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Effect of Propolis Coating on the Quality of Eggs: Microbial Contamination and Haugh Unit.

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ABSTRACT

Eggs are one of main nutrition sources in Indonesia. However, high temperature and humidity promotes growth of pathogenic microbes which reduces its shelf life and nutrition content. Application of disinfectant is considered as the best option to overcome this problem. Under those circumstances, natural substance is being considered to be use as a disinfectant for eggs. In this study, local Indonesian propolis was evaluated as disinfectant and coating agent for eggs. Various concentrations of propolis extract diluted in alcohol was sprayed to surface of 360 eggs. Weight changes, microbial contamination, and Haugh Unit (HU) of eggs were observed weekly for 5 weeks. Results indicated that 10% propolis coating had significantly prevented egg weight loss. On the other hand, application at lower concentration (2.5%) maintained Haugh Unit, and prevented microbial growth in eggshell for 3 weeks. Application of propolis did not reduce microbial contamination in egg content due to possibility of contamination prior application. Furthermore, application of higher concentrated propolis extract did not improve disinfectant action and coating effect on egg. Application of 2.5% local propolis could be recommended as natural and safe disinfectant to improve the hygiene and shelf life of eggs.
Keywords: biocoating, egg, Haugh Unit, microbial contamination, propolis.

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INTRODUCTION

Eggs are one of the best nutrition sources for human [1]. On the hand, the egg also nutritious for microorganism like bacteria. Once, the bacteria found a stable environment suitable for their growth, they start dividing and colonizing egg tissue. Consumption of such tissue could lead to food poisoning. Thus, in order to provide better nutrition for population in areas distance from egg production center, it is necessarry to maintain the quality of egg in longer time while preventing bacterial contamination that leads to a reduction in nutritional value and harms consumers [2] [3] [4].

Contamination of bacteria on eggs originated from fecal material, the environment of the laying house and storage room, and infection of the reproductive organs [5] [6] [7] [8] [9] [10]. Studies reported the growth of microbes in eggs influenced by eggshell integrity and storage environment [6] [11] [12] [13]. Nowadays, in order to prevent microbial contamination, eggs are subjects to washing, disinfecting, and cooling prior to storage and transport to the market [14]. However, this process could wash off natural shell cuticle, which is the natural defense of eggs against microbial contamination [15] [16] [17].

Eggshell of birds is a porous and breathable material which allowed movement of moisture and carbon dioxide through the shell [18] [19]. This movement may cause physical and chemical changes in albumen and yolk while at the same time increase the vulnerability of pathogens contamination and rate of egg deterioration [7] [20].

Another approach to preserving eggs has been directed to the development of coating materials, made of materials such as mineral oil, chitosan, whey protein, shellac, and edible films, to protect eggshell cuticle [11] [21] [22] [23] [24] [25]. However, most of the current use of coatings has been focused on preventing dehydration and respiration instead of inhibiting microbial activity. Moreover, coating material made of natural based material has become the focus of attention as it offers recyclability and reutilization compared with the petroleum-based synthetic coating. In this study, we applied natural substance which has both the coating and disinfectant properties like propolis.

Propolis is a sticky gummy resinous substance collected and mixed by worker honeybees (*Apis melifera*), in temperate regions, and *Trigona* sp., in tropical regions, from the young shoots and buds of certain trees and shrubs [26]. This substance is known for having strong antibacterial, antifungal, and antiviral properties [27]. Due to its beneficial effects as the disinfectant, propolis has been used on various agricultural product for protection against spoilage caused by microorganisms during storage [28] [29] [30].

This study aim to test local Indonesian propolis as a natural disinfectant and coating material for eggs which will be score based on microbial contamination level, egg weight, and physical condition of egg content by Hugh Index.

MATERIALS AND METHODS

Sample Preparation

About 300 infertile, fresh, clean, brown-shelled, large (63 ± 5 g) 3-d-old eggs from local farmers (West Java, Indonesia) were used in this study. All eggs have eggshells without blood spot, crack, and produced no sounds when shaken.

Raw propolis was collected from *Trigona laeviceps* nests in Maribaya, West Java and extracted according to the Krell Method [31]. A 5% propolis solution was prepared by mixing 1900 ml 70% ethanol and 100 g of propolis, a 10% propolis was prepared by mixing 1800 ml of 70% ethanol and 200 g of propolis while 2,5% and 15% solution were prepared based on those solutions. Solutions were kept in a sealed clean and dark bottle and shaken twice daily for one week. Each solution was filtered by filter paper separately and was kept at 4°C until use.

