ^{A,a}	4.71 ± 0.46 ^{B,c}

Data are expressed as Means ± standard deviations.

^{a-c}Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

^{A-B}Means in the same row with different capital letters are significantly different ($P < 0.05$).

^aWPI-Whey Protein Isolate; WPC-Whey Protein Concentrate.

Week 0 results were measured before coatings.

¹n = 30 eggs per mean.

spectroscopy was used by Galiş et al. (2012) for the estimation of egg freshness (530 to 1130 nm).

The control egg albumen and yolk spectra were used as a baseline to determine changes in the secondary

structure at the end of storage (Figure 1). Examples are shown of absorbance units of the albumen and yolk in the wavelength range from 800 to 2,500 nm, nearly the full NIR region. Although FT-NIR spectra are highly overlapped, the difference in the transmittance values among the coatings can be seen. Albumen becomes thinner over time which leads to a change in the transmitted spectra. Water band changes dependent on the water content were clearly observed. Water absorption bands in the NIR spectrum are influenced by effects of

solutes in water (Kampf et al., 2003; Kemps et al., 2010; Lomakina and Mikova, 2006). These spectra are in fact dominated by water absorption bands with overtone bands of the OH-bonds at 970, 1,190 and 1,450 nm and a combination band at 1,940 nm. Nicolaï, et al. (2007) reported that 1,450 nm is the first overtone of -OH stretching. The absorbance spectrum stays relatively flat from 800 to 910 nm. Prominent peaks appear at 975 nm and 1,400 to 1,450 nm (OH vibration of water), and a combination band at 1,940 nm (involving -OH

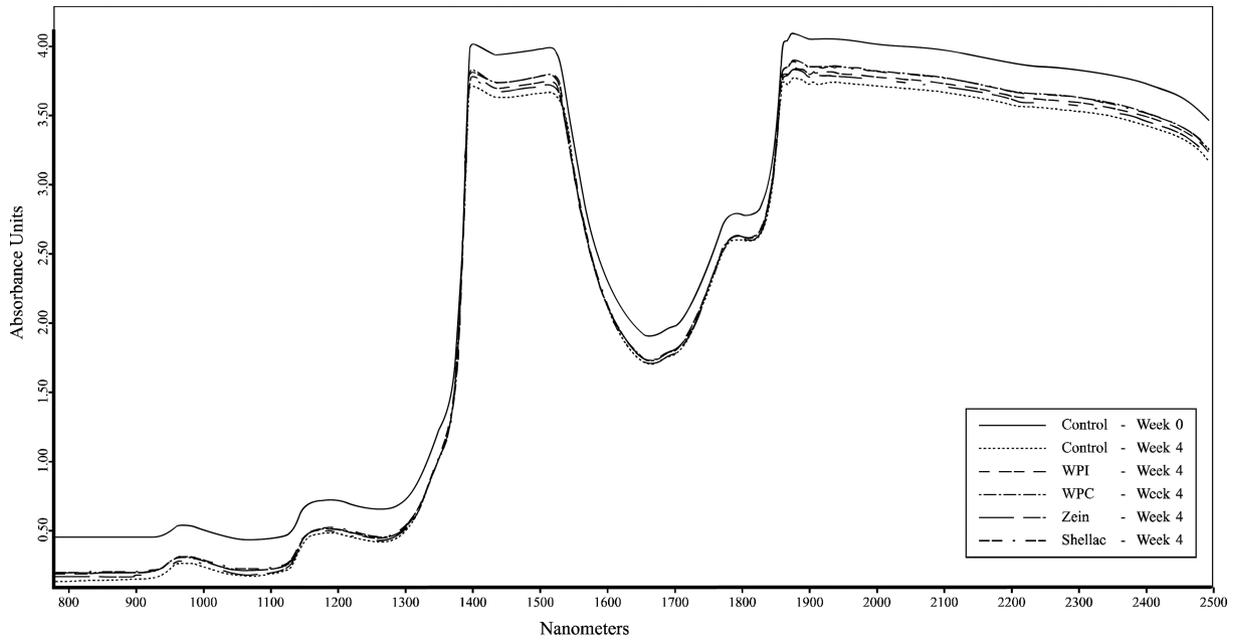


Figure 1. Effect of various coating materials [wey protein isolate (WPI), wey protein concentrate (WPC), corn zein, and shellac] and uncoated samples on average relative absorbance spectral values of albumen at the beginning (0. week) and at the end of storage (4 week) at 24°C.

