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## Soaking Salted Eggs in Gambier Liquid Waste Inhibit bacterial Growth

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**Abstract:** Gambier liquid waste containing tannin compounds were quite high and serves as an antimicrobial agent that will tanning salted eggs so that closed the pores of the egg shell and egg to be durable. This study aims to see the effect of soaking salted eggs in gambier liquid waste remaining effective in improving the quality of salted eggs. This study used a randomized block design with three replicates and ten treatments. The treatment were, A: control (no soaking), B: Immersion 49 h with a gambier liquid waste : distilled water (1:2), C: 25 h (1:2), D: 1 h (1:2), E: 49 h (1:1), F: 25 h (1:1), G: 1 h (1:1), H: 49 h (1:0), I: 25 h (1:0), J: 1 h (1:0). The variables used were water content, bacterial colony forming and shelf life. Results of this study showed a significant ( $p < 0.05$ ) on water content, bacterial colony forming and shelf life. The best treatment inhibiting bacterial growth for longer was salted eggs soaking in gambier liquid waste : water (1:0) 1 h and 25 h with a water content of 62.67%, bacterial colony forming  $0.99 \times 10^5$  CFU  $g^{-1}$  and a shelf life 63 days.

**Key words:** Gambier liquid waste, salted eggs, tannin, bacterial colony forming and shelf life

### INTRODUCTION

Eggs including livestock food was perfect because they contain quality nutrients, rich in protein, fat, minerals, vitamins and other substances needed by the body. Egg protein was a high quality protein and is easily digested so it is best eaten by children in infancy, pregnant and nursing mothers, the elderly and people who were sick or in early stages of recovery.

Duck eggs can be made salted eggs. Preservation the natural used gambier liquid waste can also extend the shelf life of salted eggs. This was because gambier liquid waste contains tannin that function tanning eggs leather so the egg shell pores closed. As a result, the shell becomes impermeable to water and gases. So that the discharge of water and gas from the eggs could be prevented as small as possible.

The boiling process gambier leaves and twigs as 30-40 kg require as much as 24 L of water. Gambier liquid waste produced as much as 6 L. If converted gambier production years 1993 - 1995 ranged between 1, 020, 900-2, 125, 800 L, the amount of tannin in the production of gambier liquid waste ranged from 204, 180-425, 160 kg, will be wasted if not used (Agricultural Information Center of West Sumatra, 1996).

Soaking eggs in gambier liquid waste for 1 h is the best in maintaining the quality of fresh eggs for a month (Novia *et al.*, 2010). The results of the pre-study soaked salted eggs in gambier liquid waste for 4 days, have not been damaged after being stored 18 days while the stench

of duck eggs was completely gone and the colour of the egg whites do not change, remain white. This study aims to see the effect of soaking salted eggs in gambier liquid waste remaining effective in improving the quality of salted eggs.

### MATERIALS AND METHODS

**Materials and equipment:** The main materials used were duck eggs aged less than 24 h of 300 duck eggs from ducks farms anduring, Padang City. Gambier liquid waste derived from Harau subdistrict, District of Cities Fifty, as much as 37, 125 mL. Then powdered brick as much as 1800 g, ash as much as 7200 g and salt as much as 3600 g were purchased at Raya Market, Padang City. Other materials are as many as 35 325 mL of water (11 700 mL for making salted eggs and 23 625 mL of dilution gambier liquid waste). While supporting material for analysis was distilled water, filter paper, PCA powder, peptone water.

The equipment used was analytic scales, spoons stainless steel, electric oven, desiccator, porcelain bowls, mortar and pestle, clamp plate, electric stove, Erlenmeyer 250, 100 mL measuring cup, oven, hot plate, spoon sterile incubator, stopwatch, Quebec Colony Counter, measuring pipettes, petri dish, hockey sticks, micropipette and autoclave.

**Draft research:** This study was conducted with the experimental method with randomized block design with 10 treatments and 3 groups as replication. As the

treatment was long soaking salted eggs with different concentrations in gambier liquid waste were, A: control (no soaking), B: Immersion 49 h with a gambier liquid waste: distilled water (1:2), C: 25 h (1:2), D: 1 h (1:2), E: 49 h (1:1), F: 25 h (1:1), G: 1 h (1:1), H: 49 h (1:0), I: 25 h (1:0), J: 1 h (1:0).

