

# Research Article

# Egg Freshness Indexes Correlations with Ovomucin Concentration during Storage

Yajun Jiang (),<sup>1,2</sup> Dandan Fu (),<sup>1,2</sup> and Ming Ma ()<sup>1,2</sup>

<sup>1</sup>College of Mechanical Engineering, Wuhan Polytechnic University, Wuhan, China <sup>2</sup>Equipment Engineering Technology Research Center for Aquatic Products Processing in Hubei Province, Wuhan, China

Correspondence should be addressed to Dandan Fu; 15527058156@163.com

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The relationship between protein changes and egg quality during storage is a critical area of investigation in both basic science and egg product preservation. Viscosity changes in albumen are a sensitive sign of the deterioration process. Ovomucin, which is present in albumen, plays an important role in the gelation of the albumen. Egg freshness indices and ovomucin concentration will alter in response to storage time. This paper analyzed the correlation and gray relational degree between the Haugh unit (HU), yolk index, albumen pH, and ovomucin concentration. We studied the differences in the ovomucin concentration at different levels of HU, yolk index, and pH during storage. We established an equivalent egg age prediction model using ovomucin concentration as the independent variable. The findings indicated a correlation between the freshness indices and the ovomucin concentration. There was a good, significantly positive relationship between HU and ovomucin concentration (r = 0.713, P < 0.01), a positive correlation between the yolk index and ovomucin concentration (r = 0.699, P < 0.01), and a negative correlation between albumen pH and ovomucin concentration (r = -0.683, P < 0.01). The highest Pearson correlation coefficient (r = 0.970, P < 0.01) was obtained between the albumen and ovomucin concentration. Significant differences in ovomucin concentration were observed when HU, yolk index, and pH were varied. The gray relational degree between each freshness parameter and ovomucin concentration was greater than 0.8. Between the HU and ovomucin concentration, there was a gray correlation degree of 0.885, indicating that the HU was the primary factor affecting ovomucin concentration variation during storage. During storage, at 22°C, the ovomucin concentration in albumen was significantly and negatively related to storage time (r = -0.926, P < 0.05). The coefficient of determination for the equivalent egg age prediction model with ovomucin concentration as the independent variable was 0.985 (P < 0.05), indicating strong reliability. The study's findings show the possibility of nondestructive prediction of an egg's internal microscopic protein composition using its freshness index value.

# 1. Introduction

Eggs are a significant source of protein in daily life, and the mechanism by which eggs lose quality during storage is complex, involving an increase in egg weight loss [1], changes in pH [2], and so on. Ovomucin accounts for roughly 3.5 per cent of total albumen protein and is critical for the albumen's gelation properties [3]. Viscosity changes are a sensitive sign of the egg deterioration process. The edible qualities and the rheological properties of eggs were governed mainly by the ovomucin concentration [4]. Ovomucin is the critical component that helps albumen retains its gelatinous shape and high viscosity. Ovomucin

has two distinct and complex glycoprotein subunits,  $\alpha$ -ovomucin, and  $\beta$ -ovomucin. The dissociation of two subunits of ovomucin leading to albumen dilution is one of the most widely accepted views. During storage,  $\beta$ -ovomucin in thick albumen gradually decreases while  $\alpha$ -ovomucin remains constant, resulting in a decrease in the proportion of water-insoluble ovomucin. Ovomucin is often present in the form of a complex in the gelatinous albumen. The ovomucin-lysozyme interaction is strongest at pH of 7 and weakens under alkaline conditions. As the pH of the albumen increases during storage, the dissociation of the lysozyme-ovomucin complex is also an important factor in the dilution of the albumen. Thick albumen is unavoidably diluted during storage, with the ovomucin level progressively lower. The relationship between variations in the protein concentration of albumen and changes in egg quality is critical for determining the pattern of egg quality changes. The study of changes in the ovomucin concentration of albumen provides insight into the pattern of egg quality variations. People are more concerned about the nutritional value of eggs, such as the protein concentration level, so finding the relationship between changes in protein concentration during storage and egg freshness indicators can provide a possible way of evaluating the internal protein concentration of eggs by quickly knowing their freshness. Overall, the study of the relationship between changes in protein concentration and egg quality during storage is a critical area of basic preservation research in egg processing.

