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Research Article

Study on enhancement of poultry egg shelf life under room storage

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Abstract

Eggs are nutritious food commodities that are consumed all around the world. Freshness is a major contribution to egg quality. However, eggs are perishable and susceptible to quality losses, and contaminated with certain microorganisms during storage. Currently, there are very few companies such as Ba Huan, CP, and Dabaco in Vietnam operating technology for processing poultry eggs and most of the eggs are sold untreated (no washing or disinfection). The study aims to determine the effect of saline, hot water, and *Litsea cubeba* essential oil (EO) on the freshness of chicken eggs including weight loss, air cell size, Haugh unit, Yolk index, pH, total viable count properties and shelf life of eggs at 37°C storage. The best preservation effect was found in the sample sprayed with *L. cubeba* EO at 4 mg/mL, followed by dipping in hot water at 65°C for 90 s and dipping in saline solution at 0.9% for 30 s to maintain the freshness of egg. The EO-treated egg could remain at average quality for up to 16 days at 37°C compared to 7 days of untreated sample. The obtained results demonstrated the potential application of natural products in food preservation.

Keywords: *egg shelf life, Litsea cubeba essential oil, Haugh unit, egg freshness*

1. INTRODUCTION

The production of poultry, along with egg production, has been increasing during the last two decades, both worldwide and in Vietnam. According to the Ministry of Agriculture and Rural Development, the production volume of eggs is rising from 6.3 billion eggs in 2010 to 19 billion eggs of all kinds in 2023. Poultry eggs are considered one of the nature's

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most complete foods because of its high nutritional value and cheap source of protein. They contain nutrients, protein, vitamins and minerals which are beneficial for the human body. They are staple foods in the human diet and are consumed globally. In addition, eggs are widely used in the food industry due to their multifunctional properties [1].

However, eggs are perishable and susceptible to contamination with certain microorganisms [2]. Although eggshells have been regarded as natural protective barriers of eggs, nearly 10,000 tiny pores present on the eggshells lead to the moisture and carbon dioxide loss of eggs, and they facilitate the penetration of certain microorganisms into the interior of eggs. The most profound factor that affects egg quality is storage temperature. Quality deterioration of eggs stored for 10 days at 27°C was comparable to that of eggs stored for several months at –1°C. Nevertheless, in some developing countries where refrigeration of eggs is seldom practiced, surface treatment is an alternative method to preserve the internal quality of eggs and to prevent microbial contamination [3]. Therefore, extending shelf-life and maintaining the quality of fresh eggs during storage by using novel methods were required.

Various methods are implemented to enhance the shelf life of eggs worldwide. For example, egg washing has established practice in the United States, Australia, and Japan [4]. For instance, egg washing can be used to decontaminate the surface of eggs and thus may lower the rate of penetration of *Salmonella* across the eggshell and decrease the incidence of food poisoning [2]. Pasteurization is also demonstrated to sanitize or improve the preservation of the eggs [5]. Sodium chloride also plays a role in reducing the growth of pathogens and organisms that cause spoilage, thereby extending shelf life. More recently, edible shell coating in order to extend the shelf-life and quality of foods during storage has been investigated [2]. Among them, essential oils (EOs) have been used widely as natural preservatives in food. They were used as an additive in edible hydrophilic films and coatings for food preservation. May Chang *Litsea cubeba* EO (Lauaceae family) is widely distributed in Vietnam and has been reported its application in meat preservation at the concentration of 0.2% w/w. The EO coating could delay food ageing and reduce its microbial load and leading to extending the shelf life of food [6].

In fact, there are very few companies in Vietnam (such as Ba Huan, CP and Dabaco) operating technology such as ozone-treated, UV-treated, and mineral oil coated of processing poultry eggs. Most of the table eggs come from the backyard/small-scale poultry producers, and the eggs are sold untreated (no washing or disinfection) and the storage of egg in refrigerator temperature is seldom practiced. Most of eggs are preserved at room temperature and the quality deteriotion happens very quickly. Therefore, the price of eggs has not made many profits for the business. In addition, to the best of our knowledge, the report on the enhancement of the shelf life of poultry eggs is still limited in Vietnam. For instance, chitosan, nanosilver and nanochitosan were investigated in enhancing the shelf life of chicken eggs [7]. The objective of this research is to investigate the effect of saline solution, hot water and *L. cubeba* EO on egg preservation at 37°C.

