EXECUTIVE SUMMARY

HALAL BIOCOATING BERBASIS PROPOLIS UNTUK PENINGKATAN UMUR SIMPAN DAN KUALITAS TELUR AYAM NEGERI PADA SUHU RUANG

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Use Potential of Local Propolis Extract as Halal Biocoating on Storage of Domestic Chicken Eggs Biocoating at Room Temperature

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Abstract— Eggs are livestock products which contribute significantly to the fullfil nutrition need of community, because of one egg contains nutrients is complete and easy to digest. However, eggs have a short shelf life and only lasted 10-14 days at room temperature. This research aims to develop a preservation method of egg which efficient and halal. In this research, egg of domestic chicken (Gallus sp.) was coated with propolis extract of Trigona sp. Propolis extracted with two methods which were ethanolic extraction followed with aquadest extraction prior to application. Propolis extract applied by immersed egg inside propolis extract for 15 seconds, 30 seconds 45 seconds and 60 seconds with eggs without any coating designated as control. Eggs were kept in room temperature and change in egg quality (e.g. shape index, shell thickness, height of air cell) measured at 0, 7, 14, 21 and 35 days after the immersion. In the same time, alcohol content were tested with ester formation and Iodoform test. The results showed that propolis coating did not effect shape index, however maintained good shell thickness and height of air cell. Furthermore alcohol test did not showed sign of alcohol all treatment groups. Based on this study, it could be concluded that application of propolis extract as egg coating may increase egg shelf life while comply with halal regulation.

Keywords—eggs, halal, biocoating, propolis extract.

Introduction

Eggs are one of most consumable livestock product due to their high nutrition value, relatively easy to cook and low cost (Hiroko, 2014). However, egg also considered as one of

perishable product with low shelf life. In tropic, average shelf life of egg in room temperature between 10 to 14 days (Lestari et al, 2011). As high nutritious organic material, egg is susceptible to microorganisme contamination i.e. *Escherichia coli*, *Salmonella typhimurium*, *Shigella* during their production (Afifah, 2013). Even though most contamination occur at egg shell, these pathogens could difuse to egg interior and caused health problem.

Since eggshells are porous and breathable material; therefore they allow movement of moisture and carbon dioxide through the shell (Wong et al. 1996). This movement may cause physical and chemical changes in albumen and yolk and also weight loss (Copur et al. 2008). Studies showed that preventing this movement minimize detoriation in interior egg (Wong et al. 1996; Bhale et al. 2003). Study by Park et al. (2003) showed by combining washing, sanitizing, and coating could significantly increase the shelf-life of the eggs. Thus, application of coating that sanitize egg while reduce the effect of shell degradation would increase the effectiveness of egg preservation procedure. One of the potential coating is propolis.

Propolis is a sticky gummy resinous substance collected by worker honeybees (*Apis melifera*), at temperate regions, and

Trigona sp., in tropical regions, from the young shoots and buds of certain trees and shrubs (Greenaway et al. 1990; Schmidt 1997). This substance known for having strong anti-bacterial, anti-fungal and anti-viral properties i.e. *Bacillus sublitis*, *Bacillis* alvei, *Proteus vulgaris*, *Proteus galangin*, *Salmonella*, *Staphyloccus aureus*, and *Escherichia coli* (Krell 1996; Bankova et al. 2000). Due to its anti-bacterial effect, propolis has been used on various agricultural product for protection during storage (Torre et al. 1990; Pastor et al. 2011; Zahid et al. 2013; Ali et al. 2014).

Previous studies showed propolis extract 2.5% could increased egg shelf life to 21 days by prevented albumin degradation and pathogen contamination (Purwati, 2015; Parwati, 2015). However, propolis extract used at previous study and most study were extracted with ethanol as solvent. This condition caused concern on the halal properties of egg. Thus, in this study we used different approach by apply aquades extract propolis (AEP) as coating for egg preservation. However since part of AEP involving extraction by ethanol, in this study beside test the effectiveness of propolis extract we also test the possibility of ethanol contamination at egg.

Material and Method

In this study, propolis extract was applied to surface of brown egg shell. Three hundred eggs, with weight between 50-60 gram, were used in this study. Propolis used in study was *Trigona* sp. propolis obtained from local *Trigona* sp. farm in North Bandung.