The majority of current research on ovomucin has been on its production mechanism, gel properties, biological activity, and potential applications [5-7]. However, only a few studies have investigated the relationship of the egg freshness indexes on ovomucin concentration [8, 9]. In this paper, the relation between egg quality and ovomucin concentration in albumen during storage is investigated, which is considered a dynamic system. The objectives of this study were to: (1) Explicate the correlation between egg freshness indexes and ovomucin concentration in albumen. (2) Elucidate the difference in the ovomucin concentration at different levels of HU, yolk index, and pH during storage. (3) Ascertain the gray relational degree between Haff value, yolk index, and pH and ovomucin concentration. (4) Develop a prediction model for equivalent egg age using ovomucin concentration as an independent variable.

#### 2. Materials and Methods

2.1. Experimental Sample. A total of 100 brown chicken eggs were obtained on their lay date from a farm on Jiu Feng Mountain in Wuhan, Hubei Province, China. The temperature on the farm was kept at a constant temperature of about 26°C. All samples were collected within two hours after the hens have laid their eggs, and then stored in an incubator (SPX intelligent biochemical incubator (Ningbo Jiangnan Instrument Factory)) at  $22 \pm 1^{\circ}$ C with a relative humidity of 65%. The selection of eggs on the farm was random, as was the selection of eggs for each experiment in the incubator. In order to have a higher reliability of the obtained results, the eggs were selected without considering a given range of egg weights, which would avoid the consistency of egg weights leading to a poor reliability of the final conclusions. Twenty eggs were randomly selected and consecutively numbered every ten days, i.e., on days 1, 11, 21, 31, and 41. The experiment was repeated twice, each time with a sample of 50 eggs. In the experiment, the eggs were detected for cracks by eye observation and simple light. If the egg is cracked, it was not selected for the experiment. It should be noted that on the 41st day of storage, two eggs dispersed their yolks while separating the albumen. The final valid sample obtained was 98 eggs.

#### 2.2. Experimental Method

2.2.1. Determination of Egg Freshness Indices. HU(Haugh unit): Each egg was weighed  $(W_i)$  separately using an electronic balance (Shanghai Puchun Measuring Instruments Co., Ltd.), after breaking the egg, and the thick albumen height in each egg was manually measured using a digital caliper (Guilin Guanglu Digital Measurement & Control Co., Ltd.). Each egg was measured three times and the average was used as the final result  $(H_i)$ . The Haugh unit of egg is determined by utilizing the following formula [10]:

$$HU_i = 100 * \lg \left[ H_i + 7.57 - 1.7 * \left( W_i^{\wedge} 0.37 \right) \right].$$
(1)

Yolk index: Each egg's yolk height and diameter were measured with a digital caliper. Three measurements were performed per egg yolk and the average was used to determine the yolk height  $(h_i)$  and diameter  $(d_i)$ . The yolk index of an egg was computed using the following formula [11]:

yolk inde 
$$x_i = \frac{\text{yolk height}(h_i)}{\text{yolk diameter}(d_i)}$$
, (2)

where i is the egg number.

2.3. Determination of Ovomucin in Albumen. The determination steps of ovomucin are as follows [12]:

- A simple device was used as the albumen separator to manually separate the albumen and yolk, and then the total mass of the albumen and the beaker was weighted by the electronic balance. The mass of the albumen is equal to the total mass minus the mass of the beaker it is in.
- (2) The albumen was stirred for 30 min at 4°C using a magnetic stirrer. Triple volume of 0.1 mol/L Nacl was added to the blended albumen mixture and stirred for 40 min at 4°C.
- (3) The pH of the mixed solution was adjusted to 6.0 with 2 mol/L HCL and stirred for 1 h at 4°C, and then left overnight. On the next day, the rested mixture was transferred to multiple 50 ml centrifuge tubes and then levelled so that the centrifuge could work properly with equal masses of samples placed on the diagonal, and then centrifuged for 15 min at 4°C and 15000 rpm. They were repeated several times under the same conditions of levelling and centrifugation until all mixed solutions could be fully centrifuged. All the sediment in the centrifuge tube was transferred to the beaker. Repeat this procedure once.
- (4) The precipitate was rinsed three times with distilled water, and then was centrifuged for 15 min at 4°C and 15000 rpm.
- (5) The washed precipitates were transported to lyophilization boxes, dried for 48 hours, weighed, and kept at  $-20^{\circ}$ C.