2. MATERIALS AND METHODS

2.1. Materials

The freshly laid eggs of one day post-lay were collected at Thuy Phuong Poultry Research Centre – National Institute of Animal Sciences, located in Hanoi. Then, the eggs were transferred immediately to the lab. Eggs then were cleaned by using a clean towel to wipe away dirt, straw, and trash adhering to the eggshell. After screening for defects and desirable weight range, eggs were individually weighed with a balance and the eggs with the same size and weight will be chosen for further studies.

L. cubeba EO was provided by the Faculty of Food Technology, School of Life Science, Hanoi University of Science and Technology. *L. cubeba* EO was prepared by dissolved in water containing 0.05% Tween 80 as emulsifier. Each 55 g egg with a surface area of about 50 cm^2 was sprayed with 1 mL of EO solution at a concentration of 4 mg/mL.

2.2. Experimental design

Four treatments were evaluated throughout the storage periods, including:

KC: control group (untreated egg)

M1: Dipping in saline solution (0.9%) for 30 s

M2: Dipping in hot water 65°C for 90 s

M3: Spraying with *L. cubeba* EO at 4 mg/mL

The parameters of treatments were chosen based on our previous studies [6] and the conditions of *Salmonella* inhibition. The eggs were then left to dry at ambient temperature. A total of 240 eggs (20 eggs per tray, four treatments, three replicates) were placed in egg trays with their small ends down in storage at 37°C – considered as room temperature in summer – until testing. To determine of egg quality, five marked eggs of each group will be taken out to weigh weekly, and were returned back right after the measurement. Another five eggs of each group will be picked up randomly to measure the Haugh unit, yolk index and albumen pH at day 4, 7, 10, 12, 14, 16, 18 at 37°C [8]. For microbiological analysis, total plate count (TPC) of all eggs was evaluated during storage [3].

2.3. Methods

2.3.1. Determination of weight loss

Weight loss (%) of the treated egg during storage was calculated as:

$$WL(\%) = \frac{M - m_i}{M} \times 100\%$$

Where:

M = initial whole egg weight (g) after coating at day 0;

m_i = whole egg weight (g) after storage at day i. The weight of whole eggs was measured with a balance [3].

2.3.2. Determination of air cell size

Candling the eggs by holding the egg from the small end where the large end was up to a light. The diameter of the eggs was determined by using a marker to draw the air cell and then using Image J program to measure (Figure 1). According to TCVN 1858:2018, eggs

can grade based on air cell as follows: AA (< 3mm), A (< 5mm), B (<8mm). The increase of air cell (%) during storage was calculated as:

$$AC(\%) = \frac{x_i - X}{X} \times 100\%$$

Where: x_i = air cell (mm) after storage at day I, X = initial air cell (mm) at day 0.

2.3.3. Determination of Haugh Unit

Haugh unit is a value related to egg weight and thick of albumen, which was calculated using Equation:

$$HU = 100 \log (h - 1.7m^{0,37} + 7.6)$$

where H is the albumen height (mm) and W is the weight of whole egg (g). According to the TCVN 1858:2018, eggs are classified into AA, A and B grade, which require Haugh unit value to be ≥ 72, ≤ 60 – 72, and < 60, respectively.

2.3.4. Determination of Yolk Index

Yolk index was calculated as the ratio of yolk height/yolk width. The egg yolk's height (mm) and width (mm) were measured using a micrometre (Figure 1).

