Effect of Beeswax and Gammalu (Pterocarpus Marsupium) Latex Coating on Internal and Sensory Attributes of Chicken Eggs Stored at Room Temperature

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Abstract — Coating of eggs is one of the methods used to preserve egg quality and extend the shelf-life. Chitosan and mineral oil are used as coating materials for eggs to preserve quality. Beeswax and Gammalu (Pterocarpus marsupium) latex (GL) have film-forming properties and much faster drying ability on egg shell. Objectives of this research were to determine the suitability of beeswax and Gammalu latex (GL) as a coating material of eggs to control egg quality and sensory attributes. Total of 540 eggs (38 weeks old ISA-Shaver Brown) assigned to six coating treatments as hot water extraction of beeswax, three GL solutions prepared by adding distilled water (DW), (GL: DW = 1:3 (w/w) GL1, 1:1 (w/w) GL2 and 3:1 (w/w) GL3), mineral oil (positive control) and uncoated (negative control) and stored at room temperature. Weight loss, Haugh unit (HU), yolk index (YI), albumen and yolk pH, yolk colour, sensory attributes and microbiological parameters were determined. Haugh unit (HU) and yolk index values (YI) decreased whereas weight loss and albumen and yolk pH increased during storage at room temperature. Non-coated eggs changed from “AA” to “C” grade after 3 weeks where the coatings maintained a “B” grade for 5 weeks except beeswax. Mineral oil and beeswax coated eggs had <1.0% weight losses. YI were decreased from 0.36 to 0.09-0.27. No significant difference (P > 0.05) in sensory evaluation up to day 28 of storage. Only beeswax coated eggs and non-coated eggs showed unacceptable sensorial properties after 2 weeks. All coated eggs were negative for Salmonella spp. Results concluded beeswax and gammalu latex coatings could not preserve the internal quality of eggs and not extend shelf life of eggs when they are used without any modifications meantime all could preserve the egg microbiologically.

Keywords — Beeswax, Chicken Eggs, Gammalu Latex, Internal, Quality, Sensory

I. INTRODUCTION

Eggs are rich source of protein and other nutrients [1]. Egg is one of the most accomplished and versatile foods available [2]. Major egg proteins like ovalbumin, ovotransferrin, ovomucoid, ovomucin and lysozyme have high potentials for industrial applications while egg provides approximately 1.6 kcal/g of egg of energy, of which 78% comes from the yolk [3, 4]. But eggs are highly susceptible to internal quality deterioration and microbial contamination during storage [1]. The internal quality of eggs begins to deteriorate after they have been laid due to loss of moisture and carbon dioxide (CO₂) via the eggshell pores and it leads to change albumen pH and albumen quality [5, 6]. Include more sentence of importance of coating materials

Low temperature refrigeration is considered as the single most important treatment for preserving eggs [1]. Also surface coating is an alternative method to preserve egg quality, although it is much less effective than refrigeration [5]. Various coating materials such as synthetic polymers, polysaccharides and proteins, oils have been applied to the surface of egg shells for preserving the internal qualities [1, 4]. Egg coating may slow down the loss of water and CO₂, causing obstruction of the eggshell pores, which hinders the penetration of microorganisms into the eggs [7].

Waxes are most commonly used hydrophobic film-forming barrier material. Many studies have investigated use of waxes (beeswax, carnauba, candelilla, paraffin and shellac) as coatings on fresh fruits and vegetables to control desiccation [8]. Beeswax is a complex mixture of saturated and unsaturated linear and complex monoenes, hydrocarbons, free fatty acids, free fatty alcohols, and other minor substances produced by the worker honeybee [9-11].

