Effects of storage temperature on the quality of eggs coated by cassava starch blended with carboxymethyl cellulose and paraffin wax

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ABSTRACT A blend of cassava starch (**CS**), carboxymethyl cellulose (**CMC**), and paraffin was prepared as a coating material to maintain the quality of eggs during 4 wk of storage at different temperatures. The efficacy of the CS/CMC/paraffin (6/1/0.5% w/v) coating was investigated in terms of the Haugh unit (**HU**), weight loss, pH, and microbial load at the end of storage. The best egg storage temperature was 4°C, which maintained an HU of grade AA in coated and uncoated eggs for 4 wk. Lower weight loss (2.14%) was observed in coated eggs at 4°C storage than at 30°C storage (3.26%). The pH in the albumen of coated and uncoated eggs at 4°C increased from 6.84 to 6.88 and 7.01 to 7.03, respectively, after 4 wk of storage. No microbes were detected in the coated and uncoated eggs at 4°C. The maximum microbial count was 728 ± 35 cfu/mL in uncoated eggs at 30°C storage. Egg coating prevented microbial contamination of eggs stored at 30°C for 4 wk. The freshness of the eggs did not affect the nutrient content. The egg-coating material effectively maintained egg quality, prevented microbial contamination of eggs, and increased the shelf life of eggs at storage temperatures of 25 and 30°C.

Key words: carboxymethyl cellulose, *Manihot esculenta*, egg quality, nutrition, microbial

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INTRODUCTION

In human diet, eggs of high nutrient quality are required because they act as a protein source (Kul and Seker, 2004). Eggs are an excellent source of high-quality protein, carotenoids, antioxidants, phospholipids, and vitamins (Lesnierowski and Stangierski, 2018). The nutritional value of eggs has influenced their popularity. The major proteins such as ovomucoid, ovotransferrin, ovalbumin, and lysozyme are present in the albumen. The storage conditions and the presence of contamination within the egg yolk or albumen influence the internal quality of the egg, whereas nutritional factors influence the quality of the albumen. When eggs are laid, the chemical, physical, and functional characteristics start aging processes inside the eggshell. Diseases caused by consuming eggs contaminated with microorganisms, such as *Salmonella*, pose a risk to consumers (De Reu et al., 2006, 2008; Cao et al., 2009; Chousalkar et al., 2010). Eggs are highly perishable because eggshells are breathable materials. It effects to distribution, storage, selling, quality, and shelf-life of eggs and food products from these eggs. Egg coating is an effective and economical technology to preserve the internal quality of eggs. Edible egg coating is harmless material which is recognized as Generally Recommended as Safe (**GRAS**).

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Haugh units (**HU**) were used to determine the interior egg quality and the rate of quality loss. Several researchers have hypothesized that there is a bias between HU and egg weight. However, egg weight does not influence the albumen index, which is better than other quality measurements. The first 1 cm of albumen from the edge of the egg yolk is used to assess the albumen quality. The height of the albumen also defines the egg quality rating. A lower viscosity of albumen represents a poor-quality rating (Karoui et al., 2006). The freshness of egg albumen is measured using albumen pH (Scott and Silversides, 2000). The gel thickness of albumen and egg yolk represents internal egg quality; moreover, high-quality eggs exhibit low microbial contamination in the egg yolk (Biladeau and Keener, 2009).

Coating the egg surface can potentially preserve the quality of the contents, increase shell strength, and decrease the microbial load on the eggshell surface (Falguera et al., 2011). The internal contents of eggs can be contaminated with microbes that enter through the pores of the eggshell. Therefore, a coating method is required to protect the internal quality of eggs with low cost and effective technology (Nongtaodum et al., 2013). The main edible coatings in the food processing industry are lipid, protein, and polysaccharide based. Lipid-based edible coatings are commonly applied to fresh fruits and vegetables because their hydrophobicity prevents moisture loss. The most common lipids used in edible coatings are fatty acids (Suppakul et al., 2010). Polysaccharide-based coatings are popular owing to their high flexibility, low thickness, and high transparency (Gast and Holt, 2001). Chitosan is an effective protein-based material for egg coating that prevents microbial contamination (Yang et al., 2019).