Propolis extraction

Propolis was extracted by ethanolic extraction in which block of raw propolis of *Trigona* sp. mixed with 70% ethanol and kept inside dark bottle. The mixture then incubated with incubator shaker for 7 days to obtained early propolis extract.

Further extraction process conducted on early propolis extract by aquades to obtain Aquades Extract Propolis (AEP). About 200 ml of ethanol extracts of propolis mixed with 0.4024 gram K_2HPO_4, 0.9228 gram KH_2PO_4 0,9228 and aquades until total volume of mixture about 500 ml for 20 min at 20°C. The mixture was centrifuged at 7000 rpm for 15 min and the supernatant was collected (Najafi et al. 2007). Collected then mixed with water to obtain 2.5% propolis-water mixture.

Propolis extract application

Eggs were dipped inside 2.5% propolis extract for 15 sec, 30 sec, 45 sec, and 60 sec. Eggs then kept at room temperature. Observation on some variables of egg quality namely shape index, shell thickness, and air cell depth were conducted at day 0, 7, 14, 21, 28, and 35. Shell index was measured by formula (Bell dan Weaver, 2002):

$$\frac{width}{height} x100\%$$

Alcohol contamination was tested by esther formation test and iodoform test. Alcohol test was conducted at day 0, 21, and 35.

Result and Discussion

Change in shape index indicated change in exterior quality of egg. In this study, we recorded value of shell index between 0.75-0.77 and that duration of immersion did not effect egg shape index (Fig. 1). Shell index recorded in this study was bigger than study of Bell and Weaver (2002) who reported shell index of 0.70-0.74 for normal eggs.

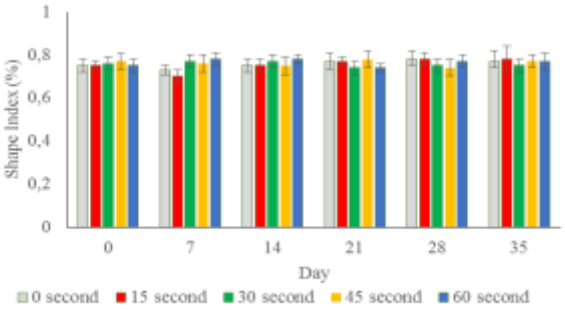

Figure 1. Change of shell index of eggs immersed inside propolis extract at different time

Egg shell thickness decrease with increase egg age. Good egg has thick shell which protect egg interior from microorganisms contamination. Thick shell also reduce rate of moisture loss. In this study we found that by immersed egg inside propolis extract for 60 sec could significantly reduce rate of shell thickness degradation.

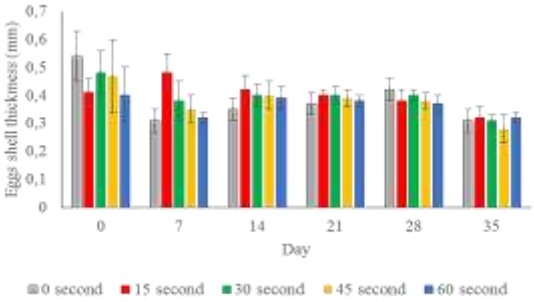

Figure 2. Change of shell thickness of eggs immersed inside propolis extract at different time

One of the component of egg quality is air cell depth. Low quality eggs has large air cell depth as result of degradation of yolk and albumin. In this study, lowest rate of air cell depth development was recorded for egg immersed inside propolis extraction for 60 sec (Fig. 3).

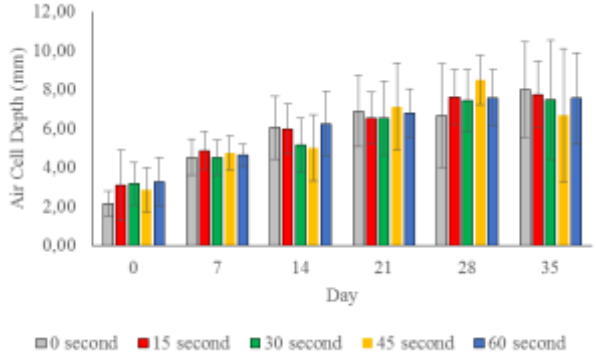

Figure 3. Change of air cell depth of eggs immersed inside propolis extract at different time

This study also tested alcohol contamination for egg immersed inside propolis extraction for 45 and 60 sec (best propolis application method) with untreated egg as control. Based on esther formation test and iodoform test, all egg used during test did not show any alcohol contamination (Table 1).

