

# Effects of packaging methods on the quality of heavy metals–free preserved duck eggs during storage

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**ABSTRACT** Preserved eggs without adding heavy metals in the pickling solution (heavy metals–free preserved eggs) have been developed, but it was found that the undesirable phenomenon such as dry shrinkage and fading occurred when they were not packaged and stored at room temperature. In this study, the effects of 5 packaging methods on the quality of heavy metals-free preserved eggs during storage were systematically studied. These methods included storage at room temperature and 4°C without packaging, wrapping with plastic bags, paraffin coating, and vacuum package. Through adopting these 5 packaging methods, the results showed that the moisture content and pH of the albumen decreased continuously, the mass loss rate increased continuously, the content of total volatile basic nitrogen increased firstly and then decreased, and the albumen hardness increased continuously. No microorganisms

were detected in all samples with the 5 packaging methods during storage. Among them, the uncoated preserved eggs suffered the most serious moisture loss and mass loss, and the pH dropped at the fastest rate, followed by the preserved eggs wrapped in plastic bags. Preserved eggs stored at low temperature tended to turn yellow during storage, and the albumen showed higher hardness. The packaging method of paraffin coating performed the best in preventing the moisture loss of the albumen and the weight loss, which only decreased by 0.34 and 1.24%, respectively, after 3 mo. The best springiness, the darkest color, and the highest sensory score were found in the vacuum-packed preserved eggs after 3 mo of storage. It was concluded that paraffin coating and vacuum packing had better effect, while plastic bag packing showed the worst preservation performance for heavy metals–free preserved eggs.

**Key words:** heavy metals–free, preserved egg, packaging method, quality

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## INTRODUCTION

The annual production of fresh duck eggs is about 4 million tons in China which is the largest producer and consumer of fresh duck eggs in the world. Moreover, the duck eggs used for processing preserved eggs account for about 40% of the fresh duck eggs.

Preserved egg is one of the traditional Chinese foods, which is popular among consumers in eastern countries. The original method of producing preserved eggs was the mud wrap method (Wang and Fung, 1996) that consumed a lot of manpower and material resources.

After continuous research and exploration, people began to produce preserved eggs with the liquid method. The liquid method was to add alkali, salt, and lead oxide into water to make the pickling liquid that was used to marinate the fresh duck eggs for about 30 d (Hou, 1981). Because the intake of lead was harmful to human health (Järup, 2003; Intawongse and Dean, 2006), compounds of other heavy metal elements such as copper and zinc have been substituted for lead oxide for pickling preserved eggs. However, Tu et al. (2013) showed that the heavy metals in the marinating liquid would penetrate into the preserved eggs through the eggshell, resulting in the residue of heavy metal compounds in the preserved eggs. Moreover, the heavy metal compounds added in the pickling liquid of preserved eggs have always left a bad impression on people, which exerted a negative impact on the healthy development of preserved eggs industry. Based on the various aforementioned factors, heavy metals–free preserved eggs were

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urgently needed by the consumers. After continuous research, our research group has developed a sort of preserved eggs without adding heavy metal compounds in the pickling solution (heavy metals-free preserved eggs). The surface of eggshell of the heavy metals-free preserved egg is bright and clean, without black spots, which is just like the clean fresh duck eggs. The quality of the heavy metals-free preserved egg is the same as with the quality of preserved eggs with traditional pickling methods. However, owing to the occurrence of undesirable phenomenon of fade and shrinkage in daily preservation, it is extremely urgent to adopt effective packaging methods to store heavy metals-free preserved eggs.

A multitude of methods to preserve eggs and egg products have been proposed, including coatings with different raw materials such as proteins, polysaccharides, fats, and ultrasonic treatment, which all have a positive impact on maintaining the quality of eggs (Caner and Yüceer, 2015a; Caner and Yüceer, 2015b; Bi et al., 2020; Gabriela da Silva Pires et al., 2020; Yüceer and Caner, 2020). Vacuum packaging, paraffin coating, plastic bag packing, and low-temperature storage are all very common and traditional packaging methods of food. Vacuum packaging keeps food in an anoxic or even anaerobic state, which can not only greatly inhibit the growth and reproduction of microorganisms but also reduce the food deterioration caused by the oxidation. When sausages and fish were vacuum-packaged, it was found that vacuum packing can more effectively control the growth and reproduction of microorganisms, prevent the loss of water, and maintain the original quality of food to a great extent than unpackaged food (Amoli et al., 2019; Canel et al., 2019). Paraffin coating is commonly used method of food preservation as well, especially for the storage of fruits and vegetables, which can effectively prevent the loss of water and reduce the respiration to effectively alleviate the loss of nutrients. It was found that adding antibacterial substances such as octanal and citral to paraffin could further improve the preservation effect of paraffin on fresh citrus (Fan et al., 2014; Tao et al., 2014). Low-temperature storage is widely used in food because the activity of enzymes and microorganisms in food can be reduced at a low-temperature condition, thereby prolonging the preservation time and ensuring the freshness of food. In addition, some foods are also stored in the way of plastic bag packing and nonpackaging at room temperature, which are 2 traditional package methods of preserved eggs currently. Although the 5 aforementioned methods are common ways of preserving food, there has been no systematic research on the effects of these packaging methods on the quality of preserved eggs. Thus, in terms of a series of problems about heavy metals-free preserved eggs during storage at room temperature, the effects of these 5 packaging methods on the quality of preserved eggs during storage were systematically studied to find out potential or effective packaging methods.

## MATERIALS AND METHODS

### Materials

Fresh duck eggs were supplied by Jiangxi Tianyun Agricultural Development Co., Ltd. Standard compounds were purchased from Shanghai Institute of Metrology and Testing Technology. The bicinchoninic acid (BCA) protein concentration determination kit was bought from Sangon Biotech (Shanghai) Co., Ltd. Peptone, agar, and yeast extract were purchased from Solarbio Chemicals Co., Ltd., and lactose was obtained from Yuanye Biotechnology Co., Ltd. The other reagents were of analytical grade and purchased from Xilong Scientific Co., Ltd.

### Pickling and Packaging of Preserved Eggs

The fresh duck eggs were cleaned, checked, and graded before soaking in the pickling solution, which was composed of NaOH (6%, m/v) and NaCl (4.0%, m/v). The eggs were pickled at 25°C for 9 d. Afterward, the eggs were taken out and marinated in the reconstituted pickling solution, which was composed of NaOH (0.3%, m/v) and NaCl (4.0%, m/v). After the eggs were completely discolored, the eggs were taken out and marinated in the reconstituted pickling solution, which was composed of NaOH (0.1%, m/v) and NaCl (4.0%, m/v). The aforementioned operations were performed at 25°C. After the preserved eggs were fully matured, they could be taken out.

Heavy metals-free preserved eggs were taken out from the pickling liquid after they were matured, then they were washed and air-dried, and the elastic ones were picked out. Heavy metals-free preserved eggs stored at 25°C and 4°C without packaging were called uncoated group (UC) and low-temperature group (LT), respectively. Heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C were named plastic bag wrapped group (PB). Heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 10 s, then removed to dry (these procedures were repeated twice), and finally stored at 25°C were called paraffin coating group (PC). Heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C were called vacuum packaging group (VP). A total of 350 preserved eggs of each group were required for experiment. Samples were determined every 2 wk.

### Determination of Albumen Moisture Content

Moisture content was determined as per the direct drying method of Chinese standard GB 5009.3-2016 (China, 2016c). The experiment was performed in triplicate.

### Determination of Mass Loss Rate

The mass loss rate is the degree of water loss in preserved eggs after a certain period of time. Ten preserved

eggs were picked randomly, and ten parallel measurements were carried out. Analytical balance (0.0001 g) (BSA224S; Sartorius, Germany) was used for weighing, and weight of every preserved egg was recorded. The formula for calculating the mass loss rate is as follows:

$$\text{Mass loss rate} = (m_1 - m_2) / m_2 \times 100\%$$

where  $m_1$  is the weight of preserved eggs before storage, g, and  $m_2$  is the weight of preserved eggs after storage, g.

