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Research Article

Study of the Molecular Structure of Proteins in Eggs under Different Storage Conditions

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The aim of this study is to reveal the relationship between changes in the physical and chemical characterization of eggs during storage and to provide a new way to assess egg freshness at the molecular chemical level. In this study, the Fourier transform infrared (FTIR) spectra of egg albumin from 60 fresh eggs (ISA Brown layer) stored for 1, 10, 20, 30, and 40 days at 4°C and 10°C were measured ($n = 6$), and the amide I band of the egg albumin spectra was quantified by Fourier deconvolution and curve fitting to obtain the composition of the protein molecular structure. The results showed that different storage temperatures and storage times led to protein denaturation, resulting in changes in the conformation of the protein secondary structure. With increasing storage temperature and storage time, the content of the ordered structures β-sheet and α-helix in the egg protein secondary structure decreased significantly ($p < 0.05$), and the content of the disordered structure β-turn and random coil increased significantly ($p < 0.05$). Based on the pattern of protein secondary structure changes during storage, it was found that fresh eggs were better stored at 4°C for up to 15 days. Spearman's correlation analysis of egg protein height, Haugh unit, egg weight, and protein secondary structure in eggs stored at 4°C showed a significant positive correlation between β-sheet and α-helix and egg weight, protein height, and Haugh unit. And a significant negative correlation between random coil and egg weight, protein height, and Haugh unit. Therefore, a curve fitting method based on Fourier transform infrared spectroscopy of deconvoluted spectra is an effective method for evaluating egg quality and freshness.

1. Introduction

Eggs are a complete source of protein, vitamins, and other substances, all of which are important in the human diet. When fresh eggs are stored at different temperatures, the variation of egg-white viscoelasticity is closely related to storage time and temperature. Such as egg-white dilution and Haugh unit diminution [1, 2]. Appropriate storage methods can inhibit the evaporation of water from fresh eggs, the growth and reproduction of microorganisms and the action of enzymes in the eggs. This slows down changes in the weight and relative density of fresh eggs and delays changes in the contents of the eggs, especially the hydration of thick proteins, thus maintaining the fresh quality of the eggs for a longer period of time, extending the shelf life of the eggs and saving costs [3]. On the other hand, the consumption of spoiled eggs can have a negative impact on the body. Therefore, it has become an urgent issue for the egg industry worldwide to test the quality of eggs during preservation, improve efficiency, and eliminate the consumption of spoiled eggs.

Long-term scientific research and extensive practical experience have shown that the quality and freshness of eggs are related to storage temperature and storage time [4, 5]. The most important indicator of egg freshness is the Haugh unit [6], but this indicator only reflects the changes in the egg during storage from the height of the concentrated protein and does not reflect the changes in the egg protein at the molecular scale [7].

FTIR is widely used to predict protein, lactose, and more detailed milk composition characteristics [8–10]. Detection of protein secondary structure by FTIR focuses on the amide I band (1700-1600 cm^{-1}) of the spectrogram [11]. This method has high resolution, sensitivity, accuracy, simplicity,

and speed of operation [12]. The FTIR method is also used for product quality testing without the need for extraction and allows direct and non-destructive testing of the sample, making the results more intuitive [13]. Therefore, unlike indicators such as physical characterization, FTIR can reveal changes in proteins during the storage of fresh eggs at the molecular level. It provides a new, fast, and easy method to assess the freshness of eggs.