Many biomaterials can be used as egg coatings. Some researchers have reported the synthesis of biomaterials such as polysaccharides from natural resource materials (Chaisuwan et al., 2020), carboxymethyl cellulose (**CMC**) (Tantala et al., 2019; Klunklin et al., 2021), and pectin (Chaiwarit et al., 2018). The properties of materials suitable for egg coatings, such as starch (Jantanasakulwong et al., 2019; Kodsangma et al., 2020; Rachtanapun et al., 2021), CMC (Suriyatem et al. 2020), carboxymethyl chitosan (Chaiwong et al., 2020), keratin (Kaewsalud et al., 2020), fibroin (Yakul et al., 2020), and pectin (Wongkaew et al., 2020), have also been investigated. Many natural products, such as propolis (Copur et al., 2008), whey protein (Caner and Yuceer, 2015), CMC (Homsaard et al., 2020), rice protein (Pires et al., 2018), chitosan (Wardy et al., 2011), and protein isolate (Pires et al., 2018), have been used as eggshell coatings to increase shelf life. The effect of coating materials and temperature storage on antibacterial properties (Yang et al., 2019) and quality of eggs (Samli et al., 2005) has been investigated.

However, the effect of temperature storage on the quality of eggs coated with cassava starch (**CS**) has not been reported. In this study, the eggs were coated with CS blended with CMC and paraffin wax. The effect of temperature (4, 25, and 30°C) on the quality of coated eggs was observed. HU, weight loss, albumen pH, microorganism, and nutritional quality of eggs were investigated during 4 wk of storage.

MATERIALS AND METHODS

Materials

Fresh eggs were obtained from R.P.M Farm & Feed Co., Ltd. (Hang Dong, Chiang Mai). CS (Dragon Fish brand; moisture content of 11% total weight, amylose/amylopectin content 17%/83%, and molecular weight of 1.34×10^8 g/mol) was obtained from Tong Chan Registered Ordinary Partnership, Bangkok, Thailand. Glycerol (99%) was purchased from Union Science Co., Ltd. (Chiang Mai, Thailand). CMC (grade 700, degree of substitution of 0.8, and molecular weight of 270,000 g/mol) was purchased from Cp Kelco Oy, Äänekoski, Finland. Paraffin wax was purchased from Hong Huat Co., Ltd. (Bangkok, Thailand).

Preparation of the Coating Solution and Egg Coating

Eggs in the weight range from 66 to 70 g were screened for surface cracks, breakage, and cleanliness on the first day at the farm before being wiped with the coating. CS was mixed with CMC and paraffin wax (6/1/0.5% w/v) at 500 rpm and 80°C for 20 min using an overhead stirrer. Eggs were wiped with the coating material by being dipped into coating material solution (separated solution in each sample). They were then placed in egg racks, left to dry at 28 to 30°C for 30 min, and then stored at 4, 25, and 30°C at 65 ± 2% RH. The internal quality of the eggs was observed for 4 wk. Uncoated eggs were also maintained under the same conditions and compared for differences in weight loss, albumen pH, and albumen quality.

Measurement of HU and Albumen pH

HU was measured with a digital egg tester (Model DET6500, NABEL Co., Ltd., Kyoto, Japan) and the pH was measured with a pH meter (Eutech Instruments pH−510, Ayer Rajah Crescent, Singapore) in both coated and uncoated eggs. Five samples from each condition were measured weekly for 4 wk. HU was calculated as 100 log (H − 1.7W0.37 + 7.6), where H and W are the albumen height (mm) and the weight of the egg (g), respectively (Cindric et al., 2007). The pH of the albumen separated from the yolk was measured over five sample using a pH meter.

Determination of Weight Loss

Five eggs from each condition were weighed immediately before and after storage at different temperatures.

