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The Physicochemical and Microbiological Quality of Pegagan Duck Eggs Immersed with Duku Fruit Peel Solution in Different Storage Period

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ABSTRACT

This study aims to determine the effect of immersing in duku fruit peel solution on physicochemical and microbiological quality of Pegagan duck eggs during storage at room temperature. This study used a complete randomized design (CRD) with a 5x3 factorial patterned. The first factor was the immersion time consisting of 5 levels, namely 0, 15, 30, 45, and 60 min. The second factor was the storage time which includes 3 levels, which were 0, 7 and 14 d. The replication used was 3 times. The observed variables were physicochemical and microbiological quality of egg, including albumen index (AI), yolk index (YI), Haugh unit (HU), moisture content (MC), protein content (PC), fat content (FC),total microbes (TM) and antioxidant activity (AA). The data were processed by analysis of variance, continued with Duncan's multiple range test (DMRT). The results showed that there was a significant interaction ($P<0.05$) between immersion time and storage time on AI, YI, HU, MC, PC, TM, and AA. Futhermore, the difference of immersion time and storage period was also significantly influenced ($P<0.05$) to all observed variables including AI, YI, HU, MC, PC, TM, and AA of duck eggs.It can be concluded that the immersion process for 60 min showed the best results on the physical, chemical, and microbiological qualities of Pegagan duck eggs up to 14 d of storage time.

Keywords: Duku fruit peel solution, Microbiology, Pegagan duck's egg, Physicochemistry, Preservation

Introduction

Pegagan duck is one type of duck originating from South Sumatra, which is mostly maintained by farmers as egg producers. Eggs are known as poultry products that are much favored by consumers because besides their low prices, eggs are also rich in nutrients. However, eggs are quite susceptible to deterioration in quality both physically and chemically, especially when they are stored at room temperature. Some facts show that there are several factors that are thought to cause egg quality to decline rapidly, such as the occurrence of contamination by microorganisms, a physical decline in egg quality, and the release of water and gases from the egg during storage (Yosi *et al.*, 2016). Related to this, efforts are needed to prevent a decrease in the quality of duck eggs during storage, one of which is the preservation process. Preservation aims to protect eggs from damage or decay by microorganisms and to extend the storage period (Yosi *et al.*, 2017). Preservation can be done by adding eggshell coating material. One of the potential coatings to use is duku fruit peel solution. It is believed that duku fruit peel solution contains a variety of active compounds that can eliminate microbes and acts as antioxidants that can increase the durability of food products.

Duku fruit, *Lansium domesticum*, is one type of seasonal fruit that is quite widely grown in South Sumatra. It was reported that the solutions of duku fruit peel were positively contained in several chemical compounds such as tannins, phenol, saponins, terpenoids, alkaloids, and flavonoids (Isfaeni *et al.,* 2012). Tannin is a complex compound in the form of a mixture of polyphenols and amorphous which has a function as tanner material that prolongs the shelf life of eggs, while phenol, alkaloids, saponins, flavonoids, and terpenoids are included in secondary metabolites that function as antioxidants to stabilize free radicals (Yuhernita and Juniarti, 2011). In addition, phenolic and terpenoid compounds are also known as substances that act as antimicrobial compounds. So far, duku fruit peel extract is more widely used as a natural insecticide (Putranta and Wijaya, 2017), while information about its use in egg preservation is still limited. Therefore, new research is needed to determine the effect of duku fruit peel solution on physicochemical and

microbiological qualities during storage at room temperature.

Materials and Methods

Materials

The materials used in the study were 135 fresh eggs of Pegagan duck (aged 1-2 d), aquades, duku fruit peel (*Lansium domesticum*), mixed indicator (bromocresol green (BCG) + methyl Red (MR) + 96% alcohol), hexane solution, mixed catalyst ($CuSO_4$: K_2SO_4), NaOH 40%, concentrated H_2SO_4, 0.1 N H_2SO_4, 0.1 N NaOH, while the tools used including plastic buckets, blenders, filters, plastic baskets, thermometers, analytical scale, spatulas, glass bases, erlenmeyer, porcelain cups, desiccators, ovens, pincers, kjedhal distillation, beaker glass, filter paper, distillation flask, stirring rods, heaters, squash destruction, soxhlet tools, aluminum foil, colony counters, petri dish, test tube, autoclaf, laminar air flow, incubator, tip pipette, and cotton.

