

The Effect of Soaking in Clove (*Syzygium aromaticum* L.) Leaf and Storage Duration on Layer Chicken Egg Quality

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Abstract— The experiment was conducted to study the effect of soaking in clove leaf extract at various concentrations and storage duration on layer chicken egg quality. The experiment was conducted from April 15th 2024 to June 3rd 2024, at the Nutrition and Animal Product Technology Laboratory, University of Mercu Buana Yogyakarta. The experimental design used a completely randomized design with a 4x4 factorial pattern with three replications in every combination. Soaking treatment in clove leaf extract at concentration of 0%, 10%, 20%, and 30%, combined with storage duration of 1 day, 11 days, 21 days, and 31 days, with each combination replicated three times. The materials used in this study were 48 eggs, 1.44 kg of clove leaves, and 9.6 liters of distilled water. The variable tested included egg weight loss, air sac value, albumen index, yolk index, and Haugh Unit. The collected data were analyzed using Analysis of Variance (ANOVA), and if there were significant differences continued by Duncan's New Multiple Range Test (DMRT). The results showed that soaking in clove leaf extract inhibited egg weight loss, reduced the air sac value, and increased the albumen index, yolk index, and Haugh Unit during storage. Based on the result of the experiment, it was concluded that there was a significant interaction between soaking in clove leaf extract at various concentrations and storage duration on layer chicken egg quality. Soaking in a 20% clove leaf extract extended the shelf life of egg up to 31 days.

Keywords— Clove leaf, egg quality, layer egg, storage duration.

I. INTRODUCTION

Layer chicken egg is one of the most popular poultry product and has high nutritional value. The egg can be utilized as a basic ingredient in processing a variety of foods due to its functional properties. However, the superiority of the egg as a nutrient-rich livestock product also presents a challenge because it is perishable. The egg has a short shelf life, lasting only up to two weeks at most (Evanuarini et al, 2021).

After 2 weeks, egg undergoes changes that lead to quality degradation. In principle, the degradation of egg quality during storage is the change in albumen viscosity (albumen becomes runny), yolk enlargement due to the movement of H₂O, CO₂, and O₂ in and out of the shell. Additionally, air pockets within the egg enlarge over time. This enlargement of air pockets can result in the absorption of off-odors and off-flavors. Therefore, the egg requires special treatment to extend its shelf life.

One special treatment for extending the shelf life of the egg is preservation using natural vegetable tanning agents, such as clove leaves. The clove (*Syzygium aromaticum* L.) plant is an agricultural commodity widely cultivated in various

regions of Indonesia. Clove plant is mostly only utilized in the flower or stalk part of the clove while the clove leaf is considered waste. Screening study by Madubuike et al (2018) showed that clove leaves contain a number of phytochemical compounds such as contain phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids. Clove leaf material has the potential to be used as a vegetable tanning agent for the preservation of layer chicken eggs.

II. MATERIALS AND METHODS

A. Materials and Tools

This experiments's materials include 48 eggs, 1.44 kg of clove leaves, and 9.6 liters of distilled water. The tools include stationery, digital scales, 0.05 mm caliper, depth micrometer, egg tray, yolk separator, knife, cutting board, plastic container, hand gloves, filter paper, measuring cups, dry tissue and glass plate.

B. Method

The research method used was experimental research. The experiment was conducted on layer chicken eggs. The initial stage was the preparation of tools and materials, including the preparation of eggs and clove leaf extract. Furthermore, various treatments were carried out in the form of soaking of broiler eggs in clove leaf extract at different concentrations for 24 hours. Eggs were stored at room temperature for 31 days and observations on egg quality were made on days 1, 11, 21, and 31.

The research design was a completely randomized design (CRD) with a 4x4 factorial pattern. This included soaking the eggs in clove leaf extract at concentrations of 0%, 10%, 20%, and 30%, with storage times of 1, 11, 21, and 31 days Each combination has 3 replication The variables observed in this study were egg weight loss, air sac value, albumen index, yolk index, and Haugh Unit value.