The average weight loss of the coated and uncoated eggs was evaluated over 5 sample according to Equation (1):

$$\left\{\frac{[\text{initial whole egg weight (g) after coating at day 0} - \text{whole egg weight (g) after storage}]}{\text{initial whole egg weight (g) after coating at day 0}}\right\} \times 100 \quad (1)$$

Detection of Micro-organisms in Eggs

A 25 mL representative egg sample (combined egg yolks and albumen) was diluted 10-fold with 0.85% NaCl. Five samples were observed under each condition. Microorganisms were observed on plate count agar (**PCA**). One milliliter of each sample was spread throughout the plate. The sample plates were incubated at 35°C for 48 h. In addition, cfu/mL of suspension was calculated from the number of counted colonies on the plates. Five replicates for each sample were observed.

Nutritional Quality of Eggs

Egg nutrient content (ash, carbohydrate, energy, fat, moisture, and protein) was determined by the AOAC method from the result of mixing albumen and egg yolk at 25°C (Kassis et al., 2010). Five samples were observed under each condition.

Statistical Analysis

Statistical analyses were performed using one-way ANOVA with SPSS software. The significance of differences found ($P < 0.05$) was evaluated using Tukey's test. Five replicates for each sample were used for the evaluation.

RESULTS AND DISCUSSION

HU

The egg coating solution was prepared by blending CS, CMC, and paraffin (6/1/0.5 w/v%) at 500 rpm and 80°C for 20 min with an overhead stirrer. Fresh eggs (on the first day at the farm) with a weight range from 66 to 70 g were wiped with the coating material by dipping into the separated coating material solution in each sample. The coated eggs were dried at 25 ± 3°C and 65 ± 2% RH for 30 min and then stored at 4, 25, and 30°C. The HU values are shown in Figure 1. The grade of the egg was determined based on the HU values as follows: AA >72, A from 72 to 60, B from 59 to 31, and C ≤30 (Pius and Olumide, 2017). The HU value decreased from 95 (grade AA) to 90−75 in the first week of storage. Storage at 4°C maintained the value of HU in grade AA for 4 wk in both coated and uncoated eggs. Coated and uncoated eggs stored at 25°C showed an HU value of 73 (grade AA) and 45 (grade B) at 4 wk of storage, respectively. The coated and uncoated eggs at 30°C storage showed HU values of 64 (grade A) and 60 (grade B), respectively, in the second week, which decreased to 47 and 37 (grade B), respectively, in 4 wk. The coating solution had no effect on the shelf life at 4°C storage temperature. This result was concordant with the observations of other studies on mineral oil-coated eggs that maintained a grade of AA for the entire 12 wk of storage at 7°C (Jirangrat et al., 2010). Uncoated eggs maintained the initial AA grade for 5 wk when stored at 4°C (Jones et al., 2002; Biladeau and Keener, 2009). Pores on the eggshell surface led to the loss of moisture and carbon dioxide, which affects the internal quality of eggs (Caner and Cansiz, 2008 ; Oliveira and Oliveira, 2013). During storage, carbon dioxide loss and the migration of water from the albumen to the egg yolk slightly change the pH of the egg yolk, which affects the albumen quality (Keener et al., 2000). The coating material was extremely effective at maintaining egg quality at high-temperature storage (25 and 30°C) because of the high water and gas barrier, strong compatibility of CS/CMC/paraffin, and eggshell pore filling of the coating material. Images of coated and uncoated eggs stored for 4 wk at 4, 25, and 30°C are shown in Figure 2. A high thickness of albumin gel was observed in the uncoated sample at 4°C and coated samples at 4 and 25°C.