2.4. Gray Relational Analysis. Gray relational analysis is a method for determining the evolution system quantitatively [13]. It is a multifactor statistical method that determines the closeness of the relationship by comparing the degree of similarity of the geometry of the reference data array and several comparison data arrays. Gray relational analysis can represent the degree of correlation between curves. It is different from Pearson correlation analysis, which examines only the degree of linear correlation between variables [14], and its connection does not imply causality. Gray relational analysis is comprised of the following steps [15].

(1) Construct a reference data series  $(x_0)$  that accurately represents the system's behavioral characteristics:

$$x_0 = \{x_0(1), x_0(2), \dots, x_0(n)\}.$$
 (3)

(2) Construct a comparative data series () that is related to the system's behavior:

$$x_i = \{x_i(1), x_i(2), \cdots, x_i(n)\}, i = 1, 2, \cdots, m.$$
(4)

(3) Calculate the transformation matrix of initialization ():

$$x'_{i} = \left\{ \frac{x_{i}(1)}{x_{i}(1)}, \frac{x_{i}(2)}{x_{i}(1)}, \cdots, \frac{x_{i}(k)}{x_{i}(1)} \right\}$$

$$= \left\{ x_{i}(1)', x_{i}(2)', \cdots, x_{i}(k)' \right\}.$$
(5)

(4) Calculate the difference series () using the following formula:

$$\Delta i(k) = |x_1'(k) - x_1'(k)|.$$
(6)

(5) Calculate the correlation coefficient ((*k*)) using the following formula:

$$\xi_{i}(k) = \frac{\min_{i} \min_{k} |x_{0}(k) - x_{i}(k)| + \varphi \max_{i} \max_{k} |x_{0}(k) - x_{i}(k)|}{|x_{0}(k) - x_{i}(k)| + \varphi \max_{i} \max_{k} |x_{0}(k) - x_{i}(k)|},$$
(7)

where  $\phi$  is the discriminant coefficient, which assists in improving the significance of the difference between the correlation coefficients,  $\phi$  is typically 0.5 [16]. Correlation coefficients indicate the degree of similarity between reference and comparison series over a certain period.

(6) Compute and rank the comprehensive gray relational degree (γ<sub>i</sub>):

$$\gamma_i = \frac{1}{n} \sum_{k=1}^n \xi_i(k).$$
(8)

The comprehensive gray relational degree is the average relational degree between the reference and comparison arrays over the system change period. The higher the comprehensive gray relational degree, the bigger the comparison array's influence on the reference array, conversely. After computing the comprehensive gray relational degree, all the correlation degrees acquired are ranked; by comparing them, the impact of multiple factors on the reference variable can be determined, allowing for the identification of the primary factor affecting the reference variable.

### 3. Results and Discussion

The ovomucin concentration was determined using relative quality, i.e., ovomucin concentration per gram of albumen  $(M_i \text{ mg/g})$ .

 $M_i = \frac{g_i}{G_i},\tag{9}$ 

where *i* is the egg number.  $g_i$  is the dry weight of ovomucin, while  $G_i$  is the egg weight.

The experiment collected data on 98 eggs, and Table 1 summarizes the descriptive information and statistical results for egg freshness indicators and ovomucin concentration.