The observation data were analyzed using ANOVA (Analysis of Variance). If the results of the analysis of variance showed significant differences between the treatments, then further tests were carried out to determine which treatment pairs were significantly different, namely with Duncan's New Multiple Range Test (DMRT) (Mardinata, 2013).

III. RESULT AND DISCUSSION

A. Egg Weight Loss

The average weight loss of eggs treated by soaking in clove leaf extract at various concentrations and for different storage durations can be seen in Table 1.

TABLE 1. Average of egg weight loss (%)

Storage Duration	Concentrations of Clove Leaf Extract				Average
	0%	10%	20%	30%	
1 day	0,00 ^p	0,00 ^p	0,00 ^p	0,00 ^p	0,0 ^a
11 days	3,19 ^{qr}	2,60 ^q	1,03 ^{pq}	1,58 ^{pq}	2,1 ^b
21 days	5,39 ^r	2,61 ^q	4,18 ^{qr}	4,29 ^{qr}	4,1 ^c
31 days	8,35 ^s	8,17 ^s	4,33 ^{qr}	5,90 ^r	6,7 ^d
Average	4,2 ^b	3,3 ^{ab}	2,4 ^a	2,9 ^a	

Notes: Average values with different superscripts in the same row and column indicate significant differences (P<0.05). There is a significant interaction (P<0.05) between the concentration of soaking in clove leaf extract and storage duration

The 0%, 10%, 20%, and 30% concentrations on day 1 of storage exhibited no significant difference in egg weight loss compared to the 20% and 30% concentrations on day 11 of storage. However, a significant difference was observed when compared with the 0% and 10% concentrations on day 11, and all concentrations on storage days ≥21. This result could be attributed to the continuous evaporation of water and gases during storage, leading to egg weight reduction. According to Thohari (2018), the decrease in egg weight was due to the evaporation of water and gasses such as CO₂, NH₃, and H₂S, with most of the evaporated gasses originating from the breakdown of organic materials within the egg.

No significant differences were observed at a concentration of 20% on days 11, 21, and 31 of storage, and at 30% on days 11 and 21 of storage. However, 20% and 30% concentrations showed significant differences compared to the 0% and 10% concentrations at 31 days of storage. These results indicate that soaking in clove leaf extract at concentrations of 20% and 30% could maintain egg weight compared to the control. This was likely due to the tannins in the clove leaf extract at 20% and 30% concentrations, which may coat the pores of the eggshell, reducing water and gas loss and prevented microbial entry during storage. Clove leaves also contain other compounds such as saponins, which have surfactant properties, that may aid in reducing evaporation by forming a layer that diminishes water diffusion from inside the egg to the outside. Saponins could act as antibacterial agents killing bacteria on the eggshell and, preventing them from entering and damaging the egg contents.

Soaking in a 10% concentration of clove leaf extract did not produce results significantly different from the control after 31 days of storage. This was probably because the 10% concentration of clove leaves was not sufficient to cover the pores of the eggs, thus failing to prevent the release of water and gas from the egg or the entry of microbes into the egg. This finding is consistent with Sari (2023), who suggested that egg weight loss is caused by microbial contamination, leading to egg deterioration. Microorganisms degrade some of the egg components, particularly the egg white, causing it to become more fluid and accelerating the evaporation of water and gases

like CO₂, NH₃, N₂, and H₂S.

