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Effect of Beeswax, Gelatin and *Aloe vera* Gel Coatings on Physical Properties and Shelf Life of Chicken Eggs Stored at 30°C

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ABSTRACT

Present study was to determine the effect of beeswax, gelatin and *Aloe vera* gel coatings on internal quality and shelf life of chicken eggs compared to uncoated and mineral oil coated eggs. Four hundred and seventy five brown shell eggs were obtained from 32 weeks old Lohmann classic brown layers and all the eggs were randomly divided into five groups as ninety five eggs per group. Mineral oil, beeswax, *Aloe vera* gel and gelatin coatings were applied on eggs as four treatments and one group of eggs were uncoated and kept as control group. Then all the eggs were stored at 30°C and relative humidity of 70% - 75% for six weeks of storage period. Beeswax and gelatin coated eggs showed significantly ($P<0.05$) lower weight loss values and preserved albumin and yolk quality of eggs than uncoated eggs. Eggs coated with mineral oil and beeswax showed similar results for weight loss, Haugh unit, yolk index, albumen and yolk pH. Based on the Haugh Unit, eggs can be classified into four grades as AA (above 72), A (72-60), B (59-31) and C (below 30). Quality of uncoated eggs, *Aloe vera* coated eggs and gelatin coated eggs dropped from AA to B and mineral oil and beeswax coated eggs changed from initial AA quality to A quality after six weeks of storage at 30°C. Results of microbiological analysis showed that all coated eggs were microbiologically safe throughout the storage period. The present study demonstrated that, in comparison to the mineral oil and the uncoated eggs, beeswax is a better novel coating material and gelatin can also be successfully used as coating material in preserving the internal quality and extending the shelf life of chicken eggs stored at 30 °C for six weeks.

Key words: Chicken eggs, Coatings, Internal quality, Shelf life, Storage time

INTRODUCTION

As an excellent source of protein, chicken eggs are among the most nutritious food consumed globally and their production has represented an important segment of the world food industry (Farrel, 2013). However, shell eggs are highly susceptible to internal quality deterioration and bacterial growth during storage. As soon as eggs are laid, the aging process begins, altering their chemical, physical, microbial and functional properties. Although the shell can be considered as natural barrier, shell eggs have short shelf life and are extremely fragile which can cause a serious economic loss to the poultry industry (Caner, 2005 and Wong et al, 1996).

Interior quality deterioration of fresh shell eggs can be delayed significantly by maintaining storage temperature near the freezing point (Zeidler, 2002). Numerous food grade coating materials have also proven to be efficient in reducing the mass transfer by sealing pores. Furthermore, such surface coatings prevent the penetration of microorganisms into the shell eggs. Thus Considerable amount of research works have been done on coating shell eggs with edible coating materials and different results in terms of efficacy of prolonging the shelf-life and improving internal qualities of eggs were obtained depending on type of the coating material (Ikame and Enelamah, 1985).

Antimicrobial-enhanced coatings, which are considered as active packaging, have been receiving increased interest since they exhibit great potential for ensuring food safety. Beeswax is a product of honey bees with natural antimicrobial substances (Zanoschiet al., 1991). Thus, it has considerable antibacterial and antifungal effect on bacteria, fungi and yeasts (Kacániová et al., 2012). Due to these anti-microbial and barrier properties against moisture and gases, beeswax has been utilized in food processing as packaging and coating material. In addition, *Aloe vera* (*A. vera*) is a tropical and sub-tropical plant having well proven its anti-microbial properties. The colorless and odorless gel obtained from *A. vera* leaves can form protective layer against oxygen and moisture and

inhibit the action of microorganisms that cause food borne illness (Serrano et al., 2006). Thus studies have shown that *A. vera* gel can be effectively used as surface coating to preserve fruits and vegetables.

Gelatin is obtained by controlled hydrolysis of fibrous insoluble protein collagen, which is widely found as a major component of the skin, bone and the connective tissues of animals. It is used to encapsulate the moisture or oil phase in food ingredients and pharmaceuticals. Due to barrier properties of gelatin it has been gained increased interest as novel surface coating material (Gennadios et al., 1994).

Mineral oil is a coating material currently used to preserve the internal quality of eggs (Waimaleongora-Ek et al., 2009 and Jirangrat et al., 2010). Even so, a problem associated with mineral oil coating is that oil dries very slowly when applied on the surface of the eggshell without wiping. However, none of the previous studies provided detailed information on internal quality and shelf life of stored chicken eggs after applying above mentioned coatings. Therefore, this study was carried out to evaluate the effectiveness of beeswax, gelatin and *Aloe vera* gel as novel coating materials in compared to mineral oil, to preserve the internal quality and shelf life of chicken eggs stored for six weeks at 30°C.