Table 1. Alcohol content test of eggs immersed inside propolis extract at 45 and 60 sec.

Treatment	Day 0		Day 21		Day 35	
	Esther formation test	Iodoform test	Esther formation test	Iodoform test	Esther formation test	Iodoform test
Control	-	-	-	-	-	-
45 sec.	-	-	-	-	-	-
60 sec.	-	-	-	-	-	-

Note: negative (-) indicated sample without alcohol contamination.

In Indonesia, definition of halal product based on Standarisasi Fatwa Halal published by Fatwa Majelis Ulama Indonesia No 4 Tahun 2003. Based on that, Ethanol as solution which not produce by khamr industry considered permissive to consume by moslem. Some islamic scholar also agree that ethanol application during food production is permissive (Najiha and Nadiah, 2014). Furthermore, result of this study also showed negative ethanol contamination which support the application of propolis extraction as solution for halal biocoating for egg preservation.

Conclusions

The results showed that propolis coating did not effect shape index, but effect shell thickness and height of air cell. Furthermore alcohol test did not showed sign of alcohol all

treatment groups. Application of propolis extract as egg coating may increase egg shelf life while comply with halal regulation.

Acknowledgment

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Comparing of effect two types of propolis of *Trigona laeviceps* coating methods on physical quality and shelf life of eggs kept in room temperature

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Abstract. The best nutrition source is chicken egg. However, studies showed in the tropical region, eggs have low shelf life, ranged from 10 to 14 days, when kept at room temperature. Low temperature is believed as the best way to extend the shelf life of eggs. Unfortunately, not all area in Indonesia has access to low-temperature method thus lead to economic loss and lower egg nutrition value due to quality loss. Previous study showed application of natural coating material, such as propolis, improved shelf life of eggs. However, due to the high cost of application it is necessary to find more efficient application methods. In this study, two types of application were tested, by brushing and spraying. Propolis was applied by brushing and spraying to 180 fresh eggs of local chicken (*Gallus* sp.) aged 24 hours and another 180 eggs without propolis coating designed as control (total numbers of eggs used were 540 eggs). All eggs were kept in room temperature for 35 days. Changes on albumin index, yolk index, and height of air sacs were measured every 7 days in order to observe the change in egg quality. This study showed different application methods did not affect value of albumin index and yolk index for each observation time, but affected haugh unit and height of air cell. However, result indicated that application of propolis by spraying maintained egg quality in longer time than brushing, 28 days and 21 days, respectively.

1. Introduction

The chicken egg is a food which has complate balance of essential nutrients for human. Furthermore, it also has low cost which increased it popularity as food in low-income population in Indonesia. However, the nutrition quality of eggs starts to deteriorate immediately after laid and during storage. Rate of deterioration affected by strain and age of hen whom egg produced, storage time and conditions [1][2]. Deterioration of egg is due to change of internal part of egg as embrio developed.

Eventhough eggshell provide protection to internal part of egg, it also a porous and breathable material; therefore they allow movement of moisture and carbon dioxide through the shell which allowing life of embrio inside the egg [3]. This may cause physical and chemical changes in albumen and yolk then reduce weight and quality of egg to consumer [4]. Studies showed that preventing movement of moisture and carbon dioxide reduce deterioration rate of interior part of egg [5]. Furthermore, combaining it with sanitizing significantly increase the shelf-life of the eggs [6]. Usually prevention of water and carbon dioxide movement conducted by application of coating material on eggshell while sanitizing by disinfectant. Thus, application of coating material that sanitize egg while reduce movement of water and carbon dioxide would increase the effectiveness of egg preservation procedure. One of the potential coating material which considered safe to human is propolis.

