p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, December 2024, 47(4):510-518
DOI: https://doi.org/10.5398/tasj.2024.47.4.510
Available online at https://journal.ipb.ac.id/index.php/tasj

Characteristics of Liquid Egg White with Addition of Forest Bee Honey During Cold Storage

K. W. Saputri[a,*], Nurliyani[b], & Widodo[b]
[a]Poultry Livestock Production Technology, Akademi Komunitas Negeri Rejang Lebong, Rejang Lebong, Indonesia
[b]Department of Animal Products Technology, Faculty of Animal Husbandry, Universitas Gadjah Mada, Yogyakarta, Indonesia
*Corresponding author: kade.wahyu@gmail.com
(Received 28-01-2024; Revised 27-05-2024; Accepted 21-06-2024)

ABSTRACT

Eggs were perishable foodstuffs during distribution to consumers. Hence, appropriate egg handling and preservation methods were needed and could be applied to farmers and the egg processing industry. This research aimed to evaluate the effect of adding forest bee honey (*Apis dorsata*) on the physicochemical and microbiological qualities of liquid egg whites at storage temperature of 4 °C. The treatments consisted of three levels of honey (0%, 5%, and 10%) with storage durations of 0, 1, 2, 3, 4, 5, 6, 7, and 8 weeks. The variables analyzed include physical properties (foam capacity and foam stability), chemical properties (S-ovalbumin and protein profile on SDS-PAGE), and total plate count (TPC). The results revealed that the interaction of storage time and the addition of honey has a significant effect ($p<0.05$) on foam capacity, foam stability, S-ovalbumin, and TPC. Egg whites with 10% honey had higher foam capacity and foam stability, but lower S-ovalbumin content and the number of microbes after eight weeks compared to egg whites with 0% honey and 5% honey. In conclusion, the addition of 10% forest bee honey could maintain physicochemical qualities and extend the shelf life of liquid egg white during 8 weeks of cold storage.

Keywords: cold storage; eggs quality; forest bee honey; liquid egg whites

INTRODUCTION

Fresh chicken eggs could decrease in quality daily and only could be stored at room temperature for 14 days, according to SNI 3926-2008 (Badan Standardisasi Nasional, 2008a). Due to several technical or non-technical matters in transportation and sales, the duration of distribution to consumers became longer, which potentially caused eggs to exceed 14 days before consumption (Akpinar & Gunenc, 2019). The decreased egg quality was caused by the evaporation of water, CO_2, and microorganism contamination; thus, eggs become damaged or rotten. In addition, generally, farmers do not apply technology to maintain egg quality. Preserving chicken eggs is needed to be carried out to reduce losses due to a decrease in the quality of eggs that are not stored properly and absorbed excess egg production at the farmer level or in the market (Ministry of Agriculture, 2022). Processed egg products in the form of liquid eggs could be used as an opportunity to increase added value and meet the needs of the food industry, such as the cake industry, angel food cakes, meringues, and shuffles (Singh *et al.*, 2019).

According to Kovacs-Nolan *et al.* (2005), the main protein in egg white consisted of ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%), and ovomucin (3.5%). Egg freshness could be measured by testing the S-ovalbumin content (Huang *et al.*, 2012; Fu *et al.*, 2019). The longer the storage, the natural ovalbumin (N-ovalbumin) would change to stable ovalbumin (S-ovalbumin). S-ovalbumin had a negative impact on the formation of gas bubbles in foaming and gel formation (Alleoni & Alentonies, 2004; Miyamoto *et al.*, 2015). Changes in the type of egg white protein could be seen using the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) method. This method could identify the main egg white protein using protein markers. N-ovalbumin and S-ovalbumin had different protein configurations with differences in electrophoretic mobility that could be detected as separate bands. N-ovalbumin would be shown with a single band, which was the same as the standard marker ovalbumin, while S-ovalbumin would be shown with multiple bands (Jiang *et al.*, 2019; Abeyrathne *et al.*, 2014).

Eggs had a complex nutritional content, especially high protein in eggs; hence, they became potential places for developing pathogenic microorganisms such as *Coliform*, *Escherichia coli*, and *Salmonella sp.*, which could cause diseases for consumers. Egg white had a natural defense mechanism with antibacterial properties, including lysozyme, ovotransferrin, proteinase inhibitors (ovomucoid, ovoinhibitor, ovostatin, cystatin), and avidin (Guyot *et al.*, 2013).

Special handling or storage of eggs was needed to extend shelf life. Cold storage could control the growth of pathogenic microorganisms. Liquid egg white could be stored at cold temperatures to extend shelf life. Liquid eggs could survive at 4 °C for 45 days (Necidova *et al.*, 2019). Total microbes during cold storage could be seen by testing the TPC. TPC showed the amount of microbial contamination that was found in egg products.

Forest bee honey was generally multiflora honey that was obtained from the nectar sources of various trees and it was widely available in Indonesia. Honey has antibacterial properties such as sugar content, polyphenol, hydrogen peroxide, 1,2-dicarbonyl, and bee defensin-1 (Almasaudi, 2021). Honey also contains antioxidants such as flavonoids and phenolic acids (Zou *et al.*, 2022). Studies showed that *Apis dorsata* honey has higher sugar, phenol, and flavonoid content than *Apis cerana* and *Apis mellifera* honey (Moniruzzaman *et al.*, 2013). The antibacterial and antioxidant content, as well as the sugar content in *Apis dorsata* honey, was higher than that of other bee honey, so it was hoped that it could be the best preservative. Honey is a natural preservative for egg products, including liquid pasteurized eggs (Yusrawati *et al.*, 2019).