Pterocarpus marsupium, or the “Gammalu” is astringent, bitter, acrid, anti-inflammatory, anthelmintic and anodyne. It is used for the treatment of elephantiasis, leucoderma, diarrhea, cough dysentery, rectalgia, and greyness of hair. It is also reported that an aqueous infusion of the wood is of use in diabetes and water stored in vessels made of the wood is reputed to have anti-diabetic qualities. The aqueous extracts of the barks of P. marsupium inhibit the growth of bacteria with minimum inhibitory concentration ranging from 0.04 mg to 0.08 mg/kg against S. aureus, K. pneuminiae and P. aeruginosa.

The objective of the present research was to examine the effect beeswax coating and Gammalu Latex (GL) on to extend the shelf life of chicken eggs in room temperature without deteriorating the internal and sensory qualities.

II. MATERIALS AND METHODOLOGY

A. Materials

Unwashed, clean, brown-shell, large (a weight range of 53-59 g, SLS 959) 540 eggs from 38 weeks old ISA-Shaver Brown hens were purchased from Government Livestock Farm at Boralandra, Sri Lanka. Fresh eggs were collected from the farm and screened for desirable weight range and then coating materials were applied within 24 hrs of laying. Mineral oil (viscosity = 26 mPas; transparent, odorless and food-grade) was obtain from local market. Bee combs were collected from bee keeping farm, Bindunuwewa, Bandarawela, Sri Lanka. Gammalu (Pterocarpus marsupium) latex was collected from Dambana and Mahiyangana, Sri Lanka.

B. Preparation of Bee Wax and Gammalu Coatings

The gammalu solutions and beeswax were prepared 48 h
before the coating experiment. Beeswax was prepared by using hot water extraction method with some modification [10]. Ground bee combs were placed in a tightly tied cotton bag and boiled overnight at different temperatures (80, 85, 90, 95 and 100 °C). As wax is lighter than water, it filtered through the cotton and rose to the surface. After all combs had melted, container was cooled down and solidified wax was collected for experiment.

Gammlau tree bark was taped for latex of the gammalu and three solutions of gammalu (Pterocarpus marsupium) latex were prepared by adding distilled water to three different weight basis ratios of gammalu latex (GL) and distilled water (DW). 1:3 GL1, 2:2 GL2 and 3:1 GL3. Gammlau latex and distilled water were weighed with a balance (Model-MXX 5001; Denver Instrument, NY, USA) and mixed with vortex mixer (Model-VM 96B, Jeio Tech, South Korea) for 10 minutes at room temperature (27 ± 2°C).

C. Sample Preparation

Five hundred and forty eggs were selected using lottery method. Eggs were individually weighed with a balance (Model TP-214, Denver Instrument GmbH, Göttingen, Germany), and coated with mineral oil (MO), three gammalu solutions prepared with distilled water (GL1, GL2 and GL3) and hot water extracted beeswax. Gammlau and mineral oil was applied using as brush and beeswax was applied by dipping for 1-2 sec and kept for drying at room temperature (27 ± 2°C). All eggs (72 eggs/treatment) were placed in a narrow-end down position in plastic egg trays and stored at room temperature (27 ± 2°C) and average of 60% RH for 6 weeks. Six treatments including the non-coated negative control sample were evaluated for quality and sensory and microbiological parameters.

D. Determination of Weight Loss

Weight loss (%) of the coated whole egg during storage was calculated as [(Initial whole egg weight at day 0 (g) – Whole egg weight after storage (g)]/ Initial whole egg weight at day 0 (g)] * 100 [12]. The weight of whole eggs was measured with a balance (Model TP-214, Denver Instrument GmbH, Göttingen, Germany).

E. Determination of Haugh Unit and Yolk Index

The height of albumen and yolk were measured with a Haugh meter (Fowler Sylvac - Digital Haugh meter, Crissier, Switzerland). The yolk width was measured with a vernier caliper (Series 530, ± 0.05 mm, Mitutoyo, USA). The Haugh unit was calculated as 100 log {H – [\(yG (30 \text{ W}^{0.37} - 100/100) + 1.9\)]}, where HU = Haugh units (no unit), H = height of albumen height (mm), G = 32.2 (constant) and W = weight (g) of egg. The yolk index was calculated as yolk height/yolk width [1].