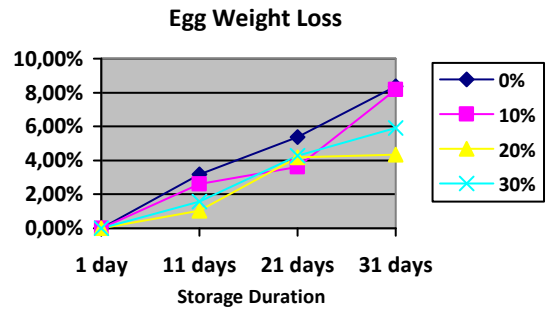


Fig. 1. Graph of average egg weight loss (%)

Figure 1 showed the graph of the effect of soaking in clove leaf extract and storage duration on egg weight loss. On day 1 of storage, there was no decrease in egg weight observed for eggs that were either not soaked or soaked in clove leaf extract at concentrations of 10%, 20%, and 30%. As storage duration increased, a decrease in egg weight was observed, with notable differences between the 20% and 30% clove leaf extracts compared to the 10% concentration and control. Soaking in clove leaf extracts at 20% and 30% concentration was more effective at maintaining egg weight than the 10% concentration and control, as reflected by the lowest decrease in egg weight during storage up to 31 days.

B. Air Sac Value

The average air sac value of eggs treated by soaking in clove leaf extract at various concentrations and for different storage durations could be seen in Table 2.

TABLE 2. Average of air sac value (mm)

Storage Duration	Concentrations of Clove Leaf Extract				Average
	0%	10%	20%	30%	
1 day	0,21	0,21	0,21	0,21	0,21 ^a
11 days	0,34	0,37	0,33	0,33	0,34 ^b
21 days	0,45	0,41	0,41	0,43	0,42 ^c
31 days	0,53	0,53	0,46	0,50	0,50 ^d
Average	0,38 ^b	0,38 ^b	0,35 ^a	0,36 ^{ab}	

Notes: Average values with different superscripts in the same row or column indicate significant differences (P<0.05).

Analysis of variance revealed that soaking in clove leaf extract and storage duration had a significant effect (P<0.05) on the air sac value of eggs. No significant interaction (P>0.05) was found between soaking in clove leaf extract and storage duration regarding air sac value.

The average results obtained during storage of 1, 11, 21, and 31 days showed significant differences in air sac value (P<0.05). This may be due to the enlargement of the air sac during storage, in line with the reduction of water due to evaporation. The air pocket in the egg enlarges due to water loss during storage and fills with gas. According with Soewarno (2013), the air cell that forms in the blunt end of the eggshell was filled with gasses produced inside the egg, as well as external air that enters the air cell through the pores.

There was a significantly effect (P<0.05) of the soaking factor in clove leaf extract on the air pocket value. The mean concentration of 20% (0.35 mm) showed a significant

difference in air sac value compared to the concentrations of 0% (0.39 mm) and 20% (0.38 mm). This may occur because the bioactive components in the 20% clove leaf extract reduce water loss and protect against microbial contamination, thereby slowing air sac enlargement.

Madubuike et al (2018) stated that clove leaves contain phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids. Tannins and saponins help form a layer on the eggshell surface, reduced water and microorganism diffusion, while glycosides and terpenoids add antimicrobial and antioxidant protection. The reduction in water evaporation and microbial protection slowing the enlargement of the air cell, resulted smaller air cell values. This aligns with Santoso (2020), who found that preserving eggs by soaking them in natural antimicrobials like papaya leaves killed pathogenic bacteria on the eggshell, prolonging egg freshness.

The average air cell value obtained from soaking eggs in a 30% clove leaf extract showed no significant difference from the control ($P>0.05$). This may be due to the 30% clove leaf concentration caused the eggshell to dry and crack, created gaps that allow gas to enter the egg, resulting in larger air cell values. This finding was consistent with Setiyaningih (2018), who reported that the non-significant result with the control was due to the turmeric extract layer on the eggshell becoming dry and cracked, allowing air to enter the egg through the pores in the cracks.

There was also no significant difference between soaking in a 10% clove leaf extract and the control ($P>0.05$). This was likely because the 10% concentration was not sufficient to fully cover the eggshell pores, allowing water and gas evaporation to continue, which led to egg weight loss and larger air cell values. This finding was supported by Jazil et al (2013), who suggested that the increase in air cell size was caused by egg weight loss due to water evaporation and gas release during storage.