The linear model of the design were:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \gamma_{ij}$$

Where:

- $Y_{ij}$  = Observations from experimental units treated to i, to j  
 $\mu$  = General median  
 $\alpha_i$  = Effect of treatment to i  
 $\beta_j$  = The effect group to j  
 $\gamma_{ij}$  = Influence of the rest of the experimental units treated to i and j group  
i = Treatment (A, B, C and D)  
j = Many groups (1, 2, 3, 4 and 5)

If significantly different between treatments ( $p < 0.05$ ) or highly significant different ( $p < 0.01$ ) then further tested by Duncan's Multiple Range Test (DMRT).

**Work procedures:** The procedure of making salted eggs as follows:

- Fresh duck eggs aged up to 48 h with a weight of 65-70 g, total 100, cleaned of dirt
- Salting medium consisting of: water, powdered red brick, ash and salt scrub. All the ingredients are mixed, then stir until blended where the ratio of brick, ash and salt scrub 3: 2: 1 means the brick was used 1200, 800 g of ash rubbing, 400 g of salt and 1950 mL water
- After that covered with duck eggs and brooded marinating media for 7 days
- Eggs that had stored 7 days, cover opened, washed thoroughly with water

- Salted eggs that have been cleared to provide treatment randomization done long soaking salted eggs with different concentrations in gambier liquid waste were, A: control (no soaking), B: Immersion 49 h with a gambier liquid waste: distilled water (1:2), C: 25 h (1:2), D: 1 h (1:2), E: 49 h (1:1), F: 25 h (1:1), G: 1 h (1:1), H: 49 h (1:0), I: 25 h (1:0), J: 1 h (1:0)
- Eggs that have been soaked then drained and boiled for 15 min in water that has been boiling, then removed and drained. Then stored for 7 days and be checked for shelf life to 7 days intervals until damage
- Analysis for water content, protein content using the white and yolk samples that have been homogenized

This research was conducted at the Animal Health Laboratory of Animal Husbandry Faculty andalas University at Padang on June 7, 2010 until the date of August 18, 2010.

#### Top of form

**The observations:** The observations made on salted eggs include: moisture content oven method, bacterial colony forming with Standard Plate Count method and shelf life by using sensory factors could use sensory parameters (colour, flavour, aroma and texture). Analysis of shelf life estimation according deadline deterioration could be tolerated at the onset of egg white mucus and foul aroma. Observations were made at intervals shelf life of 7 days until the rotten salted egg.

## RESULTS AND DISCUSSION

**Water content:** The results soaking salted eggs in gambier liquid waste were made after 7 days of storage/control was broken, found the average water content ranged from 62.67-70.02%. Average moisture content of salted egg in this study can be seen in Table 1.

Based on Table 1 could be seen the highest average water content contained on treatment A (control) with the average 70.07% while the lowest water content contained

Table 1: Average water content, bacterial colony forming and shelf life salted eggs soaking in gambier liquid waste

Treatment of long soaking, gambier liquid waste: distilled water	Water content (%)	Bacterial colony forming ( $1 \times 10^5$ ) (CFU g <sup>-1</sup> )	Shelf life (days)
A (control)	70.07 <sup>a</sup>	47.80 <sup>a</sup>	7.00 <sup>d</sup>
B ( 49 h, 1:2)	66.02 <sup>abc</sup>	1.51 <sup>b</sup>	42.00 <sup>c</sup>
C ( 25 h, 1:2)	67.22 <sup>abc</sup>	1.09 <sup>bc</sup>	53.67 <sup>b</sup>
D (1h, 1:2)	68.63 <sup>ab</sup>	1.50 <sup>b</sup>	53.67 <sup>b</sup>
E ( 49 h, 1:1)	67.61 <sup>abc</sup>	1.13 <sup>bc</sup>	46.67 <sup>c</sup>
F ( 25 h, 1:1)	65.72 <sup>abc</sup>	1.03 <sup>bc</sup>	46.67 <sup>c</sup>
G (1 h, 1:1)	66.00 <sup>abc</sup>	1.27 <sup>bc</sup>	46.67 <sup>c</sup>
H ( 49 h, 1:0)	64.96 <sup>bc</sup>	1.03 <sup>bc</sup>	56.00 <sup>b</sup>
I ( 25 h, 1:0)	62.95 <sup>c</sup>	0.88 <sup>c</sup>	63.00 <sup>a</sup>
J (1 h, 1:0)	62.67 <sup>c</sup>	0.99 <sup>bc</sup>	63.00 <sup>a</sup>