Methods

Experimental design. This study used a 5x3xfactorial complete randomized design. The first factor (A) is the time for egg immersion consisting of A0, A1, A2, A3, and A4, namely the soaking time of the eggs for 0, 15, 30, 45, and 60 min, respectively. Meanwhile, the second factor (B) is the length of storage of eggs consisting of B0, B1, and B2, namely the length of storage of eggs for 0, 7, and 14 d, respectively.

The process of making duku fruit peel solution. This process refers to Hajrawati *et al*. (2012) with some modifications. In the initial stage, the fresh peel of the duku fruit was cut to a smaller size and then agitated for 24 h. The duku fruit peel was then weighed at 50% (b / v) or as much as 2.25 kg and added with 4,500 ml of distilled water at 50-60ºC. Next, the two ingredients were blended and filtered to obtain the filtrate as a solution of duku fruit peel. Duku fruit peel solution was then taken to the laboratory for phytochemical analysis. The results of laboratory analysis showed that the solution of duku fruit peel positively contained alkaloids, phenols, and tannins, wherein the tannin concentration of 0.5%.

Preparation and immersion process of eggs. Egg samples were chosen with almost the same size and weight. The eggs were then washed with running water and drained in a plastic basket. Furthermore, the duku fruit peel solution was placed into a basin and all eggs were immersed in the solution with soaking time according to each treatment (0, 15, 30, 45, and 60 min). The eggs were drained for a few minutes and then stored with a storage time of 0, 7, and 14 d according to the treatment.

Measured variables. The variables observed were physical qualities including albumin index, yolk index, and Haugh unit according to Yosi *et al*. (2016); chemical qualities including moisture content, protein content, and fat content refer to AOAC (2005); antioxidant activity with the DPPH (diphenylpicrylhydrazyl) method; and total microbes according to AOAC (2005).

Statistical analysis. Data were processed by analysis of variance, then it will be continued with Duncan's multiple range test if there were differences between treatments at a probability of 5%. Data analysis was assisted by SPSS 17 statistical software.

Result and Discussion

The physical qualities of egg

Albumen index. Table 1 indicated that there was a significant interaction ($P<0.05$) between the time and storage period to the albumen index (AI) of duck eggs. It was known that immersion time starting from 60 min could significantly maintain the AI during the storage period. However, the AI significantly decreased with the longer storage period even though the eggs were soaked for up to 60 min.The difference of immersion time and storage period was also significantly influenced ($P<0.05$) to AI of duck eggs. Furthermore, it can be seen that there was no difference in AI between the immersion time of 0 to 45 min, which ranges from 0.090-0.094, but there was a significant increase in AI after being immersed for 60 min, which was to 0.101. This showedthat soaking eggs up to 45 min had not been able to optimize the role of tannins contained in eggs in inhibiting the process of evaporation of gas and water during storage at room temperature. This was as reported by Karmila *et al.* (2008) that tannin will react with proteins in eggshells to form brown deposits that function as egg tanners and are impermeable to water and gases such as carbon dioxide. It is clear that the longer the eggs are stored, the AI becomes lower, where the lowest AI occurs at 14 d of storage ie 0.05 or down by 61.54% from 0 d of storage. The decrease in AI was thought to be due to damage to the β-ovomucin bond during storage which resulted in the albumen becoming runny. This is as stated by Azizah *et al.* (2018) that ovomucin consists of α-ovomucin and β-ovomucin, where β-ovomucin is the most influential on albumen dilution. In line with this, Riawan *et al.* (2017) and Saraswati (2015) also reported that the longer the storage, then the higher the decrease of thick albumen and finally the AI becomes lower. Overall, the average AI of duck eggs stored up to 14 d is still good, which is around 0.05-013. This was as stated by Koswara (2009) that the AI value of eggs in good conditions ranged from 0.05-0.17.