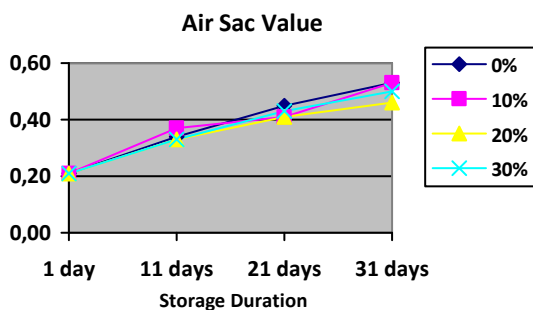


Fig. 2. Graph of average air sac value (mm)

The graph of the effect of soaking in clove leaf extract and storage duration on the air pocket value could be seen in Figure 2. On day 1 of storage, the air pocket value for all concentrations was low. However, with increased storage duration, the air pocket value tended to increase. The 20% concentration showed the lowest increase in air pocket value compared to soaking in other concentrations and the control. This indicates that the 20% concentration was able to better maintain the air sacs from enlargement due to the evaporation

rate of water and egg gas. At 31 days of storage, the lowest air sac value was obtained from 20% concentration (0.46 mm), followed by 30% concentration (0.50 mm), and highest values were from the 10% concentration (0.53 mm) and control (0.53 mm).

C. Albumen Index

The average albumen index of eggs treated by soaking in clove leaf extract at various concentrations and for different storage durations could be seen in Table 3.

TABLE 3. Average of albumen index

Storage Duration	Concentrations of Clove Leaf Extract				Average
	0%	10%	20%	30%	
1 day	0,14 ^{pi}	0,13 ^q	0,15 ^p	0,15 ^p	0,14 ^s
11 days	0,07 ^r	0,07 ^r	0,08 ^r	0,07 ^{rs}	0,07 ^b
21 days	0,02 ^t	0,03 ^t	0,05 ^s	0,05 ^s	0,04 ^c
31 days	0,01 ^v	0,02 ^{tu}	0,04 st	0,03 ^t	0,02 ^d
Average	0,06 ^b	0,06 ^b	0,08 ^a	0,07 ^a	

Notes: Average values with different superscripts in the same row and column indicate significant differences ($P<0.05$).

There was a significant interaction ($P<0.05$) between the concentration of soaking in clove leaf extract and storage duration.

The combination of soaking in clove leaf extract at concentrations of 0%, 20%, and 30% with a storage period of 1 day showed albumen index values that were not significantly different from each other but were significantly different from the 10% concentration at the same storage period, and from all concentrations tested at storage periods of ≥ 11 days. It could be concluded that the albumen index value decreases with longer storage durations. The decline in albumen index during storage was likely due to a reduction in the proportion of viscous albumen, which becomes thinner over time. As the viscous egg white height decreases, the width and length of the albumen increase, causing a decrease in the quality index of egg white. This finding is consistent with Hintono (2020), who stated that the albumen index value is influenced by the height, width, and length of the thick egg white. Thohari (2018) explained that the egg white becomes thinner due to the breakdown of ovomucin fibers in the thick egg white, caused by the evaporation of CO₂, which disrupts the buffering system formed by sodium and sodium bicarbonate. As a result, the amount of thick egg white decreases, while the amount of thin egg white increases.

The treatment combination of soaking in clove leaf extract at concentrations of 20%, 30%, and 10% with a storage period of 31 days showed significantly different results from the control at the same storage period. These results indicate that soaking in clove leaf extract can maintain the proportion of thick albumen better. Soaking in clove leaf extract was thought to slow down the evaporation of water and CO₂ gasses from the egg contents through the pores of the shell due to the presence of tannins and other polyphenolic compounds, thus slowing down the increase in pH which could cause damage to the ovomucin layer.