The cake and bread industry generally used sugar to add a sweet taste and soft texture. In addition, sugar could produce a brown color and aroma in cakes and bread due to the Maillard reaction and caramelization. Honey was used as a substitute for sugar to provide a sweet taste, producing a soft texture and providing color and aroma to cakes and bread. Liquid eggs with honey were suitable as raw materials for making cakes and sweet bread. Preserving liquid eggs with honey opened market opportunities by providing practical liquid egg raw materials with a sweet taste to the cake and bread industry. The addition of forest bee honey could maintain the quality and extend the shelf life of egg whites during cold storage. However, information on the effect of honey on the quality of liquid egg whites at cold storage was limited. This research aimed to evaluate the physicochemical and microbiological qualities of liquid egg white with the addition of honey during storage at temperature of 4 ^{o}C.

MATERIALS AND METHODS

Sample Preparation

The eggshell was cleaned using warm water, rinsed, and dried immediately. The egg was broken down, and the yolk separated from the egg white using a yolk separator. After separation, forest bee honey was added to the egg white (0%, 5%, and 10%). Samples were put into plastic standing pouch packaging using a plastic spoon each for 300 mL of egg white and then were labelled for testing every week. Egg whites with and without the addition of honey were stored in the refrigerator at temperature of 4 °C for nine storage times, namely 0, 1, 2, 3, 4, 5, 6, 7, and 8 weeks.

Foam Capacity

The capacity test of white egg foam was carried out by stirring egg whites 25 mL for four minutes with a mixer speed of 1,100 rpm. The volume of foam and the formed drain were recorded. Foam capacity was measured from the volumes of foam and the initial liquid phase (Gharbi *et al.*, 2017).

Foam Stability

Foam stability testing was carried out by stirring egg whites as much as 25 mL for four minutes with a mixer speed of 1,100 rpm. The volume of liquid separated from the foam was observed and recorded in the first 30 seconds, then continued at 60 minutes. Foam stability was measured according to Gharbi *et al*. (2017).

S-ovalbumin

The S-ovalbumin test was carried out by placing 5 g of egg white into a 100 mL beaker and adding 25 mL of 0.5 M phosphate buffer pH 7.5 (Fu *et al.*, 2019). The mixture was stirred for five minutes with a magnetic stirrer. Then 5 mL of the mixture was put into two test tubes (i_A, i_B). One test tube was heated to 75 °C for 30 minutes (i_A). After cooling, 5 mL of settling solution was added and transferred to a centrifuge tube along with 5 mL of other settling solution (i_A, i_B). The solution was left for 10 minutes and then centrifuged at 10,000 rpm for five minutes. The supernatant obtained then filtered. A total of 2 mL of supernatant was put into a test tube containing 4 mL of biuret solution. The supernatant solution was left for 30 minutes and then absorbed at 540 nm in a spectrophotometer (OD heated, OD not heated). S-ovalbumin (%) = OD heated/OD not heated x 100%. Results were expressed as the percentage of S-ovalbumin in the total amount of ovalbumin.

SDS-PAGE Protein Profile

Protein profiling employed electrophoresis Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Laemmli, 1970). 12% separating gel ingredients mixed (acrylamide 30% 2 mL, Tris HCl 1.5 M pH 8.8 1 mL, distilled water 1.6 mL, TEMED 10 µL, APS 10% 50 µL, SDS 10% 50 µL) until homogeneous. The mixture was put on a glass plate through the walls, butanol and distilled water was added. The stacking gel material was mixed (acrylamide 30% 0.5 mL, Tris HCl 1.5 M pH 6.8 0.6 mL, distilled water 1.3 mL, TEMED 2.5 µL, APS 10%, 7.5 µL, SDS 10% 25 µL) until homogeneous. The mixture was put into the separating gel until full. The comb was inserted to create a well in the buffer. A sample of 10 mg was added with 1 mL of Phosphate Buffer, and then 100 µL was inserted in another tube of Eppendorf. The sample was added with buffer five times, then heated in water for two minutes. A sample of 10 µL was inserted in a well on an electrophoresis glass plate. A marker at 8 µL was inserted into the well. Electrophoresis was run at 120 V for 60-80 minutes until the blue indicator dropped. The staining process was

carried out with Coomassie Blue for one night. Then, the washing stage was conducted.

Total Plate Count

The microbial testing procedure employed TPC according to SNI 2897-2008 (Badan Standardisasi Nasional, 2008b). A sample of 10 g was put into a sterile container, and 90 mL of physiological NaCl solution was added. Then, the solution was homogenized as a 10^{-1} dilution (P1). One mL of P1 solution was superseded using a sterile pipette into a 9 mL NaCl solution to obtain a dilution of 10^{-2} (P2), further homogenized with vortex. One mL of P2 solution was substituted using a sterile pipette into a 9 mL NaCl solution to obtain a dilution of 10^{-3} (P3). The same method was employed at each dilution up to 10^{-6} (P6). A total of 15–20 mL of plate count agar (PCA) media was added. The media in a petri dish that contained the sample was allowed to solidify. Furthermore, 1 mL of each dilution was put in different sterile petri dishes. Petri dishes containing samples were incubated at 37 °C for 24 hours by placing the dishes upside down. The calculation of the amount of microbial contamination grown was carried out using a colony counter with standard plate count calculation.

