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Preservation of duck eggs through glycerol monolaurate nanoemulsion coating

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ABSTRACT

Duck eggs have a short storage life. In this study, water-washed duck eggs and glycerol monolaurate (GML) coated duck eggs were stored at 25 °C for 70 days (away from light). The water-washed duck eggs started to lose weight from the 4th week. At the same time, Haugh unit and egg yolk index of the water-washed duck eggs started decreasing. The normal GML coating solution (NGML), the higher concentration GML diluent (HGML), and the lower concentration GML diluent (LGML) showed different preservation effects. Among them, NGML showed the strongest protection effect against spoilage of duck eggs. After 70-days storage, the weight loss rate of the NGML coated duck eggs was <6%, which was 4 times lower than that of the water-washed duck eggs; the Haugh unit and the surface morphology were also better than that of the water-washed duck eggs. Furthermore, the total colonies in NGML coated sample was >4 log CFU/g less than that was found in the water-washed samples (Control). The HGML and LGML coating agents were less effective but they might be suitable for the short storage of duck eggs due to the lower cost. Overall, this study provides a sound basis for the preparation and utilization of GML coating solution. The GML coating method is able to extend the shelf life of duck eggs by more than 6 weeks.

1. Introduction

Duck eggs are rich in proteins (1.0 mg/g), essential amino acids (46 mg/g), cholesterol (1.1 mg/g), minerals (5.5 mg/g), vitamins (4.9 mg/g), and other nutrients (Friday, 2011; Sun et al., 2019). The storage life of duck egg is < 40 days at room temperature (25 °C) and <90 days at cool temperature (4 °C) (Quan and Benjakul, 2019). In the market, duck eggs mainly exist in two forms: (1) Dark duck eggs; and (2) clean duck eggs. Dark duck eggs are covered with a layer of stratum corneum membrane, which can prevent bacterial invasion (Eddin et al., 2019). However, the dirt attached to the surface of dirty eggs is a breeding ground for microorganisms, which accelerates the decline of quality and shorten the storage life of dark duck eggs (Olsen et al., 2017). Clean duck eggs are free of surface impurities. However, washing step may destroy the protective film of the duck eggs shortening the shelf life. Therefore, it is urgent to develop a simple, effective and economical method for the preservation of duck eggs.

In recent years, coating preservation has been applied in poultry eggs. Coating material forms a stable film on the eggshell surface, preventing the transfer of oxygen and microorganisms through it. Furthermore, this uniform and dense protective film can reduce the evaporation of water from the egg, and increase the CO_2 concentration. This further reduces the respiration of the egg, which inhibits the enzyme activities, thereby reducing the life decay process (Xie et al., 2002). An effective coating should have excellent gas barrier ability, non-toxic, anti-bacterial, and film-forming properties (Davalos-Saucedo et al., 2018). Several coating materials have been applied to eggshells to extend the shelf life of poultry eggs. Davalos-Saucedo and coworkers utilized transglutaminase cross-linked whey protein-pectin coating to coat the eggshell, which maintained quality of the coated eggs after a

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15-days storage at 25 °C (Davalos-Saucedo et al., 2018). Ryu and co-workers coated eggs with different oils (Mineral-, rapeseed-, corn-, grapeseed-, olive-, soybean- and sunflower-oil) and found that the shelf life (at 25 °C) was three weeks longer than that of the uncoated ones (Ryu et al., 2011). Similarly, chitosan coating (>2 μm thickness) was also found beneficial to the storage life of eggs (Xu et al., 2018).

GML is a lipophilic non-ionic surfactant with multifunctional properties, such as anti-bacterial, anti-viral and film-forming activities (Schlievert et al., 1992). GML is naturally present in breast milk, coconut oil, American palm etc. Being a green and safe food additive (Zhang et al., 2009), GML has been approved to be used in dairy products, baked foods, and meats (Zheng et al., 2016). However, there is no research on the application of GML in the preservation of duck eggs.

