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Total Plate Count and pH of Layer Eggs Immersed in Leaf Aqueous Extract of *Averrhoa bilimbi* L.

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ABSTRACT

Layer eggs are low cost and highly nutritious, but they are perishable. Therefore, it is important applying preservation for prolong its shelf life. Preservation by using natural agent such as belimbing wuluh (*Averrhoa bilimbi* L) leaf (BWL) extract for food safety and consumer health is rationale. This study aimed to evaluate the immersion of layer eggs in an aqueous extract of BWL on the pH value and total microbial eggs. The treatments employed were eggs without soaking (P0), eggs soaked in aquadest (P1), eggs soaked in solution of BWL leaf extract (SBWLE) 15% (P2), eggs soaked in SBWLE 30% (P3), and eggs soaked in SBWLE 45% (P4). Soaking was carried out for 24 hours with the proportion of egg: water SBWLE was 1:13. The extraction process was carried out by boiling the BWL for 10 minutes at a temperature of 85 ºC. The boiled water is filtered and the results were used for treatment. The data were analyzed by ANOVA and he significant effect ($P < 0.05$) of treatments were tested by a post hoc Duncan's Multiple Range Test (DMRT). The results showed that eggs soaked in SBWLE had lower pH and total microbes than eggs without soaking and eggs soaked in aquadest. The higher concentration of SBWLE, the decreasing pH and total microbes in eggs. The conclusion was that the use of SBWLE 15-45% (w/v) was able to suppress egg microbial growth and prevent an increase in egg pH.
Keywords: *Averhoa bilimbi*, egg quality, microbes, pH

INTRODUCTION

Eggs are very popular foodstuff around the world [1]. It is due to eggs are rich in essential nutrients for humans [2] and it relatively is low prices [1, 3]. In addition, by consuming eggs, humans have the benefit of fulfilling the bioavailability of vitamin E [4] and carotenoids [5] in the human body.

Unfortunately, eggs are also a perishable food so that their quality are easily decreased in physical, chemical, and biological properties. The most important damage to eggs is caused by microbes [1]. Microbial deterioration can cause other damages, including producing toxins, physical and chemical qualities of eggs [6]. These negative effects by deterioration of consumption eggs are just not expected for layer and egg industries. Therefore, handling eggs to reduce microbial contamination is very important for prolong the shelf life of eggs.

Microbial contamination is not only in the egg shell, but they can also get involve in the inner part of egg. According to Chousalkar *et al*. [1] that microbes can come into egg by penetrating through the pores of the shell and then survive in the inner egg. Based on this contamination mechanism, it is needed attempt to minimize the contaminants in the egg shell and its penetration process. Some efforts had been conducted by washing, sanitizing, and coating [7]. It can be applied by coating natural herbal ingredients on egg shells. The use of natural materials for preservation has become an alternative to synthetic materials for safety and health reasons [7] [8]. Several studies using natural ingredients gave positive results on egg quality [9].

Immersion eggs into a solution of BWL extract is a potential alternative to be applied. It is due to BWL contains phytochemical compounds such as alkaloids, saponins, tannins, flavonoids, phenols, and triterpenoids [10]. Some of these compounds have antibacterial activity and are useful for preservation [11, 12]. The application of BWL on consumption egg is less studied.

By regarding the description above, this study aimed to evaluate the effect of immersion of layer eggs into a solution of BWL extract (SBWLE) on the pH and total plate count of eggs. It is hypothesized that the immersion can reduce the number of bacteria in eggs.

MATERIALS AND METHODS

Eggs and Extract Preparation

The layer eggs were purchased from the local layer farm in Bengkulu City. Eggs used were the fresh eggs from laying. The BWL was obtained from local area in Bengkulu City. The BWL was clean and purified from undesired materials and then it was cut into small pieces with a size of ±1 cm^2. The small pieces of BWL, then, were air-dried at a temperature range of 28-30 ºC for 72 hours. The air-dried BWL then was boiled at 85 ºC for 10 minutes. After boiled, water was filtered and the filtrate was used for study. The proportion between BWL and aquadest in treatment was adjusted according to the treatment.

Experimental Design and Treatments

The research design used was a completely randomized design (CRD) with 5 (five) level of treatments and each level was in 3 (three) replications. The treatments employed were as follows:
T0 = unimmersed eggs.
T1 = immersed eggs in distilled water.
T2 = immersed eggs in a solution of 15% (w/v) BWL extract (SBWLE).
T3 = immersed eggs in 30% (w/v) SBWLE.

T4 = immersed eggs in 45% (w/v) SBWLE.

The immersion of eggs was for 24 hours with the ratio of eggs: water or SBWLE was 1: 13. Each treatment was observed at 10 and 20 days and these were not as a factor.