Propolis is a a mixture of beeswax and resus from plant buds, leaves, and exudates [7] which collected by worker honeybees (*Apis melifera*), at temperate regions, and *Trigona* sp., in tropical regions [8]. Among natural products, this substance has recieved more attention due to its strong anti-bacterial, anti-fungal and anti-viral properties against a wide range of pathogenic microorganisms, and has been used on various agricultural product for coating material to reduce post harvest loss [9][10]. Our previous study had found that 2.5% propolis extract was the best concentration for coating. However, since the high cost of production, it is necessary to development efficient coating method to reduce material loss [11]. Thus, in this study we tested the effect of spraying and brushing as coating methods of propolis to preservation of egg quality.

2. Methods

2.1. Propolis extraction

Raw propolis used for extraction was take from the bee hives of *Trigona* sp at Dago area in Bandung, West Java. The propolis extract was prepared according to previosly method [12] with modification. Raw propolis was immersed in 70% ethanol with ratio 1:1 (w/w), kept inside dark bottle, and shake with rotator for

7 days. Ethanol Extract Propolis (EEP) obtained by filtered solution with filter paper. Before application, Aquadest Extract Propolis (AEP) was producing by adding 200 ml of EEP and 200 ml 20 mM phosphate buffer inside 500-ml flask and kept on magnetic stirrer for 20 min at 20ºC. The mixture was centrifugated at 7000 ppm for 15 min and supernatant was collected [13]. AEP then diluted with aquadest to produce 2,5% propolis solution.

2.2. Propolis application

About 540 brown and smoot eggs (no crack) were used in this study. Egg was sorted and weighed (between 40-60 g) to reduce variation of egg. Eggs were applied with 2.5% propolis extract by brushing and spraying. Eggs then kept at room temperature for 35 days.

The degradation of albumen index (AI), yolk index (YI), height of air cell, and Haugh Unit (HU) were measured each 7 days. For sampling, each eggs were broken on a flat surface, using transparant glass plate, where the height of the albumen, diameter of the albumen, height of yolk and diameter of the yolk were measured by digital calipper. Albumen index (AI) was calculated by formulae:

$$AI = \frac{H}{0.5\,(D1+D2)}$$

where:
H = Albumen height (mm)
D1 = outer diameter of thick albumen (mm)
D2 = shortest diameter of thick albumen (mm)

Yolk index (YI) was calculated by formulae:

$$YI = \frac{h}{0.5\,(d1+d2)}$$

where:
h = Yolk height (mm)
d1 = outer diameter of yolk (mm)
d2 = shortest diameter of yolk (mm)

Haugh unit were calculated from the HU formula [14]:

$$HU = 100 \log (H + 7,5 - 1,7 W^{0,37})$$

Where H = height of albumen (mm); W = egg weight (g).

2.3. Data analysis

The data were subjected to Duncan's multivariate Test to detect deference on each parameter among all treatment. The data collected was analysed using SPSS ver. 22 and STATISTICA ver. 10 software packages.

3. Results

The effect of application of propolis as egg coating did not significantly influence change of yolk and albumin condition ($p>0.05$). While Figure 1 show the trend in some quality traits as affected by aplication of propolis. This figure show that spraying method could be maintained their albumin and yolk condition up to 21 days. Low quality of eggs due to low quality nutrition received during egg production may explained this result.

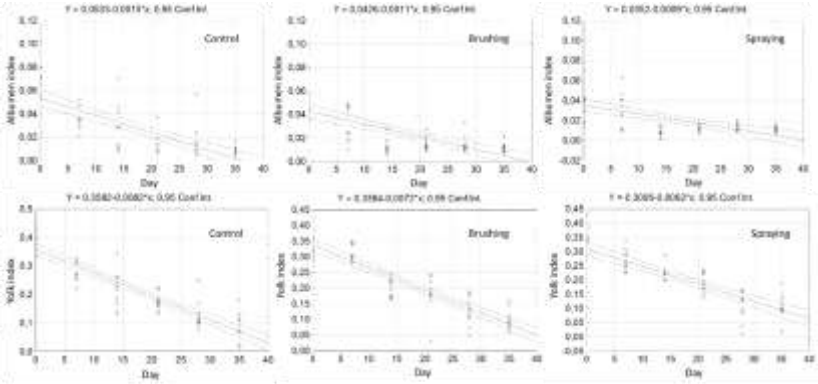

Figure 1. Changes of albumin index (above) and yolk index (below) for eggs applied with propolis extract at different methods.