The proteins in eggs are an important component of eggs, and a large part of the biological activity of eggs is represented by the proteins, the function of which is strongly related to their structure [14]. Benedé et al. [15] investigated Caco-2 cell responses induced by peptides released after digestion of heat-treated ovalbumin using FTIR. Chen [16] found the secondary structure of egg proteins after rice vinegar treatment was determined by Fourier infrared spectroscopy. The results showed that the α-helix and β-turn angles of egg proteins increased and the β-sheet decreased after rice vinegar treatment, and the secondary structure of proteins tended to be disordered. Sun [17] showed that the α-helix content decreased significantly ($p < 0.05$), the random curl content increased significantly ($p < 0.05$), and the β-turn angle and β-sheet content both increased and then decreased during the heating process. Ye [18] used infrared spectroscopy to study the effect of heating on the secondary structure of proteins in milk powder and found that whole milk powder had better temperature stability than that of infant formula. Murayama and Tomida [19] used Fourier transform infrared spectroscopy to study heat-induced changes in the secondary structure and conformation of bovine serum albumin. A previous study found that egg quality parameters were more correlated with S-albumin content than with egg mucin [20]. However, there were few reports on the relationship between the physical and chemical characterization of eggs during storage. In this study, egg albumin height, Haugh units, egg weight, and Fourier transform infrared (FTIR) spectra of egg albumin were measured at 4°C and 10°C, respectively. The composition of the secondary structure was obtained by deconvolution and curve fitting of the amide I band of egg albumin. Secondly, Spearman correlation analysis was performed on the protein height, Haugh units, egg weight, and protein secondary structure of eggs at 4°C to determine the relationship between changes in protein physicochemical properties during storage, thus enriching the theory on the mechanism of quality changes during egg storage.

2. Materials and Methods

2.1. Raw Materials. A total of 80 fresh eggs of 39-week-old ISA Brown layer were purchased from Luyuan Chicken Farm, Jinghai District, Tianjin, China, on July 1, 2019. A completely randomized block design method was used for test grouping.

2.2. Experimental Design. The test groups were randomly divided into 2 portions. 60 eggs were stored at 4°C and 10°C (Qingdao Haier Co., Shandong, China). Six eggs were removed from each temperature on days 1, 10, 20, 30, and 40 for the determination of egg weight, protein height, Haugh unit, and protein secondary structure.

3. Test Methodology

3.1. Determination of Egg Weight. The newly grouped eggs were placed on an electronic balance (Shanghai Yiheng Scientific Instruments Co., Shanghai, China) and weighed three times to obtain the average of the egg masses.

3.2. Determination of Protein Height and Haugh Unit. Place the egg on a glass plate and measure the height of the egg white at the edge of the yolk using a pycnometer. The three points of the triangle are selected as the heights of the egg white in the measurement, and the average of the three measurements is taken. The value obtained is the Haugh unit according to the following formula [1], Haugh unit = 100 Lg(H − 1.7 W + 7.57), where H is the height of the concentrated egg white (mm) and W is the weight of the egg (g).

4. Determination of the Secondary Structure of Proteins

4.1. Sample Preparation. The egg whites (albumen) and yolks (mucin) are first separated using an egg white separator, and then the separated egg whites are dried in a drying oven (45 ± 2°C; Shanghai Boxun Industrial Co., Ltd. Medical Equipment Factory, Shanghai, China), which they are crushed and stored in sealed bags.

4.2. Acquisition of FTIR Spectra. The crushed protein samples were passed through a 60-mesh standard sieve (Shangyu City Wuxing Pressed Sieve Factory, Zhejiang, China). The protein secondary structure was determined using a Fourier infrared spectrometer with a measurement range of 4000-800 cm^{-1} (Lambda Corporation, Australia), a resolution of 4$cm^{-1,}$ and 64 scans of the signal, with the sample, acquired first and then the background, a gain of 1 and 3 parallel trials for each sample.

4.3. Data Processing of FTIR Profiles. The acquired profiles were processed sequentially by the software (OMNIC 8.2) in the order of automatic baseline correction and automatic smoothing. The software (OMNIC 8.2) is then used to perform Fourier deconvolution and Gaussian function curve-fitting on the original map of the protein secondary structure. There are two general methods of curve fitting [21]: One is fit to the original Fourier spectrum, and the other is fit to the Fourier deconvoluted spectrum. Since Fourier deconvolution spectra are more likely to find overlapping narrow bands, the second method, i.e., fitting a Gaussian function to the Fourier deconvolution spectra, was used in this study, setting up multiple fits to make the results more reliable, identifying each protein secondary structure conformation according to the amide I band 1700-1600 cm^{-1}, and finally using the peak area of each subpeak to determine the percentage content of each secondary structure (α- helix, β-sheet, β-turn, and random curl) [22].

5. Data Statistics

A two-way ANOVA with Duncan's test of significance ($p < 0.05$) and Spearman's correlation analysis were performed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA) for

Table 1: Effect of different storage conditions on egg quality.