F. Measurement of Albumen pH and Yolk pH

The albumen was separated from the yolk and the thin and thick albumen were vortex mixed thoroughly for 2 minutes. Then pH of albumen and yolk were measured with a pH meter (Model- pH 700, Eutech Instruments, Singapore) that method described in [13] with modification. A 3-point calibration was performed on the pH meter using 4.0, 7.0 and 10.0 buffers before testing.

G. Colour Measurement of Egg Yolk

After measurement of weight loss (%), the eggs were broken on glass surface. Then yolk colour was measured with Roche Yolk colour Fan (DSM, Basel, Switzerland) having tags from 1 to 15 [14]. Three measurements per treatment were taken weekly.

H. Sensory Evaluation

For sensory evaluation, hard-boiled eggs were prepared by boiling eggs in a 2 L stainless steel kettle for 15 min at 100°C, and then cooled for 10 minutes in running tap water [15]. 30 untrained panelists with the age of 21-25 years in both sexes were used to evaluate the control (non-coated) and the coated eggs for aroma, colour, flavor and overall acceptability using a point hedonic scale.

I. Microbiological Analysis

According to [16], Salmonella test was done. The control (non-coated) eggs and eggs coated with mineral oil and beeswax and gammalu latex were analyzed for Salmonella at weekly intervals during storage period. The internal content (yolk and albumen) of egg samples was mixed using a magnetic stirrer (VELP, Scientifica, Italy) in a dilution of 1:9 of buffered peptone water and incubated at 37°C for 16-20 hrs. Following enrichment, subcultures were plated onto Xylose Lysine Deoxycholate Agar (XLD agar) at 37 °C for 24 hrs prior to detection.

J. Statistical Analysis

The beeswax yield, mean ± standard deviation values were analyzed using Complete Randomized Design with one way ANOVA (SAS, 2003). For internal quality (weight loss, Haugh unit, yolk index, yolk pH and albumen pH) of eggs, mean ± standard deviation values were reported based on 3 measurements (eggs) per treatment (MS Office, 2013). Data were analyzed using two way ANOVA, followed by the Tukey’s studentized range test (α = 0.05) using the SAS software (SAS, 2003). The data obtained from the sensory evaluation analyzed with Freidman test using the Minitab software (Minitab v.17, 2013).

III. RESULTS AND DISCUSSION

A. Effect of Temperature on Preparation of Beeswax

There was a relationship between beeswax yield and temperature (P < 0.05) and also at 95% confidence level, there was a strong positive linear correlation (0.840) between beeswax yield and temperature. According to tukey pairwise comparisons, the temperature 80°C is significantly differed from the other temperatures and it gives a lesser yield compared to others. No significant difference among 85, 90, 95 and 100°C temperatures. Therefore to make beeswax from bee comb at low cost 85°C was used instead of other temperature.

B. Weight Loss

Evaporation of water and loss of CO2 from the albumen through the pores of the egg shell leads to an overall weight loss of the whole egg [5, 7]. Differences in the weight loss among the control non-coated eggs and those coated with mineral oil (MO), gammalu solutions (GL: DW = 1.3, 2.2, and 3:1) were found (interaction between...
coating treatments * storage periods, P < 0.05) during 6 weeks of storage at 27 °C.

The overall, weight loss progressively increased with increased storage time. Eggs coated with mineral oil and beeswax had significantly (P < 0.05) lesser weight loss than non-coated eggs throughout the 6 weeks of storage period. There were no significant differences (P > 0.05) in weight loss among three treatments of eggs coated with gammalu latex throughout 6 weeks of storage. Weight loss of eggs coated with mineral oil (0.52%) and beeswax (0.42%) after 6 weeks of storage was lower than that (1.52%) of non-coated eggs after 1 week of storage. Similarly, [12] reported that, at 25 °C storage, the weight loss (0.85%) of eggs coated with mineral oil (26 mPas) after 5 weeks was lower than that (1.97%) of non-coated eggs after 1 week. This study demonstrated that mineral oil and beeswax coatings can equally offer a protective barrier against the loss of moisture through the eggshell, thus minimizing weight loss (Figure 1).