In this study, normal GML coating solution (NGML) and its two diluents, namely higher concentration GML (HGML) and lower concentration GML (LGML) were prepared according to the emulsion properties and cost considerations. The best coating conditions for duck eggs preservation were determined during a storage experiment (For 70-days storage at 25 °C, away from light). Through the evaluation of physico-chemical and microbial growth properties of around 240 duck eggs, the effects of GML coating on the preservation of duck eggs were investigated. Overall, this study provides a sound basis for the preservation of duck eggs at room temperature.

2. Materials and methods

2.1. Materials

Fresh duck eggs (unfertilized) of the same laying day were obtained from a duck farm (Weishan Duck, Suqian, Jiangsu). GML, Tween 80, Span 80, and ethanol were purchased from Zhenjiang Huadong Chemical Glass Co., Ltd. Plate counting agar was provided by Shanghai Ruichu Biotechnology Co., Ltd. All the chemicals used in this study were of analytical grade.

2.2. Preparation of GML coating solution

GML coating solution was prepared by adding 100 g of GML into 900 g of activated ethanol solution (containing 50 g Tween 80 and 50 g Span 80), which was equilibrated in a shaking water bath (KW-1000DC, Jintan Zhongda Instrument Factory, Jiangsu, China) at 65 °C for 30 min. Then the solution was stirred with a magnetic stirrer (IKARET basic, Dongguan Zhoushiqiaozi Electric Co., Ltd., Guangdong, China) at 600 rpm for 10 min. This was followed by a homogenizing process (IKAT18, Dongguan Zhoushiqiaozi Electric Co., Ltd., Guangdong, China) at 1000 rpm for 5 min to make the NGML coating solution (10%, w/w). A higher concentration GML diluent (HGML, 6.67%, w/w) and a lower concentration GML diluent (LGML, 3.33%, w/w) were prepared by adding 500 g and 2000 g of deionized water in 1000 g NGML solution, respectively.

2.3. Determination of the stability of the GML coating solution

Stability of the GML coating solution was determined according to Le et al. (2020) by using an ultraviolet–visible spectrophotometer (UV-1601, Beijing Ruili Analytical Instrument Company, Beijing, China). Briefly, samples were scanned at 400-190 nm wavelength. The absorbance readings at 400 nm reflects the turbidity of the GML emulsion. Meanwhile, the same samples were centrifuged at 1238×g at 25 °C for 15 min. The supernatant was collected and tested following the same protocol. The emulsion stability was calculated by using equation (1):

$$R\ (\%) = A_2/A_1 \tag{1}$$

where, R is the centrifugal stability factor (%); A_1 is the absorbance (400 nm) of emulsion before centrifugation; A_2 is the absorbance (400 nm) of emulsion after centrifugation.

2.4. Preparation and storage of GML coated duck eggs

Around 240 unfertilized duck eggs (same laying date) with uniform size, intact eggshell and average egg weight of 67.5 ± 2.5 g were selected. All the duck eggs were cleaned using distilled water and randomly divided into four groups, namely Group A, B, C, and D, which were coated with NGML solution, HGML solution, LGML solution, and distilled water (i.e., no coating), respectively. The duck eggs in the GML coated groups were soaked in the corresponding coating solutions for 30 s, and then placed on egg racks, which were tissue dried and stored in an incubator (LRH-250, Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China) at 25 °C. For the measurement purpose, five parallel duck eggs were randomly picked out from each group at 7-, 14-, 21-, 28-, 63-, and 70-days storage period and tested immediately.

2.5. Determination of weight loss rate

The weight loss rate of the duck eggs was determined according to Chen et al. (2021). The duck eggs were weighed by an analytical balance with an accuracy of 0.0001 g. The weight loss rate was calculated using equation (2) :

$$P\ (\%) = (m_2 - m_1)\ /\ m_1 \tag{2}$$

where, P is the weight loss rate (%); m_1 and m_2 are the initial weight (g) and the weight (g) after storage for a certain period of time of the duck egg, respectively.