Data Analysis

The research used a completely randomized design with a 3x9 factorial pattern with 3 replications. Treatment A was giving honey consisting of three levels (0%, 5%, and 10%). Treatment B was a storage period consisting of 9 levels (0, 1, 2, 3, 4, 5, 6, 7, and 8 weeks). Data on foam capacity, foam stability, S-ovalbumin, and TPC were analyzed using two-way ANOVA. When the variance analysis (ANOVA) indicated a real effect treatment ($p<0.05$), then the analysis continued with the Tukey test. Protein profiles using SDS-PAGE were analyzed through descriptive photo images of protein bands. Protein bands indicated the type of egg white protein detected during cold storage.

RESULTS

Foam Capacity

Storage duration had a significant effect ($p<0.05$) on the foam capacity of egg whites. The addition of honey had a significant effect ($p<0.05$) on the foam capacity of egg whites. The interaction of storage time for eight weeks and the addition of honey had a significant effect ($p<0.05$) on the foam capacity of egg whites. The quality of egg whites decreased during storage, as indicated by a decrease in viscosity every week during 8 weeks of cold storage. The foam capacity is increased if the thick egg white changes to run more quickly. The addition of honey caused the viscosity of the egg white to become thinner so that the foam capacity percentage was higher.

The foaming capacity of egg whites without honey increased from 400% to 600%, egg whites with 5% honey increased from 500% to 700%, and egg whites with 10% honey increased from 600% to 700%. Foam capacity with 0% honey showed a real difference at weeks 0, 1, 2, 3, 4, 5, but at week 8 there was no real difference from week 5. Foam capacity with 5% honey showed a real difference at weeks 0, 2, 3, 5, 6, 7, but there was no significant difference at weeks 0,1, weeks 3, 4, and weeks 7, 8. Foam capacity with 10% honey showed a real difference at week 0, 6, but there was no significant difference at weeks 0, 1, 2, 3, 4, 5, and weeks 6, 7, 8. The foam capacity of egg whites with different levels of honey addition during 8 weeks of cold storage (%) was shown in Table 1.

Foam Stability

Storage duration had a significant effect ($p<0.05$) on the foam stability of egg whites. The addition of honey had a significant effect ($p<0.05$) on the foam stability of egg whites. The interaction of storage time for eight weeks and the addition of honey also had a significant effect ($p<0.05$) on the foam stability of egg whites. Significantly different interaction superscripts were shown in terms of treatment factor means. This showed that the interaction between adding honey and storage time had effects on foam stability. The ability of ovomucin and lysozyme to maintain egg white foam bubbles decreased during 8 weeks of cold storage. The addition of honey helped increase the stability of egg white foam by maintaining more stable and uniform bubbles.

The foam stability of egg whites without honey decreased from 61.33% to 45.33%, egg whites with 5% honey decreased from 75.33% to 48.67%, and egg whites with 10% honey decreased from 81.00% to 64.00%. Foam stability with 0% honey showed a real difference at weeks 0, 2, 8, but there was no significant difference at weeks 0, 1, and weeks 2, 3, 4, 5, 6, 7. Foam stability with 5% honey showed a real difference at weeks 0, 1, 2, 4, 5, 6, but there was no significant difference at weeks 2, 3, and weeks 6, 7, 8. Foam stability with 10% honey showed a real difference at weeks 0, 2, 3, 7, 8, but at weeks 0, 1, and weeks 3, 4, 5, 6, there was no significant difference. The foam stability of egg whites with different levels of honey addition during 8 weeks of cold storage was shown in Table 2.

Table 1. Foam capacity of egg whites with different levels of honey addition during 8 weeks of cold storage (%)

Storage duration (weeks)	Honey addition		
	Egg white + honey 0%	Egg white + honey 5%	Egg white + honey 10%
0	400.00 ± 0.00^{h}	500.00 ± 0.00^{i}	600.00 ± 0.00^{k}
1	500.00 ± 0.00^{i}	506.67 ± 9.43^{i}	600.00 ± 0.00^{k}
2	600.00 ± 0.00^{k}	600.00 ± 0.00^{k}	600.00 ± 0.00^{k}
3	620.00 ± 0.00^{m}	633.33 ± 9.43^{m}	600.00 ± 0.00^{k}
4	573.33 ± 9.43^{j}	633.33 ± 9.43^{m}	600.00 ± 0.00^{k}
5	600.00 ± 0.00^{k}	613.33 ± 9.43^{l}	600.00 ± 0.00^{k}
6	600.00 ± 0.00^{k}	600.00 ± 0.00^{k}	700.00 ± 0.00^{n}
7	600.00 ± 0.00^{k}	700.00 ± 0.00^{n}	700.00 ± 0.00^{n}
8	600.00 ± 0.00^{k}	700.00 ± 0.00^{n}	700.00 ± 0.00^{n}

Note: Means in the same row/column with different superscripts differ significantly ($p<0.05$).

S-ovalbumin

Storage duration had a significant effect ($p<0.05$) on egg white S-ovalbumin. Adding honey had a significant effect ($p<0.05$) on egg white S-ovalbumin. The interaction of eight-week storage duration and the addition of honey had a significant effect ($p<0.05$) on S-ovalbumin egg whites. Significantly different interaction superscripts were shown in terms of treatment factor means. This showed that the interaction between adding honey and storage time affects the S-ovalbumin content. The change in N-ovalbumin to S-ovalbumin occurred during 8 weeks of storage, which showed a decrease in egg white quality. The addition of honey to liquid egg white reduced the percentage of ovalbumin so that the s-ovalbumin content was smaller. S-ovalbumin content of egg whites without honey increased from 38.55% to 67.40%, egg whites with 5% honey increased from 44.08% to 66.65%, and egg whites with 10% honey increased from 38.59% to 59.78%. S-ovalbumin content with 0% honey showed significant differences at weeks 0, 2, 4, 8 but at weeks 4, 6, there was no significant difference. S-ovalbumin content with 5% honey showed significant differences at weeks 0, 2, 4, 6, 8. S-ovalbumin content with 10% honey showed a significant difference at weeks 0, 4, 6, 8, but at weeks 0, 2, there was no significant difference. S-ovalbumin levels of egg whites with different levels of honey addition during 8 weeks of cold storage are shown in Table 3.