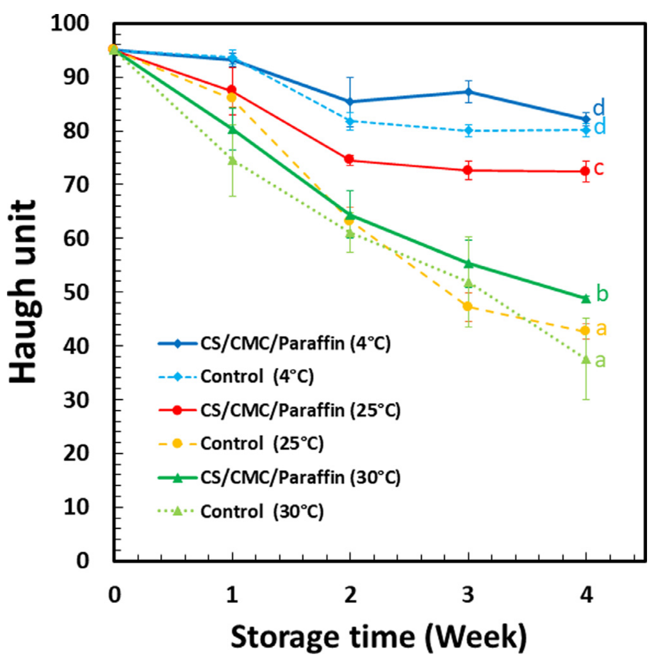

Figure 1. Variation in the Haugh unit (HU) of CS/CMC/paraffin coated and uncoated eggs from d 0 to 4 weeks at storage temperatures of 4, 25, and 30°C; n = 5. Means with different lowercase superscript letters are significantly different ($P < 0.05$).

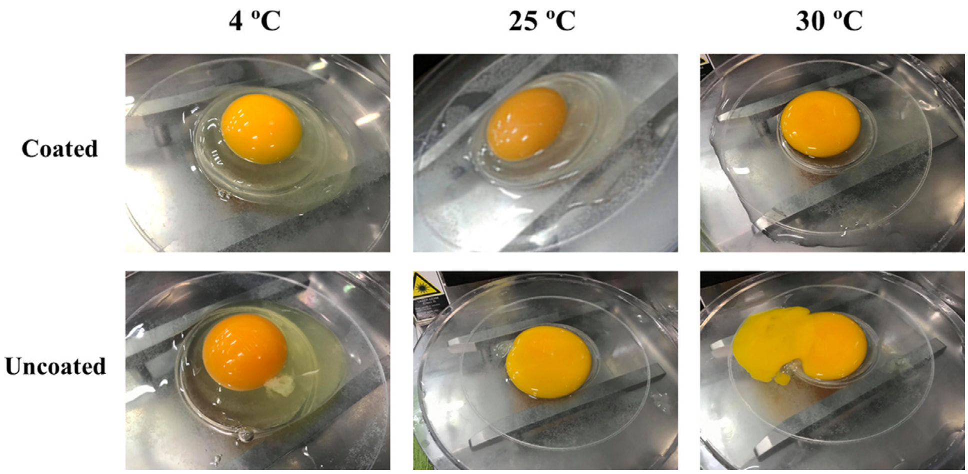

Figure 2. Images of coated and uncoated eggs stored at 4, 25, and 30°C for 4 wk.

Weight Loss

The weights of the coated and uncoated egg samples at various storage temperatures are shown in Figure 3. The weight loss of uncoated eggs was 2.8, 3.3, and 4.6% at 4, 25, and 30°C, respectively. The coating materials maintained an egg weight at a storage temperature of 4°C, whereas at 25 and 30°C, the weight loss increased significantly. Coated eggs presented lower weight loss than that of uncoated eggs at all storage temperatures. A more constant egg weight was maintained at a low temperature (4°C) than at high temperatures owing to low water evaporation (Samli et al., 2005), whereas a high penetration rate of moisture from inside to outside the egg shell was estimated in case of high-temperature storage (25 and 30°C). Reduction of egg weight loss by low evaporation rate in low-temperature storage (4–5°C) has also been previously reported (Jones and Musgrove, 2005; Samli et al., 2005). The change in egg quality during storage was caused by the storage temperature. Lower levels of weight loss due to the use of a coating material during 4 wk of storage under all conditions were caused by the high water resistance of hydrophobic paraffin wax (Guo et al, 2016). The high compatibility between CS and paraffin via interaction with CMC improved the hydrophobicity of the coating materials. The interaction between the carboxylic acid groups of CMC and hydroxyl groups has been previously reported (Jantanasakulwong et al., 2018; Rodsamran and Sothornvit, 2020). This indicates that the water permeability of the eggshell is lowered by the filling of egg shell pores with the coating material. The coated eggs in low-temperature storage demonstrated the lowest weight loss owing to the combined effect of the high water resistance of the coating material and the low evaporation rate at a low temperature (4°C).