2.6. Measurement of Haugh unit

Haugh unit (HU) of the duck eggs was measured according to the method given by Kemps et al. (2007). Briefly, the weight of the duck eggs was measured by using an analytical balance (ME204, America) with an accuracy of 0.0001 g. Then, the duck eggs were gently broken and poured on a glass plate. The thickness of egg white was measured using a Vernier caliper (S102-103, China; accuracy 0.02 mm) when it was intact with egg yolk. The HU value was calculated by using equation (3):

$$HU = 100\ lg\left(H - 1.7W^{0.37} + 7.6\right) \tag{3}$$

where, H is the height of the egg white layer (mm); is the mass of the egg (g).

2.7. Determination of yolk index

Yolk index (YI) was measured according to the method described by Caner and Cansiz (2007). For this purpose, duck eggs were broken and poured in a glass plate. While the egg yolk and egg white were intact, the height and width of the egg yolk were measured using a Vernier caliper. The yolk index was measured by using equation (4):

$$YI = h/d \tag{4}$$

where, YI is the yolk index; h is the height of the yolk (mm); d is the width of the yolk (mm).

2.8. Determination of the total number of colonies

The eggshell was fully sterilized by using an alcohol-soaked cotton ball inside a biological safety cabinet (HR1500-IIA2, Qingdao Haier Special Electric Co., Ltd., Shandong, China). Then, the shell was broken carefully and the egg was poured in a sterilized beaker. After egg white and egg yolk being mixed evenly with a sterile glass rod, 25 g of the suspension was taken and mixed with 225 ml of sterile normal saline. The total number of colonies in the sample was then determined according to a previously reported method (Leleu et al., 2011). For this

purpose, 1 ml of the above-prepared egg liquid was serially diluted with 9 ml of sterilized physiological saline for five times to make a dilution factor of 10^{-6}. The total number of colonies was counted by the pour-plate techniques, using a plate count agar (PCA). After incubating the plates at 37 °C for 48 h, the microbial colonies were counted and expressed in CFU/ml.

2.9. Monitoring the surface morphological changes of duck eggs

Duck eggs were randomly selected from all the four groups and the relatively flat part of the eggshell was selected and placed on a glass slide. The surface morphology of each group of duck eggs was observed under an optical microscope (EclipseE100, Nanjing Sigaopu Instrument Co., Ltd., Nanjing, China). The surface morphology was recorded at the beginning and the end of storage. After being stored for 70 days, three duck eggs were randomly selected from each group, broken up and poured into a small beaker to observe the difference in duck eggs surface.

2.10. Statistical analysis

SPSS software (Version 23.0) was used for the statistical analysis of data. Significant difference using one-way ANOVA test for the analysis of variance and Duncan's multiple comparison ($p < 0.05$). Each test was repeated for at least three times.

3. Results and discussions

3.1. Stability of the GML coating agent

Emulsion stability can be expressed by emulsion turbidity and centrifugal stability coefficient signs; the smaller the centrifugal stability coefficient, the higher the absorbance, the higher the turbidity, and the worse the emulsion stability. On the contrary, the larger the centrifugal stability coefficient, the lower the absorbance and the lower the turbidity, giving the better emulsion stability (Huang et al., 2020). During the centrifugal process of the emulsion, the layering may be aggravated under the action of centrifugal force, which is used to judge the stability of the emulsion (Xu et al., 2018).

As shown in Fig. 1, the GML coating agent was very stable at room temperature (25 °C). However, the stability of the coating agent decreased with the dilution. For example, no obvious change was observed in the NGML coating solution after centrifugation, whereas the HGML and the LGML showed the centrifugal stability constants of <65%. Therefore, the normal GML coating agent was found to be the best option during the preparation stage. Dilution of the GML coating agent would cut down the primary cost but it requires a faster processing operation (within 3 h at room temperature) to avoid the potential deterioration of the emulsion.