Protein Profile on SDS-PAGE

Identification of egg white protein bands that showed in SDS-PAGE was carried out based on a comparison of the molecular weight of the sample with the protein marker. The addition of honey did not remove the protein band and showed the same type of protein as egg white without honey. The protein banded of the lysozyme (10-15 kDa), ovomucoid (30-40 kDa), ovalbumin (40-50 kDa), ovotransferrin (70-75 kDa), and ovomucin >245 kDa. The addition of 5% and 10% honey reduced the concentration of egg whites so that the protein band in the SDS-PAGE results became thinner than the protein band in egg whites without honey. The protein band in the 0% honey egg white was considered larger than the 5% and 10% egg white with honey (Figure 1).

Table 2. Foam stability of egg whites with different levels of honey addition during 8 weeks of cold storage (%)

Storage duration (weeks)	Honey addition		
	Egg white + honey 0%	Egg white + honey 5%	Egg white + honey 10%
0	61.33 ± 0.94^{g}	75.33 ± 0.94^{m}	81.00 ± 1.41^{n}
1	60.00 ± 0.00^{g}	73.33 ± 0.94^{l}	79.33 ± 0.94^{n}
2	50.67 ± 0.94^{e}	69.33 ± 0.94^{j}	71.33 ± 0.94^{k}
3	50.00 ± 0.00^{e}	68.67 ± 0.94^{j}	70.67 ± 0.94^{j}
4	49.33 ± 0.94^{e}	67.33 ± 0.94^{i}	70.67 ± 0.94^{j}
5	49.33 ± 0.94^{e}	55.33 ± 0.94^{f}	70.67 ± 0.94^{j}
6	49.33 ± 0.94^{e}	51.33 ± 0.94^{e}	70.00 ± 0.00^{j}
7	49.33 ± 0.94^{e}	51.33 ± 0.94^{e}	67.33 ± 0.94^{i}
8	45.33 ± 0.94^{d}	48.67 ± 0.94^{e}	64.00 ± 0.00^{h}

Note: Means in the same row/column with different superscripts differ significantly ($p<0.05$).

Total Plate Count

Storage duration had a significant effect ($p<0.05$) on the average number of microbes in egg white. The addition of honey had a significant effect ($p<0.05$) on the average number of egg white microbes. The interaction of storage duration for eight weeks and the addition of honey had a significant effect ($p<0.05$) on the average number of egg white microbes. Significantly different interaction superscripts were showed in terms of treatment factor means. This showed that the interaction between adding honey and storage time had an effect on the total microbes. The antibacterial ability of egg whites decreased during 8 weeks of cold storage, so the number of bacteria increased. Adding honey as an antibacterial reduced the number of bacteria in egg whites.

The total number of microbes in liquid egg whites without honey increased from 2.69 log CFU/mL to 3.30 log CFU/mL, egg whites with 5% honey increased from 2.34 log CFU/mL to 3.04 log CFU/mL, and egg whites with 10% honey increased from 2.30 log CFU/mL to 2.90 log CFU/mL. The total number of egg white microbes with the 0% honey showed a real difference at weeks 0, 1, 2, 3, 4, 5, and 8, but there was no real difference at weeks 4, 6, and 7. The total number of egg white microbes with 5% honey showed a real difference at weeks 0, 1, 2, 4, and 6, but at weeks 2, 3, and weeks 4, 5, 7 and weeks 6, 8, there was no significant difference. The total number of egg white microbes with 10% honey showed a real difference at weeks 0, 1, 2, 4, 5, 6, 7, but there was no significant difference at weeks 2, 3, and weeks 7, 8. The average number of egg white microbes with different levels of honey addition during 8 weeks of cold storage is shown in Table 4.

DISCUSSION

Foam Capacity

Foam was a two-phase colloidal structure consisting of a continuous phase (water) and a gas phase (air) suspended in bubbles (Wouters *et al.*, 2018). The foam was formed when egg whites were stirred. Air was introduced into the liquid and broken into smaller bubbles by high-speed movement, resulting in the appli-

Table 3. S-ovalbumin levels of egg whites with different levels of honey addition during 8 weeks of cold storage (%)

Storage duration (weeks)	Honey addition		
	Egg white + honey 0%	Egg white + honey 5%	Egg white + honey 10%
0	38.55 ± 0.81^{h}	44.08 ± 0.89^{i}	38.59 ± 0.38^{h}
2	47.89 ± 0.66^{j}	52.73 ± 0.74^{k}	36.74 ± 1.55^{h}
4	52.34 ± 0.98^{k}	58.97 ± 0.88^{m}	47.16 ± 0.18^{j}
6	53.05 ± 0.84^{k}	54.45 ± 0.13^{l}	55.58 ± 0.34^{l}
8	67.40 ± 0.46^{n}	66.65 ± 0.94^{n}	59.78 ± 0.17^{m}

Note: Means in the same row/column with different superscripts differ significantly ($p<0.05$).