Yolk index. The results showed that the interaction between the immersion time and storage period was significant ($P<0.05$) in affecting the yolk index (YI) of duck eggs. It was seen that immersion time starting from 30 min had been able to significantly maintain the YI during the storage period. However, the longer storage time caused YI to drop significantly even though the eggs were immersed for 60 min.Both the difference in the immersion time and storage time

Table 1. The mean of albumen index, yolk index, and haugh unit in Pegagan duck eggs with different immersion time and storage period

Variables	Immersion time (min)	Storage time (d)			Mean
		0	7	14	
Albumen index*	0	0.14±0.01	0.09±0.01	0.04±0.01	0.090±0.04[a]
	15	0.12±0.01	0.11±0.01	0.05±0.01	0.094±0.03[a]
	30	0.13±0.01	0.09±0.01	0.05±0.01	0.091±0.03[a]
	45	0.11±0.01	0.10±0.01	0.07±0.01	0.091±0.02[a]
	60	0.14±0.01	0.11±0.01	0.05±0.01	0.101±0.04[b]
	Mean	0.13±0.01[c]	0.10±0.01[b]	0.05±0.01[a]	
Yolk index*	0	0.40±0.01	0.30±0.04	0.15±0.06	0.29±0.12[a]
	15	0.42±0.01	0.33±0.01	0.10±0.03	0.28±0.15[a]
	30	0.43±0.02	0.39±0.02	0.12±0.02	0.31±0.15[b]
	45	0.43±0.01	0.39±0.03	0.18±0.02	0.33±0.12[b]
	60	0.40±0.02	0.34±0.01	0.21±0.01	0.32±0.08[b]
	Mean	0.42±0.02[c]	0.35±0.04[b]	0.15±0.05[a]	
Haugh unit*	0	95.85±1.14	83.49±0.84	57.26±3.25	78.87±17.16[a]
	15	93.33±2.42	88.88±1.37	57.85±4.32	80.02±16.94[a]
	30	91.30±0.90	82.21±2.48	66.87±5.28	80.13±11.09[a]
	45	83.87±3.49	84.89±3.52	71.82±2.56	80.20 ± 6.88[a]
	60	96.55±1.40	86.74±1.18	65.78±3.32	83.02±13.74[b]
	Mean	92.18±5.04[c]	85.24±3.03[b]	63.92±6.64[a]	

ns non-significant interaction, * significant interaction
[a,b,c] different superscripts at the same column/row indicate significant different (P<0.05).

had also significant effect (P<0.05) on the YI of duck eggs (Table 1).

Furthermore, immersion up to 15 min had not shown a significant change in YI, but starting 30 min had shown a significant increase in YI. However, the immersion time from 30 to 60 min did not show the difference in YI of duck eggs, which was between 0.31-0.33. This clearly showed that the soaking time needed to significantly increase YI is two times faster than AI. The tannin contained in the duku fruit peel solution is believed to be able to inhibit the evaporation of gases and keep the vitelline membrane from being damaged so that the YI is still maintained. This was as reported by Soeparno (2011) that YI was determined by the condition of the vitelline membrane, if a lot of water moves into the yolk, this will cause the yolk diameter to become wider, which in turn can damage the vitelline membrane. Similar to AI, the data shows that the high reduction in YI is in line with the longer storage of eggs. The decrease in YI is caused by the evaporation of CO_2 and H_2O from the eggs during storage, which forms the bond of the ovomucin-lysozyme complex so that the albumen becomes runnier. This then causes the process of transferring water from albumin to yolk. The displacement of water is due to the greater yolk osmotic pressure than the albumen so that the water from albumin moves to yolk (Soeparno, 2011). The same was reported by Monira *et al.* (2003) and Miles and Henry (2004) that there was a significant decrease in YI during egg storage. Based on the average of YI, egg storage up to 7 d is still considered good, which is in the range of 0.30-0.39. This is as stated by Lestari *et al.* (2013) that the normal YI is around 0.33-0.50.