MATERIAL AND METHODS

Selection of eggs

475 brown shell eggs were obtained from 32 weeks old Lohmann classic brown layers at local producer (NEL Farm, Mangalaeliya, Sri Lanka). All the layers in the farm had been vaccinated for salmonella at chick stage so eggs were free of vertically transmitted *salmonella* spp. The eggs were obtained from battery cages therefore had lesser dirt. Furthermore, eggs were cleaned by wiping with piece of steel wool to clean any possible dirt on shell. All the selected eggs were less than six hours after laying and in the weight range of 49g- 64g. In addition eggs were unfertile, free of cracks and defects. Eggs were placed in clean egg creates at 30°C temperature after been brought to laboratory and all the eggs were randomly divided into five groups with 95 egg in each group.

Preparation of coating materials

Before preparation of coating solutions and while coating was done surgical gloves were worn to avoid any possible contaminations. In addition, 10 eggs per each group were weighted with analytical balance (AR0640, OHAUS, USA) before coating and two hours after coating to measure the mean weight of coat for single eggs for each coating material.

Preparation of Mineral oil

Mineral oil (viscosity 26.35 mPa s at 20°C, weight per ml at 25°C = 0.828 g, light absorption at 240-280 nm = 0.031, transparent, colorless, odorless, food grade) was obtained from Glorchem Enterprise (No 141, Bankshall Street, Colombo 11, Sri Lanka). For coating process, .mineral oil was put into250 ml beaker and eggs were immersed individually in mineral oil solutions by hand for one minute.

Preparation of beeswax and coating of eggs

Crude beeswax was purchased from local shop and solid beeswax was cut into small pieces by knife and put in to a clean 500 ml beaker which was set in a boiling water bathe at 40°C. Then beeswax was heated until it became a liquid and cooled up to room temperature to form semi solid beeswax that can be easily applied on to egg shell. Eggs were subsequently coated with beeswax by rubbing wax on the shell with hand.

Preparation of *Aloe vera* gel and coating of eggs

Fresh *A. vera* leaves were taken from the *A. vera* plants grown in Wayamba University, Makandura premises, Sri Lanka. Then outer cover of the *A. vera* leaves was scraped by clean knife and thin layer of gel was directly applied on egg shell.

Preparation of gelatin and coating of eggs

10% Gelatin solution was prepared by dissolving commercial gelatin powder with distilled water and heated in a water bathe (80°C) for 10 minutes to get dissolved gelatin solution. Well dissolved gelatin solution was cooled in room temperature before coating. Then gelatin solution was put into 250ml clean beaker and eggs were immersed individually by hand in the gelatin solution for one minute.

Storage of coated eggs

After coating all the coated eggs were dried at room temperature for 24 hours. Uncoated eggs served as control and mineral oil coated eggs served as positive control. Then all the eggs were subsequently placed in small end down position in labeled open molded plastic eggs trays and stored at 30°C and relative humidity of 70% - 75% for six weeks period. Twenty five eggs, as five individually marked eggs per each treatment were kept for measuring weight loss throughout the experimental period. Using three replicates per treatment Haugh unit, yolk index, albumin pH, yolk pH, were measured 24 hours after coating (0 week) and in weekly intervals for six weeks storage period. For microbial analysis, six eggs (three for total plate count and three for *Salmonella* spp. and *E. coli* detection) per each treatment were taken 24 hours after coating and then in two weeks intervals during six weeks storage period.

Determination of quality parameters of coated eggs

Determination of weight loss: Weight loss (%) of the coated whole eggs during storage were calculated as ((initial whole egg weight (g) after coating at day 0 – whole egg weight (g) after storage)/initial whole egg weight (g) after coating at day 0) × 100. Weight loss (%) of the control uncoated whole egg was calculated as ((initial whole egg weight (g) at day 0 – whole egg weight (g) after storage)/ initial whole egg weight (g) at day 0) × 100. The weight of whole eggs was measured with analytical balance (AR0640, OHAUS, USA) and

five measurements per treatment were taken in each week.

Determination of Haugh Unit and Yolk Index: Eggs were broken into flat surface and height of thick albumen and yolk were measured with tripod meter. The yolk width was measured with a digital caliper. Each parameter was estimated by averaging three measurements carried out at three different points of albumen and yolk. The Haugh unit was calculated as 100 log (H – $1.7W^{0.37}$ + 7.57), where H is the albumen height (mm) and W is the weight (g) of egg (Haugh, 1937). The yolk index was calculated as yolk height/yolk width (Stadelman, 1995a and Lee et al., 1996). Three replicates per treatment were taken at each week.