Figure 2 show that air cell sizw increased with increased storage time. Increasing height of air cell of egg caused by development of chick and change in loss of water vapour through

eggshell which replaced by mass of oxygen diffusing into the egg is balanced by carbon dioxide leaving the egg [15][16]. Air that replaces water vapour accumulates in the air cell caused increasing height of air cell [17]. Application of propolis by brushing and spraying significantly maintained quality of egg based on height of air cell ($p<0.05$) (Figure 2). Result also showed both methods did not showed as significant different result which indicated both method could provide good coating result of egg.

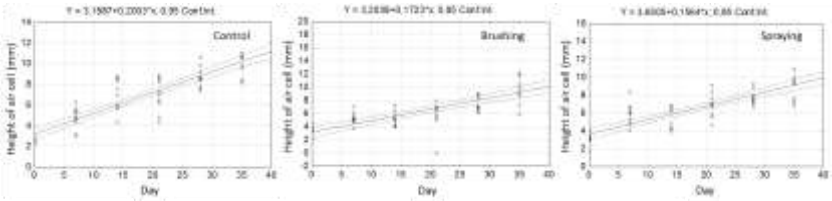

Figure 2. Changes of height of air cell for eggs applied with propolis extract at different methods.

Increasing storage time reduced HU of the eggs which consistent with other study [14][18][19][20]. This study showed both of application significantly reduced rate of HU decreasing (Figure 3). During storage, carbon dioxide is lost through the pores of the eggshell [21]. This condition increase the pH of the albumen increases [22]. Increasing pH will cause some denaturation of proteins and a decrease in HU [23]. Beside storage time, other factors that can negatively affect HU are hen genetics, hen age, and disease [24].

Similar with result in air cell, the effect did not significant between both method during study perior. However, in longer time it could be predicted that spraying group would have higher HU than brushing group.

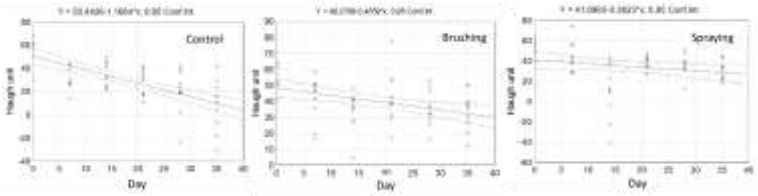

Figure 3. Changes of haugh unit for eggs immersed in propolis extract at different duration.

Based on this study, it could be conclude that spraying is better method for propolis coating on the eggshell.

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Effect of Duration of Immersion of Egg into Propolis Extract to Physical Quality and Shelf Life of Local Chicken Eggs (*Gallus* sp.)

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Abstract. The quality of eggs starts to deteriorate immediately after laid and continue decreasing during storage. One of the reason is the properties of eggshell. Air movement could cause change in physical and chemical changes in albumen and yolk resulted in weight loss, reducing shelf life of eggs. Most common practices to increase shelf life by combine washing, sanitizing, and coating could significantly increase the shelf-life of the eggs. However, various synthetic chemicals were applied in this procedure and produce additional wastes to environmental. One of the potential natural substances which may fulfilled this requirement is propolis. In this study, the effect propolis as coating for local chicken eggs was tested by immersed the egg into propolis extract with different duration (5 sec, 15 sec, 30 sec, 45 sec, and 60 sec). Egg quality measured based on degradation of albumen weight (AW) and index (AI), yolk weight (YW) and index (YI), height of air cell and Haugh unit (HU) at beginning (0), 7 days, 14 days, 21 days, 28 days, and 35 days. The result showed that duration of immersion did not significantly influence change of albumin and yolk condition of eggs. However, eggs immersed for 45 sec and more could be maintained their albumin and yolk condition up to 21 days. On the other hand, egg immersed for 60 sec maintained quality of egg based on height of air cell.

1. Introduction

Egg is considered as one of the best nutrition sources for human. However, quality of eggs starts to deteriorate immediately after laid and continue during storage. Rate of detorioration influenced by strain and age of hen whom egg produced, storage time and conditions [1][2]. Quality of egg is related to the quality of internal proportion of egg. Naturally, eggshell provides protection to internal part of egg while provide maintain necessary environment condition for development of chick. Eggshell itself is porous and breathable material; therefore they allow movement

of moisture and carbon dioxide through the shell which allowing protection and life of embrio inside the egg [3]. However, air movement may cause physical and chemical changes in albumen and yolk resulted in weight loss which indicated detorioration of egg quality [4].

