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A study of storage impact on ovalbumin structure of chicken egg

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ABSTRACT

During the egg storage, the change in structure of ovalbumin (OVA) was determined by spectroscopy of Fluorescence, Circular Dichroism and Fourier-Transform Raman. The relationships between the OVA properties and structure were also investigated in this study. Comparing with the consequence of Circular Dichroism and Fourier-Transform Raman spectra, it was obvious that the percentage of α-helix and β-sheet showed downward trends after storage. In contrast, the percentage of β-turn and random coil clearly presented raising trend. Furthermore, tryptophan residues were buried in a more hydrophobic environment. The emulsifying and foaming properties of OVA all decreased during the storage. Moreover, the correlation analysis indicated that β-sheet related to the emulsifying stability of OVA ($p < 0.01$), α-helix related to the foaming stability ($p < 0.05$) at the same time. This study provided new interesting idea for the study on the relationships between the structure and functional properties of protein during food storage.

1. Introduction

Ovalbumin (OVA), which constitutes about 55% of the total proteins in egg white, is mainly abundant protein component in avian egg white (Zhao et al., 2016). Therefore, OVA contributes to a large part of the functionality of egg white such as emulsibility, foamability and gelation, because of its quite exceptional thermal and surface properties (Croguennec et al., 2007). OVA is a typical globular protein with a diameter of 3 nm, whose molecular weight is approximately 45 kDa (containing 385 amino acid residues) and isoelectric point (pI) is 4.5 (Huntington and Stein, 2001). OVA is cross-linked by one disulfide bond and has four free sulfhydryl groups and the protein molecule has a single carbohydrate moiety bonded to Asn-292 (Iwashita et al., 2017). The protein containing the four cysteins and one cystine have been purified and sequenced, and it appears that Cys-73 and Cys-120 are apparently involved in the disulfide bond (Batra et al., 1989a). Investigations of the molecular properties have revealed that the protein exists in a compact and globular conformation (Savadkoohi et al., 2016). It was previously reported that native OVA contained 33% α-helix, 5% β-sheet, and 62% random coil (unordered non repetitive structure) (Batra et al., 1989b).

Mine et al. (1991) had discussed the influence factor of emulsifying property. They found that the emulsifying property of OVA was dependent on pH, concentration of protein dispersion, oil-phase volume, and presence of salts. In addition, Alleoni and Antunes (2004) made a research on the influential factor to foaming property of OVA. It suggested that the foaming capability of OVA could be affected by protein concentration, ionic strength, composition of the liquid phase, temperature and pH. However, accurate information about changes in OVA's structure and properties during egg storage is quantitatively limited. The characteristic of egg white would be significantly changed during the storage, on account of the physicochemical changes of egg white take place along with the lengthening of the storage period (Kato et al., 1979; Omana et al., 2011). As one of the most important components in egg white, it is necessary to know the changing of OVA's structure changes during the storage period and the effects to the protein properties. These researches would supply more information for egg products from different storage periods to apply in food industry.

In this research, the structure change of OVA was evaluated by Fluorescence spectroscopy, Circular Dichroism (CD) spectroscopy and Fourier-Transform (FT) Raman spectroscopy during the storage period. Meanwhile, the protein important function such as emulsifying and foaming properties at different stages were investigated. The relationship between the protein function and

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secondary structures of OVA was also analyzed by bivariate correlation analysis.

2. Materials and methods

2.1. Preparation

Fresh chicken eggs (60 ± 0.5 g, average weight) from Hy-line variety Brown hens laid not over 24 h were collected from the Poultry Research Centre farm of Huazhong Agricultural University. Three hundreds of eggs were divided in to 3 groups randomly and stored at 22 °C and 65% relative humidity. Each group of 10 eggs was used to determinate haugh unit and extract OVA every 10 days. Each of lyophilized OVA powder was used for structure and properties analysis. Fresh eggs (zero days) were used as control.

2.2. Measurement of haugh unit (HU)

HU was calculated by the following Formula:

$$HU = 100 \times \log(H - 1.7\ W^{0.37} + 7.6) \quad (1)$$

where H is the height of the thick albumen in millimeters. The parameter H was estimated by averaging three measurements carried out in different points of thick albumen at the distance of 10 mm from the yolk using a digital caliper (Caner, 2005). The weight (W) was measured by a balance and five eggs chose every 10 days to measure the average HU (Wardy et al., 2010). The experiment was performed three times and was reported as the mean ± standard deviation.