Determination of albumin pH and yolk pH: Albumen and yolk were separated in to 50 ml beakers and thin and thick albumen were mixed thoroughly. Then albumen pH and yolk pH were measured with pre calibrated digital pH meter (Starter 3000, OHAUS, USA) at 25°C. Three replicates per treatment were taken at each week.

Microbial analysis

Internal content of control uncoated eggs and coated eggs were analyzed for Total Plate Count (TPC), *Salmonella* spp. and *E .coli* since day one up to six weeks in two weeks intervals. TPC was done at microbiology laboratory, department of food science and technology, Wayamba university of Sri Lanka. For TPC, egg shell was sterilized with 70% ethyl alcohol before breaking the eggs. Then internal content of the egg was put into a sterilized 50 ml beaker and homogenized with sterilized glass rod. Oneml of homogenized egg sample was diluted with peptone water to prepare 10^{-1}dilute sample. Thus, Dilution series were prepared up to 10^{-3} level. Then viable cells (Colony forming units/ml of eggs) were enumerated by colony counter on plate count agar by pour plate method followed by incubation at 37°C for 48 hours.

Tests for *Salmonella* spp. and *E .coli* were done at poultry disease diagnostic laboratory, district veterinary investigation center, Wariyapola, Sri Lanka. Sample of internal egg content was taken with sterilized cotton swab by making crack on egg shell and cultured in a nutrient blood agar followed by 48 hours incubation. If microbial growth was noticed, sub culture was plated on MacConkey broth, Brilliant Green agar, Salmonella-Shigella (SS) agar, Xylose Lysine Desoxycholate (XLD) agar, Triple Sugar Iron (TSI) SI agar and Citrate media to detect the presence of *Salmonella* spp. and *E. coli*. All microbiological assays were done in duplicate for each treatment.

Statistical analysis

For Haugh unit, yolk index, albumin pH, yolk pH mean ± standard deviation values were reported based on three replicates per treatment. For weight loss, mean ± standard deviation values were reported based on five replicates per treatment. Data were analyzed using general linear model procedure considering the main effects of coating, storage time at 95% confidence level. When main effect was significant, the Tukey's comparison test was performed to identify significant differences within treatments in a particular week and differences within storage period in a particular treatment. Minitab statistical software (version15.1.1, USA) was used for analysis.

RESULTS AND DISCUSSION

Effect of beeswax, *Aloe vera* gel and gelatin coatings on weight loss

Evaporation of water and, to a much lesser extent, loss of Carbon dioxide (CO_2) from the albumen through the shell leads to overall weight loss of the whole egg (Obanu and Mpieri, 1984). This is one of the important measurements to monitor the changes in quality of fresh shell eggs during storage.

During six weeks of storage period at 30°C, differences in the weight loss among the control uncoated eggs and those coated with mineral oil, beeswax, *A. vera* gel and gelatin were found ($P<0.05$). Overall, the weight loss progressively increased with increased storage periods (Table 1). But eggs coated with mineral oil and beeswax had significantly ($P<0.05$) lesser weight loss than uncoated, *A. vera* gel, and gelatin coated eggs throughout the six weeks of storage period. However, there were no significant differences ($P>0.05$) in weight loss observed among uncoated, *A.vera* and gelatin coated eggs throughout six weeks of storage. Similarly there were no significant difference ($P>0.05$) in weight loss between mineral oil and beeswax coated eggs. After six weeks, nearly five times lesser weight losses in mineral oil (1.49%) and beeswax (1.52%) were observed than uncoated eggs (7.49%). Weight loss of *A. vera*gel (6.81%) and gelatin (6.26%) coated eggs were slightly lower than that of uncoated eggs (7.49%) but more than four times higher than mineral oil and beeswax coated eggs (1.49% - 1.52%) (Table 1).

Waimaleongora-Ek et al. (2009) reported that, at 25 °C storage, the weight loss (0.85%) of eggs coated with mineral oil after five weeks was lower than that (1.97%) of uncoated eggs after one week. Moreover, Obanu and Mpieri (1984) reported that, vegetable oil coatings significantly reduced (11 times less) the weight loss (0.013-0.016 g) of coated eggs, compared to that (0.186 g) of uncoated eggs after 35 days of storage at 25-32 °C. However it was obvious that *A. vera* gel and gelatin coatings were less effective in minimizing weight loss than mineral oil and beeswax (Table 1).