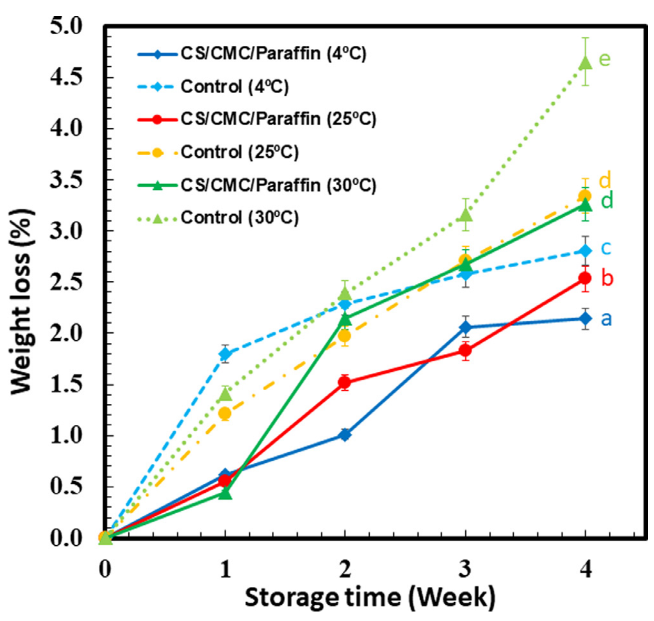

Figure 3. Variation in weight loss (%) of uncoated eggs and eggs coated with CS/CMC/paraffin from d 0 to 4 wk at storage temperatures of 4, 25, and 30°C; n = 5. Means with different lowercase superscript letters are significantly different ($P < 0.05$).

pH

Albumen pH is an indicator of chemical changes in eggs with storage time and temperature. Figure 4 shows the changes in albumen pH at various temperatures over 4 wk. Albumen pH gradually increased in uncoated eggs at 4, 25, and 30°C. Particularly, albumen pH increased at 30°C, which was enhanced from 6.8 to 7.26, in 4 wk. The increase in albumen pH was because of the hydrolysis of carbonic acid, which released CO_2 through the pores of the eggshell (Soares et al., 2021). The chemical change in albumen with storage time reduced the viscosity due to the decomposition of albumen with the change in acidity (Soares et al., 2021). The increasing of albumen pH in eggs with storage time using rice protein blend with propolis egg coating have been reported (Pires et al., 2021). The coated sample stored at 30°C showed a lower pH than that of the uncoated sample, which indicated a reduction in gas permeability, especially to CO_2. The coating material covered the pores of the eggshell, preventing moisture penetration and reducing gas permeability during long-term storage.