Haugh unit. There was a significant interaction (P<0.05) between the difference of immersion time and storage time on Haugh unit (HU) of the duck egg. It was observed that the immersion time starting from 60 min in the storage period was able to significantly maintain the value of HU, while HU continued to decrease with increasing storage time even though the egg had been soaked for the longest time of 60 min.Furthermore, the value of HU in eggs was also markedly influenced by both the soaking time and storage time (Table 1). It is clear that a significant increase in HU occurred at 60 min soaking time. This pattern is in line with what happened to AI. Basically, between HU and AI has a positive correlation. This is because HU is strongly influenced by the albumen, especially the height of the thick albumen. This is in line with Azizah *et al.* (2018) that the higher the thick albumen then the higher the HU, where the height of albumen is affected by the content of ovomucin. Regarding storage time, it is clear that the storage time is very significantly affecting the value of HU, where the longer the storage, the lower the value of HU. The decreasing viscosity of albumen causes a lower value of HU. This is as reported by Riawan *et al.* (2017) that the decrease in egg white viscosity was mainly due to changes in gel structure and physicochemical damage of ovomucin fibers, which in turn can reduce HU. There was a significant reduction in HU from 0 to 14 d, which was almost 31%. Nevertheless, the quality of duck eggs stored up to 14 d is still relatively good, where HU at storage up to 7 and 14 d is classified as AA grade (85.24%) and grade A (63.92%), respectively. This is as stated by Soeparno (2011) that eggs with HU are more than 72 and between 60-72 are included in AA and A grades, respectively.

The chemical quality of egg

Moisture content. The results showed that the interaction between the immersion time and storage time was significant (P<0.05) in influencing the moisture content (MC) of Pegagan duck eggs. It was observed that the value of MC continued to drop significantly in line with the length of the storage period even though the eggs had been soaked for 60 min. Meanwhile, the soaking time starting from 45 min had been able

to significantly reduce the evaporation process and maintain the MC in the egg during the storage period. Both the difference in the immersion time and storage time have also significant effect (P<0.05) on the MC of duck eggs (Table 2).

Furthermore, it was seen that immersion for 15 min had not been able to influence MC in duck eggs, but after 30 min it showed a significant change in MC and this change continued until the immersion time was 60 min, but the soaking time between 45 and 60 min did not show significant differences in MC, which ranged from 61.19 to 61.26%. The change in MC of duck eggs is due to the role of tannins from the solution of duku fruit peel which is able to work as tanners on eggshells so that it can inhibit the evaporation of CO2 in the egg. By inhibiting the evaporation of CO2, the egg structure of both yolk and albumen can be maintained and the formation of fluids can be inhibited. This is in line with the statement of Azizah *et al*. (2018) that tannin will form a layer on eggshells which can inhibit CO2 evaporation and prevent a decrease in the viscosity of egg contents. Regarding the length of storage, it seems clear that the longer storage time caused a significant increase in MC, which was from 36.35% at 0 d to 37.54% and 38.27% at 7 and 14 d of storage, respectively. The increase in MC during storage is due to reduced carbonate ions by evaporation of carbon dioxide gas. This condition will then affect the pH and buffer capacity of duck eggs so that the contents of the eggs, eventually, become runny. This is as reported by Saraswati (2015) that if the eggs are stored too long, the pH of the egg will increase due to CO2 evaporation, consequently the egg's buffer capacity will decrease and the albumen becomes more watery.

Protein content. Table 2 showed that there was a significant interaction (P<0.05) between the difference of immersion time and storage time on protein content (PC) of duck eggs. It was observed that the PC had been able to be maintained during the storage period with a soaking time starting from 30 min. However, the value of PC continued to decline significantly along with the increase in storage time even though the eggs had been immersed for 60 min The difference of soaking time and storage period was also significantly influenced (P<0.05) to the PC of Pegagan duck eggs. Data indicated that immersion up to 15 min had not been able to provide a significant difference to PC in duck eggs with an average of 10.12-10.41%. However, after being immersed for 30 min, there was a significant increase in the PC. In addition, the duration of immersion between 30, 45 and 60 min did not indicate a difference in the PC value of duck eggs, which was between 10.12 and 10.41%. This indicated that the significant effect of tannin contained in the solution to the egg PC occurs after 30 min of immersion. This significant effect is caused by the tannin being able to reduce the process of reforming organic matter in eggs, one of which is protein. This is as reported by Yosi *et al.* (2017) that tannins were very important because they could act as natural tanners that were able to protect organic matter in eggs from the degradation process during storage. Furthermore, it is known that the PC value of duck eggs was lower when it was stored for longer at room temperature. It was noted that there had been a decrease of 47.12% on d 14 of storage. The decline in PC value is due to the degradation of proteins during storage which can form a number of gases in the evaporation process. This is supported by the statement of Azizah *et al.* (2018) that there was a decrease in egg protein content during storage caused by decomposition and hydrolysis of proteins.