According to Food and Agriculture Organization (2003), a weight loss of 2-3% is common in marketing eggs and is hardly noticeable to consumers. This study demonstrated that beeswax similar to mineral oil ($P>0.05$) offer a protective barrier against the loss of moisture through the eggshell, thus minimizing weight loss (< 1.52%, Table 1).

Effect of beeswax, *Aloe vera* gel and gelatin coatings on Haugh unit

During storage of shell eggs, the gelatinous structure of the thick albumen gradually deteriorates, changing into thin albumen (thinning), which is

associated with either ovomucin-lysozyme interactions, disulfide bonds of ovomucin, carbohydrate moieties of ovomucin, or interrelations between α and β ovomucins (Li-Chan and Nakais, 1989). The Haugh unit, an expression relating egg weight and height of the thick albumen, is a measurement of the albumen quality. The higher the Haugh unit value, the better the albumen quality of eggs. Significant changes in the Haugh unit ($P< 0.05$) of all treatment groups during 6 week of storage at 30°C were observed (Table 2). Generally, the Haugh unit gradually decreased with increased storage periods; however, this decrease progressed at a much slower rate for eggs coated with beeswax, gelatin and mineral oil than for *A. vera* gel coated and uncoated eggs. Compared with uncoated and *A. vera* gel coated eggs, eggs coated with beeswax, gelatin and mineral oil had significantly higher Haugh units ($P<0.05$) throughout six weeks of storage at 30°C.

At the beginning of the experiment (0 week) all the eggs were having Haugh unit between 86.55 – 86.74 and after six weeks storage, it had dropped to 41.03, 49.72, 62.41, 57.13 and 61.31 in uncoated, *A. vera* gel, beeswax, gelatin and mineral oil coated eggs respectively (Table 2). These results were substantiated by previous observations for mineral oil coated eggs (Waimaleongora-Eket al., 2009 and Jirangratet al., 2010). Based on the Haugh unit, eggs can be classified into four grades: AA (above 72), A (72-60), B (59-31), and C (below 30) (Lee et al., 1996). At 30°C, the grade of uncoated, *A. vera* gel and gelatin coated eggs decreased rapidly from an initial AA to B grade after six weeks of storage (Table 2). However, eggs coated with beeswax and mineral oil, (which was in AA grade at beginning) had maintained A grade after six weeks of storage period. These results revealed that, beeswax was better in preserving albumen quality during six weeks of storage period, which was similar to the mineral oil.

Effect of beeswax, *Aloe vera* gel and gelatin coatings on yolk index

During storage of shell eggs, the yolk index value (an indicator of freshness) declines as a result of a progressive weakening of the vitelline membrane, reduction of the total solid and liquefaction of the yolk caused mainly by the osmotic diffusion of water from the albumen (Obanu and Mpieri, 1984 and Stadelman, 1995a). In present study, the yolk index values of uncoated and coated eggs decreased significantly ($P<0.05$) with increased storage periods (Table 3). But decrease progressed in a higher rate in uncoated and *A. vera* gel coated eggs than beeswax, gelatin and mineral oil coated eggs. As indicated in table 3, at the beginning of the study all the eggs had yolk index of 0.43 – 0.45. Although yolk index values dropped to 0.25 and 0.24 in uncoated and *A.vera* gel coated eggs after two weeks, other coated eggs with beeswax, gelatin and mineral oil maintained 0.40, 0.36 and 0.39 respectively. After six weeks of storage period, uncoated (0.14) and *A. vera* gel coated (0.15) eggs had significantly lower yolk index values ($P<0.05$) than that of beeswax (0.35), gelatin (0.26) and mineral oil (0.33) coated eggs. Yolk index values of beeswax, gelatin and mineral oil at sixth week were even higher than the yolk index values of control group and *A. vera* gel coated eggs at second week.

These results indicated that, beeswax coating has enhancement effect in maintaining yolk quality similar to the mineral oil during storage. Moreover, both beeswax and gelatin minimized yolk quality loss, as they effectively reduced the rate of water and Carbon dioxide (CO_2) loss from the albumen through the egg shell, thereby inhibiting albumen liquefaction and water uptake by the yolk. Similarly Caner (2005) and Obanu and Mpieri (1984) had noticed significant differences in yolk index of eggs coated with groundnut, cottonseed and coconut oils after 36 days of storage under ambient conditions.