3.1. Correlation between and Ovomucin Concentration Egg Freshness Indices. Pearson correlation analysis was conducted using SPSS software (version 26), and the findings are presented in Table 2. As shown in Table 2, a good significant positive correlation between the Haugh unit and ovomucin concentration was found (r=0.713,P < 0.01). This result corroborates Wang's findings [6]. A significant positive correlation was found between ovomucin concentration and the albumen height (r = 0.729, P < 0.01), and egg weight was found to be irrelevant (r=0.139). Irrelevant could be because the ovomucin concentration is computed by the protein concentration per gram of albumen. A weak but significant positive correlation existed between the volk index and ovomucin concentration (r = 0.699, P < 0.01) while a significant negative correlation appeared between pH and ovomucin concentration (r = -0.683, P < 0.01). The results reveal that the strongest correlation was found among the egg freshness indicators between the albumen height and ovomucin concentration, which may be related to the fact that ovomucin plays a significant role in the gel characteristics of albumen.

TABLE 1: Descriptive information and statistical results for egg freshness indicators and ovomucin concentration.

	Minimum	Maximum	Mean	Standard deviation
Egg weight (g)	53.390	72.140	62.329	3.890
The albumen height (mm)	2.177	10.480	5.212	2.377
HU	24.824	101.272	64.575	20.694
Yolk height (mm)	10.650	18.740	14.805	1.816
Yolk diameter (mm)	34.3467	47.723	41.983	3.172
Yolk index	0.233	0.507	0.357	0.067
pH	7.940	9.860	9.040	0.601
Ovomucin concentration (mg/g)	0.253	4.203	1.489	0.918

TABLE 2: Results of Pearson correlation analysis between ovomucin concentration and each egg freshness indexes.

	Egg weight (g)	The albumen height (mm)	HU	Yolk height (mm)	Yolk diameter (mm)	Yolk index	рН	Ovomucin concentration (mg/g)
Egg weight (g)	_	0.212*	0.089	0.259*	0.021	0.158	$-0.278^{**}$	0.139
The albumen height (mm)		_	0.970**	0.803**	$-0.844^{**}$	0.870**	$-0.834^{**}$	0.729**
HU			_	0.831**	$-0.873^{**}$	$0.888^{**}$	$-0.769^{**}$	0.713**
Yolk height (mm)				_	$-0.834^{**}$	0.967**	$-0.643^{**}$	0.660**
Yolk diameter (mm)					_	$-0.941^{**}$	0.643**	-0.666**
Yolk index						_	-0.696**	0.699**
pH							—	-0.683**
Ovomucin concentration (mg/								
g)								—
$*P \le 0.05; **P \le 0.01.$								

#### 3.2. Differences in the Ovomucin Concentration at Different Levels of Egg Freshness Indexes

3.2.1. Differences in the Ovomucin Concentration at Different Levels of the HU. The HU is an indicator used to determine the freshness of eggs as specified by the USDA egg quality standards (United States standards, grades, and weight classes for shell eggs. AMS 56, USDA, 2000) and the People's Republic of China agricultural industry standards (fresh egg grading, NY-T 1758-2009). According to the standard, a HU of more than 72 is an AA grade, a HU of 60 to 72 is an A grade, and a HU of less than 60 is a B or C grade [17]. To determine the differences in the ovomucin concentration at different levels of HU, the HU were classified into three categories, greater than 72, between 60 and 72, and less than 60, according to the general classification criteria for HU. An ANOVA analysis using SPSS software (version 26) was used to determine the differences in the ovomucin concentration at different levels of HU. Table 3 summarizes the results of an analysis of variance for ovomucin concentration at various HU values.

As shown in Table 3, when the HU value was greater than 72, a total of 35 egg samples satisfied the condition, and the mean value of ovomucin concentration for these 35 eggs was 2.171 mg/g (minimal variance, standard deviation of 0.172 mg/g), 1.595 mg/g (maximal variance, standard deviation of 1.359 mg/g) for 20 eggs' average when the HU was between 60 and 72, and 0.737 mg/g for 43 eggs' average when the HU was less than 60. The resulting p value was approximately 0.000 for the three groups with equal means. Taken together, these results suggest that there was

TABLE 3: Results of analysis of variance for the ovomucin concentration at various HU values.

HU	Ν	Mean	Std	F	ħ
110	11	mean	ota	1	P
Greater than 72	35	2.171	0.172		
Between 60 and 72	20	1.595	1.359	30.966	$\leq 0.001$
Less than 60	43	0.476	0.737		

a significant difference ( $p \le 0.001$ ) between the three groups. In other words, there was a significant difference in the ovomucin concentration at different levels of HU.