### Determination of pH

The pH was determined as per the method of Chinese standard GB/T 5009.47-2003 (China, 2003) with a slight modification. Five preserved eggs were cleaned, shelled, and divided into albumen and yolk. Distilled water was added to the albumen (yolk) in the proportion of 2: 1, and put it in a beater for beating. Albumen (yolk) homogenate (15 g) was diluted with water to 150 mL and then homogenized with a homogenizer (T18; IKA, Germany). The homogenate was filtered with double-layer gauze to obtain the filtrate. Filtrate (50 mL) was taken out to measure pH with a pH meter (PHS-25, Shanghai Yidian Scientific Instrument Co., Ltd., China). The experiments were carried out 3 times.

### Determination of Total Volatile Base Nitrogen

Total volatile base nitrogen (TVB-N) was determined as per the microdiffusion method of Chinese standard GB 5009.228-2016 (China, 2016d). Three preserved eggs were cleaned and shelled. Distilled water was added to the preserved eggs in the proportion of 2: 1 and then put it in the beater for beating. Preserved egg homogenate (15 g) was weighed accurately and placed in an Erlenmeyer flask. One hundred milliliter of water was added, and the flask was shaken periodically and filtered after 30 min.

The water-soluble glue was coated on the edge of the diffuser dish. One milliliter of  $\text{H}_3\text{BO}_3$  solution (20 g/L) and 1 drop of mixed indicator (1 part of  $\text{C}_{15}\text{H}_{15}\text{N}_3\text{O}_2$  solution [1 g/L] and 5 parts of  $\text{C}_{21}\text{H}_{14}\text{Br}_4\text{O}_5\text{S}$  solution [1 g/L]) were added in the central chamber of the dish. One milliliter filtrate was added into the outer chamber of the dish, and the dish was covered with a frosted glass lid. Then, 1 mL of saturated  $\text{K}_2\text{CO}_3$  solution was added to the outer chamber of the diffusion dish from the gap, and the diffusion dish was covered with the frosted glass cover. Then, it was gently rotated on the workbench to mix the filtrate and saturated  $\text{K}_2\text{CO}_3$  solution thoroughly. Then, it was placed in a thermostat at  $37^\circ\text{C} \pm 1^\circ\text{C}$  for 2 h, was taken out, and cooled to room temperature. Finally, the standard titration solution of HCl was subjected to a titration test. The experiments were carried out in triplicate.

### Determination of Texture Characteristics

The albumen was cut into cubes with the length and height both of 1 cm (Ganasen and Benjakul, 2011) and performed by a texture analyzer (TA-XTPLUS, SMS,

UK). A P36 cylindrical probe was used to compress the sample twice, and the compression ratio was 50%. The pretest speed, test speed, and post-test speed all were 2 mm/s. The experiments were carried out 6 times.

### Determination of Intermolecular Interaction Forces

The protein solubility of the albumen was measured as per the method of Chen et al. (2015). Three preserved eggs were randomly selected to be cleaned and shelled, and the albumen was placed in a beater for beating. Albumen homogenate (0.6 g) was added to 5.4 mL S1 (0.6 mol/L NaCl) solution and homogenized with a homogenizer at 12,000 rpm for 2 min and then was placed at room temperature for 30 min. Homogenate of the sample was then centrifuged in a high-speed centrifuge (TGL-20B; Shanghai Anting Instrument Company, China) at 10,000 rpm for 20 min, and the supernatant was placed in an EP tube for later use. The precipitation part was added to 5.4 mL of S2 (0.6 mol/L NaCl + 1.5 mol/L urea) solution and the aforementioned operations were performed repeatedly. The supernatant was taken out for later use. A total volume of 5.4 mL of S3 (0.6 mol/L NaCl + 8 mol/L urea) was added to the precipitation part, then the previous operation was repeated, and the supernatant was taken out for later use. A total volume of 5.4 mL of S4 (0.6 mol/L NaCl + 8 mol/L urea + 0.5 mol/L  $\beta$ -ME) was added to the precipitation part, and the previous operation was repeated. The supernatant obtained this time was placed in S1 and dialyzed for 24 h before it was used. The aforementioned 4 supernatants were tested for protein concentration with BCA method. The BCA method referred to that 2 mg/mL BSA bovine serum protein standard was diluted to 0.5 mg/mL so as to make a standard curve to determine protein concentration, which was measured using a microplate reader (K3; Thermo Fisher Scientific). The parallel experiments were carried out 3 times.

### Determination of Color

The albumen was cut into small cubes with the length, width, and height all of 1 cm. Three random points were selected on the yolk. A spectrophotometer (NS810; Shenzhen Sannshi Technology Co., Ltd., China) was used to determine the color of the albumen and yolk. The experiments were performed 6 times.

### Determination of Microorganisms

*Escherichia coli* count was measured as per Chinese standard GB 4789.3-2016 (China, 2016b).

The total number of colonies was measured as per Chinese standard GB 4789.2-2016 (China, 2016a).

### Sensory Evaluation

Sensory evaluation table was set up as per Chinese standard GB 9694-2014 (China, 2014), as shown in

Table 1, which included color, appearance, texture, smell, and taste. Eight students majoring in food scored the quality of preserved eggs as per Table 1, with a score of 0–100.

## Statistical Analysis

The research results were processed by Origin 8.5 software. All data were shown in the manner of mean  $\pm$  SD, and SPSS25 statistical software was used for 1-way ANOVA Duncan multirange test. A  $P$  value of  $<0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

### Changes in Moisture Content of the Albumen With Different Packaging Methods

Fresh duck eggs contained about 70.83% water (Hester, 2017). But, the moisture content constantly changed during the process of pickling preserved eggs. The moisture content of the albumen decreased from 87.69% before the curing to 83.52% when it was matured (Guo et al., 2019). The moisture content of the albumen is closely related to the quality of preserved eggs. Preserved eggs with severe water loss will not only show an unacceptable shrinkage phenomenon but also gradually lose elasticity, resulting in a bad taste experience.

The changes of the albumen moisture content with different packaging methods are shown in Figure 1. There was no significant difference ( $P > 0.05$ ) in the moisture content between groups during the early stage. During 6 to 14 wk, the albumen moisture content of UC was significant lower ( $P < 0.05$ ) than that of LT, PC and VP, which indicated that low temperature, paraffin coating, and vacuum packaging had a certain effect on preventing water loss of the albumen. There was no significant difference ( $P > 0.05$ ) in the water content of each group during 0 to 2 wk and 10 to 14 wk, but a significant downward trend ( $P < 0.05$ ) was shown in 2 to 10 wk. After 14 wk of storage, the albumen moisture content of PC, LT, VP and PB was 83.36, 82.78, 81.49, and 81.15% respectively. Therefore, it can be seen that paraffin coating was more efficient to inhibit the moisture loss of the albumen.

During the pickling process, the free water content of preserved eggs reduced significantly. After the preserved eggs were matured, water in the albumen showed multi-layer binding state in which a single layer of water

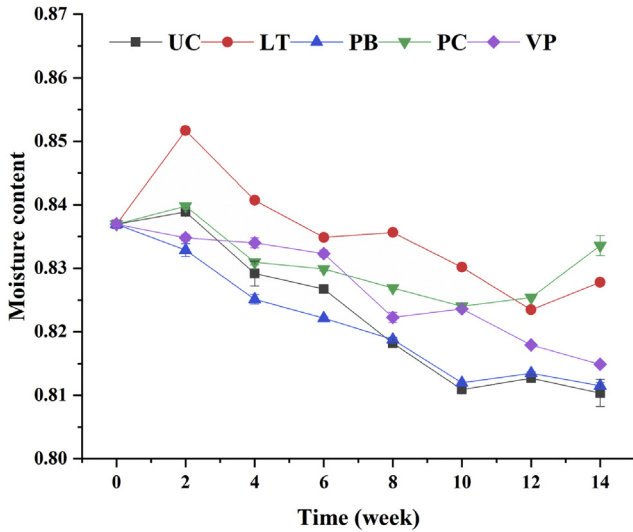
molecules bound to polar or nonpolar groups on the protein or amino acid peptide chain through hydrogen bonding was possibly included (Zhao et al., 2016). The moisture content of preserved eggs in each group decreased during storage mainly owing to the high moisture content of the albumen and the low humidity in the environment, thus water drained away from the albumen through the pores of the eggshells into the air during storage (Hickson et al., 1982). The moisture loss of preserved eggs with paraffin coating was less compared with other packing methods. On the one hand, this was due to the good barrier properties of paraffin to water vapor (Nowacka et al., 2018). On the other hand, Pires et al. (2019) found that the application of mineral oil coating effectively reduced the porosity of the egg shell. At low temperature, the internal thermal kinetic energy of the albumen gel decreased and more stable noncovalent bonds were further formed between the exposed functional groups on the protein molecules (Fennema, 1996), and the gel network and water-holding capacity became more stable, resulting in that the moisture in the albumen stored at low temperature evaporated less easily than at room temperature. The albumen moisture content of VP showed a downward trend as a whole. It was possibly because of big difference in moisture content between the albumen and yolk, which caused the gradual shift of water from the albumen to yolk. Preserved eggs of PB can exchange gases directly with the environment, which resulted in poor water resistance. However, owing to the reduction of their contact area with the air, the moisture loss in the albumen of PB was still slightly smaller than UC.