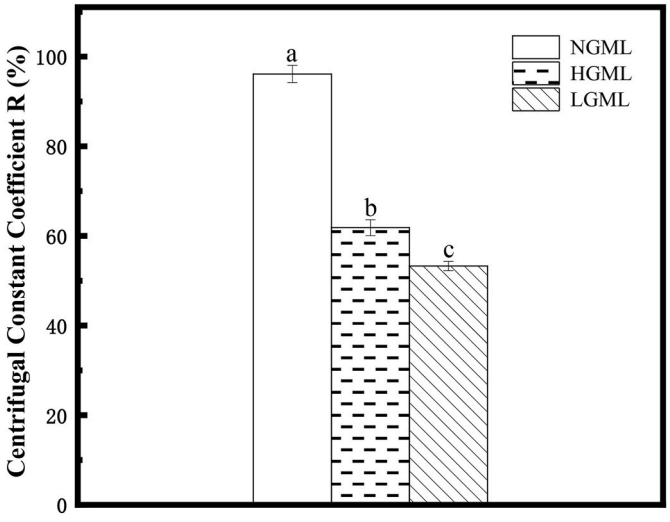

Fig. 1. Centrifugal stability constants of GML coating agent. NGML, HGML, and LGML represents the normal GML coating solution, the higher concentration GML diluent and the lower concentration GML diluent.

3.2. Effect of GML coating on the weight loss rate of duck eggs

During storage, water vapor and carbon dioxide in the egg white escape through the pores of the eggshells, resulting in the decrease of weight (Biladeau and Keener, 2009). As shown in Fig. 2, the weight loss rate of duck eggs increased with the storage time. The control sample (washed by distill water) and the LGML group started to lose weight from the 4th week. The weight loss rate of these two groups reached 20–25% by the end of the study (70 days), while the HGML and the NGML groups showed much lower weight loss rate, which was <11% and <6% at the end of the 70-days storage, respectively. Furthermore, the weight of the NGML coated duck eggs after 70-days storage was not significantly different ($p > 0.05$) to the initial ones (0-days storage). In summary, NGML and HGML coating was noted to reduce or even prevent the weight loss of duck eggs during storage (25 °C, away from light), which was suitable for the long storage of duck eggs. Moreover, at the short storage period (<4 weeks), GML coating did not significantly affect the weight loss rate of duck eggs ($p > 0.05$). Compared with other coating materials, GML coating liquid had a longer time in preventing the loss of ingredients in the eggshell. Caner and Cansiz (2007) reported that chitosan coating reduced the weight loss rate of fresh eggs stored for 4 weeks at room temperature; Xu et al. (2017) reported that soy protein isolate and montmorillonite coating can maintain the weight of eggs within 3 weeks; while NGML coating can still maintain the weight of the duck eggs during the 10 weeks.

3.3. Effect of GML coating on Haugh unit (HU) of duck eggs

HU value is an index for analyzing and expressing the freshness of eggs stipulated by the US Department of Agriculture. It is a recognized method for assessing egg quality. HU value is associated with the weight of the eggs and the height of egg white, reflecting the protein quality