Measurement of pH

Measurement of egg pH was carried out using the AOAC method [13]. The yolk and albumen were mixed and shaken to be a homogeny mixture. The mixture was taken as much as 2 ml as a sample, then added 20 ml of distilled water. The sample was homogenized for 3-5 minutes by using a vortex. Furthermore, the sample was transferred into a measuring cup. Measurement was by dipping the electrode of pH-meter into sample approximately 2-4 cm, and allowed to stand until the indicator on the screen was constant (1-2 minutes). The measurements were carried out on 10 days and 20 days of storage eggs.

Total Microbes

The microbiological quality of eggs was detemined by using the Total Plate Count (TPC) method based on SNI 2897: 2008 [14]. The egg shells are mashed and weighed as much as 25 grams and then ground first. For the yolk and albumen, first homogenized and taken as much as 25 ml. Each sample of egg shells and yolk-albumen mixture were put into an Erlenmeyer containing 225 ml of sterile Buffered Peptone Water (BPW, Himedia M614S) solution then homogenized by vortexing for 1-2 minutes to obtain a dilution (10-1). Subsequent dilution 1 ml of the dilution suspension from Erlenmeyer (10^{-1}) was put into a test tube containing 9 ml of BPW solution to obtain a dilution (10^{-2}). For dilutions 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} are made in the same way as previous.

Put 1 ml of suspension from each dilution sample into a petri dish in duplicate. Add 15-20 ml of sterile Plate Count Agar (PCA, Himedia M091) at a temperature of ± 45ºC to each cup containing suspension and the cup was shaken evenly, then let stand to be solid. After solidification, the petri dish was put into the incubator at 37ºC for 24-48 hours. The colonies were counted in each dilution series. The selected dish was a cup that had colonies of 25-250. The number of colonies formed (colony forming units) was calculated by multiplying the average number of colonies by the dilution factor. The total of microbes in egg shells was expressed in log CFU/g and in yolk-albumen was CFU/ml. The observation was carried out on 10 days and 20 days of storage eggs.

Data Analysis

The data were analyzed by ANOVA (Analysis of Variance). The significant effect ($P < 0.05$) of treatments were analyzed by a post hoc test DMRT [15].

RESULTS AND DISCUSSIONS

Eggs pH

The pH value of inner egg of the study is shown in Table 1. The results showed that immersion of the eggs resulted in a decrease the pH value. The higher percentage of BWL extract used, the pH value decreased significantly ($P<0.05$) toward neutral pH. The pH at 10 days of storage were 7.51, 7.35, 7.29, 7.23, and 7.13 for T0, T1, T2, T3, and T4 respectively. Each pH value for each treatment is significantly different. Whereas, at 20 days of storage, it was obtained pH value as 7.64, 7.54, 7.32, 7.26, and 7.19 for T0, T1, T2, T3, and T4 respectively. The decrease in pH value occurred in eggs of 10 and 20 days of storage with a relatively similar pattern of decline, except for the T2 and T3 treatments on the 20^{th} day of storage which showed no significant difference ($P>0.05$).

Theoretically, the pH value of albumen and yolks is actually different. In fresh eggs, the pH of the albumen is about 7 and in the yolk is about 6 [16]. By longer the storage time, the pH value will increase [16]. The increase of egg pH occurs due to evaporation of CO_2 gas out through egg shell pores [17]. The phenomenon in this study indicated that the immersion of eggs in the SBWLE resulted in the lower pH than unimmersed eggs. It was most probably that the immersion into SBWLE caused the pores of the egg shell to be coated with tannins contained in the BWL extract. The coated eggshell with tannins prevented evaporation process and the pH of the inner eggs could be maintained. The higher concentration of BWL extract, the higher tannin coating level, so that the pH of inner eggs was lower. The results of this study were in line with the research of Riawan *et al*. [17] that soaking eggs in a solution of Moringa leaves could slow down the evaporation of water and CO_2 gas.

The longer eggs were stored, the pH value increased (Table 1). However, pH in eggs immersed in a SBWLE tended to be lower. Table 1 at the last row shows that the different increments of the pH from 10 to 20 days of storage are higher in T0 (0.13) and T1 (0.19) than T2 (0.02), T3 (0.03), and T4 (0.06). Rather, the eggs that immersed in the SBLWE are lower increments of pH changes. It confirmed that immersion of eggs in SBWLE can decrease eggs pH value. It was most presumably caused by tannin content in BWL extract coating the pores of eggshell, thereby inhibiting the evaporation of water and CO_2. Thus, the evaporation of eggs immersed in SBWLE was apparently to be lower and the pH could be maintained along with the length of storage time. These decrease of pH value of eggs remain in normal range which at least 7.1 [18].

In addition, the lower pH of eggs that immersed in the SBWLE (T2, T3, and T4) could be caused by the phenolics compounds found in BWL extract. Setiawan *et al*. [19] reported that BWL contains polyphenolics compounds which have acidic properties. This acidic property affected the solution pH and finally it decreased inner egg pH.