All eggs were divided into 6 treatment groups: (1) control without any treatment grouped as control (negative), (2) coating with alcohol 70%, coating with (3) 2.5%, (4) 5%, (5) 10%, and (6) 15% propolis extract in

ethyl alcohol. Each group consisted of 60 eggs. Propolis was sprayed to surface egg with a hand sprayer. The distance between the head of sprayer and egg was between 10-15 cm. Coated eggs then air-dried and kept at room condition (26-32 °C, 60-72% RH) for 21 d. Control and treated eggs were stored for 35 d at room condition (26-32 °C, 60-72% RH).

Data Collection

Determination of Weight Change and Haugh Unit.

The weight of eggs were measured with a balance every week for 35 d. At the same time the height of albumen was measured by a digital caliper. Both egg weight (W) and height of albumen (H) were applied to calculate Haugh Unit (HU). The Haugh Unit was calculated as 100 log (H + 7.5-1.7 W0.37) [32].

Bacteria Count

The microbial contamination of external (eggshell) and internal (albumen and yolk) were measured at 0, 7, and 14 d after application. Microbes from the eggshell surface were collected with a sterile swab previously damped in 1 mL sterile distilled water. The swab was placed in a glass tube filled with sterile distilled water and homogenized for 30 seconds. On the other hand, albumen and yolk were homogenized inside a sterile plastic bag.

Serial dilution of the sample in sterile distilled water was performed for both external and internal samples. Aliquot of 0.1 mL from control and each treatment group were planted on MacConkey's broth. The plates were incubated at 37^{o}C for 48 h when the reading of bacterial colony forming unit (CFU/mL) was performed.

Statistical analysis of data was performed using analysis of variance (ANOVA) and continued by Duncan's New Multiple Range Test (DNMRT) at 5% significant level. The results of data processing was further verified by literature and previous research. [33] [34].

RESULTS AND DISCUSSION

General Egg Quality

Application of alcohol, 10%, and 15% propolis alcohol prevented egg weight loss (Fig. 1). In average, highest weight loss after 5 weeks were recorded for propolis 2.5% with 10.32% followed by control (7.34%), propolis 15% (6.03%), propolis 5% (5.27%), alcohol (4.25%), and propolis 10% (1.73%). Among all groups, only alcohol and propolis groups showed insignificant weight loss ($P<0.05$).

Prevention of eggs weight loss due to the application of propolis [35] [36]. Propolis might produce a protective barrier that prevented water evaporation and loss of CO_2 from egg interior [35] [37]. Weight loss around between 8.28 to 10.05%, after 14 days, with the application of Turkish propolis [35]. On the other hand, the application of Egypt propolis, resulted in weight loss between 9.79 to 11.90% after 18 days [36]. This study then showed differences in the composition of propolis that produced in different regions, will produce different protection effect [38] [39] [40] [41]. Compare with other coating material, application of Indonesian propolis 10% was better than glycerol (3.73% loss after 35 days) [42] and chitosan (3.45% loss after 28 days) [43] while it slightly less than mineral oil (0.85% loss after 35 days) [21]. However, coating material alone could not explain the difference in weight loss as variation storage period, temperature, egg size, and shell porosity also influences weight loss.

Application of higher concentrated propolis increased the rate of HU reduction and egg coated with propolis 2.5% showed the highest quality (based on HU value) at the end of study period (Fig. 1). HU inversely related to albumin thickness and tend to decline as egg ages [44] [45]. Multiple hypotheses have been proposed to explain this relation including the breakdown of ovomucin lysozyme, loss of carbohydrate of ovomucin, and increasing pH due to movement of CO_2 to outside of egg across eggshell [46].