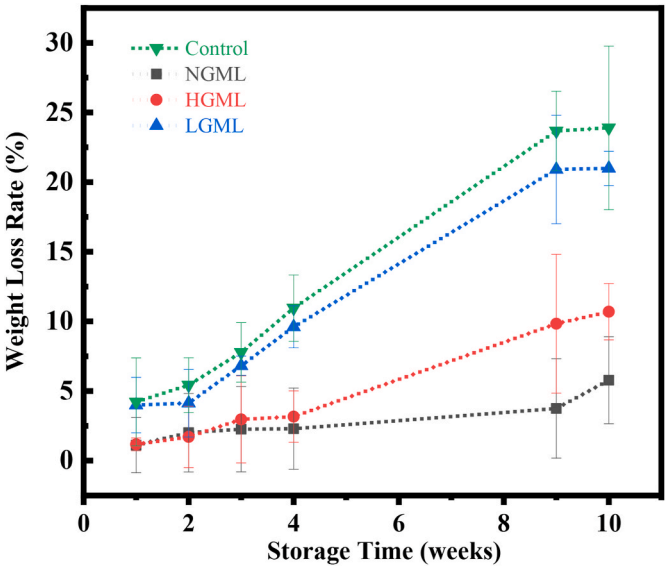

Fig. 2. Weight loss rate of duck eggs. Control, NGML, HGML, and LGML represents the water washed duck eggs, the normal GML coated duck eggs, the higher concentration GML diluent coated duck eggs and the lower concentration GML diluent coated duck eggs, respectively.

(Nongtaodum et al., 2013). The higher the HU, the better the egg quality. The HU value of fresh duck eggs is generally above 72; when it is lower than 60, the egg quality is considered as inferior (Si et al., 2013). The control duck eggs and the GML-coated duck eggs both had the HU value of around 75 with no significant differences ($p > 0.05$) among the samples. The HU value of these duck eggs gradually declined during the 70-days storage (Fig. 3A), indicating that the thick protein layers in duck eggs gradually became thinner during storage, which might be caused by the decomposition of organic compounds in the egg and the loss of carbon dioxide through the eggshell. This finally caused an increase in the pH of proteins (Caner and Cansiz, 2008). Moreover, the water-washed duck eggs started to show significant differences in HU on the 3rd week ($p < 0.05$); the GML-coated duck eggs started to show significant differences on the 4th week ($p < 0.05$). These differences were amplified with the prolonged storage. The findings were similar to the results reported by Suresh et al. (2015). After 10 weeks, the HU of the water-washed duck eggs dropped from 75 to 58, which was below the marketing standard of poultry eggs (Si et al., 2013). On the contrary, the HU of the NGML-coated duck eggs was in the range of 65–70, which was considered as medium fresh. The diluted GML coating solution (Both HGML, and LGML) did not show obvious protection in the freshness of duck eggs. It implied that the NGML coating solution had much better barrier effects than its diluents. Water instead of ethanol was used as the diluting agent to reduce the prime cost of the GML coating method. However, the emulsion system was not stable after the dilution and the protein preservation effect of the water-diluted GML solution was also destroyed.