Study showed that preventing this movement minimize detoriation rate of interior part of egg [5]. On the other hand, in order to prevent microbial contamination washing technology was introduced in many countries and applied by many egg producers. Study by Park et al. [6] showed by combining washing, sanitizing, and coating could significantly increase the shelf-life of the eggs. During that procedure, various synthetic chemical substances usually applied which increase environmental pressure. Thus, application of coating substances that provide all benefit of washing, sanitizing, and coating would increase the effectiveness of egg preservation procedure. Moreover, application of natural substances would fulfilled market demand on healthy food. Among natural substances available, propolis is considered as substance with big potential.

Propolis is a sticky gummy resinous substance collected by worker honeybees (*Apis melifera*), at temperate regions, and *Trigona* sp., in tropical regions, from the young shoots and buds of certain trees and shrubs [7]. This substance known for having strong anti-bacterial, anti-fungal and anti-viral properties and has been used on various agricultural product for post harvest protection [8].

Previous study had found that 2.5% propolis extract was the best concentration for coating. In that study also showed that application of propolis coating by spraying able to mantain the quality off eggs but not increased storage time of egg [9]. In this study we tested the effect propolis from *Trigona* sp. as coating for local chicken eggs by immersed the egg into propolis extract in order to improve the effectiveness of coating procedure.

2. Methods

2.1. Propolis extraction

Propolis extract was produced by extraction of propolis substance from raw propolis of *Trigona* sp. Raw propolis was immersed in

70% ethanol with ratio 1:1 (w/w), kept inside dark bottle, and shake with rotator for 7 days. Solution then filtered with filter paper to obtain Ethanol Extract Propolis (EEP). Further activity was producing Aquadest Extract Propolis (AEP). This extract produced by adding 200 ml of EEP and 200 ml 20 mM phosphate buffer inside 500-ml flask and kept on magnetic stirrer for 20 min at 20^{o}C. The mixture was centrifugated at 7000 ppm for 15 min and supernatant was collected [10]. AEP then diluted with aquadest to produce 2,5% propolis solution.

2.2. Propolis application

About 300 brown eggs were used in this study. Egg was sorted and weighed to reduce variation of egg. Only smooth egg (no crack) and egg with weight between 40-60 g were used.

Eggs were dipped inside 2.5% propolis extract for 15 sec, 30 sec, 45 sec, and 60 sec. Eggs then kept at room temperature for 35 days. Egg quality measured each 7 days based on degradation of albumen index (AI), yolk index (YI), height of air cell, and Haugh Unit (HU).

Eggs were broken on a flat surface, using transparant glass plate, where the height of the albumen, diameter of the albumen, height of yolk and diameter of the yolk were measured by digital calipper. Albumen index (AI) was calculated by formulae:

$$AI = \frac{H}{0.5\,(D1+D2)}$$

where:
H = Albumen height (mm)
D1 = outer diameter of thick albumen (mm)
D2 = shortest diameter of thick albumen (mm)

Yolk index (YI) was calculated by formulae:

$$YI = \frac{h}{0.5\,(d1+d2)}$$

where:
h = Yolk height (mm)
d1 = outer diameter of yolk (mm)
d2 = shortest diameter of yolk (mm)

Following equation was used for Haugh unit (HU) [11]:

$$HU = 100 \log (H + 7{,}5 - 1{,}7\ W^{0,37})$$

Where H = height of albumen (mm); W = egg weight (g).

2.3. Data analysis

The results were subjected to analysis of variance ($p > 0.05$) was applied to detect deference on each parameter among all treatment. Significant means were calculated by the Duncan's multivariate Test. The data collected was analysed using SPSS ver. 22 and STATISTICA ver. 10 software packages.

3. Results

The result showed that application propolis as egg coating did not significantly influence change of yolk and albumin condition. However, eggs immersed for 60 sec and more could be maintained their albumin and yolk condition up to 21 days (Figure 1). Low quality of eggs due to low quality nutrition received during egg production may explain this result.