Items	1 day	10 day	20 day	30 day	40 day
Albumen height					
4°C	7.26 ± 0.60^{a}	4.97 ± 0.28^{b}	4.72 ± 0.05^{b}	4.64 ± 0.83^{b}	4.29 ± 0.92^{b}
10°C	7.26 ± 0.60^{a}	6.06 ± 0.60^{b}	5.06 ± 0.24^{c}	4.44 ± 0.33^{c}	2.67 ± 0.30^{d}
Haugh unit					
4°C	84.86 ± 1.48^{a}	67.09 ± 2.53^{b}	65.42 ± 1.12^{bA}	62.53 ± 3.87^{b}	53.82 ± 0.96^{cA}
10°C	84.86 ± 1.48^{a}	61.65 ± 2.79^{b}	60.22 ± 2.01^{bcB}	56.46 ± 0.92^{c}	48.15 ± 0.45^{dB}
Egg weight					
4°C	67.40 ± 1.22^{a}	61.16 ± 1.11^{b}	60.98 ± 3.21^{b}	56.81 ± 0.95^{c}	53.85 ± 1.62^{c}
10°C	67.40 ± 1.22^{a}	61.13 ± 1.27^{b}	56.95 ± 0.17^{c}	56.31 ± 2.11^{c}	55.09 ± 2.01^{c}

Note: data are expressed as Means ± standard deviations of triplicate measurements. $^{a\text{-}c}$Means in the same row with different lowercase letters are significantly different ($p < 0.05$). $^{A\text{-}B}$Means in the same column with different capital letters are significantly different ($p < 0.05$).

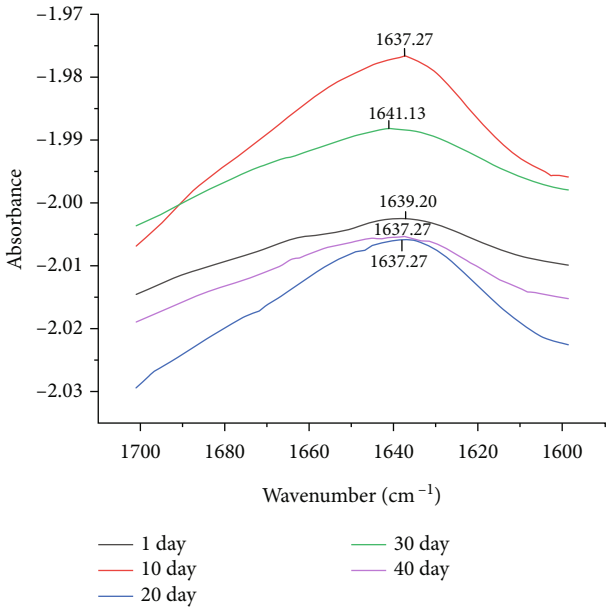

Figure 1: FT-IR infrared spectrum at 4°C.

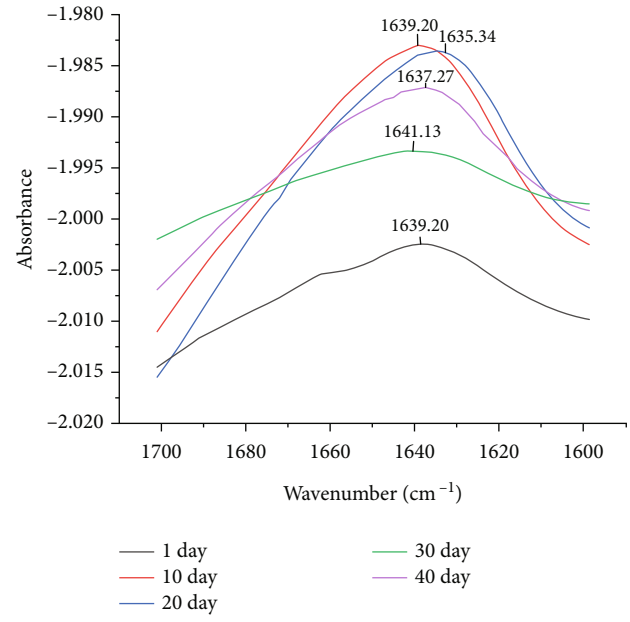

Figure 2: FT-IR infrared spectrum at 10°C.

egg quality and protein secondary structure in egg white, secondary structure as the dependent variable, time and temperature as factors, and plotting was done using Origin 2020 (OriginLab Corp., Northampton, MA, USA), OMNIC 8.2 (Thermo Nicolet Corporation, Madison, WI, USA) software.