According to Torrico et al. (2011), Haugh unit, weight loss and yolk index are highly correlated. In this study, Table 1 (weight loss), Table 2 (Haugh unit) and Table 3 (yolk index) collectively imply that coating with beeswax and gelatin can preserve both albumen and yolk quality for at least three more weeks compared with observed for uncoated eggs at 30 °C.

Effect of beeswax, *Aloe vera* gel and gelatin coatings on albumen pH

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

In the beginning of the study, all the eggs were in 8.91 – 8.97 pH range and since then, pH of the uncoated and *A. vera* gel coated eggs were significantly ($P<0.05$) increased than beeswax, gelatin and mineral coated eggs during six weeks of storage period. This implies that beeswax, gelatin and mineral oil as coating materials could retard loss of Carbon dioxide (CO_2) through eggshell pores by acting as a gas barrier (Obanu and Mpieri, 1984 and Stadelman, 1995b). There were no significant differences ($P>0.05$) in albumen pH among uncoated and *A. vera* gel coated eggs and neither were among beeswax and mineral oil coated eggs during six weeks of storage at 30°C (Table 4).

The pattern for changes in albumen pH during the storage periods differed between five treatment groups. The albumen pH of uncoated and *A. vera* gel coated eggs increased from 8.93-8.95 to 10.21 and 10.26 respectively after six weeks of storage. However, the opposite was observed for eggs coated with beeswax, gelatin and mineral oil. Whereas, the pH gradually decreased from 8.93-8.97 to 8.48, 8.62 and 8.29 after five weeks and thereafter slightly increased to 8.40, 9.47 and 8.80 respectively after six weeks of storage at 25 °C (Table 4). Similarly, Jirangrat et al. (2010) observed that the albumen pH of uncoated eggs markedly ($P<0.05$) increased from 8.71 to 9.42 while, that of mineral oil coated eggs slightly decreased (but not significant, $P>0.05$) from 8.71 to 8.64 after five weeks of storage at 25°C.

The decrease in albumen pH during storage may be due to the continuing breakdown of the constituents in egg white and/or a change in the bicarbonate buffer system (Obanu and Mpieri, 1984; Biladeau and Keener, 2009). However, differences in initial egg quality, egg size, and storage conditions (temperature, humidity, and period) may affect albumen pH before and after storage (Goodwin et al., 1962; Sabrani and Payne, 1978). These results implies that beeswax had better barrier properties similar to mineral oil to avoid CO_2 loss via shell pores and which lower the albumen pH incensement during long storage, similarly gelatin was also better in avoid CO_2 loss compared uncoated eggs.

Table 1. Weight loss (g) of control and coated eggs during six weeks of storage at 30°C.

Coating	Day 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	$1.47\pm0.24^{A,a}$	$2.58\pm0.43^{B,a}$	$3.80\pm0.65^{C,a}$	$5.04\pm0.87^{D,a}$	$6.23\pm1.07^{D,a}$	$7.49\pm2.18^{E,a}$
Aloe vera gel	$1.36\pm0.04^{A,a}$	$2.40\pm0.11^{B,a}$	$3.48\pm0.14^{C,a}$	$4.59\pm0.19^{D,a}$	$5.68\pm0.25^{E,a}$	$6.81\pm0.31^{F,a}$
Beeswax	$0.31\pm0.06^{A,b}$	$0.53\pm 0.12^{AB,b}$	$0.77\pm0.19^{ABC,b}$	$1.02\pm 0.27^{BCD,b}$	$1.27\pm0.36^{CD,b}$	$1.52\pm0.43^{D,b}$
Gelatin	$1.32\pm0.17^{A,a}$	$2.27\pm0.21^{B,a}$	$3.29\pm0.31^{C,a}$	$4.29\pm0.41^{D,a}$	$5.27\pm0.51^{E,a}$	$6.26\pm0.62^{F,a}$
Mineral oil	$0.34\pm0.14^{A,b}$	$0.82\pm0.25^{AB,b}$	$0.84\pm0.43^{AB,b}$	$1.07\pm0.56^{AB,b}$	$1.29\pm 0.66^{AB,b}$	$1.49\pm0.76^{B,b}$

Means ± standard deviations of 3 measurements. $^{A-D}$ Means with different superscripts within a row indicate significant differences ($P<0.05$). $^{a-c}$ Means with different superscripts within a column indicate significant differences ($P<0.05$).

Table 2. Haugh unit of control and coated eggs during 6 weeks of storage at 30°C.