This is in accordance with the basic principle of egg preservation using plant tannins, as stated by Ora (2015), where the tanning reaction on the outer eggshell by the tannin causes the eggshell to become impermeable to water and

gasses. By preventing the egg contents from evaporating and releasing gases, this process slows down the pH increase that can harm the ovomucin layer.

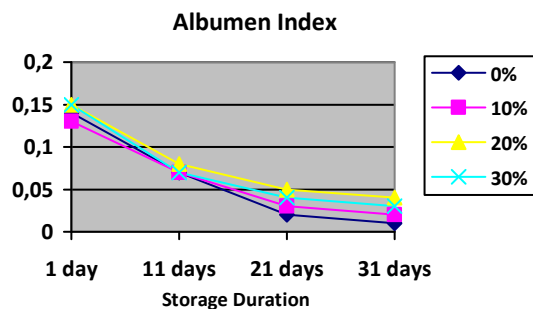


Fig. 3. Graph of average albumen index

Figure 3 shows the response pattern graph of the effect of soaking in clove leaf extract and storage duration on albumen index. From the graph, it could be observed that the albumen index consistently decreased with increasing storage duration in all treatments, indicating a decrease in egg white quality, with the egg white becoming more liquid. On the first day of storage, the highest albumen index was observed in eggs treated with 30% concentration, followed by 20%, 10%, and 0%. A sharp decline in albumen index occurred on day 11, especially in eggs not soaked in clove leaf extract (0%), indicating a more rapid deterioration in egg white quality. By day 31 of storage, the graph shows that eggs soaked in clove leaf extract at concentrations of 20% and 30% tended to maintain a higher albumen index compared to soaking at lower concentrations or not soaked at all. The treatment with 20% concentration gave the highest average albumen index, with a slower decline than the other treatments. These results indicate that soaking in clove leaf extract at higher concentrations such as 20% and 30% concentrations is effective in maintaining egg white quality during storage, slowing the decline in albumen index compared to the control.

D. Yolk Index

The average yolk index of eggs treated with soaking in clove leaf extract with various concentrations and different storage durations can be seen in Table 4. Both the soaking factor in clove leaf extract and storage duration each showed a significant effect ($P < 0.05$) on the yolk index. However, there was no significant interaction ($P > 0.05$) between soaking in clove leaf extract and storage duration on the yolk index.

TABLE 4. Average of yolk index

Storage Duration	Concentrations of Clove Leaf Extract				Average
	0%	10%	20%	30%	
1 day	0.50	0.49	0.51	0.49	0.49 ^a
11 days	0.28	0.27	0.30	0.34	0.29 ^b
21 days	0.19	0.20	0.21	0.22	0.21 ^c
31 days	0.14	0.17	0.21	0.18	0.18 ^d
Average	0.28 ^b	0.28 ^b	0.31 ^a	0.30 ^a	

Notes: Average values with different superscripts in the same row or column indicate significant differences ($P < 0.05$).

There was a significant difference in the average yolk index values during storage at 1, 11, 21, and 31 days. The

highest yolk index was obtained on the first day of storage (0.49), followed by 11 days (0.29), 21 days (0.21), and 31 days (0.18). This shows that as storage duration increases, the yolk index value decreases which is influenced by the enlargement of yolk diameter and the decrease of yolk height. The osmotic pressure difference between the yolk and the albumen can cause water to flow from the egg white into the egg yolk, resulting in this. This is consistent with Lestari (2013), who stated that egg storage causes water transfer from the albumen to the yolk, increasing the yolk's osmotic pressure compared to the egg white, leading to water movement from the albumen to the yolk. This results in a softer yolk and a decrease in the yolk index.