6. Results

6.1. Effect of Different Storage Conditions on Egg Quality. Egg protein height, Haugh unit, and egg weight decreased significantly after 40 days of storage at 4°C and 10°C, respectively, with the greatest changes occurring at 10°C ($p < 0.05$), suggesting that prolonged storage decreases the quality of eggs (Table 1). In addition, the value of the Haugh unit was evidently decreased on day 40 at 10°C ($p < 0.05$), compared to 4°C, low temperature storage was more suitable for maintaining egg quality than that at high temperature (Table 1).

7. FTIR Infrared Spectrogram

It can be seen that the amide I band of the egg albumin sample was composed of a series of broad peaks with overlapping amino acid residues (Figures 1 and 2). The amide I band (1700-1600 cm^{-1}) caused by the stretching vibration of C=O is the most interesting for the study of the protein secondary structure [23]. The peak shape of the secondary structure IR spectra of the protein at 10°C is sharper than that of the secondary structure IR spectra at 4°C, and the wavelength of the amide I band changes with storage conditions (Figures 1 and 2). At 4°C and 10°C, the peak shape of the secondary structure IR spectra on day 1 of the storage is flatter than that of the secondary structure IR spectra on days 10, 20, and 40; with storage time extended, the peak shape of the amide I band on the day is 30. The amide I band shifts from maximum

Table 2: Relative percentages of ovalbumin secondary structure in eggs under different storage conditions.

Items	1 day	10 day	20 day	30 day	40 day
β-Sheet					
4°C	47.95 ± 0.67^{a}	45.76 ± 0.55^{bA}	36.46 ± 0.54^{c}	30.50 ± 0.65^{eB}	35.15 ± 0.49^{dA}
10°C	47.95 ± 0.67^{a}	44.54 ± 0.53^{bB}	37.26 ± 0.52^{c}	32.93 ± 0.73^{dA}	26.73 ± 0.33^{eB}
Unordered					
4°C	0.00 ± 0.00^{e}	2.62 ± 0.22^{dB}	14.60 ± 0.96^{cA}	38.19 ± 0.58^{aA}	25.18 ± 0.74^{bB}
10°C	0.00 ± 0.00^{e}	$9.90 \pm 0.00d^{A}$	13.07 ± 0.12^{cB}	15.85 ± 0.16^{bB}	26.59 ± 0.25^{aA}
α-Helix					
4°C	25.51 ± 0.92^{a}	20.31 ± 0.12^{bA}	20.34 ± 0.27^{bA}	14.56 ± 0.19^{cB}	11.17 ± 0.22^{dB}
10°C	25.51 ± 0.92^{a}	19.08 ± 0.51^{bB}	17.27 ± 0.35^{cB}	16.08 ± 0.37^{dA}	12.76 ± 0.18^{eA}
β-Turn					
4°C	26.54 ± 0.19^{d}	31.31 ± 0.42^{bA}	28.61 ± 0.92^{cB}	35.13 ± 0.14^{a}	28.50 ± 0.55^{cB}
10°C	26.54 ± 0.19^{d}	26.49 ± 0.90^{dB}	32.39 ± 0.87^{cA}	35.13 ± 0.37^{a}	33.90 ± 0.59^{bA}

Note: data are expressed as Means ± standard deviations of triplicate measurements. $^{a\text{-}c}$Means in the same row with different lowercase letters are significantly different ($p < 0.05$). $^{A\text{-}B}$Means in the same column with different capital letters are significantly different ($p < 0.05$).

absorption at long wavelengths to long wavelengths on day 30, and from maximum absorption at long wavelengths to short wavelengths on days 20 and 40.