Coating	Day 1	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	$86.55\pm2.58^{A,a}$	$70.27\pm4.67A^{B,a}$	$57.62\pm4.64^{BC,a}$	$51.31\pm3.30^{BC,a}$	$49.29\pm3.85^{C,a}$	$47.56\pm3.30^{C,a}$	$41.03\pm3.60^{C,a}$
Aloe vera gel	$86.71\pm2.37^{A,a}$	$65.67\pm2.01^{AB,ab}$	$53.47\pm3.19^{B,a}$	$52.12\pm2.97^{B,a}$	$51.57\pm3.46^{B,ab}$	$50.72\pm4.13^{B,a}$	$49.72\pm3.95^{B,ab}$
Bees wax	$86.72\pm1.17^{A,a}$	$79.43\pm3.23^{AB,a}$	$71.59\pm2.44^{AB,b}$	$67.19\pm4.58^{AB,b}$	$66.10\pm5.56^{AB,b}$	$63.69\pm5.62^{AB,b}$	$62.41\pm4.25^{B,c}$
Gelatin	$86.74\pm2.80^{A,a}$	$72.08\pm4.65^{AB,ab}$	$68.13\pm2.46^{AB,b}$	$65.83\pm2.55^{B,b}$	$64.87\pm4.95^{B,b}$	$62.66\pm2.48^{B,b}$	$57.13\pm4.52^{B,bc}$
Mineral oil	$86.62\pm1.19^{A,a}$	$74.07\pm4.60^{AB,ab}$	$68.78\pm3.91^{AB,b}$	$64.17\pm3.72^{B,b}$	$63.22\pm3.55^{B,b}$	$62.02\pm2.81^{B,b}$	$61.31\pm2.94^{B,c}$

Means ± standard deviations of 3 measurements. $^{A-D}$ Means with different superscripts within a row indicate significant differences ($P<0.05$). $^{a-c}$ Means with different superscripts within a column indicate significant differences ($P<0.05$).

Table 3. Yolk index of control and coated eggs during 6 weeks of storage at 30°C.

Coating	Day 1	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	$0.43\pm0.01^{A,a}$	$0.32\pm0.01^{B,a}$	$0.25\pm0.02^{C,a}$	$0.18\pm0.02^{BC,a}$	$0.16\pm0.01^{D,a}$	$0.15\pm0.01^{D,a}$	$0.14\pm0.01^{D,a}$
A. vera gel	$0.44\pm0.01^{A,a}$	$0.30\pm0.01^{B,a}$	$0.24\pm0.03^{C,a}$	$0.18\pm0.02^{D,a}$	$0.17\pm0.02^{D,a}$	$0.15\pm0.01^{D,a}$	$0.15\pm0.00^{D,a}$
Bees wax	$0.44\pm0.01^{A,a}$	$0.42\pm0.01^{AB,b}$	$0.40\pm0.01^{AB,b}$	$0.39\pm0.03^{AB,b}$	$0.37\pm0.03^{AB,b}$	$0.36\pm0.03^{B,b}$	$0.35\pm0.03^{B,b}$
Gelatin	$0.45\pm0.00^{A,a}$	$0.41\pm0.01^{AB,b}$	$0.36\pm0.02^{BC,b}$	$0.34\pm0.02^{CD,b}$	$0.29\pm0.02^{DE,c}$	$0.27\pm0.02^{E,c}$	$0.26\pm0.03^{E,c}$
Mineral. Oil	$0.43\pm0.01^{A,a}$	$0.43\pm0.02^{A,b}$	$0.39\pm0.02^{AB,b}$	$0.38\pm0.03^{AB,b}$	$0.29\pm0.02^{AB,b}$	$0.34\pm0.01^{B,b}$	$0.33\pm0.02^{B,b}$

Means ± standard deviations of 3 measurements. $^{A-D}$ Means with different superscripts within a row indicate significant differences ($P<0.05$). $^{a-c}$ Means with different superscripts within a column indicate significant differences ($P<0.05$).

Table 1. Albumen pH of control and coated eggs during 6 weeks of storage at 30°C.