### Changes in Mass Loss Rate of Preserved Eggs With Different Packaging Methods

The changes in mass loss rate of preserved eggs with different packaging methods are shown in Figure 2. The trend of the mass loss rate was opposite to that of the albumen moisture content. There was a little difference in the first 2 wk between groups. Then, the mass loss rate of LT, PC, and VP was significantly lower than that of UC ( $P < 0.05$ ) during 2 to 14 wk. The mass loss rate of heavy metals-free preserved eggs that were stored with each packaging method increased significantly ( $P < 0.05$ ). It showed that the mass loss rate of preserved eggs with the 5 packaging methods was getting faster and faster, but the decline of LT, PC and VP was much slower than other groups.

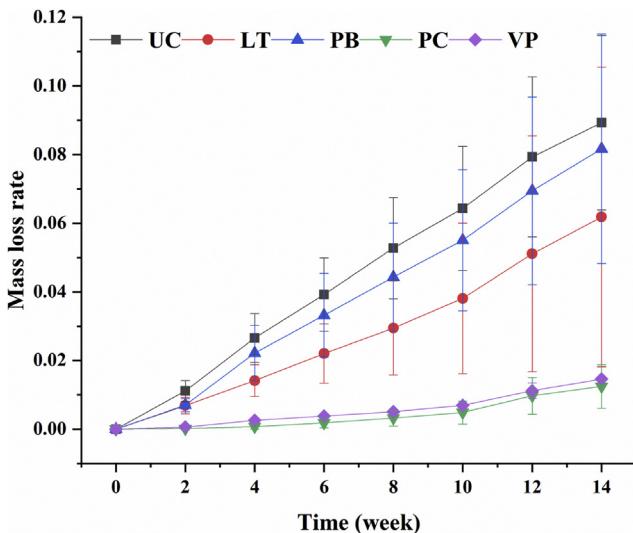
Table 1. Sensory evaluation.

Score	Color	Appearance	Texture	Smell	Taste
0–5	Yellow	Severe shrinkage Shape change	Dry hard almost inelastic	Oxidized or musty smell	Serious weird taste
6–10	Yellow-green	Severe loss Noticeable shrinkage	High hardness low elasticity	No smell of preserved eggs	Obvious alkaline or strange taste
11–15	Dark green	Fuller shape Slight loss	Less elastic Increased hardness	Slight smell of preserved egg	Slight alkaline taste
16–20	Dark brown	Full shape	Moderately hard	Rich flavor of preserved egg	Moderate taste

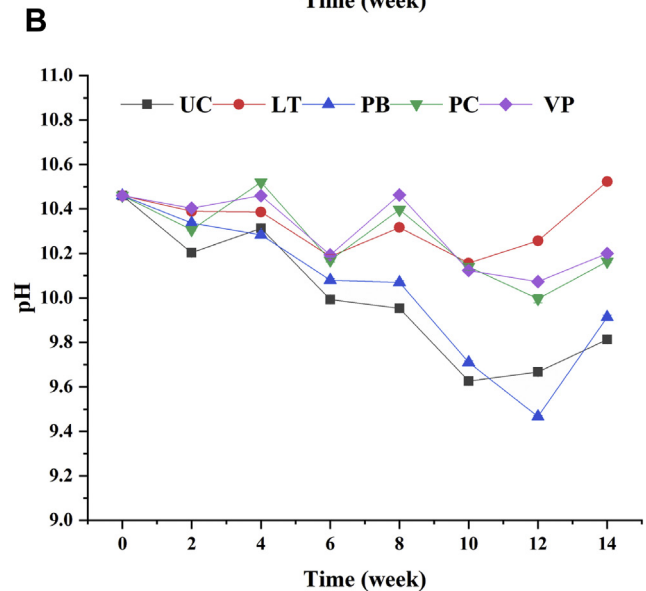
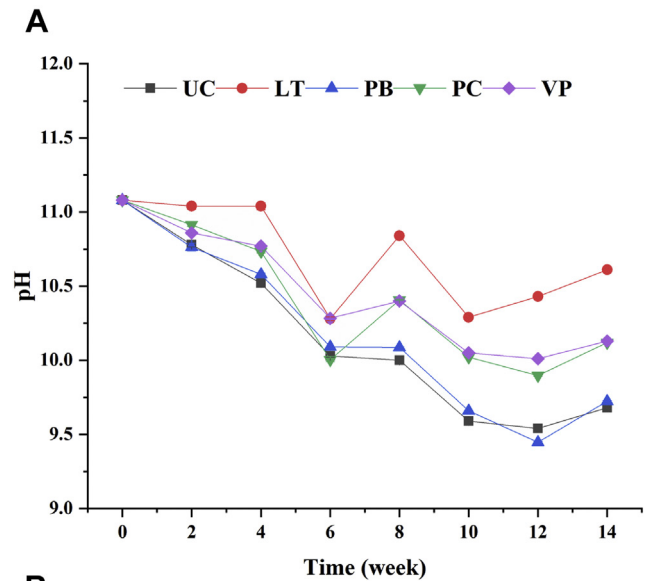


**Figure 1.** Changes of moisture content of albumen with different packaging methods. Abbreviations: LT, heavy metals-free preserved eggs stored at 4°C at low-temperature; PB, heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C; PC, heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 10 s, then removed to dry, and finally stored at 25°C; UC, heavy metals-free preserved eggs stored at 25°C without packaging; VP, heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C.

Among them, the most serious mass loss happened in UC mainly because of the loss of water in the preserved eggs, which echoed the trend of the albumen moisture content in UC (Figure 1). Interestingly, the albumen moisture content of LT was higher than that of VP during storage, but the quality of LT decreased faster than that of VP. On the one hand, it might be related to the



**Figure 2.** Changes in the mass loss rate of preserved eggs with different packaging methods. Abbreviations: LT, heavy metals-free preserved eggs stored at 4°C at low-temperature; PB, heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C; PC, heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 10 s, then removed to dry, and finally stored at 25°C; UC, heavy metals-free preserved eggs stored at 25°C without packaging; VP, heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C.



**Figure 3.** Changes of pH value of albumen (A) and yolk (B) with different packaging methods. Abbreviations: LT, heavy metals-free preserved eggs stored at 4°C at low-temperature; PB, heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C; PC, heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 10 s, then removed to dry, and finally stored at 25°C; UC, heavy metals-free preserved eggs stored at 25°C without packaging; VP, heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C.

changes in the moisture content of the preserved egg yolk because the yolk of LT became hard more quickly. On the other hand, it was related to the loss of some volatile substances inside the preserved egg during storage.