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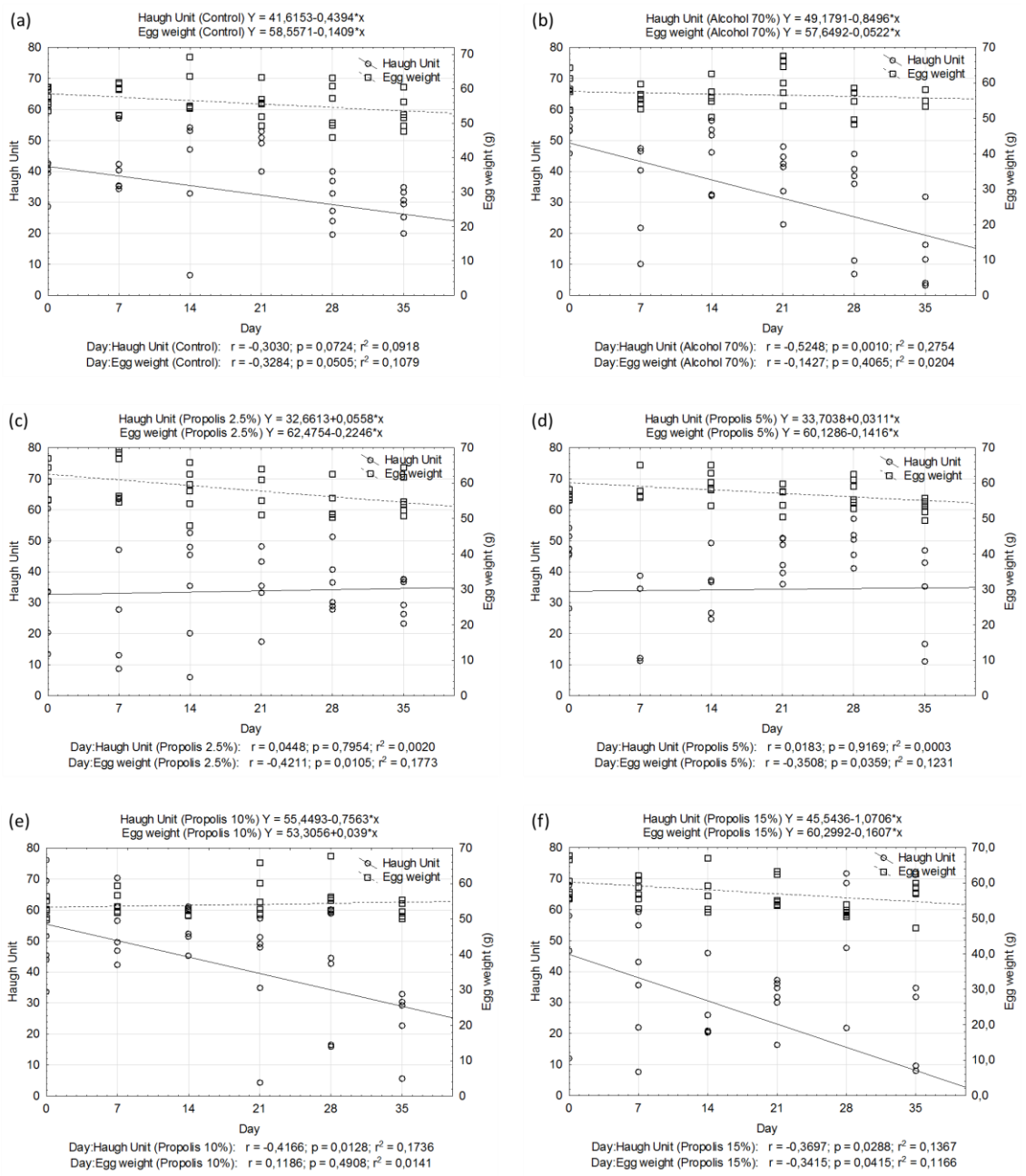

Fig.1. Changes in weight and Haugh Unit of egg treated with propolis coating (c-f), alcohol 70% coating (b) and no treatment (a)

Propolis is a resinous mixture consist of flavonoid, acid, and aromatic esters [41]. The higher acid content of higher concentrated propolis might react with calcium carbonate and protein matrix of eggshell and reduced structure stability of eggshell thus increased carbon dioxide and moisture loss from egg's interior. Significant increase of egg porosity due to alteration or removal of cuticle [47].

Microbial Safety of Egg

Eggshell Surface

The eggs coated with propolis had significantly lower numbers of coliform colonies recovered from egg surface than control and alcohol group at day 21 (Table 1, Fig.2). In addition, application of 2.5% propolis maintained a low microbial contamination at eggshell. This result confirmed that the antibacterial activity of propolis to reduce bacterial contamination at eggshell [48].

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Studies reported about strength of the antibacterial activity of propolis against Gram-positive bacteria [49] [50]. Fluctuation of the microbial population might relate with the interaction between coliform bacteria with other Gram negative and positive bacteria or accumulation of waste. After application of both disinfectant and propolis, no bacteria population found at eggshell of all treatment groups. After one week, only eggs coated with 2.5% propolis showed no bacteria contamination which could be caused by the continuous disinfectant effect of propolis. At the second week, highest numbers of bacteria recorded from all groups which follow growth curve of bacteria population and ended by a drop in quantity, probably due to waste accumulation.