7.1. Effect of Different Storage Conditions on the Molecular Structure of Egg Proteins. Based on the results of previous studies [24], the following criteria were used to designate the protein secondary structure for the amide I band: β-sheet is 1610 to 1640 cm^{-1}, the random coil is 1641 to 1650 cm^{-1}, α-helix is 1651 to 1660 cm^{-1}, and β-turn is 1661 to 1700 cm^{-1}.

Storage of fresh eggs at 4°C and 10°C, the β-sheet and α-helix contents of the egg albumin secondary structure were significantly reduced on day 40, and random coil and β-turn content was significantly increased ($p < 0.05$,Table 2, Figure 3). It indicated that the molecular conformation of the protein secondary structure of eggs gradually changed from this point onwards. The freshness of eggs became less and less, and the proteins began to denature, culminating in an aqueous dilution of the proteins. Increased content of random coils and β-turn and decreased content of α-helix in the protein secondary structure of egg albumin when stored at 10°C (p <0.05,Table 2).

7.2. Correlation between Egg Quality and the Molecular Structure of Egg Albumin. The β-sheet and α-helix in egg whites showed a significant positive correlation with egg weight, protein height, and Haugh unit (Figure 4). Random coil showed a significant negative correlation with egg weight, protein height, and Haugh unit (Figure 4). It probably results from a conformational change in the structure of the protein molecule, which in turn affected the Haugh unit. This suggests that the FTIR-based study of the molecular structure of egg white proteins can be a useful method to evaluate egg quality.

8. Discussions

The secondary structure of a protein does not need to consider the spatial configuration of the whole peptide chain and the stereo structure of the side chains, but the local spatial arrangement of the backbone of the polypeptide chain. Common secondary structures include regular structures such as β-sheet and α-helix; semiregular structures, such as β-turns; and random coils [25]. The various types of secondary structures are mainly supported by hydrogen bonds between oxygen and hydrogen atoms. Natural proteins were subjected to a protein denaturation process in which solubility was reduced and biological activity was lost when they were subjected to external environmental influences. During this process, the protein secondary structure was also changed, and small changes within the protein secondary structure can be precisely observed using FTIR spectroscopy [26].

Using a Gaussian fit to Fourier raw infrared spectra, Shengnan Wei [5] found that the α-helix and β-sheet content of eggs decreased with increasing storage temperature, and the β-turn and random coil showed an increasing trend. In this study, a Gaussian function fitting method was used to fit the deconvoluted spectra to compare the changes in protein secondary structure during storage at 4°C and 10°C, respectively. The results showed that the content of α-helix and β-sheet in the protein secondary structure gradually decreased with increasing storage time, while the content of the content and β-turn gradually increased, and the findings are consistent with those of previous authors [27, 28]. As a result, the molecular structure of egg proteins changes in response to storage conditions, and FTIR-based techniques can provide a clearer picture of the exact changes that occur.

The α-helix is an ordered sequence in the secondary structure of a protein and is maintained by hydrogen bonds between protein molecules. The reduction in the α-helix content of the secondary structure of egg proteins at elevated