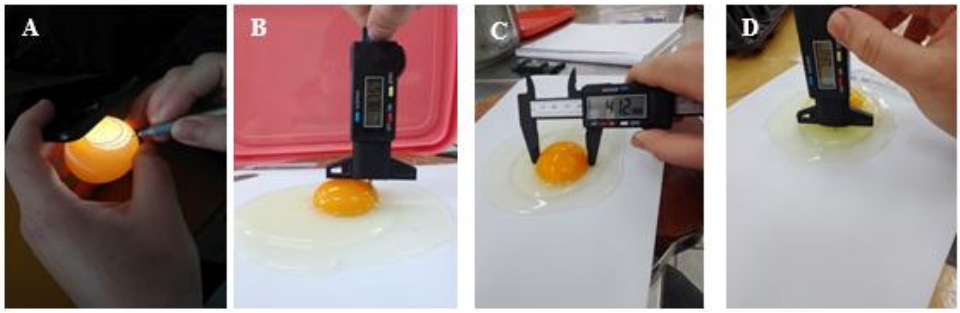

***Figure 1**. (A) Candling egg; determination of (B) the height and (C) diameter of egg yolk;(D) determination the height of egg white*

2.3.5. Determination of pH of albumen and yolk

After measurement of Haugh unit and yolk index, the albumens will be separated from the yolk. pH values will be measured by a digital pH meter.

2.3.6. Microbiological analysis

The eggshell and mixture of yolk and albumen the of egg was homogenized in a dilution of 1:10 of 0.9% saline water. For total plate count, viable cells (CFU/g) were enumerated on plate count agar (PCA) by the pour plate method followed by incubation at 30°C for 48 h [3].

2.3.7. Data analysis

Data were analyzed using SPSS 18 software at a significant level of 0.05.

3. RESULTS AND DISCUSSION

3.1. Effect of treatment methods on the weight loss of eggs during storage

The results of weight loss during storage were shown in Figure 2. Indeed, the weight loss of all treatments increased over time, but the degree of decline varies in different samples. Up to day 18, the highest weight loss was observed at KC (11.69%), corresponding

to a decrease from 57.37 g to 50.79 g), followed by M1, M2 and M3 samples. Significant differences were found between treated samples.

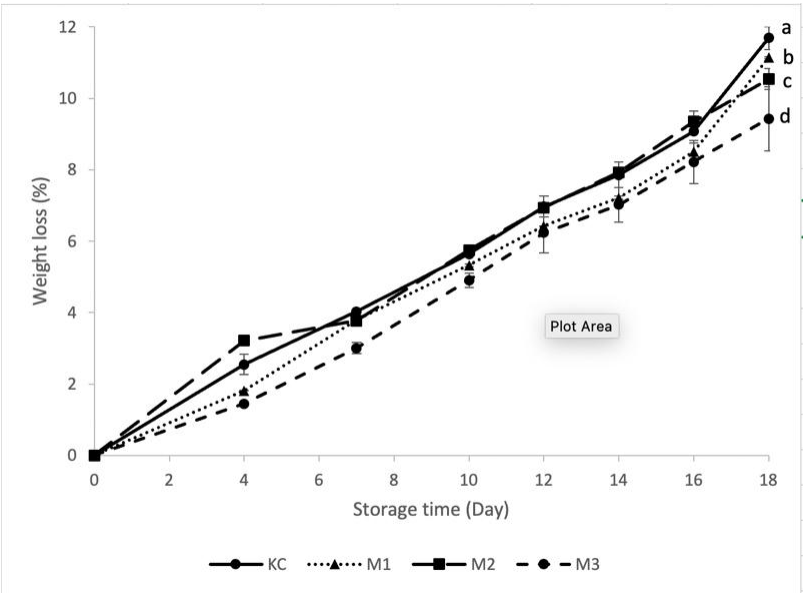

***Figure 2**. Weight loss (%) of eggs during storage*

Values with different lower-case letters are significantly different

Due to the escaping of CO_2 and water vapor in albumen through pores on the eggshells during storage, the weight of eggs was decreased. Therefore, weight loss rate of eggs is an important index of egg quality. The permeability coating layer on the eggshell will minimize the evaporation from the egg. Rachtanapun P. et al. (2022) reported that the weight loss of uncoated eggs and coated eggs with cassava starch + carboxymethyl cellulose + paraffin at 30°C were 4.7% and 3.3% after 4 weeks, respectively. Higher weight loss during storage found in this study could be due to the difference in storage temperature [9].