Mean with different superscripts indicate significantly different ( $p < 0.05$ )

in the D treatment (1 h soaking gambier liquid waste: distilled water was 1:0) with a mean of 62.67%. Water content affected significantly ( $p < 0.05$ ) by a long soaking and dilution gambier liquid waste. The shorter immersion and the lower the dilution will reduce levels of water content.

Duncan's range test results showed that the treatment A (control) did not affect significantly ( $p > 0.05$ ) on treatment B, C, D, E, F and G and the real effect to treatment H, I and J. The lowest water content contained in the treatment J (1 h immersion concentration gambier liquid waste and distilled water 1:0). During the tanning process by submersion occurs tannins derived from gambier liquid waste with eggshell membrane lining. The process of tanning causes attraction of free water between the fibers of the cell, so that the moisture content was reduced. 1 h immersion was effective tanning membrane egg grading 8.33% (data pre-research in the Laboratory of Agricultural Technology, Agriculture Faculty andalus University) wherein the low water content in this treatment. This is in line with the opinion of Ibrahim *et al.* (2005) on the diffusion process of tanning substances through the skin from the surface into the fiber woven structure, so that the free water between the fibers of the skin was out. Based on data from 0 days, A the water content of 62.17% and after treatment A faulty control shows the average water content of as much as 62.67%.

In fact no different moisture treatment A with salted eggs with all treatments except treatment A (control) and D (1 h immersion concentration gambier liquid waste and distilled water 1:2) was caused by the egg shell pores are covered with keratin protein has tanned by the tannins so the egg pores closed. Closed pores eggs thus inhibiting the circulation water, gas and microorganisms into the salted egg, water content obtained different unreal. In treatment D by 1 h immersion concentration gambier liquid waste and distilled water was 1:2 lead to penetration of tanning leather tannin immature eggs. According to Ibrahim *et al.* (2005), factors affecting the concentration of substances such as tanning and tanning substance particle size molecules. In treatment D tannin levels only a third of the treatment J (tannin content of 8.33%), so no treatment D black as well as the particle size becomes smaller, causing tannins holding capacity was reduced. Added by Ibrahim *et al.* (2005) particle size smaller tanning substances cause holding capacity was reduced but the power of penetration will increase. So time was 1 h tanning causes tanning rudimentary compared with other treatments remainder soaked in gambier liquid waste.

In treatment A (control) had the highest water content. In this treatment the egg is broken because it has undergone a change with the onset of mucus and smell. Data 0 day treatment A (control) showed levels of water and as much as 65.05% after 7 days of treatment A (control) shows the average moisture content of as much as 70.07%. Increased the water content of treatment A (control) was also due to the influence metabolism by microorganisms during storage of 7 days. According to Buckle *et al.* (2009), one of the factors that influence bacterial growth was water. Added Fardiaz (1992) that the metabolism of the bacteria using the nutrients in the food as a source of carbon and energy for growth, where the breakdown of organic substances will result in the final product in the form of water, causing the water content of food was increasing.

Wulandari (2004) resulted that salted water levels are influenced significantly by the immersion method and length of storage and the water evaporating and the amount was determined by the diameter of the pores of the egg shell. Added by Novia and Andalusia (2011) in the process of soaking salted onion skin extract for 1-7 days generate 67.51-67.09% moisture content was measured after control was broken. According to Lai *et al.* (2010) water content white and yolk eggs were decline of 8 and 7% to salted eggs for 3 weeks. Research (Kaewmanee *et al.*, 2011) The longer the marinating process, an increase in water content and a decrease for salted eggs cooked in egg yolk and white.