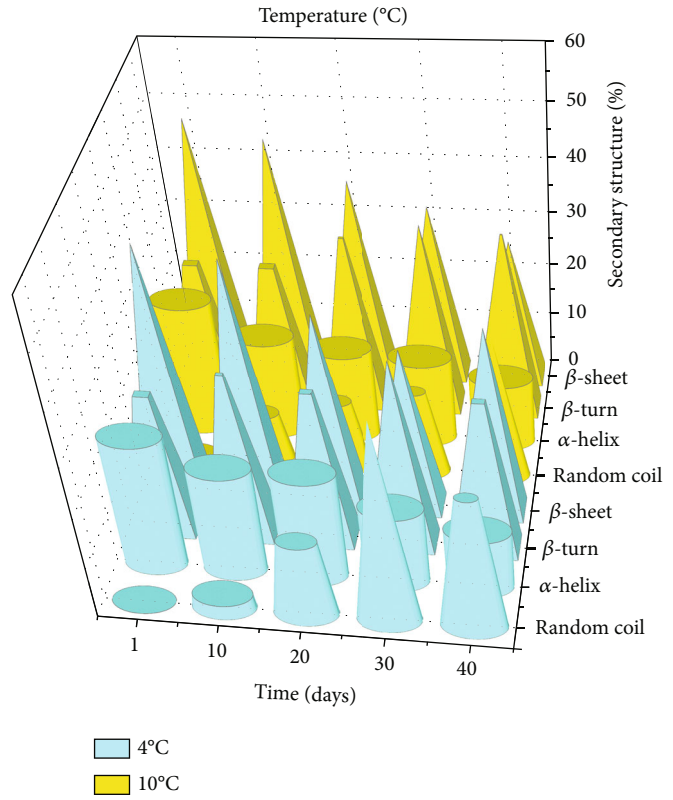

Figure 3: Changes in the secondary structure of egg albumin under different storage conditions.

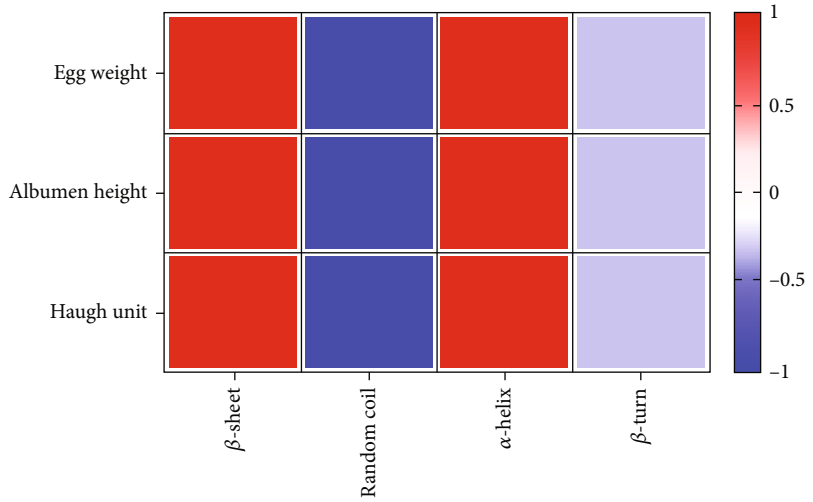

Figure 4: Correlation between egg quality and the molecular. The color represents a significant correlation ($p < 0.05$). Red represents a significant positive correlation, and blue represents a significantly negative correlation ($n = 6$).

temperatures indicates a weakening of the hydrogen bonds and a gradual unfolding of the protein molecules. The decrease in α-helix content with increasing storage temperature and time in the test is therefore due to the weakening of intermolecular hydrogen bonding in egg albumin as a result of the high temperature over time [27–29].

The decrease in β-sheet content with increasing storage temperature and time in this study is due to the denaturation of the protein caused by the increase in temperature and the formation of a new antiparallel β-anti-sheet structure containing molecular hydrogen bonds in the denatured protein structure, which is completely different from the β-sheet of the natural protein, so the two are inversely proportional, and this test result has been confirmed in many protein thermal denaturation experiments [30–32].

In addition, the content of the random coil and the β-turn angle in this study gradually increased with increasing storage temperature and time, suggesting that during storage, the protein secondary structure changes from a regular and ordered structure to a disordered structure [33]. The

protein denaturation of egg albumin increases and the quality of eggs decreases at higher temperatures and longer storage times, and eggs stored at higher temperatures are more prone to The eggs were more likely to deteriorate at higher temperatures than at lower temperatures. Spielman's correlation analysis also revealed that the β-sheet and α-helix structures in egg white were significantly positively correlated with egg weight, protein height, and Haugh unit, and negatively correlated with the random coil, indicating that the use of FTIR combined with second-order derivatives, deconvolution, and curve fitting was effective in evaluating egg freshness.

9. Conclusion

Better preservation of egg quality was at low temperatures and during short-term storage. The β-sheet and α-helix structures in the molecular structure of the egg were significantly positively correlated with egg weight, protein height, and Haugh unit, and negatively correlated with the irregularly convoluted structure. Therefore, curve fitting of deconvoluted spectra based on Fourier transform infrared spectroscopy was a valid and feasible method to evaluate egg freshness.

Data Availability

Data is available on request from the authors. The datasets generated during the experiment that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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