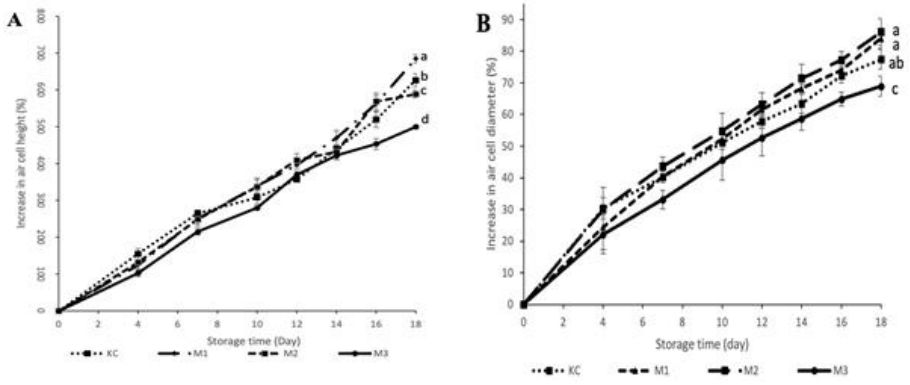

***Figure 3**. Change in (A) air cell height and (B) air cell diameter of eggs during storage*
Values with different lower-case letters are significantly different

Air chamber size is one of the indicators of fresh eggs. Figure 3A and 3B show that all egg sample' air cell size (including height and air cell diameter) increased with storage time. The lowest increase in air cell size was obtained in EO-treated samples, followed by M2,

M1 and KC. The egg dipping in saline solution or hot water could increase the osmotic pressure or expand holes in the eggshell, leading to the loss of water and CO_2. Meanwhile, spraying with EO could help penetrate of microorganism through the holes in the eggshell.

3.2. Effect of treatment methods on the Haugh unit of eggs during storage

Haugh unit is an important parameter of egg freshness. As given in Table 1, one-day laid eggs have an HU of 84 (grade as AA class). The untreated egg was downgraded to A and grade B at the day 4 and day 7 of preservation, respectively. Whereas saline-treated, hot-water treated eggs maintained at grade B until days 12 and 14, respectively. The EO-treated showed the most effective effect with the egg grade classified as B until day 10 at 37°C preservation (Table 1). The trend of HU gradually decreasing with storage time is consistent with the results of previous studies [10, 11]. However, egg quality in this study declined faster than the ones used for other coatings at lower temperatures. Specifically, P. Okiki P. and O. Almed (2017) demonstrated that vegetable oil-coated egg could help to maintained quality at grade A until day 50th at 28°C [10] or W.E. Pamarin et al. (2009) reported that the quality of coated eggs with mineral oil help to keep eggs at grade A for 3 weeks at 25°C [11]. This can be explained by the oil's ability to block the air pores of the eggshell.

Table 1. *Change in Haugh unit of eggs during storage*

Storage time (day)	KC	M1	M2	M3
0	$84.46\pm0.49^{Aaa*}$	84.46 ± 0.49^{Aaa}	84.46 ± 0.49^{Aaa}	84.46 ± 0.49^{Aaa}
4	56.95 ± 0.35^{Ba}	61.20 ± 0.63^{Ab}	64.36 ± 0.66^{Ac}	68.83 ± 0.62^{Ad}
7	**43.67 ± 1.46^{Ba}**	56.11 ± 1.44^{Bb}	58.88 ± 0.46^{Bb}	65.29 ± 1.44^{Ac}
10	-	49.56 ± 1.33^{Ba}	55.78 ± 1.33^{Bb}	59.34 ± 0.48^{Bc}
12	-	**42.96 ± 1.47^{Ba}**	50.17 ± 1.63^{Bb}	56.18 ± 1.36^{Bb}
14	-	-	**44.72 ± 1.23^{Ba}**	50.66 ± 1.16^{Bb}
16	-	-	-	**41.47 ± 1.46^{Ba}**
18	-	-	-	-

(-): not determined because the egg white is liquid.