We also found that the higher the HU value, the higher the ovomucin concentration. Several studies have revealed that HU is determined by thick albumen height and egg weight, that the bigger the thick albumen height, the better the gel characteristics of albumen, and that ovomucin is a protein-related to the gel characteristics of fresh albumen [6], that it is the key protein responsible for maintaining the viscosity and height of thick albumen.

3.2.2. Differences in the Ovomucin Concentration at Different Levels of Yolk Index. Due to the lack of an industry standard for yolk index classification, the international standard for rating egg freshness is often the Haugh unit. Therefore, a linear regression relationship was established between the yolk index as the dependent variable (y) and the Haugh unit as the independent variable (x), according to the data obtained from the experiment.

$$y = 0.0029x + 0.1707 (R^2 = 0.789, P < 0.05).$$
(10)

The yolk index was classified by internationally accepted criteria for HU categorization, namely, when x is 72, y equals 0.3795, and when x is 60, y equals 0.3447. As a result, the yolk index can be classified as greater than 0.380, between 0.345 and 0.380, or less than 0.345. The ANOVA analysis was performed, and the analysis of variance for the ovomucin concentration at various yolk index values is shown in Table 4.

From Table 4, we found that when the yolk index was greater than 0.380, a total of 40 egg samples satisfied the range, and the mean ovomucin concentration for these 40 eggs was 2.208 mg/g (maximal variance, standard deviation of 0.894 mg/g), 1.212 mg/g (minimal variance, standard deviation of 0.491 mg/g) for 21 eggs' average when the yolk index was between 0.345 and 0.380, and 0.868 mg/g for 37 eggs' average when the yolk index was less than 0.345. The resulting *p* value was approximately 0.000 for the three groups with equal means. Therefore, a one-way ANOVA revealed a statistically significant difference between the three groups ( $p \le 0.001$ ). Specifically, there was a significant difference in the ovomucin concentration at different levels of the yolk index.

We also found that a drop in the yolk index resulted in a significant decrease in the ovomucin concentration (P < 0.05). It may be because a small amount of ovomucin is also found in the yolk membrane and egg frenulum, where it acts as a barrier against the yolk spreading out. With an increased storage period, the elasticity of the yolk membrane gradually reduces, the yolk's diameter increases, the yolk's height decreases, and the ovomucin partially depolymerizes [18].

3.2.3. Differences in the Ovomucin Concentration at Different Levels of pH. Similarly, using data from the experiment, a binomial regression relationship was built between the pH value as the dependent variable (y) and the Haugh unit as the independent variable (x).

$$y = 0.0005x^{2} + 0.043x + 8.5558(R^{2} = 0.789, P < 0.05).$$
(11)

According to globally accepted criteria for HU classification, the pH was divided into three categories: less than 9.089, between 9.089 and 9.360, and greater than 9.360. Table 5 provides the results of the analysis of variance in ovomucin concentration at various pH values.

As shown in Table 5, when the pH was less than 9.089, a total of 39 egg samples satisfy the range, and the mean value of ovomucin concentration was 2.210 mg/g (maximal variance, standard deviation of 0.889 mg/g), 0.831 mg/g (minimal variance, standard deviation of 0.523 mg/g) when the yolk index was between 9.089 and 9.360, and 1.112 mg/g when the yolk index was greater than 9.360. These results were statistically significant at the p = 0.05 level. In conclusion, it was determined that pH had a significant impact on the ovomucin concentration.

The results of the study also indicate that the ovomucin concentration does not increase in a predictable manner as the pH increases. However, we noticed that when the pH was greater than 9.089, the concentration of ovomucin was

TABLE 4: Results of analysis of variance for the ovomucin concentration at various yolk index values.

Yolk index	Ν	Mean	Std	F	P
Greater than 0.380	40	2.208	0.894		
Between 0.345 and 0.380	21	1.212	0.491	38.397	$\leq 0.001$
Less than 0.345	37	0.868	0.506		

TABLE 5: Results of the analysis of variance in ovomucin concentration at various pH values.