Coating	Day 1	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	$8.95\pm0.24^{A,a}$	$9.18\pm0.14^{A,a}$	$9.25\pm0.19^{A,a}$	$9.20\pm0.04^{A,a}$	$9.30\pm0.08^{A,a}$	$9.95\pm0.48^{B,a}$	$10.21\pm0.16^{B,a}$
A.vera gel	$8.91\pm0.20^{A,a}$	$8.98\pm0.50^{A,a}$	$9.26\pm0.20^{AB,a}$	$9.15\pm0.05^{AB,a}$	$9.07\pm0.15^{AB,ab}$	$9.87\pm0.42^{BC,ab}$	$10.26\pm0.07^{C,a}$
Bees wax	$8.97\pm0.29^{A,a}$	$8.14\pm0.08^{B,bc}$	$7.95\pm0.07^{B,bc}$	$7.74\pm0.19^{B,b}$	$8.48\pm0.36^{ABC,bc}$	$8.48\pm0.01^{ABC,c}$	$8.40\pm0.02^{BC,b}$
Gelatin	$8.92\pm0.07^{A,a}$	$8.45\pm0.45^{A,ac}$	$8.70\pm0.03^{A,bd}$	$8.48\pm0.30^{A,c}$	$8.62\pm0.21^{A,abc}$	$8.98\pm0.18^{AB,bc}$	$9.47\pm0.19^{B,c}$
Mineral Oil	$8.93\pm0.07^{A,a}$	$7.88\pm0.08^{A,bc}$	$7.87\pm0.07^{A,bcd}$	$7.76\pm0.21^{A,b}$	$8.29\pm0.46^{B,ac}$	$8.54\pm0.36^{AB,c}$	$8.90\pm0.51^{AB,bc}$

Means ± standard deviations of 3 measurements.$^{A-D}$ Means with different superscripts within a row indicate significant differences ($P<0.05$). $^{a-c}$ Means with different superscripts within a column indicate significant differences ($P<0.05$).

Effect of beeswax, *Aloe vera* gel and gelatin coatings on yolk pH

At day 1 no significant difference in yolk pH between treatments was noticed, hence all the eggs were having yolk pH in the range of 6.33- 6.37 (Table 5). During 6 weeks of storage period yolk pH was slightly increased from the initial value in all uncoated and coated eggs. Increase of pH was significantly higher ($P<0.05$) in uncoated eggs than that of coated eggs until week 2 and controversy, there was no significant difference in yolk pH ($P>0.05$) of all treatments at week 3.During storage, pH of the albumen increases due to Carbon dioxide (CO_2) loss and water from the albumen migrate into the yolk, leading to increased pH of the yolk as well (Biladeau and Keener, 2009). After six weeks of storage initial yolk pH value of uncoated (6.36), *A. vera* gel (6.37), beeswax(6.37), gelatin (6.33) and mineral oil (6.34) were increased to

7.64, 7.50, 7.46, 7.17 and 7.59 respectively (Table 5), whereas ultimate pH value of gelatin coated eggs was significantly lower ($P < 0.05$) than that of uncoated and other coated eggs.

Microbiological analysis

Results of Total Plate Count (TPC) and detection of *Salmonella* spp. and *E. coli* for internal content of uncoated and coated eggs with beeswax, gelatin and mineral oil during the storage period are shown in table 6 and table 7 respectively. Up to two weeks storage period, no TPC was detected in all uncoated and coated eggs. After four weeks, TPC of 2.5 log CFU/ml and 2.2 log CFU/ml were detected in uncoated eggs and gelatin coated eggs respectively. Ultimately after six weeks of storage period, TPC of 3.2 log CFU/ml, 2.1 log CFU/ml and 2.6 log CFU/ml were detected in uncoated, *A. vera* gel and gelatin coated eggs respectively, whereas no TPC was detected in beeswax and mineral oil coated eggs. As shown in table 7, no *salmonella* spp. was detected in all uncoated and coated eggs during six weeks of storage period at 30°C. In addition *E. coli* was not detected in all uncoated and coated eggs up to two weeks of storage period but *E. coli* colonies were detected in uncoated and gelatin coated eggs after four weeks of storage. After six weeks of storage eggs *E. coli* colonies were detected in all uncoated and other coated eggs except beeswax. This may be due to antimicrobial properties of the beeswax.

According to Ricke et al. (2001), eggs products should meet the specification of less than 5.0×10^4 CFU/g for TPC and absence of *Salmonella* spp. The International Commission on Microbiological Specification of Foods (1986) has mentioned microbiological safety parameters for eggs as absence *Salmonella* spp. and $1.0\text{-}5.0 \times 10^4$ CFU/ g for TPC value. According to these standards, present results (Table 6 and 7) indicated that all uncoated and coated eggs were microbiologically safe throughout the six weeks of storage period at 30°C.

Table 5. Yolk pH of uncoated and coated eggs during 6 weeks of storage at 30°C.