Fat content. The results indicated that the interaction between the immersion time and storage duration was not significant (P>0.05) in affecting the fat content (FC) of Pegagan duck eggs. However, both the soaking time and storage time have a significant effect (P<0.05) on the FC of duck eggs (Table 2). In general, it is known that FC in duck eggs without immersion was higher

Table 2. The mean of moisture content, protein content, and fat content of Pegagan duck eggs with different immersion time and storage duration

Variables	Immersion time (min)	Storage time (d)			Mean
		0	7	14	
Moisture content (%)*	0	63.45±1.06	68.84±0.33	66.35±1.06	66.21±2.47^{c}
	15	61.10±0.55	70.48±1.14	68.73±1.11	66.77±4.40^{c}
	30	62.08±0.57	67.57±0.52	66.21±0.64	65.29±2.52^{b}
	45	51.77±0.78	64.38±1.18	67.64±0.60	61.26±7.30^{a}
	60	53.19±0.65	62.32±0.54	68.05±0.48	61.19±6.51^{a}
	Mean	58.32±5.06^{a}	66.72±3.15^{b}	67.40±1.22^{c}	
Protein content (%)*	0	12.65±0.51	8.48±1.02	7.06±0.96	9.40±2.63^{a}
	15	12.83±0.55	8.59±1.16	5.70±0.44	9.04±3.18^{a}
	30	13.09±0.29	10.18±0.37	7.08±0.57	10.12±2.63^{b}
	45	12.83±0.44	10.93±0.51	7.46±0.35	10.41±2.39^{b}
	60	12.91±0.55	10.94±0.26	6.70±0.41	10.18±2.77^{b}
	Mean	12.86±0.43^{c}	9.83±1.29^{b}	6.80±0.80^{a}	
Fat content (%)^{ns}	0	36.68±1.16	39.09±0.61	39.11±1.75	38.29±1.63^{b}
	15	36.49±0.50	37.40±0.49	38.43±0.10	37.44±0.91^{a}
	30	36.12±1.17	36.95±0.70	37.34±0.05	36.80±0.65^{a}
	45	36.30±1.78	37.18±0.27	38.41±0.18	37.30±1.29^{a}
	60	36.14±0.45	37.08±1.12	38.04±0.20	37.09±1.03^{a}
	Mean	36.35±0.87^{a}	37.54±1.00^{b}	38.27±0.90^{c}	

ns non-significant interaction, * significant interaction
^{a,b,c} different superscripts at the same column/row indicate significant different (P<0.05).

Table 3. The mean of total microbes and antioxidant activity of Pegagan duck eggs with different immersion time and storage period

Variables	Immersion time (min)	Storage time (d)			Mean
		0	7	14	
Total microbes (10^5 CFU/g)*	0	0.34±0.07	1.80±0.25	4.03±0.57	2.06±1.64^{c}
	15	0.32±0.02	1.35±0.04	4.33±0.30	2.00±1.81^{bc}
	30	0.34±0.04	1.38±0.12	3.82±0.11	1.85±1.55^{b}
	45	0.24±0.07	0.95±0.07	3.30±0.17	1.49±1.39^{a}
	60	0.31±0.06	0.86±0.07	2.81±0.12	1.33±1.14^{a}
	Mean	0.31±0.06^{a}	1.27±0.37^{b}	3.66±0.62^{c}	
Antioxidant (IC_{50}) (%)*	0	4.85±0.14	4.70±0.07	4.72±0.16	4.76±0.13^{b}
	15	2.49±0.06	3.64±0.14	4.79±0.15	3.64±1.00^{a}
	30	2.44±0.15	3.60±0.12	4.75±0.06	3.60±1.01^{a}
	45	2.49±0.05	3.65±0.14	4.80±0.08	3.65±1.00^{a}
	60	2.65±0.09	3.74±0.10	4.63±0.06	3.67±0.86^{a}
	Mean	2.98±0.97^{a}	3.87±0.45^{b}	4.74±0.11^{c}	