рН	Ν	Mean	Std	F	P
Less than 9.089	39	2.210	0.889		
Between 9.089 and 9.360	21	0.831	0.523	35.250	$\leq 0.001$
Greater than 9.360	38	1.112	0.554		

dramatically reduced. This is mostly due to the fact that ovomucin is 252 times more soluble in an alkaline environment (pH > 9) than in a neutral one [19]. The pH of albumen increased with storage time, and the solubility of ovomucin steadily increased and transferred to thin albumen, resulting in a decrease in the ovomucin concentration of thick albumen [20].

3.3. Gray Relational Analysis between the Egg Freshness Index and the Ovomucin Concentration. The changes in egg quality and ovomucin concentration during storage were considered as a dynamic system, and the major factors affecting ovomucin concentration were determined using gray relational analysis. Gray relational analysis was used to calculate and analyze the gray relational degree between HU, yolk index, pH, and ovomucin concentration. A quantitative analysis of the relationship between several egg freshness parameters and ovomucin concentration was then performed.

Since each egg freshness factor has unique dimensions and it is impossible to compare factors with different dimensions, the normalization approach was applied to make each freshness indicator dimensionless before completing the gray correlation analysis, which was calculated as follows:

$$M_{ij}' = \frac{M_{ij}}{\overline{M_j}}, \qquad (12)$$

where  $M'_{ij}$  is the dimensionless value of in the *i* th row and *j* th column,  $M_{ij}$  is the value in the *i* th row and *j* th column, and  $\overline{M_i}$  is the average in the *j* th column.

The reference array was the ovomucin concentration, and the comparison array was each egg freshness index. The gray relational degree between egg freshness parameters and ovomucin concentration was computed using the gray relational analysis approach, as shown in Table 6.

As presented in Figure 1, the gray relational degree between each egg's freshness index and ovomucin concentration altered continuously during storage. On day 11 of storage, the biggest gray relational degree between HU and ovomucin concentration (with a gray relational degree larger than 0.90) was attained; the gray relational degree between

TABLE 6: The comprehensive gray relational degree between each egg freshness index and ovomucin concentration.



FIGURE 1: The changing trend in the relative gray relationship degree between each egg freshness indicator and ovomucin concentration for various storage days.

the two was greatest at this time, and their changing curves were nearly synchronous. Additionally, on day 21 of storage, the gray relational degree between the yolk index and pH and the ovomucin concentration were at their maximum values.

From Table 6, we found that the comprehensive gray relational degree between each freshness index and ovomucin concentration during storage was greater than 0.8. They were ranked in the following order: HU is greater than pH, which is greater than the yolk index. By and large, the bigger the comprehensive gray relational degree, the closer the two are related. A comprehensive gray relational degree of more than 0.8 indicates a strong connection between the reference and the comparison arrays; 0.3 to 0.8 suggests a minor relationship; and less than 0.3 implies a poor relationship.

Taken together, these findings indicate that the synchronous variation trend between each egg freshness index and the ovomucin concentration was strong, and the synchronous variation trend between the HU and the ovomucin concentration was greatest (a comprehensive gray relational degree of 0.885). That is, of the influential factors listed above, the HU has the greatest impact on the variation in ovomucin concentration. In contrast, the yolk index had the least relation to a synchronous variation on the change in ovomucin concentration.

3.4. Establishment of a Prediction Model for Equivalent Egg Age. Not only do eggs provide a significant amount of highquality protein, but they are also economical and indispensable in customers' daily lives, and their freshness has a significant impact on consumers' food safety. The



FIGURE 2: Changes in ovomucin concentration during storage.

evaluation and prediction of egg quality during storage, as well as its relationship to storage time, have been a hot topic of research in the field of food processing and preservation, as egg age (i.e., the storage time of eggs) does not accurately reflect their true freshness under different storage conditions [21]. Therefore, it has been proposed to determine the freshness of eggs under various storage conditions by calculating their equivalent egg age [22]. The amount of ovomucin in albumen from eggs held at 22°C was significantly correlated with the storage time (r = -0.926, P < 0.05). From Figure 2, it can be found that with the increase of storage time, the ovomucin concentration showed a decreasing trend.