Coating	Day 1	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	$6.36\pm0.19^{A,a}$	$7.43\pm0.40^{B,a}$	$7.43\pm0.41^{C,a}$	$6.54\pm0.37^{A,a}$	$6.82\pm0.20^{ABC,a}$	$7.66\pm0.25^{BC,a}$	$7.64\pm0.16^{BC,a}$
Aloe vera gel	$6.37\pm0.30^{A,a}$	$6.84\pm0.31^{AB,ab}$	$6.48\pm0.38^{AB,b}$	$6.57\pm0.18^{ABC,a}$	$7.54\pm0.22^{B,b}$	$7.66\pm0.42^{B,a}$	$7.50\pm0.42^{B,a}$
Bees wax	$6.37\pm0.12^{A,a}$	$6.72\pm0.31^{AB,ab}$	$6.66\pm0.34^{ABC,b}$	$6.32\pm0.19^{ABCD,a}$	$7.68\pm 0.06^{BCD,a}$	$7.19\pm0.19^{BCD,a}$	$7.46\pm0.35^{D,a}$
Gelatin	$6.33\pm0.07^{A,a}$	$6.55\pm0.29^{AB,b}$	$6.39\pm0.14^{ABC,b}$	$6.56\pm0.35^{ABCD,a}$	$6.64\pm0.26^{ABCDE,ac}$	$7.07\pm0.17^{BDE,a}$	$7.17\pm0.30^{BDE,a}$
Mineral oil	$6.34\pm0.12^{A,a}$	$6.73\pm0.10^{B,ab}$	$6.19\pm0.08^{C,b}$	$6.59\pm0.25^{ABCD,a}$	$6.76\pm 0.08^{BD,ac}$	$7.29\pm0.23^{E,a}$	$7.59\pm0.26^{E,a}$

Means ± standard deviations of 3 measurements. $^{A-D}$ Means with different superscripts within a row indicate significant differences ($P<0.05$). $^{a-c}$ Means with different superscripts within a column indicate significant differences ($P<0.05$).

Table 6. Total plate count of uncoated and coated eggs in 2 weeks intervals from first day to six weeks of storage period at 30°C.

Treatment	Day 1 log CFU/ml	Week 2 log CFU/ml	Week 4 log CFU/ml	Week 6 log CFU/ml
Control	Not detected	Not detected	2.5	3.2
Aloe vera gel	Not detected	Not detected	Not detected	2.1
Beeswax	Not detected	Not detected	Not detected	Not detected
Gelatin	Not detected	Not detected	2.2	2.6
Mineral oil	Not detected	Not detected	Not detected	Not detected

Table 7. Detection of *Salmonella* spp. and *E. coli* in uncoated and coated eggs within 2 weeks intervals from first day to six weeks of storage period at 30°C.

Treatment	***Salmonella* spp.**				***E. coli***			
	Day 1	**Wk 2**	**Wk 4**	**Wk 6**	**Day 1**	**Wk 2**	**Wk 4**	**Wk 6**
Mineral oil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Positive
Aloe vera gel	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Positive
Beeswax	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Gelatin	Nil	Nil	Nil	Nil	Nil	Nil	Positive	Positive
Control	Nil	Nil	Nil	Nil	Nil	Nil	Positive	Positive

Nil: Negative for *Salmonella* spp. and *E. coli*

CONCLUSION

Coating was effective in preserving internal quality and improving shelf life of chicken eggs for six week of storage period. All coated eggs except *A. vera* gel showed better results in weight loss, Haugh unit, yolk index and pH compared to uncoated eggs. During six week storage period, highest weight loss (7.49%) was observed in uncoated eggs, whereas beeswax showed the lower weight loss (1.52%) next to mineral oil (1.49%). Thus beeswax coated eggs had lower moisture and CO_2 loss by effective sealing of pores in egg shell. Beeswax coated eggs as same as with mineral oil coated eggs maintained "A" quality during entire

storage period compared to B quality in uncoated, *A. vera* gel and gelatin coated eggs. Whilst uncoated eggs showed lower yolk index values after six weeks of storage, higher yolk index values were observed in beeswax and gelatin coated eggs. Although, there were no significant differences (P>0.05) in yolk pH value among treatments, beeswax and gelatin coated eggs had low albumen pH than uncoated and *A. vera* gel coated eggs. Results of microbiological analysis showed that, all coated eggs were microbiologically safe throughout the six weeks of storage period of at 30°C. Beeswax was desirable coating material to increase the shelf life and preserve internal quality of chicken eggs. Moreover, good consumer acceptability could also be achieved in beeswax coated eggs by adopting proper egg coating methods. Gelatin was with high potential to use as egg coating material and it is essential to study different concentrations of gelatin solutions to achieve best internal quality preservation and improved shelf life.

Competing interests

The authors have no competing interests to declare.

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