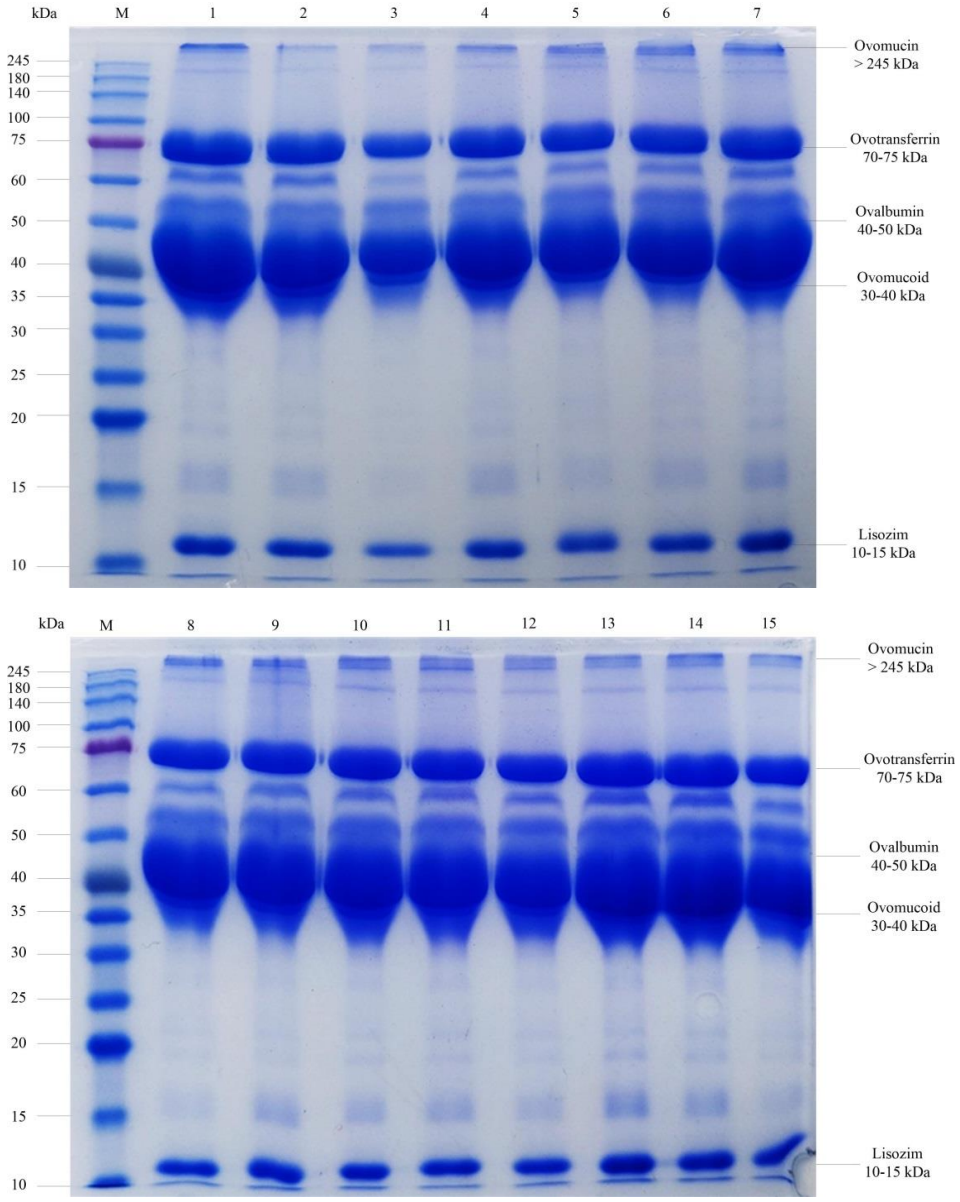

Figure 1. Protein profile of egg white with different levels of honey addition during 8 weeks of cold storage. 1=egg white + honey 0%, 0 week; 2=egg white + honey 5%, 0 week; 3=egg white + honey 10%, 0 week; 4=egg white + honey 0%, 2 week; 5=egg white + honey 5%, 2 week; 6=egg white + honey 10%, 2 week; 7=egg white + honey 0%, 4 week; 8=egg white + honey 5%, 4 week; 9=egg white + honey 10%, 4 week; 10=egg white + honey 0%, 6 week; 11=egg white + honey 5%, 6 week; 12=egg white + honey 10%, 6 week; 13=egg white + honey 0%, 8 week; 14=egg white + honey 5%, 8 week; 15=egg white + honey 10%, 8 week. The marker protein (M) covers a wide range of molecular weights from 10 to 245 kDa.

cation of shear and turbulence to the gas-liquid mixture (Narsimhan & Xiang, 2018). Foam power was formed after shaking due to open ovomucin-lysozyme protein bonds. One measurement of the functional properties of foam formation was foam capacity. Foam capacity was the ability of egg whites to form foam when stirred and expressed in percentage. The increasing foam volume indicated that the power of egg white foam was also increasing.

Egg whites changed during storage. CO_2 evaporation occurred during storage so that bicarbonate ions decrease. As a result, the buffer system also decreased, which caused the pH value to rise. An increase in pH caused a decrease in ovomucin's ability to maintain the viscosity of egg whites so that the egg whites become thinner. The water content in egg whites was high, reaching 80% (Rehault-Godbert *et al.*, 2019). Egg white proteins that played a role in foam formation were ovomucin and lysozyme. Egg white protein would undergo a chemical reaction resulting in changes in the protein structure. Changes in egg white protein during 12-24 days of storage increased foam capacity (Wang *et al.*, 2019). Breaking of interchain disulfide bonds and exposure of sulfhydryl groups via lysozyme-ovomucin dissociation, thus, the foaming power was higher from 40 to 60 days (Chen *et al.*, 2019).