*Egg grade: AA, excellent (≥72); A, high quality (71–60); B, average quality (59–31)
Values with different lower-case letters in a row are significantly different.

The decrease in the Haugh unit during storage is due to thinning of albumen. This thinning is due to the increase of clusterin and Ovoinhibitor. It is also due to the disordered structure of ovalbumin. These protein changes have been mainly attributed to the proteolysis of dense protein or to the increase of albumin pH, which is influenced by the losses of water and CO_2 during storage [5].

3.3. Effect of treatment methods on the yolk index of eggs during storage

An important indicator of egg freshness in addition to the Haugh unit is the yolk index. Similar to the Haugh unit, eggs' yolk index decreased gradually with storage (Table 2). Osmotic pressure-driven continuous absorption of water from albumen to yolk through the vitelline membrane was identified as the cause of the reduced values of the yolk index with storage time. This resulted in the liquefaction and flattening of the yolk. The albumen

viscosity was correlated with the osmotic pressure between the albumen and yolk, and this was diminished when the ovomucin-lysozyme complex broke down. Therefore, when EO coating reduced the loss of CO_2 and water vapor slowed down the structural changes in albumen, the increase in osmotic pressure between albumen and yolk would be slowed down, resulting in improved yolk quality [5].

Table 2. *Change in Yolk index of eggs during storage*

Storage time (day)	KC	M1	M2	M3
0	0.46±0.01^{a}	0.46±0.01^{a}	0.46±0.01^{a}	0.46±0.01^{a}
4	0.25±0.01^{a}	0.28±0.01^{b}	0.26±0.02^{ab}	0.32±0.01^{c}
7	0.15±0.01^{a}	0.19±0.01^{b}	0.19±0.01^{b}	0.22±0.02^{b}
10	-	0.17±0.01^{a}	0.15±0.02^{a}	0.16±0.02^{a}
12	-	0.13±0.01^{a}	0.14±0.01^{a}	0.14±0.02^{a}
14	-	-	0.13±0.00^{a}	0.13±0.01^{a}
16	-	-	-	0.12±0.01^{a}
18	-	-	-	-

Values with different lower-case letters in a row are significantly different

In an older egg, the air cell will be greater, and the yolk will move freely when the egg is manipulated. Additionally, once an egg is cracked open, a firm, high-sitting yolk with a tight surrounding white is a good sign of a fresh egg as opposed to a flattened and pale yolk with a runny white (Figure 4). When eggs were cracked, no effect of *L. cubeba* EO on the sensory properties of the egg was observed. It can be explained that during preservation, EO was evaporated and did not cause the sensory quality to deteriorate.

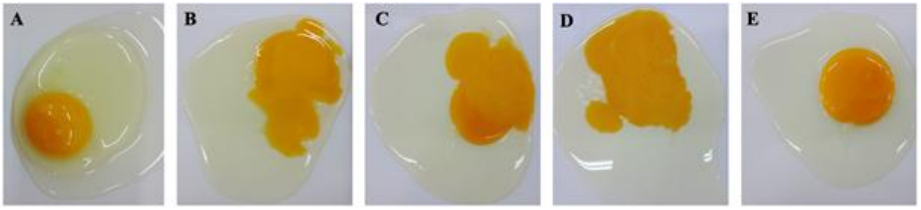

Figure 4. *The freshly laid egg (A) and older eggs at day 16th of KC (B); saline (C); hot water (D) and essential oil (E) treated samples*

3.4. Effect of treatment methods on the pH of eggs during storage

Since the albumen of fresh-laid eggs is saturated with CO_2, the evacuation of CO_2 through eggshells led to an increase in the albumen pH with increasing storage time [5, 8]. pH of albumen and yolk of fresh-laid egg were 8.38 and 6.20, respectively. As given in Figure 5A, a sharp increase of pH albumen in the first 4 days of storage was observed and remained at this value (around 9) during storage time in all treatments. pH of yolk was also increased in all treatments during preservation due to the absorption of water from the egg white into the yolk and the proteolytic activity of microorganisms. The lowest pH of yolk was presented in EO-treated, whereas the highest was presented in KC sample. The obtained results demonstrated the effect of EO in egg preservation.