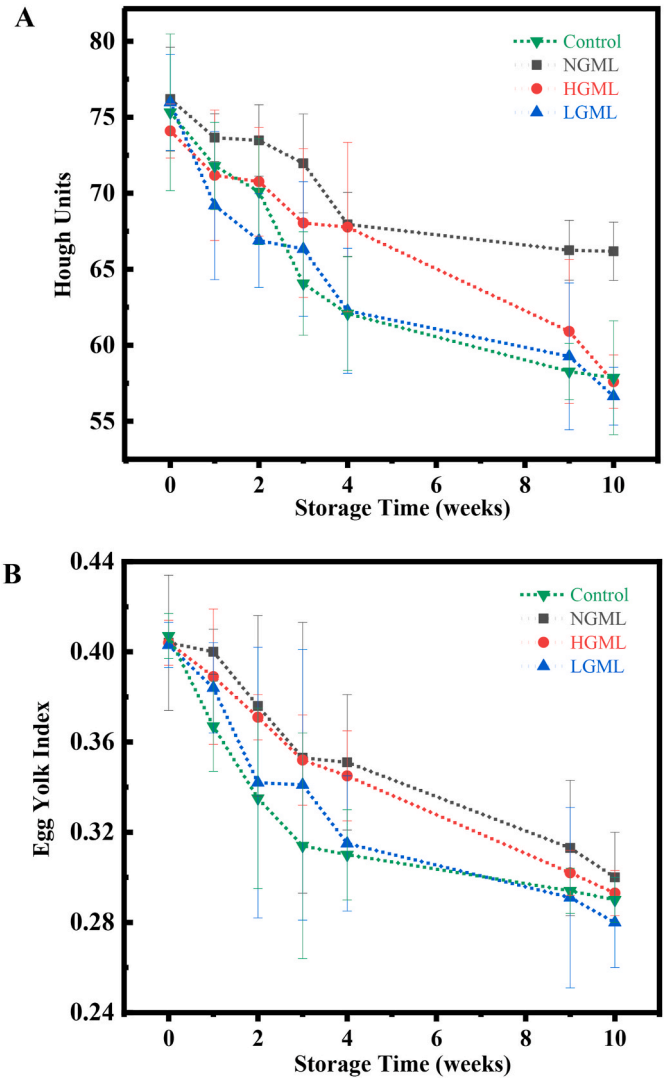

Fig. 3. (A) HU and **(B)** YI value of the duck eggs during storage. Control, NGML, HGML, and LGML represents the water washed duck eggs, the native GML coated duck eggs, the high concentration GML diluent coated duck eggs and the low concentration GML diluent coated duck eggs, respectively.

3.4. Effect of GML coating on yolk index (YI) of duck eggs

Yolk index (YI) is another important indicator to measure the freshness of duck eggs. The YI value of fresh poultry eggs is in the range of 0.3–0.5 (Pires et al., 2021); the yolk of unqualified eggs covers a large area, giving the YI value of less than 0.3. The YI value of the water-washed duck eggs and the GML-coated duck eggs were 0.40–0.41 (Fig. 3B) with no significant differences ($p > 0.05$). During storage, the YI of duck eggs was gradually decreasing, as found by Pires et al. (2019). This is because the water in the egg white gradually migrated to the yolk, reducing the elasticity of the yolk membrane (Si et al., 2013). After 10 weeks of storage, the YI value of the water-washed duck eggs reduced to 0.29. The GML coated duck eggs also dropped to the similar value with very slight differences; the highest YI value was observed for the NGML coated duck eggs (0.30), followed by the HGML coated duck eggs (0.29) and the LGML coated duck eggs (0.28). These results indicated that the NGML coating solution had much better effects than its diluent, the former could slow down the decline of YI in the short-term storage process, but the latter had no significant effect on the migration of the moisture inside the duck eggs due to its low dilution concentration.

3.5. Antibacterial effect of the GML coating

3.5.1. Effect of GML coating on the microbial growth rate of the duck egg

During storage, colonies might appear in duck eggs due to contamination and invasion of external microorganisms. The main ways of microbial pollution are endogenous and exogenous pollution (Mn et al., 2021). Endogenous pollution refers to the pollution that occurs during the formation of eggs in the oviduct or ovary of an infected hen. Exogenous pollution is the pollution of the eggshell, bacterial species in the farm environment or in the process of transportation through the supply chain, as well as microorganisms that penetrate through the eggshell (Musgrove et al., 2005). The detection index of the total number of colonies is a mandatory criterion for quality assurance (Rivas et al., 2014). In general, the total number of colonies should be below 50000 CFU/ml in poultry eggs.

In this study, the total number of colonies of the water-washed duck eggs and the GML-coated duck eggs was measured at the beginning and the end of the storage. At the initial stage, all the duck eggs were fresh (no colonies were detected). After 10 weeks of storage, bacteria was detected in the samples (Fig. 4). However, the GML-coated duck eggs showed significantly different bacterial growth behavior to the water-washed duck eggs. The total number of colonies in the NGML coated samples was 4 lg less than that was found in water-washed duck eggs. The antibacterial effects of the HGML and LGML coating solution were slightly lower than that of the NGML coating solution. Overall, GML coating solution has shown its antibacterial effect during 70-days storage at room temperature (25 °C, away from light). This might be because GML coating liquid formed an effective protective film, which blocked the eggshell pores and prevented microorganisms invasion (Biladeau and Keener, 2009). The water diluted HGML and LGML coating solutions were not as effective as the NGML, owing to their lower emulsion stability and filming ability.