### Changes in pH of Preserved Eggs With Different Packaging Methods

pH was one of the important indicators to measure the quality of preserved eggs (Gabriela da Silva Pires et al., 2020). Because the alkali concentration of pickling solution was far beyond that of inside of egg during the pickling process and  $\text{OH}^-$  from the marinade would slowly

**Table 2.** Changes of TVB-N in preserved eggs with different packaging methods.

Group	0	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk	14 wk
TVB-N (mg/100g)								
UC	0.69 ± 0.56 <sup>d,A</sup>	0.70 ± 0.33 <sup>d,B</sup>	0.68 ± 0.32 <sup>d,A</sup>	5.4 ± 0.66 <sup>c,C</sup>	7.52 ± 1.25 <sup>b,c,A</sup>	11.96 ± 3.93 <sup>a,A</sup>	6.36 ± 2.11 <sup>c,A</sup>	8.57 ± 3.19 <sup>b,c,A</sup>
LT	0.69 ± 0.56 <sup>d,A</sup>	0.70 ± 0.16 <sup>d,B</sup>	1.34 ± 0.83 <sup>d,A</sup>	4.15 ± 0.56 <sup>c,C</sup>	8.55 ± 0.54 <sup>b,A</sup>	11.26 ± 1.94 <sup>a,A</sup>	2.72 ± 1.47 <sup>c,d,B</sup>	2.09 ± 0.57 <sup>c,d,B</sup>
PB	0.69 ± 0.56 <sup>c,A</sup>	1.04 ± 0.16 <sup>c,A,B</sup>	3.01 ± 3.12 <sup>d,c,A</sup>	12.56 ± 2.28 <sup>a,B</sup>	9.98 ± 0.64 <sup>a,b,A</sup>	11.87 ± 1.64 <sup>a,A</sup>	8.21 ± 0.88 <sup>b,c,A</sup>	5.57 ± 1.14 <sup>c,d,A,B</sup>
PC	0.69 ± 0.56 <sup>c,A</sup>	1.63 ± 0.75 <sup>c,A,B</sup>	1.62 ± 0.33 <sup>c,A</sup>	6.82 ± 1.20 <sup>b,C</sup>	7.18 ± 1.68 <sup>a,b,A</sup>	10.16 ± 2.85 <sup>a,A</sup>	7.45 ± 1.84 <sup>a,b,A</sup>	2.70 ± 0.00 <sup>c,B</sup>
VP	0.69 ± 0.56 <sup>d,A</sup>	1.96 ± 0.28 <sup>d,A</sup>	1.62 ± 0.33 <sup>d,A</sup>	18.36 ± 2.92 <sup>a,A</sup>	4.12 ± 1.94 <sup>c,d,B</sup>	9.04 ± 2.05 <sup>b,A</sup>	6.22 ± 0.51 <sup>b,c,A</sup>	4.18 ± 1.97 <sup>c,d,B</sup>

<sup>a-d,A-C</sup> Lowercase and uppercase letters represent significant differences in the same line and column, respectively, and the same letter means no significant difference.

Abbreviations: LT, heavy metals-free preserved eggs stored at 4°C at low-temperature; PB, heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C; PC, heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 10 s, then removed to dry, and finally stored at 25°C; TVB-N, total volatile base nitrogen; VP, heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C.

penetrated into the duck egg through the eggshell pores, the pH value of the albumen was significantly higher before pickling (Zhang et al., 2015).

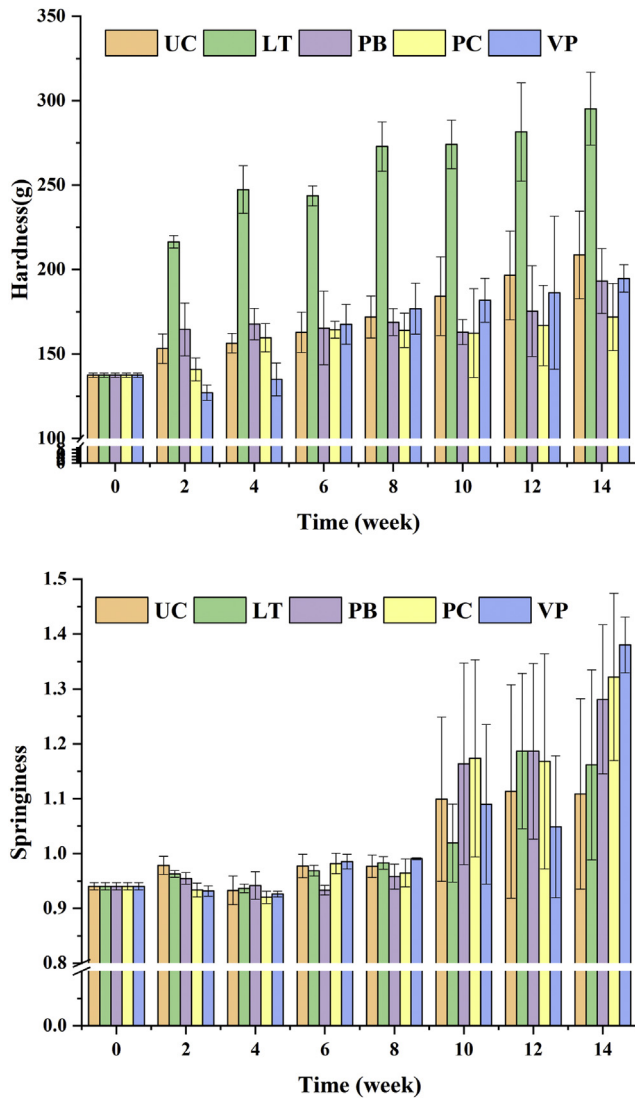
The changes in the pH of preserved eggs with different packaging methods are shown in Figure 3. There was a significant difference ( $P < 0.05$ ) in the pH of the albumen between UC, LT, PC, and VP during the whole storage period. And, a significant difference ( $P < 0.05$ ) was shown in the pH of the preserved egg yolk between UC and the other 4 packaging methods. A significant downward ( $P < 0.05$ ) trend of pH was shown in the albumen and yolk of UC during the whole storage period. The lowest pH values of the albumen and yolk were shown in UC after 14 wk of storage.

On the one hand, the decrease in the pH of the albumen may be because of the difference of pH between the albumen and yolk during storage, resulting in that the alkaline substances were transferred to the yolk. On the other hand, it may be related to the continuous production of volatile materials such as amines, heterocycles, and lipids in preserved eggs during the storage (Deng et al., 2012; Zhao et al., 2013). After 14 wk of storage, the drop in the pH of LT was smallest, followed by VP and PC. This possible reason was that the vacuum packaging and paraffin coating inhibited the contact between oxygen and preserved eggs, which reduced the oxidation reaction. At the same time, tight packaging made it difficult for alkaline volatile substances produced by the oxidation reaction to spill out of the eggshell, which maintained the alkalinity of the preserved eggs. Low temperature slowed down the oxidation rate of preserved eggs, thus playing a role in maintaining the internal pH of the preserved eggs.

### Changes in TVB-N of Preserved Eggs With Different Packaging Methods

The changes of TVB-N in preserved eggs with different packaging methods were shown in Table 2. The trend of first rising and then declining of TVB-N content was shown in each group. Among them, the TVB-N content of VP increased at the fastest rate in the early stage, reaching a maximum of 18.36 mg/100 g in the sixth wk, followed by PB, reaching a maximum of 12.56 mg/100 g in the sixth wk. The TVB-N content of UC, LT and PC all reached their maximum values in week 10 with 11.96 mg/100 g, 11.26 mg/100 g, and 10.16 mg/100 g, respectively. In the 14th week, the TVB-N content of UC was the highest, followed by PB, VP, PC, and LT.

Preserved eggs are rich in proteins. During the storage process, proteins will be degraded owing to the action of enzymes or microorganisms and then ammonia and amines were produced, which might have a negative effect on human health and cause unpleasant flavor to food. The growth and reproduction of microorganisms will be limited under low-temperature or low-oxygen conditions. Therefore, the TVB-N content of LT, PC, and VP can be effectively controlled.



**Figure 4.** Changes in hardness, elasticity and chewiness of albumen with different packaging methods. Abbreviations: LT, heavy metals-free preserved eggs stored at 4°C at low-temperature; PB, heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C; PC, heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 10 s, then removed to dry, and finally stored at 25°C; UC, heavy metals-free preserved eggs stored at 25°C without packaging; VP, heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C.

### Changes in Texture Characteristics of the Albumen With Different Packaging Methods

Texture analysis of food was very important for the presentation of food texture because the texture analysis objectively connected the texture characteristics of food with the human sensory characteristics of food through data (Pons and Fiszman, 1996). In the study, hardness and springiness of the albumen were tested to explore the texture characteristics of the albumen during storage. Hardness refers to the maximum peak that occurs during the first compression process, as well as the peak that occurs with the maximum deformation of the sample. Springiness refers to the height or volume ratio of the deformed sample after being compressed

restoring to the condition before deformation (Mochizuki, 2001).