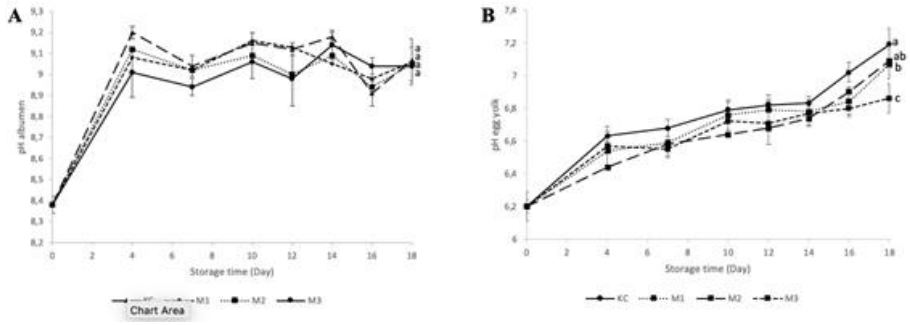

***Figure 5**. Change of (A) pH albumen and (B) pH egg yolk during storage Values with different lower-case letters are significantly different*

3.5. Effect of treatment methods on the total viable microorganisms number of eggs during storage

Most eggs when laid contain very few or no bacteria, and the entrance of these microorganisms usually takes place because of carelessness or neglect on the part of those handling the eggs. Microbial growth is one of the important causes of egg spoilage. The results on the effects of protection methods on the TPC on eggshells and in eggs were presented in Fig. 5. In fact, the number of bacteria on the eggshell in all treatments increased along with the storage time. On the 18th day, KC sample had the highest number of microorganisms at 4.49 log CFU/mL, followed by M1 (4.48 log CFU/mL), M2 (4.3 log CFU/mL) and M3 (3.67 log CFU/mL) (Figure 6A). Thus, using preservation measures has helped significantly reduce the number of microorganisms clinging to eggshell. In addition, the microorganism was not detected in KC, M1 and other treatments after 4, 7 and 10 days of preservation in eggs, respectively (Fig. 6B). Based on the results of TPC, the use of EO was inhibited significantly the growth of bacteria in eggshell and eggs leading to prolong the freshness of the eggs compared to control sample. Previous studies showed that the total viable microorganisms of 4-week storage carboxymethyl cellulose and paraffin-coated eggs at 4°C and 30 were < 1 log CFU/mL and 2.83 log CFU/mL, respectively [9]. Differences in results may be due to the differences in storage temperature and the initial number of bacteria in eggs that are affected by the condition of farming.

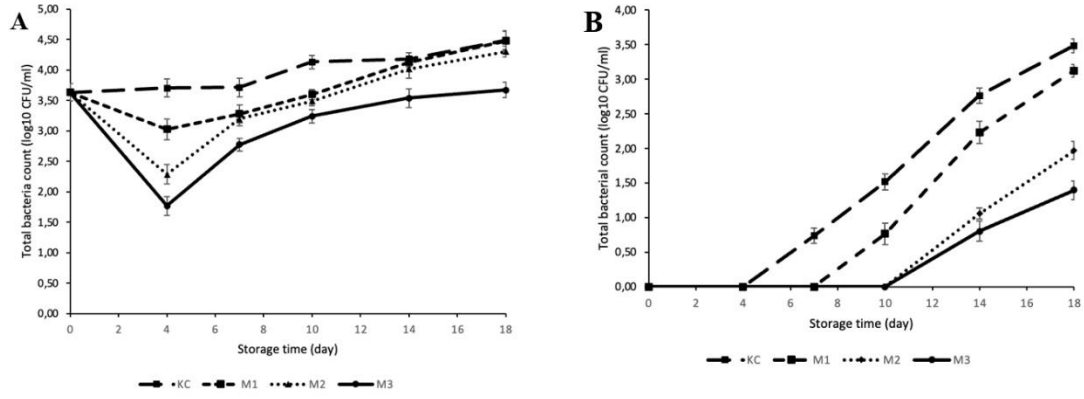

***Figure 6.** The total bacterial count of (A) eggshell and (B) mixture of egg whites and egg yolk during storage at 37℃*