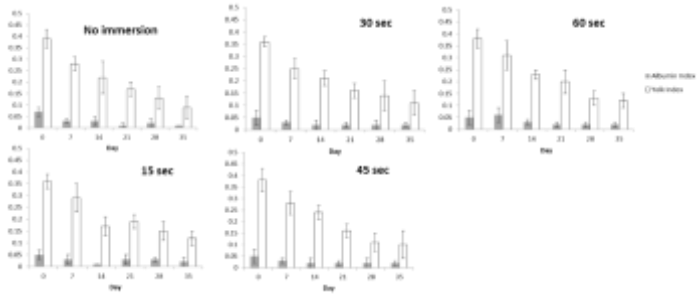

Figure 1. Changes of albumin and yolk index for eggs immersed in propolis extract at different duration.

On the other hand, egg immersed for 60 sec maintained quality of egg based on height of air cell (lower height mean good egg) (Figure 2). Increasing height of air cell of egg caused by development of chick and change in loss of water vapour through eggshell which replaced by mass of oxygen diffusing into the egg

is balanced by carbon dioxide leaving the egg [12][13]. Air that replaces water vapour accumulates in the air cell caused increasing height of air cell [14].

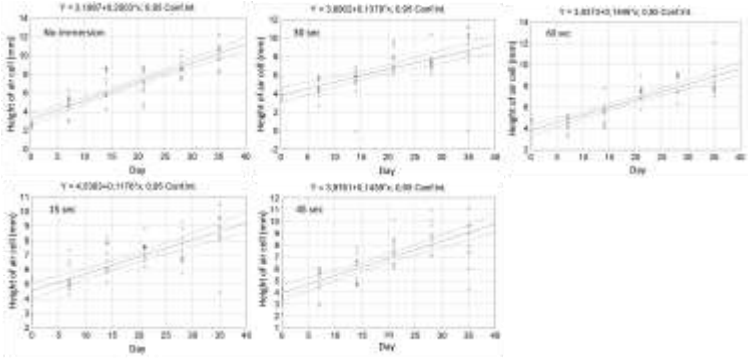

Figure 2. Changes of height of air cell for eggs immersed in propolis extract at different duration.

Increasing storage time reduced HU of the eggs which consistent with other study [11][15][16][17]. This study showed immersing egg for 60 sec reduced rate of HU decreasing (Fig. 3). We also found that most of eggs obtained from local egg producer had low HU, less than 60. This value much lower than value for standard of excellent quality egg in North America which is 72 [18] and minimum standar of egg prior release to consumer which is 60 [19].

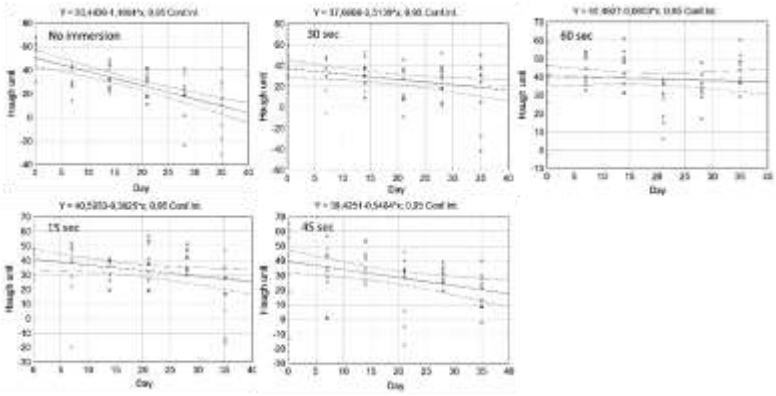

Figure 3. Changes of haugh unit for eggs immersed in propolis extract at different duration.

During storage, carbon dioxide is lost through the pores of the eggshell [20]. This condition increase the pH of the albumen increases [19]. Increasing pH will cause some denaturation of proteins and a decrease in HU [21]. Beside storage time, other factors that can negatively affect HU are hen genetics, hen age, and disease [22].

Based on the result, it could be concluded that longer immersion may increase to possibility of surface of eggshell perfectly coated which improve shelf life of eggs. Better coating will reduce the rate of gas exchange between internal part and environment outside egg. On the other hand, this study also indicated lack of screening process for egg on farm which could provide more challenge for develop strategy to preserve local egg quality.

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