The research explored eggs stored at 22°C and 65 percent relative humidity, and the ovomucin concentration per gram of albumen was used to develop a prediction model of equivalent egg age for evaluating the freshness of commercial eggs stored at this temperature and relative humidity during their shelf life.

$$y = 9.42x^2 - 52.279x + 73.262(R^2 = 0.985, P < 0.05).$$
(13)

The coefficient of determination for the equivalent egg age prediction model with ovomucin concentration as the independent variable was 0.985 (P < 0.05), it is higher than that of the equivalent egg age model developed by Wang at 25°C [8].

# 4. Conclusions

This study investigated the link between the egg freshness index and the ovomucin concentration in albumen using Hyland brown eggs. The dominant factors affecting ovomucin concentration during storage were determined using gray relational analysis, and an equivalent egg age prediction model was developed. The study identified a good, significant positive correlation between HU and ovomucin concentration (r = 0.713, P 0.01), a positive correlation between the yolk index and ovomucin concentration (r = 0.699, P < 0.01), and a negative correlation between albumen pH and ovomucin concentration (r = -0.683, P < 0.01). The highest Pearson correlation coefficient (r = 0.970, P < 0.01) was found between the albumen height and egg ovomucin concentration among the egg freshness indices. The HU, yolk index, and pH all had a significant effect on the ovomucin concentration, with the HU having the highest influence (a comprehensive gray relational degree of 0.885) and the most synchronous change trend with the ovomucin concentration. In contrast, the yolk index had a minor synchronous change trend with the change in ovomucin concentration. The ovomucin concentration in the albumen of eggs held at 22°C was significantly correlated with storage time (r = -0.926, P < 0.05). The coefficient of determination for the equivalent egg age prediction model with ovomucin concentration as the independent variable was 0.985 (P < 0.05), indicating high reliability. This study provides the possibility to reveal the mechanism of egg quality changes from a biochemical point of view without breaking the eggs and lays the theoretical foundation for developing a nondestructive and rapid method for egg quality detection and effective nondestructive monitoring of egg quality in the future.

# **Data Availability**

The experimental data used to support the findings of this study have not been made available because of the needs for the future study.

## **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

# **Authors' Contributions**

Dandan Fu has made substantial contributions to acquisition of data, analysis, interpretation of data, and drafted the manuscript; Yajun Jiang has made substantial contributions to experiment design; Ming Ma has been involved in revising it critically for important intellectual content. All of them agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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#### References

- P. V. Suresh, K. R. Raj, T. D. Nidheesh, G. K. Pal, and P. Z. Sakhare, "Application of chitosan for improvement of quality and shelf life of table eggs under tropical room conditions," *Journal of Food Science & Technology*, vol. 52, no. 10, pp. 6345–6354, 2015.
- [2] H. Nagashima and T. Ichiro, "Relationships between height, diameter and weight distributions of *Chenopodium album* plants in stands: effects of dimension and allometry," *Annals* of *Botany*, vol. 75, no. 2, pp. 181–188, 1995.
- [3] W. Xiaocu, "Ovomucin:Structure composition, physicochemical properties, the role in egg white thinning and

nutritional modulation," *Chinese Journal of Animal Nutrition*, vol. 27, no. 2, pp. 327–333, 2015.