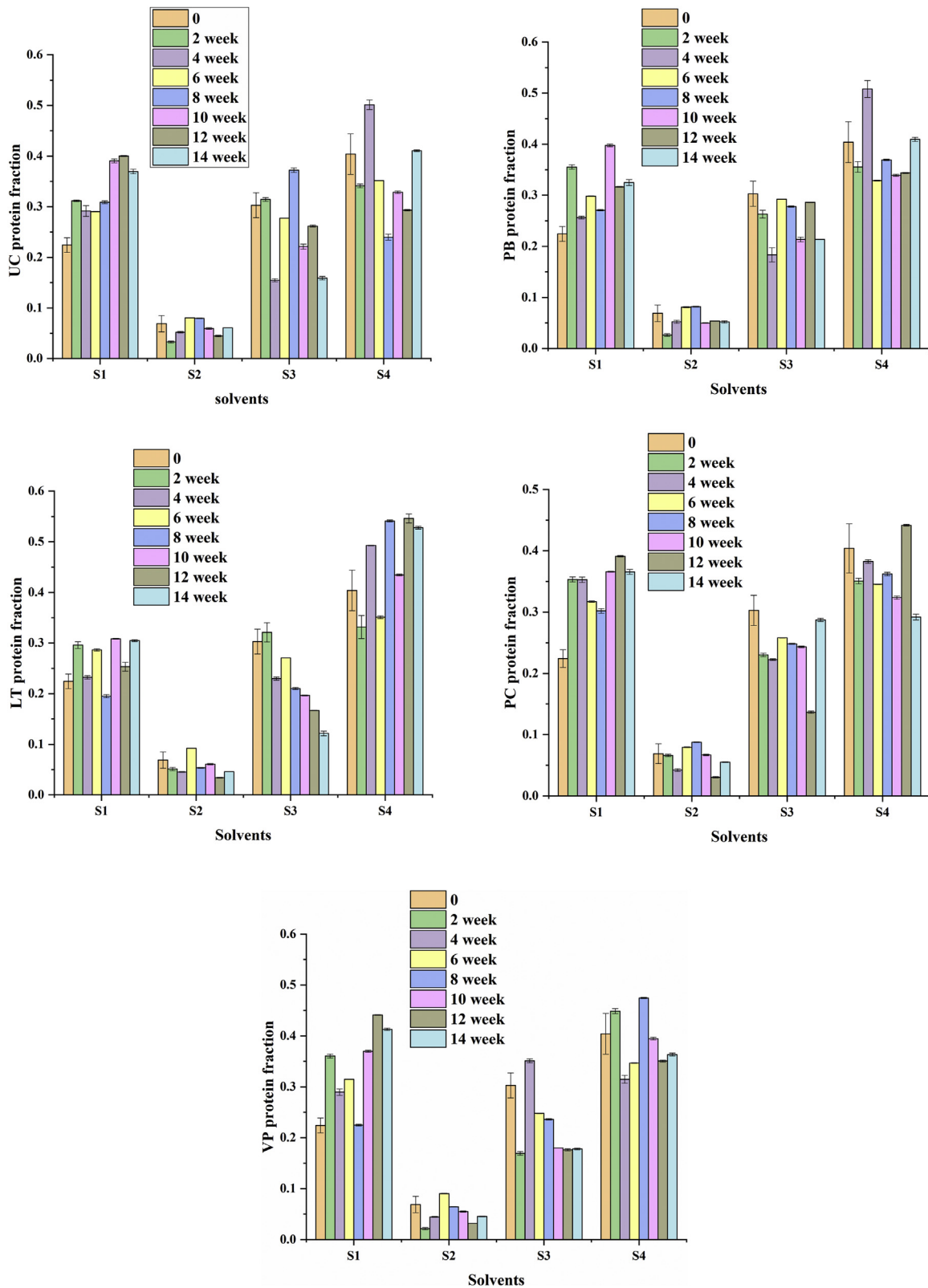
Changes in texture characteristics of preserved eggs with different packaging methods were shown in Figure 4. The albumen hardness of UC, LT, and PB showed a general upward trend during storage, indicating that the strength of the albumen gel was getting stronger with time passing by. Significant increase of hardness was shown in PC during 0 to 6 wk ( $P < 0.05$ ), and then, there was no significant change. There was a slight decrease in VP during 0 to 2 wk and then a distinct increase afterward ( $P < 0.05$ ).

The increase of albumen hardness of UC was related to the changes in moisture content and pH of the albumen in UC. The decrease of water content led to an increase in the concentration of sodium chloride in the albumen, and the  $\text{Na}^+$  binds to protein molecules with negative charge. The decrease of pH resulted in the reductions of negative charges on protein molecules. These results all weakened the electrostatic repulsion between protein molecules and made the network structure between protein molecules denser, thereby leading to the increase of hardness (Ganaseen and Benjakul, 2011; Zhang et al., 2013; Zhao et al., 2014). The denser network structure of protein molecules in the albumen gel meant that the pores of the network structure of the protein gel were reduced, which may explain the slight change in albumen moisture content of UC during the later storage period. After 14 wk of storage, the hardness of the albumen in UC, LT, PB, PC, and VP was 208.7 g, 295.3 g, 193.3 g, 172.0 g, and 194.7 g, respectively. The largest hardness was shown in LT, followed by UC. This meant that lower temperature can significantly enhance the strength of albumen gel. This might be attributed to the decrease of the thermal kinetic energy between albumen gel molecules at low temperatures, the gradual stabilization of the intermolecular interaction, and the mutual combination of the exposed functional groups between molecules, resulting in a more stable protein structure in the albumen gel. Owing to the tightness of the paraffin coating and the vacuum packaging, the moisture in the albumen gel was effectively retained, and the pH was maintained. Therefore, the changes of hardness were smaller than UC.

Better springiness of the albumen was one of the characteristics of high-quality preserved eggs. As shown in Figure 4, there was slight change in springiness of the albumen between groups during storage. However, after 14 wk of storage, the vacuum-packaged preserved eggs white showed the best springiness, followed by the PC, PB, LT, and UC. These might be related to their hardness.

### Changes in Intermolecular Forces of the Albumen With Different Packaging Methods

Proteins showed different solubility in different solvents, and proteins representing different bonding interactions could be respectively dissolved in S1, S2, S3, and S4 (Matsumoto, 1980; PerezMateos et al., 1997). Specifically speaking, proteins can be dissolved in S1, S2, S3, and S4



**Figure 5.** Changes of intermolecular force of albumen with different packaging methods. Abbreviations: LT, heavy metals-free preserved eggs stored at 4°C at low-temperature; PB, heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C; PC, heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 10 s, then removed to dry, and finally stored at 25°C; UC, heavy metals-free preserved eggs stored at 25°C without packaging; VP, heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C.

when they interact through ionic bonds, hydrogen bonds, and hydrophobic cross-link, and disulfide bonds, respectively.

As shown in [Figure 5](#), the protein content in the albumen of UC dissolved in S1 showed an upward trend during storage and increased significantly ( $P < 0.05$ ) in 6