C. Haugh Unit

The higher the Haugh unit value, the better the albumen quality of eggs [1]. Changes in the Haugh unit of non-coated and coated eggs during 6 weeks of storage at 27 °C were observed (interaction between coating treatments * storage periods, P < 0.05). Overall, the Haugh unit decreased with increased storage periods; however, this decrease progressed at a much slower rate for eggs coated with mineral oil and beeswax than for gammalu latex coated eggs. Compared with non-coated eggs, eggs coated with mineral oil had significantly higher Haugh unit throughout 6 weeks of storage (P < 0.05) and beeswax had significantly higher Haugh unit within first 3 weeks of storage (P < 0.05). On the other hand there were no significant differences (P > 0.05) in gammalu latex coated eggs and non-coated eggs throughout 6 weeks of storage.

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). The grade of non-coated eggs decreased rapidly from AA to B and C after 1 and 4 weeks, respectively. However, eggs coated with mineral oil changed from AA to B grade after 3 weeks, thus preserving the A grade quality up to 3 weeks. Gammalu latex coated eggs and Beeswax coated eggs changed from AA to B grade from 1 weeks. Results of the study strongly concluded and agreed with previous findings of [12], mineral oil can preserve the eggs for 3 weeks of duration where it turns the AA egg as B grade. Haugh unit of non-coated eggs could not be measured after 4 week because those beeswax coated eggs yolk were attached to the egg shell.

D. Yolk Index

Generally, the yolk index values decreased with increased storage periods. This decrease was affected by the coating treatments and storage period at 27°C (interaction between coating treatments * storage periods, P < 0.05). All eggs coated with mineral oil had significantly higher yolk index values (0.27) than non-coated eggs (0.09). [12] It has reported similar findings with the mineral oil coatings. Further in the present study the yolk index values was varied from 0.27 to 0.31 for eggs coated with mineral oil, after 6th week of storage it was higher than that (0.24) of non-coated eggs at after 2nd week of storage and results of this study further sustained that gammalu latex and beeswax coating have not significant affected on the yolk quality of the eggs in terms of yolk index.

E. Albumen pH

Besides the Haugh unit, albumen pH can also be used as an indicator for the albumen quality of eggs [1]. Freshly laid eggs contain 1.44-2.05 mg CO₂/g of albumen and have an albumen pH value of 7.6-8.7 [1, 12]. The pattern for changes in albumen pH during 6 weeks of storage slightly differs with coating treatments. The albumen pH of non-coated and gammalu latex coated eggs gradually increased. However, the opposite was observed for the albumen pH of eggs coated with mineral oil and beeswax, with decreasing pH moreover albumen pH values of all eggs coated with mineral oil and beeswax were significantly lower (P < 0.05) than that of non-coated eggs. Further no significant differences in albumen pH values were observed among gammalu latex coated eggs and non-coated eggs throughout 6 weeks of storage. Similarly, [17] observed that the albumen pH of mineral oil coated eggs decreased from the initial value of 8.35 to 7.96 after 12 weeks of storage at 27 °C. Differences in initial egg quality, egg size and storage conditions (temperature and period) may affect albumen pH before and after storage [18].

F. Yolk pH

The pH of the yolk of a freshly laid egg is 6.0 but during the storage, the pH increased up to 6.4-6.9 [1]. The yolk pH of non-coated and coated eggs gradually increased from an initial value of 6.19 to 7.01 and 6.25-7.07, respectively, after 6 weeks of storage. The yolk pH of non-coated eggs was not significantly different (P > 0.05) from those of eggs coated with any coating treatments throughout 6 weeks of storage period. Previous research has documented a maximum increase in yolk pH of 6.0 to 6.9 [1]. This is expected because the pH of the albumen increases during storage due to CO₂ loss and water from the albumen migrates into the yolk during storage.