The decrease in egg white viscosity during 8 weeks of storage resulted in an increased in the egg white foam capacity. The foam capacity of egg whites without honey after one week of storage was 400%. The foaming

Table 4. Total number of egg white microbes with different levels of honey addition during 8 weeks of cold storage (log cfu/mL)

Storage duration (weeks)	Honey addition		
	Egg white + honey 0%	Egg white + honey 5%	Egg white + honey 10%
0	2.69 ± 0.75^{h}	2.34 ± 0.09^{f}	2.30 ± 0.56^{e}
1	2.75 ± 0.14^{i}	2.63 ± 0.98^{g}	2.46 ± 0.09^{f}
2	2.92 ± 0.09^{k}	2.80 ± 0.55^{i}	2.69 ± 0.73^{h}
3	3.07 ± 0.41^{l}	2.82 ± 0.51^{i}	2.64 ± 0.51^{h}
4	3.17 ± 0.43^{m}	2.91 ± 0.98^{k}	2.63 ± 0.91^{g}
5	3.11 ± 0.50^{l}	2.92 ± 0.44^{k}	2.83 ± 0.49^{j}
6	3.14 ± 0.34^{m}	2.96 ± 0.77^{l}	2.81 ± 0.49^{i}
7	3.14 ± 1.00^{m}	2.94 ± 0.80^{k}	2.91 ± 0.95^{k}
8	3.30 ± 0.39^{n}	3.04 ± 0.96^{l}	2.90 ± 0.63^{k}

Note: Means in the same row/column with different superscripts differ significantly ($p<0.05$).

capacity of egg whites with or without honey began to increase with storage time. Even though egg whites were stored in cold storage, they still caused changes in the egg protein bonds and the egg whites became increasingly runny. The watery condition of the egg white, which contained a lot of water, made it easier for air to be captured and form gas bubbles. The foam capacity began to increase after 12 days at 4 °C storage due to the decomposition of the egg white protein from the complex to a higher protein solubility (Guo *et al.,* 2022). Egg white protein had the characteristics of absorbing quickly at the interface, undergo structural changes at the interface and formed a congestive viscoelastic layer (Campbell *et al.,* 2003).

The foaming capacity of egg whites increased until week 8, however, the foaming capacity of egg whites with 10% honey content was higher than the foaming capacity of egg whites with concentrations of 0% and 5% honey. The addition of honey to egg whites did not inhibit foam formation but caused a change in the viscosity of the egg whites. The process of stirring or mixing honey with egg white caused the viscosity from thick to thinner. This stirring process would also cause changes in protein bonds so that it was easier to form foam. The higher the percentage of honey added, the thinner the egg white would be. Decreased viscosity caused the water content of the egg white to increase, thereby increasing the foam capacity. Apart from that, adding sugar to egg whites had an important role in foam formation and stability. Added sugar could shorten the hydrogen protein relaxation time by the hydration effect between sugar and water. Research by Sun *et al*. (2022) showed that the addition of saccharides to egg whites increased surface hydrophobicity and reduced the surface tension of the egg white solution, thereby increasing foam capacity. The higher the concentration of honey was given, the higher the egg white foam capacity with high foam stability.

Foam Stability

Foam stability was the ability of the foam to maintain its shape, remain strong, not melt within a certain time, and be tight and identical. The process of beating egg whites caused stereometric changes due to the presence of hydrophobic groups on the surface, surface tension was reduced, thus, foam was formed, and the foam became stable. Ovalbumin was a type of egg white protein that could form foam (Alleoni & Antunes, 2004). The stability of egg white foam decreased after eight weeks of storage. Foam capacity was inversely proportional to foam stability. The stability of the foam decreased with increasing shelf life. According to Gharbi & Labbafi (2019), there were three main mechanisms of foam instability: drainage, coalescence, and disproportion. Drainage is a reduction in the liquid phase of the foam so that the foam becomes drier. Coalescence was the merging of two adjacent bubbles. Disproportion was the movement of gas from small bubbles to large bubbles so that the small bubbles disappear (Zhao *et al.,* 2021).

The stability of egg white foam without honey decreased from 61.33% to 45.33%, the stability of egg white foam with 5% honey also decreased from 75.33% to 48.67% and the stability of egg white foam with 10% honey decreased from 81.00% to 64.00%. Research by Guo *et al*. (2022) revealed a decrease in the stability of liquid egg white foam stored at 4 °C after 40 days of storage. The decrease in foam stability during storage was influenced by the binding of ovomucin to lysozyme. The ovomucin lysozyme binding played an important role in maintaining foam stability because it formed a protective layer on the surface of the foam bubble (Zhao *et al.,* 2021). Thohari *et al.* (2020) stated that the structure of ovomucin that had not been broken would produce low foam power but was able to maintain the volume of foam formed. During storage, the binding strength of ovomucin lysozyme would decrease, resulting in the egg white becoming watery so that the stability of the foam also decreased.

Although the stability of egg white foam with or without the addition of honey decreased during storage, the stability of egg white foam with the addition of 10% honey had a high level of stability for 8 weeks. The addition of honey could increase the stability of egg white foam. The percentage of egg whites added with honey produced a smaller volume of egg whites, so the S-ovalbumin content formed during storage was also lower. The increasing S-ovalbumin content during storage inhibited the formation of a protective layer of gas bubbles. It was caused the foam formed to become unstable and melt easily. The stability of egg white foam slightly decreased at 40 days of cold storage due to the increase in less hydrophobic S-ovalbumin disrupting the formation of a cohesive film at the air-water interface and it caused a decrease in foam stability (Chen *et al.,* 2019).