Table 1. Comparison of bacterial contamination (Cfu/ml-log10) recorded at eggshell among control and treatment group

Treatment	Day			
	0	7	14	21
Control	4.3 ^{a}	3.5 ^{a}	7.5 ^{a}	4.5 ^{ab}
Alcohol 70%	0 ^{b}	5.0 ^{a}	6.1 ^{ab}	4.0 ^{ab}
Propolis 2.5%	0 ^{b}	0 ^{b}	6.4 ^{ab}	0 ^{c}
Propolis 5%	0 ^{b}	4.6 ^{a}	5.0 ^{b}	4.6 ^{a}
Propolis 10%	0 ^{b}	5.4 ^{a}	6.8 ^{a}	2.3 ^{b}
Propolis 15%	0 ^{b}	5.7 ^{a}	6.9 ^{a}	4.2 ^{ab}

Different letter within the same column indicated statistically different data with $P < 0.05$

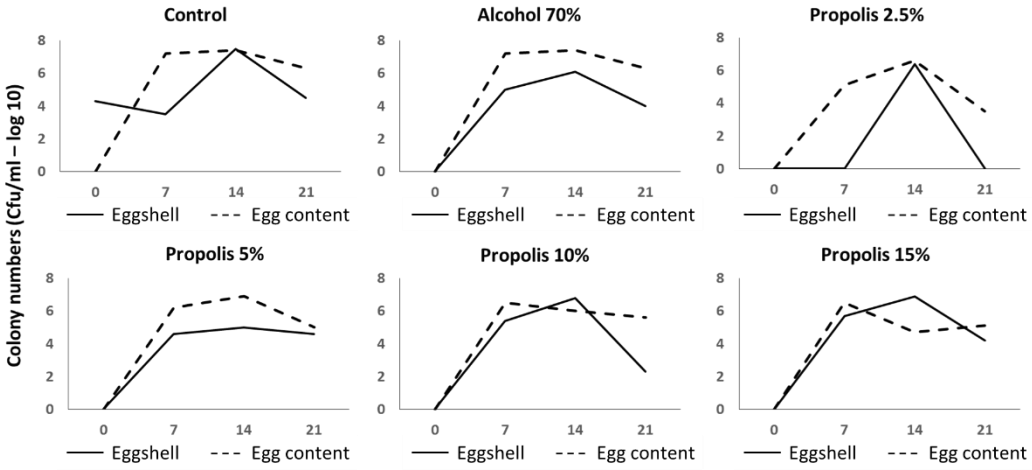

Fig.2. Changes in colony numbers recorded at eggshell and egg content of egg treated with propolis coating

Egg Content

Eggs coated with propolis had significantly lower numbers of coliform colony recovered from the content of the eggs than control (Table 2, Fig.2). Similar to the result of eggshell, the best result showed for group of 2.5% propolis followed by 5% propolis even though it is not significant.

The egg has a natural defense system against bacterial contamination in albumen and vitelline membrane. Once bacteria penetrates both albumen and vitelline membrane during storage, it will growth rapidly in yolk [8] [51].

Application of disinfectant with fast action effect produced instant effect of bacteria count on egg content [52] [53]. However, the effect was temporary compared with propolis as all four propolis coating concentration continued to provide conditions that inhibited the bacteria over the time period studied. Result also indicate that propolis provides a physical barrier which impact bacterial growth. By the end of first week, even though bacterial contamination was not found at eggshell of 2.5% propolis group, bacterial contamination was recorded inside the egg. There was a possibility that a naturally occurred air inside the egg provide resource for bacterial

growth after the eggs were coated [54]. High microbial contamination of egg content might indicated hygiene problems in farms.

Table 2. Comparison of bacterial contamination recorded (Cfu/ml-log10) at egg content among control and treatment group

Treatment	Day			
	0	7	14	21
Control	0 [a]	7.5 [a]	7.4 [a]	6.3 [a]
Alcohol 70%	0 [a]	5.2 [a]	6.1 [ab]	5.6 [ab]
Propolis 2.5%	0 [a]	5.1 [a]	6.6 [ab]	3.5 [b]
Propolis 5%	0 [a]	6.2 [a]	6.9 [ab]	5.0 [ab]
Propolis 10%	0 [a]	6.5 [a]	6.0 [ab]	5.6 [ab]
Propolis 15%	0 [a]	6.5 [a]	4.7 [b]	5.1 [ab]

Different letter within the same column indicated statistically different data with $P < 0.05$

This study indicated the importance of on farm sanitation in order to improve shelf life of highly nutritious eggs. Results also indicated that natural disinfectant could be apply as protective coating material for egg however special concern should be applied to the possibility of detrimental effect to eggshell component as a natural protective structure of eggs. This study only focused on protection of eggs from common gram positive bacteria which was detected only based on culture media. Future studies should be conducted for the efficiency of propolis to prevent contamination by gram negative bacteria with better techniques which allow better identification of microbe contamination.

CONCLUSION

Local Indonesian *Trigona laeviceps* propolis ethanolic extract, when used as disinfectant of eggs, presented antibacterial effect and maintain quality of eggs. This extract is an alternative as a natural product, to the use of washing agent or another non-organic based disinfectant to improve shelf life of nutrituous eggs.

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