**Table 3.** Changes in the color of the preserved egg whites with different packaging methods.

Group	0	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk	14 wk
L*	38.74 ± 0.93 <sup>a,A</sup>	27.83 ± 2.68 <sup>f,C,D</sup>	45.24 ± 1.47 <sup>b,B</sup>	41.66 ± 1.36 <sup>d,B</sup>	43.48 ± 0.88 <sup>c,B</sup>	47.53 ± 0.87 <sup>a,B</sup>	49.07 ± 2.04 <sup>a,C</sup>	49.04 ± 2.13 <sup>a,A</sup>
LT	38.75 ± 0.93 <sup>d,A</sup>	36.93 ± 4.16 <sup>d,A</sup>	49.51 ± 2.36 <sup>b,A</sup>	44.46 ± 1.73 <sup>a,A</sup>	49.21 ± 1.35 <sup>b,A</sup>	48.42 ± 1.03 <sup>b,A</sup>	55.02 ± 3.41 <sup>b,A</sup>	48.21 ± 1.55 <sup>b,A</sup>
PB	38.75 ± 0.93 <sup>e,A</sup>	33.77 ± 2.38 <sup>f,B</sup>	45.70 ± 1.70 <sup>b,c,B</sup>	45.09 ± 1.13 <sup>a,A</sup>	42.37 ± 0.55 <sup>d,C</sup>	46.78 ± 0.94 <sup>b,B</sup>	51.37 ± 2.34 <sup>b,B</sup>	43.46 ± 0.84 <sup>d,B</sup>
PC	38.75 ± 0.93 <sup>a,A</sup>	30.18 ± 1.76 <sup>b,C</sup>	27.66 ± 0.79 <sup>c,D</sup>	25.10 ± 1.32 <sup>d,C</sup>	23.77 ± 0.38 <sup>e,E</sup>	22.74 ± 0.99 <sup>d,D</sup>	26.09 ± 1.98 <sup>d,D</sup>	23.81 ± 0.26 <sup>c,C</sup>
VP	38.74 ± 0.93 <sup>a,A</sup>	26.68 ± 2.38 <sup>e,D</sup>	30.25 ± 2.68 <sup>b,C</sup>	23.63 ± 0.44 <sup>d,D</sup>	24.66 ± 0.95 <sup>d,D</sup>	27.40 ± 0.70 <sup>c,C</sup>	28.06 ± 0.51 <sup>c,D</sup>	24.27 ± 0.64 <sup>d,C</sup>
a*	2.05 ± 0.60 <sup>a,A</sup>	10.75 ± 2.20 <sup>a,A</sup>	5.55 ± 0.86 <sup>d,e,B</sup>	6.40 ± 1.17 <sup>c,d,B</sup>	5.23 ± 0.71 <sup>e,B</sup>	7.98 ± 0.85 <sup>b,B</sup>	8.36 ± 1.06 <sup>b,A</sup>	7.42 ± 1.17 <sup>a,b,B</sup>
LT	2.05 ± 0.60 <sup>a,A</sup>	1.42 ± 0.96 <sup>c,C</sup>	-1.92 ± 0.37 <sup>d,D</sup>	1.96 ± 0.63 <sup>b,d,e,g,C</sup>	-0.27 ± 0.63 <sup>e,G,C</sup>	0.10 ± 0.58 <sup>d,f,D</sup>	-0.89 ± 0.37 <sup>f,D</sup>	0.81 ± 0.15 <sup>f,D</sup>
PB	2.05 ± 0.60 <sup>a,A</sup>	8.17 ± 1.11 <sup>a,B</sup>	3.74 ± 0.84 <sup>d,C</sup>	6.94 ± 1.08 <sup>b,B</sup>	5.43 ± 1.08 <sup>b,B</sup>	8.10 ± 0.46 <sup>b,B</sup>	4.39 ± 1.20 <sup>d,B,C</sup>	8.69 ± 0.84 <sup>b,A</sup>
PC	2.05 ± 0.60 <sup>a,A</sup>	8.24 ± 0.82 <sup>b,B</sup>	11.74 ± 1.01 <sup>a,A</sup>	11.54 ± 1.00 <sup>a,A</sup>	5.75 ± 0.94 <sup>c,B</sup>	5.02 ± 1.74 <sup>c,C</sup>	5.24 ± 1.39 <sup>e,B</sup>	3.29 ± 0.85 <sup>d,C</sup>
VP	2.05 ± 0.60 <sup>d,A</sup>	11.61 ± 2.14 <sup>a,A</sup>	11.66 ± 1.26 <sup>a,A</sup>	12.41 ± 1.04 <sup>a,A</sup>	7.61 ± 1.04 <sup>b,A</sup>	11.44 ± 1.26 <sup>b,A</sup>	3.91 ± 0.85 <sup>c,C</sup>	3.92 ± 0.54 <sup>c,C</sup>
UC	22.63 ± 2.10 <sup>d,A</sup>	45.21 ± 5.85 <sup>a,B</sup>	25.33 ± 0.98 <sup>c,A</sup>	17.44 ± 0.99 <sup>b,B</sup>	22.00 ± 0.70 <sup>d,A</sup>	28.86 ± 1.80 <sup>b,A</sup>	29.35 ± 1.98 <sup>b,A</sup>	27.51 ± 1.65 <sup>b,c,A</sup>
LT	22.63 ± 2.10 <sup>d,A</sup>	22.43 ± 3.33 <sup>b,C</sup>	13.26 ± 2.45 <sup>d,C</sup>	11.16 ± 0.83 <sup>c,C</sup>	15.40 ± 1.42 <sup>d,B</sup>	18.95 ± 1.04 <sup>d,B</sup>	18.68 ± 1.56 <sup>c,C</sup>	18.84 ± 1.24 <sup>b,C</sup>
PB	22.63 ± 2.10 <sup>d,A</sup>	51.30 ± 2.92 <sup>a,A</sup>	25.73 ± 0.84 <sup>c,A</sup>	21.92 ± 0.87 <sup>d,A</sup>	22.76 ± 0.87 <sup>d,A</sup>	27.83 ± 1.29 <sup>b,A</sup>	27.84 ± 1.39 <sup>b,B</sup>	25.71 ± 0.73 <sup>c,B</sup>
PC	22.63 ± 2.10 <sup>a,A</sup>	17.87 ± 1.64 <sup>b,D</sup>	14.49 ± 2.10 <sup>c,C</sup>	3.77 ± 0.51 <sup>d,D</sup>	3.00 ± 0.49 <sup>d,D</sup>	4.31 ± 1.37 <sup>d,D</sup>	3.35 ± 0.85 <sup>d,D</sup>	0.18 ± 0.89 <sup>e,E</sup>
VP	22.63 ± 2.10 <sup>a,A</sup>	20.42 ± 3.80 <sup>b,C,D</sup>	17.30 ± 2.32 <sup>c,B</sup>	3.78 ± 0.98 <sup>e,d,D</sup>	5.18 ± 1.02 <sup>e,C</sup>	7.79 ± 1.07 <sup>d,C</sup>	3.03 ± 0.66 <sup>d,D</sup>	2.34 ± 0.87 <sup>d,D</sup>

<sup>a-g,A-D</sup>Lowercase and uppercase letters represent significant differences in the same line and column, respectively, and the same letter means no significant difference.

Abbreviations: LT, heavy metals-free preserved eggs stored at 4°C at low-temperature; PB, heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C; PC, heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 10 s, then removed to dry, and finally stored at 25°C; VP, heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C.

to 12 wk. No significant differences of protein content dissolved in S2 and S4 were shown in the albumen of UC as a whole, and the protein content dissolved in S3 of the albumen in UC showed an overall downward trend. These results indicated that the ionic bond increased and the hydrophobic interaction decreased in the albumen of UC during storage. A small amount of dissolved protein in S2 was found with the other 4 packaging methods, basically less than 10%, and had no significant difference. The content of dissolved protein in S3 decreased with time and decreased significantly ( $P < 0.05$ ) in 6 to 14 wk. The dissolved protein content of the albumen of LT in S4 showed an overall upward trend. These findings meant that low temperature can promote the formation of disulfide bonds in the albumen and reduce hydrophobic interactions. The overall changes in the content of dissolved protein in S1 and S3 of the albumen in PB and VP were similar to UC. The content of ionic bonds was generally on the rise, hydrophobic interactions generally reduced, and there was no significant change in the content of disulfide bonds as a whole. The proteins content of the albumen of PC soluble in S2 and S3 had no significant difference as a whole. The content of proteins soluble in S1 showed an upward trend as a whole, while the amount of protein soluble in S4 showed a downward trend. These findings showed that the content of ionic bonds in albumen gel increased as storage time went by, while the content of disulfide bonds decreased.

The increase of ionic bonds in the albumen of UC should be related to a decrease of the water content and a decrease of pH value. As stated in the previous texture analysis, the decrease in pH resulted in the reduction of negative ions on the protein molecules and the decrease of water content led to the increase of the concentration of sodium ions, which were beneficial to reduce the electrostatic repulsion between protein molecules, thus electrostatic interactions and the content of ionic bond increased. The weakening of the hydrophobic interaction may be because of the gradual decrease of the water content in the albumen during storage, which led to the gradual exposure of the hydrophobic groups in the protein molecule. Disulfide bonds, as covalent side chains naturally existing in proteins, could stabilize the folded structure of proteins once it was formed and had extremely important effects on the formation and maintenance of protein gels (Fennema, 1996; Chen et al., 2015). The disulfide bonds content of the albumen in LT increased, which was consistent with the phenomenon that the hardness of the albumen increased significantly at low temperatures.

### Changes in the Color of the Albumen and Yolk With Different Packaging Methods

The brown albumen and the blackish-green yolk are representative features of preserved eggs. The heavy metals-free preserved eggs developed by our research group were prone to fade (yellowish) during the storage

Table 4. Changes in the color of the preserved egg yolk with different packaging methods.