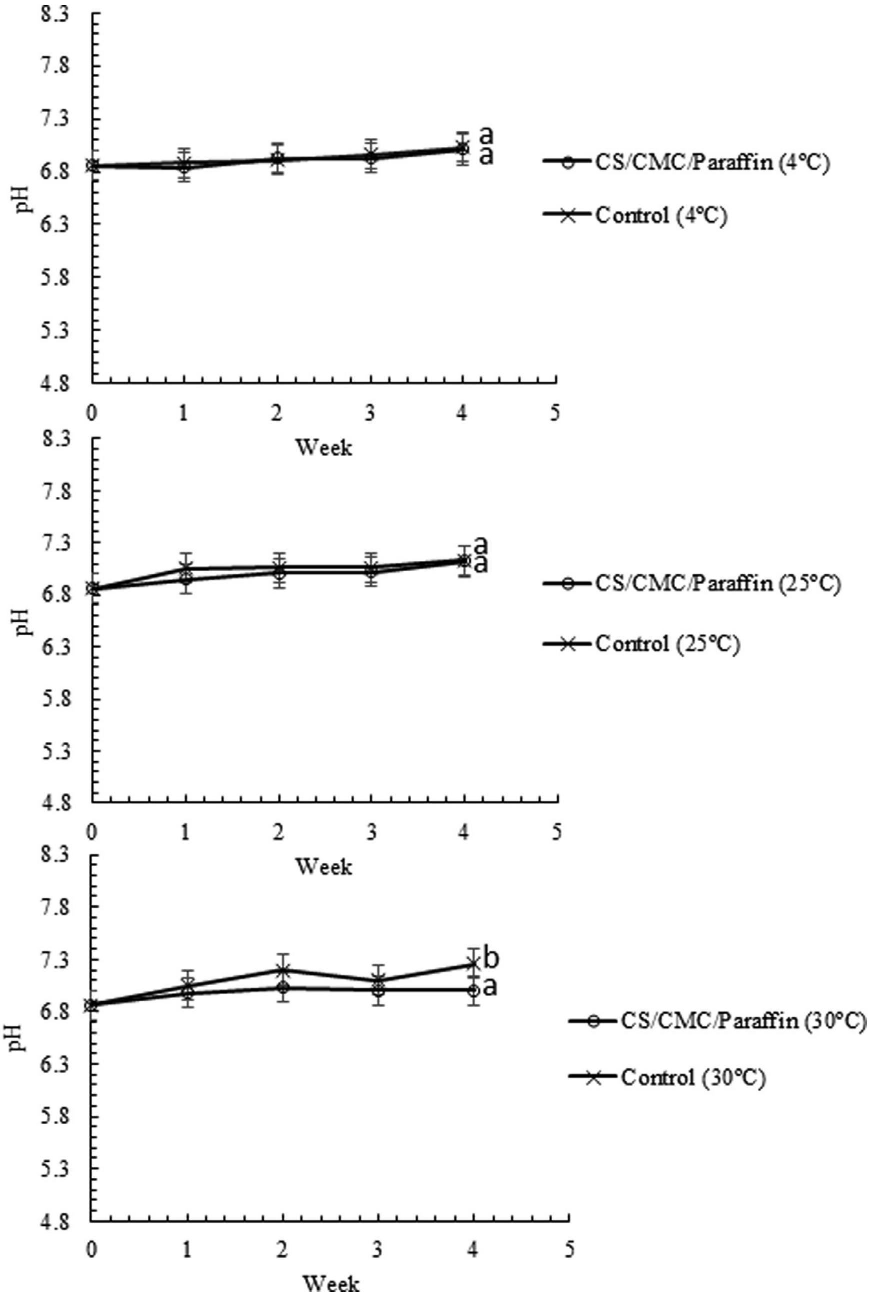

Figure 4. Variation in pH of CS/CMC/paraffin-coated and uncoated eggs from d 0 to 4 wk at storage temperatures of 4, 25, and 30°C; n = 5. Means with different lowercase superscript letters are significantly different ($P < 0.05$).

Microorganisms

The microbial counts in coated and uncoated eggs at 4, 25, and 30°C after 4 wk of storage are presented in Table 1. Total microbial counts in coated and uncoated eggs were observed on PCA in samples stored for 4 wk. Microbial counts were not detected in fresh eggs or coated and uncoated eggs stored at 4°C. The absence of detectable microbial counts of coated and uncoated eggs stored at 4°C for 4 wk was because of the inhibition of microbial replication at 4°C. The coated and uncoated samples stored at 25°C presented microbial counts of less than 10 cfu/mL. Total microbial counts at 728 ± 35 cfu/mL were detected in uncoated eggs stored at 30°C

Table 1. Microbial counts in coated and uncoated eggs at 4°C, 25°C, and 30°C at 4 wk; n = 5.

