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# Investigation of conformation change of glycated ovalbumin obtained by Co-60 gamma-ray irradiation under drying treatment

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ABSTRACT

Irradiation can significantly improve the incorporation of glucose into protein. In this study, the efficacy on conformation change and glycation extent of ovalbumin (OVA) powder was investigated during Co-60 irradiation treatment. Spectrophotometer was used to monitor the conformation and structure change of glycation OVA under various irradiation doses condition. A dose dependent increase in UV absorbance and development of fluorescence was observed. Free amino groups content showed that up to 5 kGy irradiation could accelerate the glycation reaction between OVA and glucose, while there were inapparent differences among the other various doses. Intrinsic, synchronous and 3D fluorescence spectra showed that the glycation reaction caused the dynamic quenching of OVA and changed the polarity of the hydrophobic microenvironment (Trp and Tyr residues) of OVA with unfolding conformation change.

## 1. Introduction

Irradiation, as a powerful cold pasteurization technology has been applied to food sterilization, food packaging, and medical products (Lee, Lee, & Song, 2003). Researchers have reported that irradiation could enhance shelf life and improve the microbiological safety of raw and processed food materials without compromising nutritional quality. Compared with traditional food processing, irradiation could prevent the deterioration of food flavor (Jin et al., 2017). However, hydroxyl and superoxide, which were generated during irradiation, could induce chemical changes in proteins, such as fragmentation, cross-linking, aggregation, and oxidation (Lee et al., 2003). Therefore, the majority of chemical changes during food irradiation processing is directly related to the structure change and chemical reactions, which were mainly caused by the electron beams and free radicals derived from radiolysis of water (Zanardi, Caligiani, & Novelli, 2017).

One of the most thoroughly studies have been shown that chemical reactions among the food components were the Maillard reaction, which was a non-enzymatic glycation initiated by the carbonyl group of reducing sugar reacting with amino group in protein (Milkovska-Stamenova & Hoffmann, 2017). The products of Maillard reaction have been extensively studied in recent years with regard to their effects on the physicochemical properties, digestibility, bioavailability, and concentration of the essential amino acids of proteins (Milkovska-Stamenova & Hoffmann, 2016; Zhong, Tu, Liu, Luo, & Liu, 2015). Therefore, when the processing treatment is harsh, the number of glycation sites is considered an indication of the glycation degree and the relative quantities of targeted glycation sites (Birlouez-Aragon et al., 2004; Guan et al., 2010; Huang et al., 2013). Previous studies had suggested that ionizing irradiation could successfully applied to produce Maillard reaction products (MRPs) in whey protein suspension, which was similar to the heat-induced Maillard reaction (Chawla, Chander, & Sharma, 2009). Irradiation is a novel tool that can improve the Maillard reaction of proteins and form protein conjugates with novel functional properties, which are initiated via irradiation and the free radicals generated by the radiolysis of water (Zarei, Bahreinipour, Eskandari, MousaviZarandi, & Ardestani, 2017). However, further studies are necessary to elucidate the mechanism behind this phenomenon. In particular, several studies have been conducted in suspension, where a large number of free radicals exist due to the radiolysis of water (Gaber, 2005). Therefore, the mechanism of the Maillard reaction in suspension under irradiation is more complex than powder directly irradiated due to the greater effect of oxygen radicals on molecular structures. To the best of our knowledge, no study has yet monitored the conformation change of dry protein under various irradiation doses.

Ovalbumin (OVA) is the predominant protein in egg white and

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comprises approximately 54% of its total protein. As a thermally and chemically stable protein, OVA is one of only two pure proteins that can adequately meet the nutritional requirements for amino acids (Huang et al., 2013). OVA also is an important food ingredient with high functionality, including foaming stability and gelling ability. Mine (Mine, 1995; Mine, Noutomi, & Haga, 1991) published a series of papers on the functionality, structure, and nutritional value of OVA. Li (Li, Enomoto, Hayashi, Zhao, & Aoki, 2010) performed several studies on the phosphorylation of OVA to explore its emulsifying ability (Lv & Chi, 2012), foaming ability (Li et al., 2009) and thermal stability (Li et al., 2005). Therefore, chemical reaction among the components of a food system is an important factor that affects the functionality and nutrition of food products, which require further research in the food processing field. Radiation processing could improve the formation of Maillard reaction products, which exhibit antioxidant activity in a solution (Chawla et al., 2009). However, minimal information regarding to the effects of Maillard reaction products from the mixture powder of OVA and glucose on the structure and conformation during irradiation has been reported.

In this work, to characterize the structure change and the glycation reaction information, we performed the experiment in a stepwise fashion: first, irradiation was employed to treat the mixture powder of OVA and D-glucose; then, spectrometry was used to elucidate the conformation and structure change of the Maillard reaction products under irradiation processing. The results of this research will enhance our understanding of the influence of Co-60 irradiation treatment on Maillard reaction, as well as providing information for application of Co-60 irradiation in the food industry.

## 2. Materials and methods

### 2.1. Materials

OVA (Grade V, A-5503) and D-glucose were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were analytical grade. Ultrapure water from water purification system (Millipore, Bedford, MA, USA) was used throughout this study.