In addition, the sugar content of honey also affected the stability of the foam. Forest bee honey contains a total of 63.60% sugar (Moniruzzaman *et al.,* 2013). The sugar component with a high concentration consisted of 38.5% fructose and 31% glucose. The sugar content of honey changed during storage. The fructose content increased by 4% and glucose increased by 1.1% at a storage temperature of 4 °C (da Silva *et al.,* 2016). It was high

sugar content functions as a foam stabilizer by binding water and forming a stable foam. Adding sugar to egg whites could reduce the number of large bubbles and increase small bubbles, make the foam bubbles denser and more uniform (Sun *et al.*, 2022). The addition of sugar had been widely applied in the food industry, taking advantage of the functional properties of foam. Sugar functions to prevent the foam from melting, making the foam texture soft and the foam stability high. The stable foam structure could provide good texture and excellent taste to foods such as ice cream, bread, and beer (Zhan *et al.*, 2021). Egg white with the addition of honey had better foam stability, characterized by a gradual change in foam volume.

S-ovalbumin

Ovalbumin was an egg protein with a content of 55% of the total protein. Ovalbumin gradually converts to S-ovalbumin during storage. S-ovalbumin was result of the natural configuration of ovalbumin (N-ovalbumin) into an S configuration with changes in molecular, physical, and chemical properties. The S-ovalbumin content affected 3 main egg freshness indicated: Haugh Units, egg yolk index, and egg white pH. Evaluation of S-ovalbumin as a prediction index for egg freshness observed at the age of the egg and the kinetics of S-ovalbumin formation in egg white, resulted in a high correlation coefficient between S-ovalbumin and storage time at a certain temperature (Huang *et al.*, 2012). The longer eggs were stored, the S-ovalbumin content increased, as indicated by an increase in egg white pH, a decrease in egg yolk index and a decrease in Haugh Units. Thus increased in S-ovalbumin indicated a decrease in egg white ovalbumin content. The S-ovalbumin formed was a percentage of the total ovalbumin. S-ovalbumin could be an indicator of egg freshness (Fu *et al.*, 2019).

Egg whites experienced an increase in pH during storage due to CO_2 evaporation, so the variosity decreased and the egg whites became runny. The addition of honey could reduce the pH value of honey because honey was acidic with a pH of 4. The higher the concentration of honey given, the lower the pH value due to the organic acid content (da Silva *et al.*, 2016). The addition of honey could reduce the pH value of egg whites, but the pH value continued to increase during storage. The faster the pH raised, the faster the change of N-ovalbumin to S-ovalbumin. Honey also experiences chemical changes during storage, which affect its nutrition and sensory properties. The change reaction that occurred, was the Maillard reaction due to heating and storage for too long, up to one year at room temperature (Moreira *et al.*, 2010). The brown colour in the Maillard reaction was caused by the presence of NH_2 and CaO reactive groups due to the chemical reaction of reducing sugars and primary amino acids (da Silva *et al.*, 2016). In this study, the age of the honey used was still within one month so it had not experienced any significant changes. The cooling treatment in storing egg whites and honey also inhibited the Maillard reaction so that the colour of the honey did not darken or remain the same during 8 weeks of storage.

In this study, the S-ovalbumin content of egg whites increased after eight weeks of storage. The S-ovalbumin value in egg white without honey increased from 38.55% to 67.40%, egg white with 5% honey also increased from 44.08% to 66.65%, and egg white with 10% honey, from 38.59% to 59.78%. This aligned with research by Alleoni & Antunes (2004), which stated that the S-ovalbumin content became 86% after six months of storage at cold temperatures. Cold storage could inhibit S-ovalbumin formation compared to storage at room temperature. The S-ovalbumin content of egg white at room temperature was higher than the content at cold temperatures in this study. Huang *et al*. (2012) revealed that S-ovalbumin reached 91.86% after 27 days of storage at 25 °C and 91.24% after 12 days of storage at 37 °C. Even though there was an increase in S-ovalbumin during storage, the S-ovalbumin content of egg whites with 10% honey was lower. The addition of honey could reduce the S-ovalbumin content. This was influenced by the smaller percentage of egg white when added to honey. The volume of egg whites without honey was higher than the volume of egg whites with honey. The higher the egg white volume, the higher the ovalbumin protein content. This resulted in the greater potential for changing ovalbumin to S-ovalbumin.

Protein Profile on SDS-PAGE

The Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) method could separate protein profiles. SDS-PAGE testing was a technique for separating molecules based on their size by applying an electric current. The SDS-PAGE method in this study was used to determine the effect of storage time and honey on the protein produced. Proteins in the emerging band would be identified based on protein markers. According to Al-Shuhaib *et al*. (2019), the egg white protein profile consisted of ovalbumin 45 kDa, ovotransferrin 76-78 kDa, lysozyme 14.4 kDa, ovomucin 220-270 kDa and ovomucoid 28 kDa. The main egg white proteins in the identified protein bands were ovomucin 100-250 kDa, ovotransferrin 70 kDa, ovalbumin 35 kDa, and lysozyme 15 kDa (Ji *et al.*, 2020). The SDS PAGE method could not directly show the difference between N-ovalbumin and S-ovalbumin. This was because the amino acid composition between S-ovalbumin and N-ovalbumin was not different (Alleoni & Antunes, 2004). SDS PAGE identified the ovalbumin protein profile band, where the ovalbumin protein band may contain S-ovalbumin. Methods that could predict S-ovalbumin visually could use machine vision, near-infrared spectroscopy, and hyper-spectral imaging technology (Fu *et al.*, 2019).