The average yolk index of eggs soaked in clove leaf extract of 20% and 30% concentration showed insignificant differences, but was significantly different when compared to 10% concentration and control. Tannin content in clove leaves at 20% and 30% concentrations is thought to effectively inhibit water migration from egg white to egg yolk. This effect may be due to the combination of antimicrobial, antioxidant, astringent, and surfactant properties of the bioactive components in clove leaves, which help maintain egg quality by protecting eggs from microba, reducing water evaporation, and preventing oxidation of egg components.

Muchtadi et al (2011) explained that the yolk contains comprehensive components, making it easily utilized by microbes. The antimicrobial compounds in clove leaves inhibit bacterial growth and reproduction on the surface and inside the egg, reducing the risk of microbial contamination and deterioration, especially from bacteria. The reduction in bacterial count and activity by the antibacterial compounds in clove leaves helps prevent physical and chemical degradation of the yolk. Bacterial infection in the yolk can cause nutrient degradation, such as lipids and proteins, leading to a decline in quality.

The average yolk index obtained from the soaking treatment in clove leaf extract of 10% concentration and the control showed no significant difference ($P > 0.05$). It is suspected that the tannin content in 10% concentration of clove leaves is not enough to cover the pores of the shell effectively. This may allow gas to escape and microbes to enter the egg, reducing the elasticity of the vitelline membrane and thinning the yolk. This finding is in line with Sari (2023), who suggested that the decrease in the egg's vitelline membrane elasticity is due to microorganisms entering through the egg white and producing proteolytic enzymes, weakening the vitelline membrane. Nova et al. (2014) explained that the decrease in vitelline membrane elasticity is due to the continuous osmotic pressure difference between the egg white and the yolk, leading to a reduction in the yolk index.

Figure 4 shows a graph of the response pattern of the effect of soaking in clove leaf extract and storage duration on yolk index. In general, the graph shows that the yolk index decreased with increasing storage duration, indicating a decline in yolk quality, characterized by increasingly flat yolks. On the first day of storage, all treatments had a relatively high yolk index, with the 30% concentration

showing a slight advantage over the other concentrations. After 11 days of storage, there was a significant decrease in all treatments. However, this decreasing was more pronounced in the control and 10% concentration, while the 20% and 30% concentrations were slightly better at maintaining yolk index. On days 21 and 31, yolk index values continued to decline significantly, with all treatments showing a similar pattern of decline. Nonetheless, eggs soaked in 20% and 30% concentrations tended to have higher yolk index values compared to soaking in lower concentrations of 10% or no soaking. This suggests that soaking in clove leaf extract, particularly at higher concentrations, may help slow down the deterioration of egg yolk quality during storage.

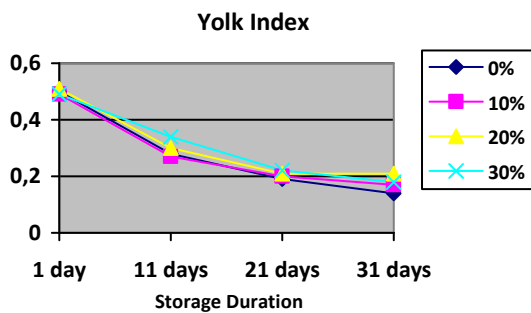


Fig. 4. Graph of average yolk index

E. Haugh Unit

The average haugh unit of eggs treated with soaking in clove leaf extract with various concentrations and different storage durations can be seen in Table 5.

Table 5. Average Haugh Unit value

Storage Duration	Concentrations of Clove Leaf Extract				Average
	0%	10%	20%	30%	
1 day	94,69 ^p	93,55 ^p	99,90 ^p	99,29 ^p	96,91 ^a
11 days	71,80 ^{qf}	76,41 ^q	68,40 ^f	75,10 ^q	72,81 ^b
21 days	42,84 ^e	34,26 ^u	61,42 ^s	62,03 ^s	46,08 ^c
31 days	19,15 ^v	31,93 ^u	60,40 ^s	44,44 ^t	37,67 ^d
Average	51,71 ^c	59,04 ^b	72,53 ^a	70,21 ^a	

Notes: Average values with different superscripts in the same row and column indicate significant differences ($P < 0.05$).