2.3. Extraction of OVA

OVA was purified by the following two steps: (1) Egg whites were well mixed with 3 vol of 50 mM NaCl solution by stirring for 2 h. The pH of the solution was adjusted to 6.0 with 2 M HCl, and PEG-8000 was added while stirring (final w w^{-1} = 10%). The dispersion was allowed to settle for 2 h, followed by centrifuging the homogenate at 15,000 *g* at 4 °C for 10 min and collecting the supernatant. (2)The supernatant was applied to a Q Sepharose Fast Flow column (60 mm × 16 mm), Automatic low pressure liquid chromatography system (JiaPeng Technology Co., Ltd., Shanghai, China). The flow-through fraction was eluted using Tris−HCl buffer (pH 8.0, 20 mM), followed by isocratic elution using 20 mM Tris−HCl buffer (pH 8.0) successively containing 0.08, 0.18 and 0.30 M NaCl, at a flow rate of 2 mL/min. The peak of 20 mM Tris−HCl buffer (pH 8.0) containing 0.18 M NaCl was pooled and dialyzed against distilled water for 48 h and then lyophilized (Geng et al., 2012). Approximately 28 g OVA was obtained from 10 eggs. The purity of OVA obtained from anion-exchange chromatography was measured to be 97.2% by reverse phase HPLC.

2.4. Fluorescence spectroscopy

Fluorescence measurements were obtained using an IF-5401 spectrofluorimeter (Hitachi, Japan) equipped with a xenon lamp source and a 1.0 cm quartz cell. Each sample was dissolved in 10 mM phosphate buffer (pH 7.2) at a protein concentration of 10 μg mL^{-1}. The fluorescence spectra of the solutions were scanned at emissions from 290 to 450 nm excited at a wavelength of 280 nm. The widths of both excitation slit and emission slit were set at 5 nm.

2.5. CD spectroscopy

CD spectra were determined on a Jasco J-810 spectropolarimeter (Jasco, Japan). Each sample was dissolved in 10 mM phosphate buffer (pH 7.2) and OVA concentration in the solution was adjusted to 0.2 mg mL^{-1}. The sample solutions were scanned from 190 to 250 nm using a cell with a 1.0 mm path length. Each spectrum was obtained as an average of three scans to reduce noise before structure analysis was performed.

2.6. FT Raman spectroscopy

FT Raman spectra were recorded on an INVIA laser Raman spectrometer (Renishaw, UK) at room temperature. Spectral resolution was set at 4 cm^{-1}, laser wavelength at 785 nm, grating density at 600 grades cm^{-1}, laser power at 25 mW, slit width at 200 μm, exposure time at 60 s. Spectra were obtained in the Raman shift range between 400 and 4000 cm^{-1}. The original spectra were baselined and the intensity was normalized using the phenylalanine peak at 1006 cm^{-1}. Gaussian was selected as input parameters for a least-squares curve-fitting procedure.

2.7. Measurement of emulsifying property

The emulsifying properties were determined by the Pearce and Kinsella method (Pearce and Kinsella, 1978). To prepare the emulsion, 40 mL of OVA (1 mg mL^{-1}) was mixed with 10 mL of corn oil. The mixture was homogenized with a high-speed centrifuge at 10000 rpm for 1 min at 25 °C. Emulsion sample of 25 μL was taken from the bottom of the container diluted with 5 mL of 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted emulsion was measured at 500 nm. The emulsifying activity (EAI) was determined by the absorbance measured immediately after emulsion formation, and the emulsion stability (ESI) was estimated by measuring the half-life (seconds) of the turbidity of the emulsion.

2.8. Measurement of foaming property

The foaming properties were determined by the Hammersh and Qvist method (Hammersh and Qvist, 2001). Foams were produced of 20 mL OVA (1 mg mL^{-1}) by shaking at a frequency of 3 Hz for 25 s in closed 100 mL cylinders, the foaming activity (FA) and foam stability (FS) were recorded:

$$FA = VB / VL \times 100 \quad (2)$$

$$FS = VB_1 / VL \times 100 \quad (3)$$

VB represents foam volume after homogenization; VL represents the liquid volume before homogenization; VB_1 represents the foam volume standing after 30 min.

2.9. Statistics analysis

All experiments were performed three times and were reported as the mean ± standard deviation. A bivariate correlation analysis of the linkage between OVA's secondary structure and functional properties was implemented through a software named statistical product and service solutions (SPSS) program. Pearson correlation coefficient was performed for correlation analysis, variance analysis for comparison of the inter group, Least Significant Difference (LSD) method for multiple comparisons, and $p < 0.05$ considered as significance.