Group	0	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk	14 wk
L*	UC	49.07 ± 0.69 <sup>a,A</sup>	56.09 ± 1.90 <sup>c,A</sup>	62.08 ± 0.38 <sup>a,A</sup>	61.79 ± 0.46 <sup>a,A,B</sup>	58.21 ± 4.50 <sup>b,B</sup>	61.09 ± 0.50 <sup>a,B</sup>	58.90 ± 0.80 <sup>b,C</sup>
	LT	49.07 ± 0.69 <sup>a,A</sup>	49.36 ± 3.32 <sup>c,B</sup>	59.77 ± 0.54 <sup>d,B</sup>	62.51 ± 0.69 <sup>b,c,A</sup>	62.11 ± 0.98 <sup>c,A</sup>	64.29 ± 0.44 <sup>b,A</sup>	66.43 ± 1.37 <sup>a,E</sup>
	PB	49.07 ± 0.69 <sup>d,A</sup>	56.73 ± 0.73 <sup>c,A</sup>	59.47 ± 0.89 <sup>b,B</sup>	61.18 ± 1.44 <sup>a,B</sup>	60.01 ± 0.83 <sup>b,A,B</sup>	60.33 ± 0.38 <sup>a,b,C</sup>	60.42 ± 1.18 <sup>a,b,D</sup>
	PC	49.07 ± 0.69 <sup>a,A</sup>	33.77 ± 0.17 <sup>b,C</sup>	27.94 ± 0.48 <sup>f,C</sup>	29.36 ± 0.83 <sup>c,D</sup>	31.53 ± 1.95 <sup>c,C</sup>	29.38 ± 0.72 <sup>e,E</sup>	30.48 ± 0.95 <sup>d,A</sup>
	VP	49.07 ± 0.69 <sup>a,A</sup>	25.43 ± 0.94 <sup>a,D</sup>	26.71 ± 1.30 <sup>f,D</sup>	31.46 ± 1.06 <sup>d,C</sup>	30.19 ± 0.37 <sup>c,C</sup>	34.81 ± 0.33 <sup>c,D</sup>	36.33 ± 0.35 <sup>b,B</sup>
a*	UC	-3.44 ± 0.34 <sup>f,A</sup>	-0.75 ± 0.30 <sup>c,B</sup>	0.53 ± 0.22 <sup>d,A</sup>	2.02 ± 0.35 <sup>b,A</sup>	2.60 ± 0.70 <sup>b,A</sup>	1.31 ± 0.46 <sup>c,A</sup>	2.23 ± 0.38 <sup>a,b,E</sup>
	LT	-3.44 ± 0.34 <sup>e,A</sup>	-3.36 ± 0.29 <sup>c,C</sup>	-2.16 ± 0.27 <sup>d,B</sup>	-0.23 ± 0.20 <sup>c,A</sup>	-0.14 ± 0.21 <sup>c,C</sup>	0.01 ± 0.12 <sup>b,c,B</sup>	0.84 ± 0.43 <sup>a,B</sup>
	PB	-3.44 ± 0.34 <sup>d,A</sup>	0.09 ± 0.50 <sup>c,A</sup>	0.31 ± 0.67 <sup>c,A</sup>	1.82 ± 0.44 <sup>b,C</sup>	1.41 ± 0.55 <sup>b,B</sup>	1.63 ± 0.25 <sup>b,A</sup>	1.52 ± 0.34 <sup>b,D</sup>
	PC	-3.44 ± 0.34 <sup>c,d,A</sup>	-4.96 ± 0.34 <sup>d,D</sup>	-5.29 ± 0.69 <sup>e,D</sup>	0.89 ± 0.37 <sup>a,B</sup>	-2.80 ± 0.55 <sup>b,D</sup>	-2.96 ± 0.64 <sup>b,C</sup>	-3.69 ± 0.59 <sup>c,A</sup>
	VP	-3.44 ± 0.34 <sup>c,d,A</sup>	-5.23 ± 0.60 <sup>f,D</sup>	-4.15 ± 0.40 <sup>e,C</sup>	1.00 ± 0.38 <sup>a,B</sup>	-3.73 ± 1.03 <sup>d,e,E</sup>	-3.24 ± 0.64 <sup>c,d,C</sup>	-2.83 ± 0.59 <sup>c,B</sup>
b*	UC	18.83 ± 0.62 <sup>f,A</sup>	39.79 ± 1.37 <sup>a,A</sup>	27.46 ± 0.81 <sup>b,C</sup>	22.65 ± 0.98 <sup>c,B</sup>	22.45 ± 0.88 <sup>c,B</sup>	21.81 ± 0.63 <sup>c,d,B</sup>	21.39 ± 0.72 <sup>d,e,B</sup>
	LT	18.83 ± 0.62 <sup>d,A</sup>	27.55 ± 1.64 <sup>c,B</sup>	27.45 ± 1.08 <sup>c,A</sup>	28.30 ± 1.24 <sup>c,A</sup>	31.58 ± 1.55 <sup>b,A</sup>	33.15 ± 1.15 <sup>b,A</sup>	32.96 ± 0.96 <sup>b,A</sup>
	PB	18.83 ± 0.62 <sup>e,A</sup>	38.60 ± 0.81 <sup>a,A</sup>	27.76 ± 1.50 <sup>b,A</sup>	23.36 ± 1.04 <sup>c,B</sup>	21.07 ± 0.71 <sup>c,C</sup>	21.89 ± 0.58 <sup>d,B</sup>	19.33 ± 0.33 <sup>c,C</sup>
	PC	18.83 ± 0.62 <sup>a,A</sup>	7.02 ± 0.93 <sup>b,C</sup>	3.71 ± 0.75 <sup>d,B</sup>	1.33 ± 0.63 <sup>c,D</sup>	4.16 ± 0.90 <sup>c,d,D</sup>	4.71 ± 0.94 <sup>c,C</sup>	4.43 ± 0.85 <sup>c,d,D</sup>
	VP	18.83 ± 0.62 <sup>a,A</sup>	6.29 ± 1.43 <sup>b,C</sup>	2.28 ± 0.48 <sup>b,C</sup>	3.16 ± 0.52 <sup>c,C</sup>	3.37 ± 1.33 <sup>c,D</sup>	4.97 ± 0.42 <sup>d,C</sup>	5.05 ± 0.46 <sup>d,D</sup>

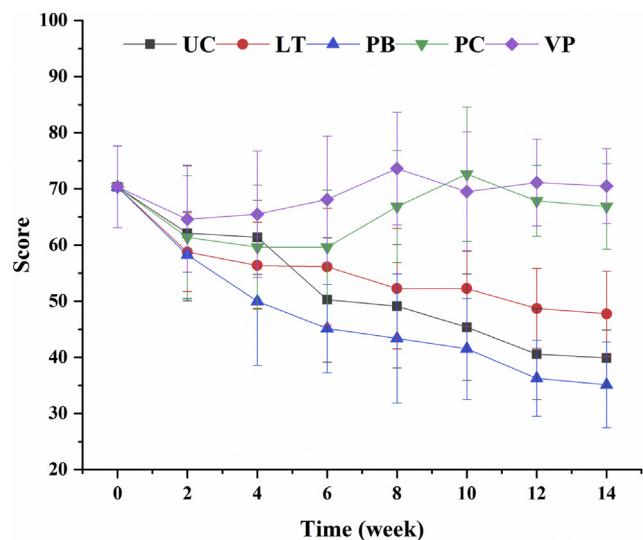
<sup>a-f,A-D</sup> Lowercase and uppercase letters represent significant differences in the same line and column, respectively, and the same letter means no significant difference.

Abbreviations: LT, heavy metals-free preserved eggs stored at 4°C at low-temperature; PB, heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C; PC, heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 10 s, then removed to dry, and finally stored at 25°C; VP, heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C.

process, so it was important to control the color change of the preserved eggs during storage. Three parameters were tested for color: L\* (1-100), a\* (-100-100), and b\* (-100-100). L\* value stands for lightness and darkness. Larger the L\* value is, the brighter the color is and vice versa. The parameter a\* stands for red and green. Larger the a\* value is, the deeper red becomes, while smaller it is, the deeper green becomes. The parameter b\* stands for yellow and blue. Larger the b\* value is, the deeper yellow becomes, while smaller it is, the deeper blue becomes.