G. Yolk Colour

Colour of egg yolk is important to consumers [19]. There were no significant difference (interaction between coating treatments vs. storage periods, P > 0.05) of yolk colour during storage periods. The highest and lowest
Values for the yolk colour recorded were 5.00 and 2.00 respectively. However [14] reported that, there was significant difference in yolk colour between 3 and 5 day after receiving eggs from the market. Results of this study did not agree with the findings of [14], reason may be the storage conditions of the coated eggs.

H. Microbiological Analysis

Bacteria including <i>Salmonella</i> can readily penetrate the shell and membranes of an intact hatching egg [20]. No suspected <i>Salmonella</i> colonies were detected in all non-coated and coated eggs throughout 5 weeks of storage. Thus, present investigation indicated that non-coated and coated eggs were microbiologically safe throughout 5 weeks of storage at 27°C. However most of the eggs coated with gammalu latex and beeswax had spoiled during the storage period than mineral oil coated eggs and non-coated eggs.

I. Sensory Evaluation

One of the most important quality parameters in determining consumer acceptance of any food item is the sensory characteristic. In this study, sensory evaluation was done from 1 to 4 week for all coated and non-coated hard boiled eggs. There were significant different (P < 0.05) at day 14 sensory evaluation for all sensory parameters (Figure 2).

![Sensory evaluation of coated eggs at day 14](image)

Figure 2: sensory evaluation of coated eggs at day 14

Gamamlu latex (GL): Distilled water (DW) (1:3) = GL1, GL: DW (2:2) = GL2, GL: DW (3:1) = GL3

Sensory results at day 14 revealed beeswax coated egg had low acceptance than all other eggs because it had some off flavor of beeswax after peel off the egg shell. Chemically, beeswax consists mainly of esters of fatty acids and various long-chain alcohols [21]. Contamination of these elements may be the reason to give off flavor to the bee wax coated eggs after peeled off.

IV. CONCLUSION

This study concluded that mineral oil is an effective coating material in preserving the internal quality of eggs where as gammalu latex solutions and beeswax do not have the same capacity without any modifications. Comparatively, extended drying time associated with mineral oil coating was identified as a main problem.

Results further revealed that even though all coatings gave microbiologically safe eggs, beeswax and gammalu latex coatings are not suitable for extending shelf life of eggs at room temperature storage.

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REFERENCES


AUTHOR’S PROFILE

Dr. E.D.N.S. Abeyrathne, corresponding author and the supervisor of this study is an eminent academic and a researcher who has completed Bachelor of Science in Agriculture in University of Peradeniya, Master in Science in Dairy and Meat Product Technology in Post Graduate Institute of Agriculture, University of Peradeniya, Sri Lanka and subsequently his PhD in Biomodulation in Seoul National University, Seoul, South Korea (2010- 2013). His major field of study includes isolation and separation of bio active compounds from poultry eggs and fresh water fish. Currently he has been serving as a Senior Lecturer attached to the Department of Animal Science, Uva Wellassa University of Sri Lanka, Badulla since 2007. Further he has contributed his valuable service as a Research Assistant, Protein chemistry laboratory, Seoul National University, Seoul, Korea (09- 2010to08-2013) and Research Assistant, Poultry Meat Laboratory, Iowa state University, Ames, IA, USA(11-2012 to 02-2013). Meantime he has published many scientific papers in various reputed journals and conferences both locally and internationally. Few important publications are as follows. Abeyrathne E.D.N.S., Lee, H.Y., Jo, C., Suh, J.W. and Ahn, D.U.. Enzymatic hydrolysis of ovomucin and the functional and structural characteristics of peptides in the hydrolysates. Food Chemistry. 2016, 192: 107-113. Ishani A.H.M.E. Herath, Jayasinghe J.M. Priyanath, Dong U Ahn, E.D. Nalaka S. Abeyrathne.. Use of lysozyme from chicken egg white as a nitrite replacer in an Italian-type chicken sausage. Functional Food in Health and Diseases. 2015, 5(9): 319-329. Abeyrathne E.D.N.S., Lee, H.Y. Jo, C., Suh, J. W. and Ahn, D.U.. Enzymatic hydrolysis of ovomucoitin and the functional properties of its hydrolysates. 2015;94 (9).2880-2287. His current research interests basically focus on development of functional food to control type II diabetes and obesity, development of value added products from poultry eggs and fresh water fish, identifying natural antioxidants from livestock products, identifying hazard points in food processing and minimizing the hazards with cost effective methods.