ns non-significant interaction, * significant interaction
^{a,b,c} different superscripts at the same column/row indicate significant different (P<0.05)

than those with immersion, while among various immersion periods does not show a difference in FC values, which ranged from 36.80-37.44%. The low value of FC in immersion treatment is because the solution of duku fruit peel contains phenol compounds as antioxidants which can inhibit the formation of oxidative products due to the process of fat oxidation. This is in line with Ahmad *et al*. (2012) and Handayani *et al.* (2014) that antioxidants are compounds that can prevent lipid oxidation and the occurrence of free radical reactions in the oxidation of lipids. Furthermore, it was also noted that the longer storage time caused the FC of duck eggs to increase significantly. This increase is suspected because, during storage, there is an oxidation process of unsaturated fatty acids in eggs that produce many oxidative products such as fat peroxide.

The microbiological quality of egg

Total microbes. There was a significant interaction (P<0.05) between the difference of immersion time and storage period to total microbes (TM) of duck eggs. It was known that microbial growth was significantly inhibited during the storage period with immersion time starting from 30 min, but the lowest TM was obtained when immersed for 60 min. Meanwhile, along with the increase in storage period, TM continued to increase significantly even though the eggs had been immersed for 60 min.Furthermore, the value of TM in duck egg was markedly (P<0.05) influenced both by the immersion time and the storage period (Table 3).

Data indicate that duck eggs soaked for up to 15 minutes using duku peel solution have not been able to show different results on TM. However, immersion starting from 30 min had been able to reduce TM significantly and this decline continues with soaking for 45 and 60 min, which was between 1.33-1.49x10^5 CFU/g. The reduction in TM in eggs is inseparable from the role of several chemical compounds such as alkaloids and phenols contained in solutions, which act as antimicrobials in suppressing the growth of microorganisms that can reduce egg quality. This is in line with the statement of Silva and Junior (2010) that phenol compounds play an important role in destroying bacterial cell structures and damaging bacterial cell walls. This indicated that the optimal activity of antimicrobial compounds in solution decreased along with storage time. Related to this, Prihharsanti (2009) also reported that TM in food products would increase even though they were stored in low temperatures.

Antioxidant activity. The results indicated that the interaction between the immersion time and storage period was significant (P<0.05) in affecting the antioxidant activity (AA) of duck eggs. It was observed that immersion time starting from 15 min had significantly increased the AA during storage. Meanwhile, along with the increase in storage period, the AA continued to decline significantly to the lowest activity at 14 d which was marked by an increase in IC_{50} even though the eggs had been immersed for 60 min.Both the difference in the immersion time and storage time had also significant effect (P<0.05) on the AA of duck eggs (Table 3). Overall, the data showed that eggs immersed in the duku peel solution had a lower IC_{50} value than those without immersion, but there were no significant differences in IC_{50} values in eggs soaked with different immersion times, which ranged from 3.60 to 3.67%. This clearly indicated that the eggs with immersion treatment had higher antioxidant activity. This increased antioxidant activity is due to the role of several chemical compounds that function as antioxidants such as phenols, alkaloids, saponins, flavonoids, and terpenoids contained in duku fruit peel solutions in order to stabilize free radicals. It was known that the smaller the IC_{50} value, the higher the antioxidant ability (Mu'addimah *et al.*, 2015). This is because the IC50 is closely related to concentration and inversely proportional to the high antioxidant activity. Furthermore, it was seen that the antioxidant activity of eggs continued to decrease along with the longer storage time, which was marked by a increase in IC_{50}. This increase showed that the phenol activity contained in the solution as an antioxidant becomes decreased.

Conclusions

It was concluded that there were significant interactions between immersion time and storage

time on the albumen index, yolk index, Haugh unit, water content, protein content, microbial total, and antioxidant activity. Furthermore, the immersion process for 60 min showed the best results on the physical, chemical, and microbiological qualities of Pegagan duck eggs up to 14 d of storage time.

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