Values with different lower-case letters are significantly different

Thus, the quality deterioration of egg during storage at 37°C can be evaluated as follows:

- Untreated egg: the egg deteriorated (grade B) after only 7 days
- Saline treatment: eggs can keep at grade B after 12 days
- Hot water treatment: eggs can keep at grade B after 14 days
- L. cubeba EO treatment: eggs can keep at grade B after 16 days

4. CONCLUSION

In conclusion, spraying with *L. cubeba* EO (4 mg/mL) on eggshell showed the best effect in maintaining the freshness of egg at average quality for up to 16 days at 37°C in terms of physical (weight, air chamber, HU, YI), pH and microorganisms parameters compare to saline and hot water treatment. Meanwhile, the untreated eggs sample deteriorated (average quality) after only 7 days under the same storage condition. Furthermore, due to the limitation in research design, EO coating has been proposed to be tested at an industrial model. The above preliminary results demonstrated the potential application of natural products in food preservation.

REFERENCES

[1]. C. Caner and M. Yüceer, "Efficacy of various protein-based coating on enhancing the shelf life of fresh eggs during storage," *Poultry science*, vol. 9, no. 7, pp. 1665-1677, 2015.

[2]. A. S. Eddin, S. A Ibrahim and R. Tahergorabi, "Egg quality and safety with an overview of edible coating application for egg preservation," *Food Chemistry*, vol. 296, pp. 29-39, 2019.

[3]. D. D. Torrico, W. Wardy, K. M. Carabante et al., "Quality of eggs coated with oil–chitosan emulsion: Combined effects of emulsifier types, initial albumen quality, and storage," *LWT-Food Science and Technology*, vol. 57, no. 1, pp. 35-41, 2014.

[4]. S. Leleu, W. Messens, K. De Reu et al., "Effect of egg washing on the cuticle quality of brown and white table eggs," *Journal of Food Protection*, vol. 74, no. 10, pp. 1649-1654, 2011.

[5]. P. M. De Souza, R. de Melo, M. A. de Aguilar Santos, F. R. Lima, and K. H. Vieira, "Risk management of egg and egg products: Advanced methods applied," *Food Enginering*, Intech Open publishing house, pp. 82691, 2019.

[6]. Nguyen Hai Van, Luu Viet Ha, Hoang Thi Kim Anh, Phan Thanh Tam "Research on the ability to preserve fresh pork with a mixture of (*Litsea cubeba*) essential oil and chitosan at cold temperatures," *Vietnam journal of Agricultural and Rural Development*, vol. 9, pp. 81-88, 2021 (in Vietnamese).

[7]. Vu Thi Thuy Dung, Nguyen Thi Mai Lien, Pham Thi Phuong, and Luong Hung Tien, "Research on the effects of low molecular weight chitosan coating combined with

nano silver on fresh chicken eggs during storage," *TNU Journal of Science and Technology*, vol. 225, no. 1, pp. 134-139, 2020 (in Vietnamese).

[8]. D. Xu, J. Wang, D. Ren, and X. Wu, "Effects of chitosan coating structure and changes during storage on their egg preservation Performance," *Coatings*, vol. 8, no. 9, pp. 317, 2018.

[9]. P. Rachtanapun, N. Homsaard, A. Kodsangma et al., "Effects of storage temperature on the quality of eggs coated by cassava starch blended with carboxymethyl cellulose and paraffin wax," *Poultry Science*, vol. 101, no. 1, pp. 101509, 2022.

[10]. P. Okiki and O. Ahmed, "Preservation of Quality of Table Eggs Using Vegetable Oil," *International Letters of Natural Sciences*, vol. 63, pp. 27-33, 2017.

[11]. W. E. Pamarin, M.G. Karen, K.N. Hong, P. Witoon and R.I. Dennis R., "Selected Quality and Shelf Life of Eggs Coated with Mineral Oil with Different Viscosities," *Journal of Food Science*, vol. 74, no. 9, pp. 423-429, 2009.

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