The addition of honey did not affect egg white protein during storage. This was shown in Figure 1, which shows the protein bands in egg whites with honey, the same as the protein bands in egg whites without honey. The protein profiles identified were ovalbumin, ovomucin, ovotransferrin, ovalbumin, and lysozyme. Honey contained 0.2%-1.6% protein with the main protein being proline, which was sourced from plant pollen (da Silva *et al.*, 2016). This honey protein percentage was smaller than that of egg white protein, which reaches 10% (Rehault-Godbert *et al.*, 2019). This resulted in the addition of honey that not having a major effect on the SDS PAGE protein profile. Storage at a cold temperature

of 4 °C could preserve egg white proteins. The protein banded lysozyme (10-15 kDa), ovomuciod (30-40 kDa), ovalbumin (40-50 kDa), ovotransferrin (70-75 kDa), and ovomucin >245 kDa were still able to persist until the 8th week. The proteins lysozyme, ovotransferrin, and ovomucoid acted as antibacterials (Guyot *et al.,* 2013). Ovalbumin, ovotransferrin, ovomucin, and lysozyme were antioxidants (Nimalaratne & Wu, 2015). In addition, cold storage could inhibit changes in ovalbumin.

Total Plate Count

Unpasteurized liquid egg products should consider food safety, thus, they would not be contaminated with pathogenic bacteria that caused foodborne diseases such as *Salmonella* and *E. coli.* Liquid eggs separated from shells could be easily contaminated if the equipment was not sterile (Zhu *et al.,* 2021). The average number of microbes in liquid egg whites increased during eight weeks of storage at 4 °C. The total number of microbes in the 0 week in egg whites without honey, egg whites with 5% honey, and egg whites with 10% honey, respectively, were 2.69 ± 0.75 log CFU/mL (4.9 x 10^2 CFU/mL), 2.34 ± 0.09 log CFU/mL (2.7 x 10^2 CFU/mL), and 2.3 ± 0.56 log CFU/mL (2.0 x 10^2 CFU/mL). The number of microbes was lower than the limit of microbial contamination of egg consumption according to Food Agricultural Organization, which was 6 log CFU/mL.

Egg whites had a defense mechanism through the antibacterial content of egg protein. The antibacterial contained of egg whites include lysozyme, ovotransferrin, proteinase inhibitors (ovomucoid, ovoinhibitor, ovostatin, cystatin), and avidin (Guyot *et al.,* 2013). However, the quality of egg whites decreased during storage, resulted in a decrease in antibacterial and antioxidant capacity, so eggs were easily damaged. In this study, the average number of microbes in egg whites increased during eight weeks of storage at 4 °C. The average number of microbes in 0%, 5%, and 10% honey egg whites were 3.30 ± 0.39 log CFU/mL (2.0 x 10^3 CFU/mL), 3.04 ± 0.96 log CFU/mL (1.1 x 10^3 CFU/mL), and 2.90 ± 0.63 log CFU/mL (8.1 x 10^2 CFU/mL). The liquid egg whites in this study were able to survive for eight weeks with the numbers of honey egg white bacteria of 0%, 5%, and 10% lower compared to Wang *et al*. (2019) who stated that pasteurized egg whites were able to survive for 16 days in cold storage with the number of bacteria reaching 6 log CFU/mL. Cold storage could inhibit the growth of microbes in egg whites compared to room temperature. Liquid egg white stored in plastic at room temperature could survive for six days with a bacterial count of more than 1 x 10^6 (Guo *et al.,* 2022).

The addition of honey could inhibit the growth of microbes in egg whites. The average number of microbes in egg whites without and with honey both increased during 8 weeks of storage, but the average number of microbes in egg whites with 10% honey was lower. Honey has high antibacterial and antioxidant content. Honey components as antioxidants consist of phenolic acids and flavonoids (Martinello & Mutinelli, 2021). Honey's antibacterial components consist of sugar, polyphenols, hydrogen peroxide, and others (Almasaudi, 2021). The antibacterial and antioxidant contents in honey could function as a defense mechanism like the antibacterial properties in egg whites.

The addition of honey could reduce the pH of egg whites because honey had a low pH and contains aspartic acid, butyric acid, citric acid, acetic acid, formic acid, and others (da Silva *et al.,* 2016). The organic acid content in honey could inhibit the growth of microbes in egg whites. The higher the acidity of honey, the higher the organic acid content that inhibited bacterial growth. In addition, honey has a high sugar content, which causes osmotic pressure, so that bacteria become dehydrated and die. The antibacterial content and organic acids in honey were used as food preservatives. Several studies had used honey to inhibit the growth of microorganisms. Honey added for marinating beef can reduce lipid oxidation and microbial counts, extending the shelf life of beef without affecting meat quality (Ayoob *et al*., 2023). The increase in the number of microbes in egg whites without honey was higher than in egg whites with 5% honey and 10% honey.

CONCLUSION

The addition of 10% honey to liquid egg whites maintained physicochemical qualities, inhibited microbial growth, and extends shelf life up to 8 weeks during cold storage. Forest bee honey had the potential to be a liquid egg white preservative.

CONFLICT OF INTEREST

We certify that there was no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

The author would like to thank the Faculty of Animal Husbandry, Master of Animal Science Study Program, Gadjah Mada University, for research support.

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