Moreover, Dr. Abeyrathne is a life time member in Sri Lanka Association of Animal Production (SLAAP) and Sri Lanka Institute of Food Science and Technology (IFST-SL). Further he has been awarded with several titles including Young Scientist research award in WCU Biomodulation, 2013, Third place awarded in the poster retreat of WCU Biomodulation Major 2011 under PhD category, 4th International Biomodulation Symposium, Seoul national University, Second place awarded in the poster retreat of WCU Biomodulation Major 2012 under PhD category in WCU Biomodulation Major, Department of Agricultural. Additionally, Dr. Abeyrathne’s outstanding findings in the doctoral studies have been rewarded with patents and he has bagged two patents in Separation of ovotransferrerin from chicken egg white using environmental friendly techniques (Patent number :10-2012-0143250) and sequential separation of lysozyme and ovalbumin from chicken egg white (Patent number : 10-2013-0003215).

Dr. S.M.C. Himali, served as a co-supervisor in the study is an eminent academic and a researcher who has completed Bachelor of Science in Agriculture, Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka and Master of Science in Meat Science and Technology, Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka. Moreover she has successfully completed her Doctor of Philosophy at Department of Animal Science, College of Agriculture and Live Sciences, Iowa State University, Ames, Iowa, USA. Currently she is a Senior Lecturer, engaging with teaching mainly in meat science and egg technology attached to the Department of Animal Science, University of Peradeniya, Sri Lanka. Meantime, she has been awarded several awards for the outstanding performances of her career including, Kundalal Scholarship (1998/1999) for meritorious performance at the Second Year Examinations in Agriculture Faculty of Agriculture, University of Peradeniya, Sri Lanka, R.R. Appadurai Memorial Prize (2001) for the best performance in Animal Science at the first, second and third examinations in Agriculture, Faculty of Agriculture, University of Peradeniya, Sri Lanka, E.M.R.B. Ekanyake Memorial Prize (2001)For the best performances at the final examinations in Agriculture, Faculty of Agriculture, University of Peradeniya, Sri Lanka and D. S. Senanayake Memorial Gold Medal (2001)For the overall best performances in Agriculture, Faculty of Agriculture, University of Peradeniya.

Moreover she has continuously been publishing her outstanding research findings in many scientific papers in reputed SCI indexed journals over the past years. Several publications are,


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Few recently published scientific findings are as follows:


Mr. E.D.M.T. Edirisinghe contributed in experimental designing, investigation, sample collection, preparation and analyzing for the study and writing the paper as a part of his undergraduate research study. He completed his (BASc) in Animal Science, Department of Animal Science, Uva Wellassa University of Sri Lanka. (2011-2015).

Moreover, Mr. Edirisinghe has shown strong interest in researching...
on utilization of locally available coating materials for egg preservation, products development and sensory evaluation. He has completed his internship at CIC Agribusiness and currently he is working as a Production Executive at Crown Feeds (Pvt) Ltd, Sri Lanka.