- [4] H. Takada, T. Katoh, and T. Katayama, "Sialylated O-Glycans from hen egg white ovomucin are decomposed by mucindegrading gut microbes," *Journal of Applied Glycoscience*, vol. 67, no. 2, pp. 31–39, 2020.
- [5] M. Offengenden and J. Wu, "Egg white ovomucin gels: structured fluids with weak polyelectrolyte properties," *RSC Advances*, vol. 3, pp. 910–917, 2013.
- [6] Z. Wang, A. Tu, D. Tang, and Y. Shan, "Effectively preparing soluble ovomucin with high antiviral activity from egg white," *International Journal of Biological Macromolecules*, vol. 118, pp. 504–510, 2018.
- [7] E. Abeyrathne, H. Y. Lee, and D. U. Ahn, "Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—a review," *Poultry Science*, vol. 92, no. 12, pp. 3292–3299, 2013.
- [8] Y. Wang, Z. Wang, and Y. Shan, "Assessment of the relationship between ovomucin and albumen quality of shell eggs during storage," *Poultry Science*, vol. 98, no. 1, pp. 473–479, 2019.
- [9] Y. Shan, D. Tang, R. Wang et al., "Rheological and structural properties of ovomucin from chicken eggs with different interior quality," *Food Hydrocolloids*, vol. 100, Article ID 105393, 2020.
- [10] Z. Nemati, H. Ahmadian, M. Besharati et al., "Assessment of dietary selenium and vitamin E on laying performance and quality parameters of fresh and stored eggs in Japanese quails," *Foods*, vol. 9, p. 1324, 2020.
- [11] M. E. Abd El-Hack, M. Alagawany, K. M. Mahrose et al., "Productive performance, egg quality, hematological parameters and serum chemistry of laying hens fed diets supplemented with certain fat-soluble vitamins, individually or combined, during summer season," *Animal Nutrition*, vol. 5, no. 1, pp. 49–55, 2019.
- [12] H. Abd Elgalil, A. M. Osman, A. Abo Eita, and S. El Saadany, "Isolation and characterization of duck egg white ovomucin," *Zagazig Journal of Agricultural Research*, vol. 45, no. 5, pp. 1721–1728, 2018.
- [13] J. Hafsa, A. Naseer, H. Uzair, and M. Rajesh, "Parametric optimization of durable sheeting fabric using taguchi grey relational analysis," *Journal of King Saud University - Science*, vol. 34, Article ID 102004, 2022.
- [14] K. Oğuz, E. Mehmet, R. Çakıroğlu, and A. Adem, "Optimization of melting time of solar thermal energy storage unit containing spring type heat transfer enhancer by Taguchi based grey relational analysis," *Journal of Energy Storage*, vol. 47, Article ID 10367, 2022.
- [15] N. H. Abou-Taleb, D. T. El-Sherbiny, N. M. El-Enany, and H. I. El-Subbagh, "A new grey relational analysis application in analytical chemistry: natural deep eutectic solvent as a green extractant for HPLC determination of lamotrigine in plasma," *Microchemical Journal*, vol. 172, Article ID 106918, 2022.
- [16] R. Mu, Y. Zheng, A. Lambertz, R. G. Wilks, M. Bar, and Y. Zhang, "A spectrum deconvolution method based on grey relational analysis," *Results in Physics*, vol. 23, Article ID 104031, 2021.
- [17] V. G. Narushin, M. N. Romanov, and D. K. Griffin, "A novel Egg Quality Index as an alternative to Haugh unit score," *Journal of Food Engineering*, vol. 289, Article ID 110176, 2021.
- [18] K. Mann, "Proteomic analysis of the chicken egg vitelline membrane," *Proteomics*, vol. 8, no. 11, pp. 2322–2332, 2008.

- [19] J. Wang and J. Wu, "Effect of operating conditions on the extraction of ovomucin," *Process Biochemistry (Oxford, United Kingdom)*, vol. 47, no. 1, pp. 94–98, 2012.
- [20] X. Sun, M. G. Gänzle, C. J. Field, and J. Wu, "Effect of proteolysis on the sialic acid content and bifidogenic activity of ovomucin hydrolysates," *Food Chemistry*, vol. 212, pp. 78–86, 2016.
- [21] F. Wang, H. Lin, P. Xu, X. Bi, and L. Sun, "Egg freshness evaluation using transmission and reflection of NIR spectroscopy coupled multivariate analysis," *Foods*, vol. 10, no. 9, p. 2176, 2021.
- [22] J. Coronel-Reyes, I. R. Morales, E. Fernández-Blanco, R. Daniel, and P. Alejandro, "Determination of egg storage time at room temperature using a low-cost NIR spectrometer and machine learning techniques," *Computers and Electronics in Agriculture*, vol. 145, pp. 1–10, 2018.