Condition of coated and uncoated eggs	Quantity of microorganisms (cfu/mL)
Fresh eggs	<10
CS/CMC/paraffin (4°C)	<10
Control (4°C)	<10
CS/CMC/paraffin (25°C)	<10
Control (25°C)	<10
CS/CMC/paraffin (30°C)	<10
Control (30°C)	728* ± 35

*Values are presented as mean ± SD.

Table 2. Nutrient analysis of coated and uncoated eggs during 4 wk of storage at 25°C (n = 5).

Specification	Nutritional content			
	Fresh eggs*	CS/CMC/paraffin (25°C)*	Control (25°C)*	Units
Ash	0.92[a] ± 0.03	0.94[b] ± 0.05	0.97[c] ± 0.04	g/100 g
Carbohydrate	2.78[a] ± 2.12	2.89[a] ± 3.06	2.82[a] ± 4.04	g/100 g
Energy	127.32[a] ± 4.03	136.84[b] ± 6.21	139.43[b] ± 5.48	kcal/100 g
Fat	7.20[a] ± 2.73	7.68[a] ± 3.41	7.79[a] ± 4.35	g/100 g
Moisture	76.25[a] ± 3.72	74.45[a] ± 3.35	73.91[a] ± 3.43	g/100 g
Protein	12.85[a] ± 2.13	14.04[a] ± 3.10	14.51[a] ± 3.25	g/100 g

*Values are presented as mean ± SD.
[abc]Different lowercase superscript letters on the same line are significantly different ($P < 0.05$).

but not in coated eggs. Total microbial counts in uncoated eggs at 30°C were attributed to the low activity of enzymes in eggs with high pH albumen (7.26) (Miyazaki, 1997), microbial cell internalization growth, and microbial replication during storage via egg pores in the shell. Coating eggs prevented microbial replication during storage by filling the eggshell pores and through the water resistance of the coating material. Microbial contamination can be caused by many factors, such as *Salmonella* infection in poultry or salmonellosis. Animals have bacteria in their fallopian tubes, allowing *Salmonella* contamination during egg generation. Contamination of eggs with *Salmonella* (Leleu et al., 2011; McWhorter and Chousalkar, 2020) and the use of egg coating materials to prevent microbial contamination (Yang et al., 2019) have been reported. Reduction of *Escherichia coli* contamination of eggs by pulsed light technology with vaseline coating have also been reported (Wang et al., 2021). In addition, packaging, storage, transportation, and distribution are factors that affect the microbial contamination of eggs.

Egg Nutrition

The nutrients (ash, carbohydrate, energy, fat, moisture, and protein) in eggs were analyzed in a mixture of albumen and egg yolk. The nutritional content of fresh eggs was compared with that of coated and uncoated eggs stored at 25°C, as shown in Table 2. The nutrient content of fresh eggs showed an energy value of 127.32 kcal/100 g, a moisture content of 76.25 g/100 g, and a high protein content (12.85 g/100 g). The nutrient content of coated and uncoated eggs at 25°C after 4 wk of storage was similar to that of fresh eggs. It was found that the coating material, temperature storage at 25°C, and egg freshness did not affect the nutritive value.

In conclusion, the freshness of both coated and uncoated eggs during storage for 4 wk at 4°C was assigned grade AA. The CS/CMC/paraffin coating prevented egg weight loss during low-temperature storage (4°C). The egg coating material effectively maintained HU and weight loss during storage at 25°C for 4 wk. The CS/CMC/paraffin coating and low-temperature storage (4°C) maintained HU, reduced egg weight loss, and prevented microbial contamination inside the eggshell. In addition, the maintenance of egg freshness by the coating material did not affect the nutritional value of eggs. Thus, an effective egg coating technology was successfully developed with high transparency and low cost of edible polymers. This coating material and storage condition can be applied to egg production, storage, and distribution.

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DISCLOSURES

The authors have no conflicts of interest to report.

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