### 2.2. Sample irradiation

OVA (18 g) and an equal mass of D-glucose were dissolved in 180 mL distilled water and split into 18 aliquots in centrifuge tubes. Then, each aliquot was dried using a LGJ freeze-dryer (Yataikerong Scientific; Beijing, China) at −80 °C for 48 h. The moisture content of the mixture was 2.60%. The dried sample mixtures were irradiated at room temperature using a Co-60 gamma-ray irradiation source, which was provided by a commercial irradiator (Jiangxi keyuan irradiation sic & tech, Co., Nanchang, China). The dose of irradiation was controlled from 0 to 25 kGy (0, 5, 10, 15, 20, and 25 kGy) at a dose rate of 0.5 kGy/h with duration of 0, 10, 20, 30, 40, 50 h, respectively. The OVA with 0 kGy irradiation treatment was selected as the control. Three repetitions were performed for each sample.

Each sample in the tube was dissolved in 10 mL distilled water and filtered with a centrifugal filter unit (3000 Da cutoff, Merck Millipore Ltd., Darmstadt, Germany) to remove salts and free glucose. The concentration of protein was adjusted to 100 mg/mL for subsequent assays.

### 2.3. Determination of free amino groups

The glycation content was evaluated by measuring the free amino groups using the ortho-phthalaldehyde (OPA) method (Fayle et al., 2001). The OPA reagent was freshly prepared and protocol as follows: firstly, 4 mg OPA was dissolved in 1 mL of methanol and then mixed with 100 μL 2-mercaptoethanol, 25 mL 0.1 M sodium borate, 2.5 mL of 20% (w/v) sodium dodecyl sulfate (SDS). Finally, the volume was adjusted to 50 mL using distilled water. The sample solution contained 50 μg protein was mixed with the OPA reagent and incubated for 2 min at 37 °C. Absorption was measured at 340 nm against a blank that contained the OPA reagent. Working standards were prepared through the serial dilution of leucine to final concentrations ranging from 0 mg/mL to 0.4 mg/mL. Three repetitions were performed for each sample.

### 2.4. Surface hydrophobicity

1-Anilinonaphthalene-8-sulfonate (ANS) was fluorescent when it is associated with hydrophobic residues/groups, and thus, it is used to determine hydrophobic domains in proteins (Xiang, Ngadi, Ochoa-Martinez, & Simpson, 2011). In the present work, samples were diluted to 1, 0.5, and 0.25 mg/mL using phosphate-buffered saline (PBS; 10 mM, pH 7.4), respectively. Then, 4 mL of the samples were mixed with 20 μL bis-ANS with 8 μM. Fluorescence emission scan was obtained from 400 nm to 600 nm, with excitation at 370 nm using a Hitachi fluorophotometer (F-7000, Hitachi, Tokyo, Japan). The surface hydrophobicity of the sample was defined as the ratio of the fluorescence absorption to different concentrations.

### 2.5. SDS-page

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by following the method of Laemmli (Laemmli, 1970). Samples were diluted in a loading buffer [pH 6.8, 60 mM Tris-HCl, 2% (w/v) SDS, 10% (v/v) glycerol, and 0.025% (w/v) bromophenol blue]. The sample solutions were loaded onto 12% polyacrylamide gel that contained 0.1% SDS. Electrophoresis was performed in a running buffer (which contained 0.025 M Tris-HCl, 0.192 M glycine, and 0.1% SDS) with currents of 15–35 mA. Gels were stained with Coomassie Brilliant Blue R250 and then destained in a mixture of 5% ethanol and 7.5% acetic acid.

### 2.6. UV absorption spectroscopy

The ultraviolet (UV) absorption spectra of the samples were recorded on a UV/Vis array spectrophotometer (U-2910, Hitachi, Tokyo, Japan) using a quartz cuvette with a concentration of 1 mg/mL in PBS (10 mM, pH 7.4). The UV absorption spectra were recorded and scanned from 240 nm to 400 nm (Hu et al., 2011).

### 2.7. Fluorescence spectroscopy

Tryptophan (Trp) fluorometric determination, synchronous fluorescence analyses, and 3D fluorescence analyses were performed using a Hitachi spectra fluorophotometer (F-7000, Hitachi, Tokyo, Japan) with a protein concentration of 0.1 mg/mL in PBS (10 mM, pH 7.4). The excitation wavelength was 280 nm. The emission spectrum was scanned from 300 nm to 450 nm using a bandwidth of 5 nm at a scan speed of 1200 nm/min. The synchronous fluorescence spectra were recorded within the scanning ranges, and the wavelength interval was fixed individually at $\Delta_\lambda$ = 15 nm and 60 nm. Then, the 3D fluorescence spectra of proteins were recorded. The scanning range of the emission wavelength was between 200 nm and 450 nm and that of the excitation wavelength was between 200 nm and 450 nm at 10 nm increments.

### 2.8. Data analysis

The data are expressed as the mean ± standard deviation. The analysis was performed using Origin-Pro 8.0 (OriginLab Corp., Northampton, MA). The value of DSP ± standard deviation was determined from three separate experiments. Statistical data were determined based on a two-tailed *t*-test using standard deviation.