The color changes of the albumen are shown in Table 3. There was a significant decline ( $P < 0.05$ ) of the L\* value of the albumen in UC in 0 to 2 wk, while the a\* value and b\* value showed a significant increase ( $P < 0.05$ ) during this period, which indicated that the color of the albumen became darker during this period, and turned red or yellow. The color change in the albumen of PB was the same as with UC described previously. Subsequently, the L\* value of the albumen of UC and PB showed an overall upward trend. There was no significant difference ( $P < 0.05$ ) in the overall change of the a\* value in 2 to 14 wk, and the b\* value decreased significantly ( $P < 0.05$ ) in 2 to 4 wk, and its change was relatively stable. On the whole, the brightness of the albumen of UC and PB were higher than that of fresh preserved eggs, but their color became redder and yellower. The L\* value of the albumen in LT showed an overall upward trend, while the a\* value did not show an obvious change. The b\* value decreased significantly ( $P < 0.05$ ) in 2 to 6 wk and increased significantly ( $P < 0.05$ ) in 6 to 10 wk, and then, its value fluctuated slightly but had no significant difference. Overall, the brightness of the albumen in LT increased significantly, but the color did not show obvious change. The L\* value of the albumen of PB and VP showed a trend of first decline and then increase. The value of a\* augmented distinctly ( $P < 0.05$ ) in 0 to 6 wk and then displayed a declining trend, whereas the b\* value was on a falling trend as a whole. In general, the albumen brightness of PC and VP decreased, yellow gradually disappeared, and color turned deeper.

The color change of yolk surface layer is shown in Table 4. The L\* value of the yolk of UC increased first and then leveled off. A significant increase ( $P < 0.05$ ) of the a\* value of the yolk of UC was shown in 0 to 6 wk, and then, its change was relatively stable. Significant increase ( $P < 0.05$ ) of the b\* value of the yolk in UC was shown in 0 to 2 wk. The color change in the yolk of PB was the same as with the UC described previously. The b\* value of the yolk of UC manifested an obvious declining tendency ( $P < 0.05$ ) in 2 to 14 wk. However, the b\* value of the yolk in PB decreased significantly in 2 to 8 wk ( $P < 0.05$ ), then its change was steady. As a whole, the brightness of the yolk in UC and PB increased, and their colors turned red or yellow. The values of L\*, a\*, and b\* of the yolk in LT were on the rise, indicating that the brightness of the yolk in LT was significantly enhanced, and their color obviously turned red or yellow. There was a distinct reduction



**Figure 6.** Changes in sensory scores of preserved eggs with different packaging methods. Abbreviations: LT, heavy metals-free preserved eggs stored at 4°C at low-temperature; PB, heavy metals-free preserved eggs wrapped in plastic bags, tightened at the seal, and stored at 25°C; PC, heavy metals-free preserved eggs immersed in liquid paraffin completely for 5 to 110 s, then removed to dry, and finally stored at 25°C; UC, heavy metals-free preserved eggs stored at 25°C without packaging; VP, heavy metals-free preserved eggs packed with a vacuum packaging machine, tightly sealed, and finally stored at 25°C.

( $P < 0.05$ ) of the  $L^*$  value of the yolk in PC in 0 to 4 wk and significant increase ( $P < 0.05$ ) in 4 to 8 wk, and then, it became stable. As for the  $L^*$  value of the yolk in VP, it showed a significant decline ( $P < 0.05$ ) in 0 to 2 wk and a slight change afterward. The  $a^*$  value of the yolk preserved with these 2 packaging methods fluctuated slightly, while the value of  $b^*$  decreased first and then increased. In general, the yolk brightness of UC, PC, and VP were reduced during storage, the yellow gradually disappeared, and the color turned dark.

Through the aforementioned analysis, we know that the brightness of UC, LT, and PB was enhanced, and the color was faded gradually, while the brightness of PC and VP was significantly reduced and the color was significantly darkened.

From the aforementioned experimental data, it can be seen that the direct contact between air and heavy metals-free preserved eggs had an important effect on the change of its color. The albumen and yolk were darkened significantly in the absence of air contact during storage, while colors gradually returned yellow in the presence of air contact. Ganesan and Benjakul (2014) believed that the brown color of the albumen was mainly caused by Maillard reaction. Ganasen and Benjakul (2011, 2014) added glucose to the feed solution to marinate preserved eggs and found that the color of the albumen was significantly deeper than that preserved in solution without glucose, indicating that the Maillard reaction was one of the reasons to explain the phenomenon that preserved eggs turned brown. However, because of the complication of Maillard reactions, it was unknown whether oxygen had an effect on the Maillard reactions. Perhaps, it can be speculated

that Maillard reactions may be able to carry out a reverse reaction in the presence of oxygen, which may cause albumen fading. Wang and Fung (1996) believed that the formation of the blackish green of the yolk was because of the decomposition of sulfur-containing proteins under strong alkaline conditions to produce  $S^{-2}$ , which reduced  $Fe^{3+}$  in the high-phosphorus protein in the yolk to  $Fe^{2+}$ , then  $Fe^{2+}$  combines with  $S^{-2}$  to form FeS. The blue-green of FeS combines with the original yellow of the yolk to form blackish-green.

### **Changes of the Total Number of Colonies and Coliforms in Preserved Eggs With Different Packaging Methods**

During the 14-week storage, the total number of colonies and coliforms was not detected in each group. On the one hand, it may be owing to the high pH of the preserved eggs, which inhibited the growth and reproduction of microorganism; on the other hand, it may be because the protein of the preserved eggs was denatured during the pickling process, which caused the production of some antibacterial peptides or other antibacterial substances to restrain the growth of microorganism.

### **Changes in Sensory Evaluation of Preserved Eggs With Different Packaging Methods**

The level of sensory evaluation means people's acceptability of the food. The criteria for evaluation here included appearance, color, texture, smell, and taste of preserved eggs. These standards all reflect the popularity of preserved eggs and greatly affect the sales of preserved eggs in the market. The total score of sensory evaluation is set as 100 points. The results of sensory evaluation in each group are displayed in Figure 6. It can be clearly seen that there were 2 trends in the sensory evaluation of the 5 groups of preserved eggs. The sensory scores of UC, LT, and PB showed a significant decrease ( $P < 0.05$ ) during storage, while those of PC and VP made no difference, and their quality was steady. Besides, PB scored 35.12 points after 14 wk of storage which was the lowest compared with other 4 packing methods, while VP attained the highest score of 70.5.

The reasons for the significant decrease in the scores of UC, LT, and PB during storage can be analyzed from the following 3 aspects. First of all, the preserved eggs packaged in these 3 ways gradually shrunk and shrunk more severely as time went by. The water evaporation decreased with paraffin coating and vacuum packaging method owing to the tightness of the package, so no obvious shrinkage occurred with these 2 methods. Second, the color of the albumen and yolk packaged in these 3 ways gradually turned yellow and lost the inherent color of the preserved egg. But the color of the PC and VP became deeper and turn dark brown during storage. Finally, the preserved egg of UC and PB produced more obvious oxidized odor and other odors, and the

odors got worse with time, which might be caused by the oxidation reaction in the preserved eggs. The sensory score of PC and VP decreased slightly in the early stage. This was because the alkaline smell of preserved eggs cannot be emitted under sealing conditions, thus producing more pungent smell. But as time went by, the alkaline taste changed to the umami of the preserved eggs, so the sensory score increased and maintained.

## CONCLUSIONS

No microorganisms were detected in heavy metals-free preserved eggs throughout the storage period by adopting the methods in this study. In the whole storage period, the 4 packaging methods, namely, low-temperature storage, plastic bags packaging, paraffin coating, and vacuum packaging all showed certain preservation effects from different aspects of quality compared with UC.

There is a limitation on the preservation effect of plastic bags packaging and low-temperature storage. Plastic bags packaging performed poorly in maintaining the pH, moisture loss, and mass loss of preserved eggs. With the treatment of plastic bags, the pH value of preserved egg showed a rapid decline with severer mass and moisture loss. The original color of preserved eggs turned yellow at low temperature.

Vacuum packaging and paraffin coating had a better effect on maintaining the quality of heavy metals-free preserved eggs. These 2 methods delayed the changes in the pH of the preserved eggs and reduced the loss of albumen moisture and mass of preserved eggs. These 2 ways also reduced the content of TVB-N in heavy metals-free preserved eggs, maintained the good hardness, springiness, and chewiness of the albumen, and effectively prevented the preserved eggs from turning yellow.

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## DISCLOSURES

The authors declare no conflicts of interest.

## SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.psj.2021.101051>.

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