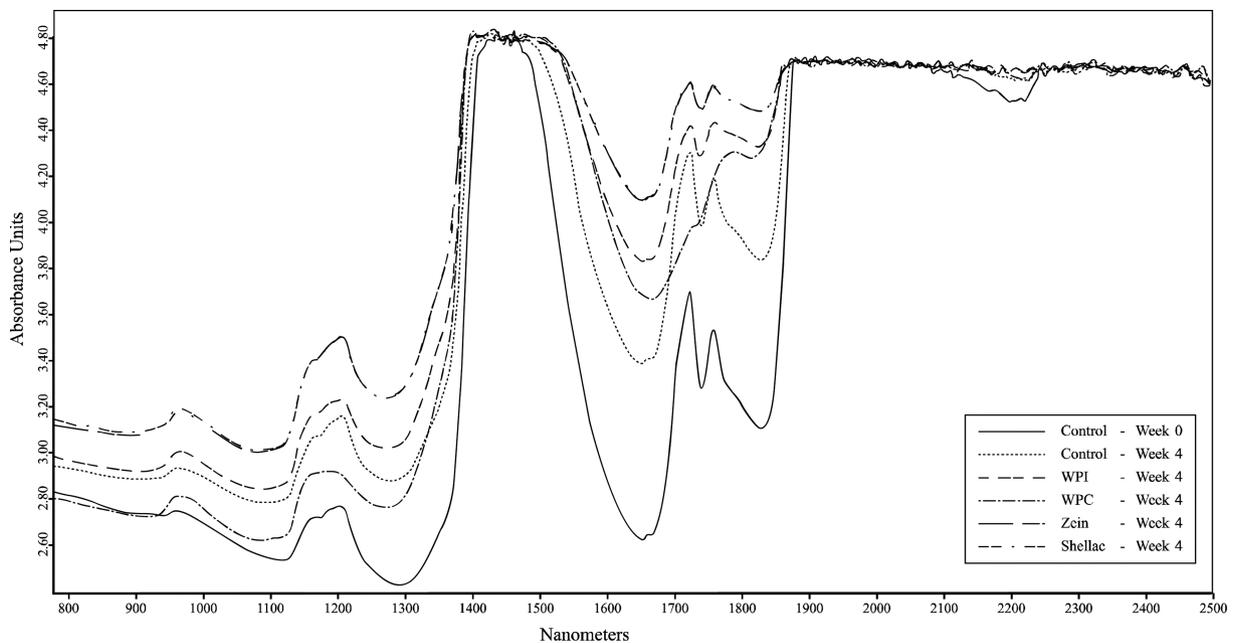


Figure 2. Effect of various coating materials [wey protein isolate (WPI), wey protein concentrate (WPC), corn zein, and shellac] and uncoated samples on average relative absorbance spectral values of egg yolk at the beginning (0. week) and at the end of storage (4 week) at 24°C.

stretching and –OH deformation) which is due to absorption by water and carbohydrate (Kemps et al., 2010). The characteristic bands were identified: 1,135 to 1,200 nm (2nd overtone of the C–H stretch of CH₂ group); 1,450 nm (1st overtone of the O–H stretch); the region of 1,660 to 1,760 nm (1st overtone of the symmetric C–H stretch of CH₂ and CH₃ group); 1,940 nm (a combination of the O–H bend and the stretching band of water (Figures 1 and 2) (Aday and Caner, 2010; Yukihiro and Berry, 2002). The increasing of the absorbance of –OH stretching during conservation could denote a structural change of proteins; the increase of absorbance for the wavelength 1946 nm corresponds to water bound to protein (Nicolai et al., 2007). Eggs coated with shellac or zein displayed a higher absorbance at 970 and 1,197 nm (OH vibration of water) compared with those coated with WPI or WPC and the uncoated group at the end of storage. The sharp absorption band at 1,190 and 1,420 nm increased in intensity due to loss of water (Figures 1 and 2). These results could be related to corresponding differences in weight loss.

The yolk represents 33% of the liquid weight and its composition consists of fat and proteins. There were clear differences between the typical average absorbance spectra corresponding to the yolk of the various coated eggs and also the control eggs at the end of storage. In addition we note the characteristic Amide I C–O stretch (1,654 (s)cm⁻¹), Amide II NH₂ deformation (1,632 (s)cm⁻¹) and Amide II (1,542 (s)cm⁻¹) absorbances associated with the proteins of egg. All of these amide features are clearly present in the naturally aged sample of egg yolk after storage (Figure 2). Interestingly, spectral features attributed to the triglyceride ester linkages (1,746, 1,239, 1,164, 1,098 cm⁻¹) and to the unsaturation (3,006 and 722 cm⁻¹) in the fresh egg do not appear in the spectrum for the naturally aged sample. The band pattern at 1,734 and 1,714 cm⁻¹ may be due to triglyceride derived aldehydes and acids, respectively, or it may suggest the presence of an imide linkage derived by oxidation of protein amide linkages.

CONCLUSIONS

This work demonstrates that it is possible to use coatings to stabilize egg albumen retaining most of the technologically relevant properties. We can conclude from the present study that WPI, WPC, zein, and shellac coatings are effective in preserving the interior quality of fresh eggs due to mass transfer properties during storage for 6 weeks. Coatings can be a viable alternative to existing techniques for maintaining functional properties (HU, YI, pH, viscosity, total solids, and RWC) that are adversely affected by length of storage. The resistance to gas exchange of coated eggs is strongly influenced by the coating's ability to block pores on the surface of the eggs. The stability of the foam is enhanced as a result of coatings. Overall the effects of coatings on egg albumen and yolks were favorable. These param-

eters are used to determine albumen quality, a measure of the freshness of the egg. The dry matter, RWC, and FT-NIR measurements could be used to accurately predict the internal quality parameters for egg freshness. Coating with shellac was more effective in preventing weight loss than with zein, WPI, or WPC.

Protein-based coatings, especially zein coating (as compared to WPI and WPC), and shellac are promising and are the most effective coatings since they delay the deterioration of internal quality and increase shell strength which would in turn reduce eggshell breakages. This technology using protein based and shellac coatings may assist industries in reducing economic losses and can help to alleviate many of the problems encountered with perishable foods.

Further studies should use different and better coating formulations such as those containing antioxidants and antimicrobial compounds on various perishable food products. Shelf-life studies of coated eggs in inoculated eggs also need to be performed.

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