Table 1. The average pH value of inner egg among unimmersed, immersed in distilled water, and Immersed in a solution of BWL extract

Storage	Treatments					P
	T0	T1	T2	T3	T4	
Day 10th	7.51±0.02[a]	7.35±0.03[b]	7.29±0.02[c]	7.23±0.01[d]	7.13±0.01[e]	0.00
Day 20th	7.64±0.01[a]	7.54±0.03[b]	7.31±0.04[c]	7.26±0.03[c]	7.19±0.03[d]	0.00
Different between 10 and 20 days of storage	0.13	0.19	0.02	0.03	0.06	

The different superscript in same row indicates significant different (P<0.05); T0 = unimmersed eggs; T1 = immersed eggs in distilled water; T2 = immersed eggs in a solution of 15% (w/v) BWL extract (SBWLE); T3 = immersed eggs in 30% (w/v) SBWLE; T4 = immersed eggs in 45% (w/v) SBWLE. P = Probability

Total Microbes

The results showed that the treatment had a significant effect on the total microbial shell and inner egg (P<0.05) as shown in Table 2. The results of this study also illustrated that immersion of eggs in SBWLE caused the total plate count to be lower than without immersion and SBWLE immersion. This phenomenon occurred on the 10th and 20th days of storage with a similar pattern in both the shell and inner eggs both in egg shell and inner eggs.

The total microbes in egg shell and inner eggs both at the 10 and 20 days of storage, the eggs without any immersion (T0) and immersed in aquadest (T1) were similar. They were also lower than total microbes of eggs immersed in 15% of SBWLE (T2), immersed in 30% of SBWLE (T3), and immersed in 45% of SBWLE for both 10 and 20 days of storage. Whereas T2 and T3 were similar. There was a tendency that the higher concentration of BWL extract used, the lower the total microbe found.

Theoretically, total microbes in egg shell and in inner eggs are correlated to each other. They are connected through a channel pore at egg shell. In this study, total microbes in egg shell were higher than in inner egg. However, the total microbes in inner egg for eggs immersed in SBWLE were lower significantly (P<0.05) than eggs without immersed and immersed in aquadest. It was indicated that the polyphenolic content of BWL, mainly tannin played a vital role in coating and antimicrobial activity.

The low total microbes shell and inner egg in eggs SBWLE immersion was probably to be due to the content of secondary metabolites in BWL extract. These compounds acted as antimicrobials [11, 12] so that they inhibited and killed microbes found in eggshells and inner eggs. Several studies have shown that BWL extract was able to inhibit the growth of *Salmonella typhi* [20], *Enterococcus faecalis* [21], and *Staphylococcus aureus* [22] [23], as well as being able to inhibit the growth of other pathogenic bacteria.

On the other hand, the low total microbes in eggs immersed in SBWLE was presumably to be due to secondary metabolites entering the eggs and acted as antimicrobials [11, 12]. In

addition, it was hypothesized that the role of tannins coating the pores could prevent the penetration of microbial contaminants into inner eggs. The coating mechanism of tannin in the inner eggs is as follow. Tannin reacts with protein under the eggshell and then the protein is denaturized and forms coagulation [24]. Contrary, the immersion of egg in *Gnetum gnemon* leaves infuse just increased the total plate count of eggs [25]. It indicates that the use BWL is better than *Gnetum gnemon* leaf for decreasing microbial total. Based on the Indonesian National Standard (SNI) 2897-2008 [14], eggs have good microbiological quality and are safe for consumption, which contaminated by microbes less than 1×10^5 CFU/ml [14]. The results of the study indicated that the total microbial of the egg showed that it fitted to the SNI's category so it was safe for consumption.

Table 2. The average total microbes of shell and inner egg among unimmersed, immersed in distilled water, and Immersed in a solution of BWL extract

Parameters	Storage	Treatments					P
		T0	T1	T2	T3	T4	
		---------- Log CFU/mg ----------					
Egg shell	Day-10	5.93±0.01^{a}	5.94±0.01^{a}	5.84±0.02^{b}	5.82±0.04^{b}	5.74±0.02^{c}	0.00
	Day-20	6.02±0.02^{a}	6.01±0.02^{a}	5.94±0.01^{b}	5.88±0.00^{c}	5.84±0.01^{d}	0.00
		---------- Log CFU/mg ----------					
Inner eggs	Day-10	2.80±0.04^{a}	2.81±0.05^{a}	2.71±0.04^{b}	2.63±0.04^{bc}	2.56±0.03^{c}	0.00
	Day-20	2.93±0.01^{a}	2.94±0.01^{a}	2.80±0.03^{b}	2.67±0.03^{c}	2.58±0.01^{d}	0.00

The different superscript in same row indicates significant different (P<0.05); T0 = unimmersed eggs; T1 = immersed eggs in distilled water; T2 = immersed eggs in a solution of 15% (w/v) BWL extract (SBWLE); T3 = immersed eggs in 30% (w/v) SBWLE; T4 = immersed eggs in 45% (w/v) SBWLE; P = Probability

CONCLUSIONS

The immersion of layer eggs in a solution of 15-45% (w/v) BLE extract could suppress microbial growth as indicated by lower total microbes and decreased pH of eggs compared to unimmersed eggs, or immersed egg in distilled water.

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