**Bacterial colony forming:** The results soaking salted eggs in gambier liquid waste were made after 7 days of storage at the control corrupt or average bacterial colony forming obtained ranged from  $8.80 \times 10^2$ - $47.80 \times 10^5$  CFU g<sup>-1</sup>. Bacterial colony forming significantly influenced by long soaking and gambier liquid waste and dilution water, the shorter the time of immersion and the more concentrated gambier liquid waste would reduce bacterial colony forming.

Bacterial colony forming lowest was in treatment I (25 h soaking in gambier liquid waste and distilled water 1: 0). Data 0 days treatment G showed bacterial colony forming  $1.04 \times 10^3$  CFU g<sup>-1</sup> after 7 days was  $0.88 \times 10^5$  CFU g<sup>-1</sup>. I was the optimal treatment as indicated by the bacterial colony forming least. Based on the pre-research content of tannins in gambier liquid waste was as much as 8.33%. Tannin serves as a tanner eggs which could inhibit the activity of microorganisms from the outside. According Zulaekah and Widyaningsih (2005) contains tannins phenol compounds that are antibacterial and functions cover the pores of the egg shell that inhibits the entry of microorganisms into the egg.

Unlike no real treatment I (25 h soaking in gambier liquid waste with water and distilled water 1:0) with E, F, G, H and J the bacterial colony forming caused by water immersion treatment was done gambier liquid waste gambier liquid waste with time not too long with a high tannin content. This maintains durability salted eggs produced, bringing the bacterial colony forming generated in the range  $0.49 \times 10^5$  CFU  $g^{-1}$  until the  $1.55 \times 10^5$  CFU  $g^{-1}$ , where the range was still safe to eat.

Data 0 day treatment A (control) showed bacterial colony forming  $1.12 \times 10^3$  CFU  $g^{-1}$  and after treatment of a broken control showed bacterial colony forming  $47.80 \times 10^5$  CFU  $g^{-1}$  in which the pores of the egg shell was not covered by the tannins resulting in the evaporation of water, gas and air, facilitate the entry of bacteria into the egg and lead to increased activity of microorganisms compared with treatment using a solution of gambier liquid waste. The results of Novia *et al.* (2011) that salted eggs soaking in onion skin solution that the longer it would increase the bacterial colony forming produced. Immersion 1-7 days of data showed bacterial colony forming between  $0.49 \times 10^5$ - $1.55 \times 10^5$  CFU  $g^{-1}$  were calculated after control was broken.

**Shelf life:** The results soaking salted egg in gambier liquid waste were made after 7 days of storage/control foul, obtained averaging 7-63 days. Salted egg shelf life significantly influenced by long soaking and dilutions were performed. The shorter and more intense immersion gambier liquid waste would extend the salted eggs shelf life.

The test results further showed that treatment J (1 h soaking in gambier liquid waste and distilled water 1: 2) do not differ significantly with treatment I (25 h immersion in gambier liquid waste and distilled water 1: 0). This shows that the treatment I and J by using gambier liquid waste without more effective for curing salted eggs. This was because the tannins contained in gambier liquid waste able to tan the skin pores eggs well and maintain the quality salted eggs. In accordance with the opinion Zulaekah and Widyaningsih (2005) that compounds containing tannins are phenol compounds as anti-bacterial that can inhibit the growth of bacteria that food products be durable.

Based on Table 1 can be seen the highest average shelf life were on treatment J (1 h immersion in gambier liquid waste and distilled water 1:0) and I (25 h immersion in gambier liquid waste and distilled water 1:0) with averaging 63.00 days while the lowest shelf contained on treatment A (control) with the average 7.00.

While the results of the study Novia *et al.* (2011) making oven salted eggs way to 70°C temperature, 6 h, broke down after 25 days of storage. Added by Novia *et al.* (2012) smoke salted egg was still in good condition after 37 days of storage, characterized by the absence of changes in protein and fat.

## CONCLUSION

With the effects of long soaking salted eggs in gambier liquid waste showed significant effect on the moisture content, bacterial colony forming and shelf life. The best treatment was contained in 1 h soaking in gambier liquid waste: distilled water (1:0), with a water content of 62.67%, bacterial colony forming  $0.99 \times 10^5$  CFU  $g^{-1}$  and a shelf life of 63 days.

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