There is a significant interaction ($P < 0.05$) between the concentration of soaking in clove leaf extract and storage duration.

Both the soaking factor and storage duration, as well as their interaction, each showed a significant effect ($P < 0.05$) on the Haugh Unit value. Haugh Unit value obtained from various combinations of soaking in clove leaf extract with concentrations of 0%, 10%, 20% and 30% with the same storage duration of 1 day was significantly different from the combination of various concentrations with storage durations of 11, 21, and 31 days. The longer the storage time, the lower the Haugh Unit value. This indicates that the longer the egg storage, the more the freshness will decrease. The decrease in Haugh Unit value during storage is thought to be due to the shrinkage of egg weight caused by the release of gases and water from the egg, as well as microbial entry, which leads to the degradation of thick albumen.

Ora (2015) stated that egg deterioration during storage can be caused by microbial infiltration and the evaporation of

water and gasses like carbon dioxide (CO_2), ammonia (NH_3), nitrogen (N_2), and hydrogen sulfide (H_2S) from within the egg, leading to weight loss and thinning of the egg white, ultimately reducing egg freshness. Hintono (2022) added that certain microorganisms might also contribute to the thinning of egg white during storage.

The combination of soaking in 20% clove leaf extract and 31 days of storage resulted in significantly different Haugh Unit values compared to combinations of 30%, 10%, and 0% concentrations with the same storage duration. The 30% concentration was significantly different from 10% and 0%, while the 10% concentration was significantly different from 0% at 31 days storage. This indicates that soaking in clove leaf extract is more effective than the control.

The 20% clove leaf extract concentration was thought to be effective in preventing water evaporation and gas release through the eggshell pores, thus inhibiting the thinning of the egg white. Additionally, the phytochemicals in clove leaves with antimicrobial properties can prevent microbial invasion that may degrade the chemical composition of the egg white, particularly ovomucin, leading to thinning and damage. According to Bale-Therik et al. (2013), phytochemicals in plants can act as antimicrobials.

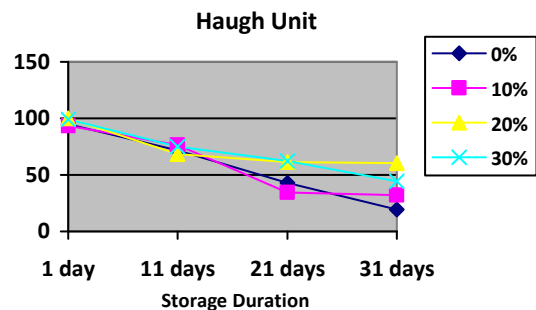


Fig. 5. Graph of Haugh Unit value

Figure 5 shows the response pattern of the effect of soaking in clove leaf extract at difference concentration and storage duration on Haugh Unit value. In general, Haugh Unit values tended to decrease with increasing storage duration, indicating a decrease in egg white quality. However, the rate of decrease varied depending on the concentration of clove leaf extract used. In unsoaked or 0% concentration, the decrease in Haugh Unit value was the sharpest, especially after 11 days of storage, with a significant decrease up to 31 days. In contrast, soaking in clove leaf extract showed a slower decline, especially at concentrations of 20% and 30%. This suggests that soaking in clove leaf extract, particularly at higher concentrations, is effective in maintaining egg white quality during storage, with higher Haugh Unit values than the control

IV. CONCLUSION

Based on the results of the experiment, it was concluded that there was a significant interaction between soaking in clove leaf extract at various concentrations and storage duration on layer chicken egg quality. Soaking in a 20% clove leaf extract extended the shelf life of the eggs up to 31 days. It

was recommended that further research be conducted to evaluate the chemical quality of layer chicken egg.

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