3.5.2. Effect of GML coating on the surface and internal structure of duck eggs

The surface morphology of eggshells was observed under a 100X optical microscope (Fig. 5A). All the duck eggs were washed by distill water before storage; while some of the water cleaned duck eggs were

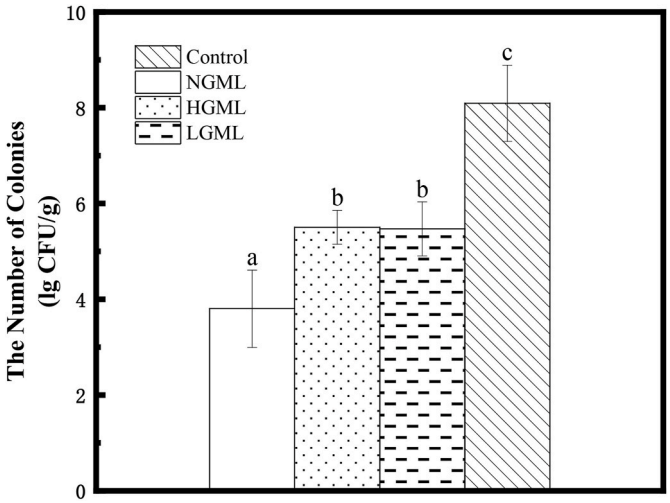

Fig. 4. Total number of colonies of duck eggs at the end of the 70-days storage. Control, NGML, HGML, and LGML represents the water washed duck eggs, the normal GML coated duck eggs, the higher concentration GML diluent coated duck eggs and the lower concentration GML diluent coated duck eggs, respectively.

further coated by the GML solution. As shown in Fig. 5A, the gloss surface of the eggshells was damaged (scratches was observed) after cleaning. This indicated that the biological protective film of duck eggs was destroyed. The GML-coated duck eggs showed much glossier surface compared to the water-washed ones. No scratches were observed on the NGML coated duck eggs. This implies that GML can fill the scratches on the eggshell and form a protective film to compensate the surface damage (induced by the water washing process). As a consequence, the GML-coated duck eggs showed a smoother and more intact surface, compared to the water-washed duck eggs after 10-weeks storage. These findings also supported the antibacterial and quality protection effect of the GML coating.

The images of the duck eggs contents are presented in Fig. 5B. After 10 weeks storage, the control and the LGML-coated duck eggs showed obvious spoilage contents. The turbid, thin egg liquid, and strong odor all suggested that these samples reached the highest stage of poultry egg spoilage (Quan and Benjakul, 2018). On the contrary, the NGML-coated duck eggs still showed a bright color; the yolk and the egg white were clearly distinguished; and the yolk membrane was intact. This indicated that the NGML coating not only protected the surface of the duck eggs, but also prevented the spoilage of duck eggs content. The HGML-coated duck eggs were between the stages of the NGML-coated duck eggs and the water washed duck eggs. Its protein layer became thinner, the white and egg yolk boundary began to blur, and the yolk lost elasticity. This might be the effect of microbial invasion in the early stage of poultry egg spoilage. In summary, the GML coating solution has a good effect on maintaining the internal quality of duck eggs and the appearance looks no different from fresh duck eggs. The protection effect of HGML diluent and LGML diluent was not that satisfactory though they prolonged the shelf life of duck eggs to a certain extent.

4. Conclusions

GML coating has a positive effect on the preservation of duck eggs. Compared with its diluents (HGML and LGML), the NGML coating emulsion is more stable, more effective but unfortunately, more expensive. However, NGML coating method was able to extend the shelf life of the duck eggs by more than 6 weeks (25 °C, away from light). The weight loss rate, HU, and YI of duck eggs and the total colony counts results all supported the findings. Moreover, the surface and internal content of the NGML-coated duck eggs were maintained after 70 days storage. Although the higher or lower diluents of GML coating were less effective, they might be suitable for a short storage of duck eggs due to their lower primary cost. The GML coating methods may also be applicable in the preservation of other food products.

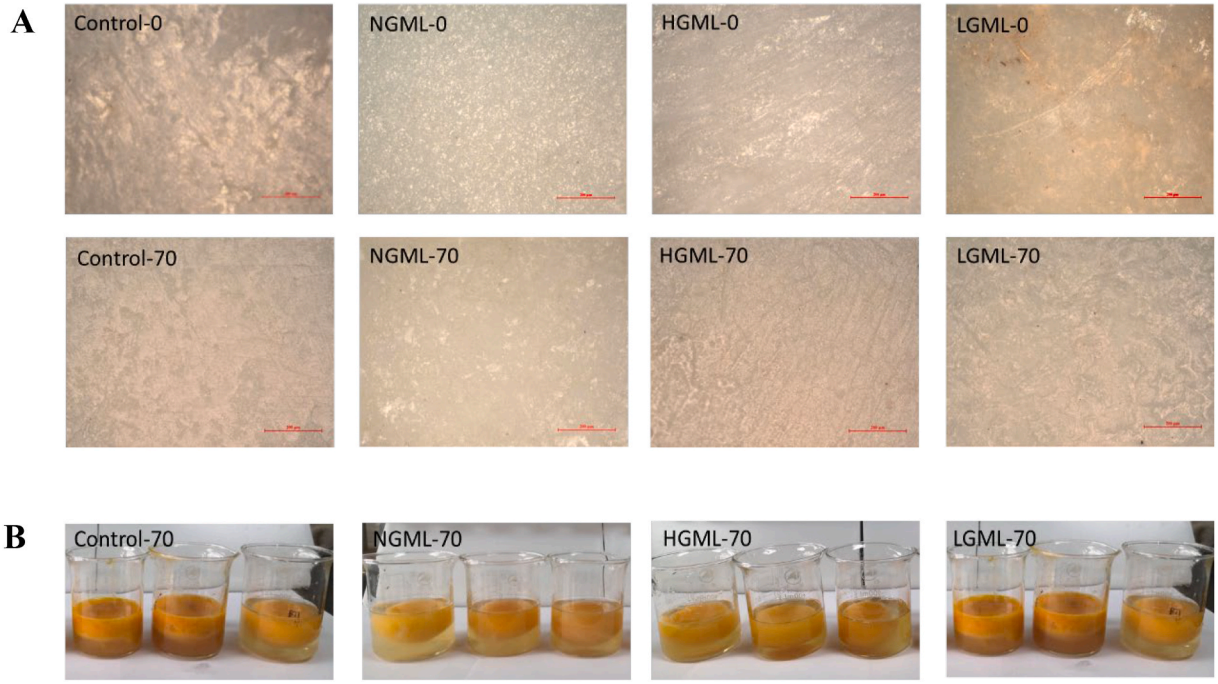

Fig. 5. (A) Optical microscopic images of the surface of the duck eggs and **(B)** direct view of the contents of the duck eggs. Control, NGML, HGML, and LGML represents the water washed duck eggs, the normal GML-coated duck eggs, the higher concentration GML diluent-coated duck eggs and the lower concentration GML diluent coated duck eggs, respectively. The numbers after hyphen indicate the storage time (days) of the duck eggs.

CRediT authorship contribution statement

Bo Wang: Literature review, manuscript edition, graph and figures, response to the reviewers' comments, funding support. **Jingwen Zhang:** Experiments, Writing – original draft, Manuscript edition, response to the reviewers' comments. **Yuchuan Wang:** Guidance on the topic selection, Manuscript edition. **Tiantian Xu:** Experimental design, Grammar check, Manuscript edition, response to the reviewers' comments. **Cunshan Zhou:** literature review, manuscript edition, laboratory support.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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