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Ultrahigh pressure promotes colorless bovine serum albumin-glucose conjugates generation

Wencheng Wang *, Liqing Huang

Department of Food and Biology Engineering, Zhangzhou Institute of Technology, Zhangzhou, People's Republic of China

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ABSTRACT

Browning generation always limits the application of protein-carbohydrate conjugates. In the present study, we found that ultrahigh pressure can promote bovine serum albumin (BSA)-glucose conjugation without browning generation. BSA-glucose conjugates were fabricated at 120, 240 and 360 MPa for 4 h. The intermediate products, browning intensity, glucose concentration and secondary structure of conjugates were tested. Results suggested that BSA-glucose conjugates were generated at pressures of 240 and 360 MPa with significant increase of absorbance at 294 nm (Abs294) from 0.88 to 0.93 and 1.17, and marked consumption of glucose to 87 and 85%. While, insignificant change was found in browning (Abs420) or the secondary structure of BSA during conjugates formation with 40–42% α-helix, 5–10% β-sheet and 21–23% β-turns. Near no BSA-glucose conjugates were formed at lower pressure of 120 MPa. This study indicated that ultrahigh pressure is a potential method to generate colorless BSA-glucose conjugates.

Industrial Relevance

The color generation is a serious problem limiting the industrial application of protein-carbohydrate conjugates. There is nearly no report about inhibiting the color formation during fabricating protein-carbohydrate conjugates. Our study suggested an effective method to fabricate colorless BSA-glucose conjugates, which may provide potential improvement in food processing.

1. Introduction

The Maillard-type conjugation, as one of the most important carbohydrate chemical reactions in food processing, has been used to fabricate protein-reducing sugar conjugates (Mulcahy, Park, Drake, Mulvihill, & O'Mahony, 2016; Nooshkam & Madadlou, 2016a, 2016b). During this reaction, reducing sugar can react with amino groups of protein, and generate stable Amadori rearrangement product (Kaufmann, Meissner, Pelke, Mügge, & Kroh, 2016), which is the primary basic composition of protein-reducing sugar conjugates. However, many recent researchers found that it was avoidless to generate browning during protein-reducing sugar conjugates fabrication (de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016; Guan, Chen, Yu, Tang, & Yan, 2014).

High pressure was found to affect the Maillard reaction recently. It was found that high pressure accelerated the initial stage of the Maillard, but inhibited the intermediate and advanced stages via suppressing the degradation of the Amadori rearrangement product, resulting in slight browning in sugar-amino acid or sugar-ammonium salt model reaction systems (Guan et al., 2011; Moreno, Elena, Agustian and Rosina, 2003). Significant inhibition of whey protein-sugar advanced stage products was also found by high pressure high temperature (HPHT) method in recent study (Avila Ruiz et al., 2016).

This objective of the present study was to fabricate colorless BSA-glucose conjugates using ultrahigh pressure method. The second objective was to theoretically investigate the effects of ultrahigh pressure on BSA-glucose conjugation especially on the changes of intermediate products, aggregated products of advanced stage and the secondary structure of conjugates.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), glucose and Coomassie Brillant Blue R-250 were purchased from Sigma-Aldrich (Sigma, MO, USA). Other chemicals were analytical reagents.

* Corresponding author at: Department of Food and Biology Engineering, Zhangzhou Institute of Technology, 1 Maanshan Avenue, Zhangzhou, Fujian 363000, People's Republic of China.
E-mail address: wenchengwang16@gmail.com (W. Wang).

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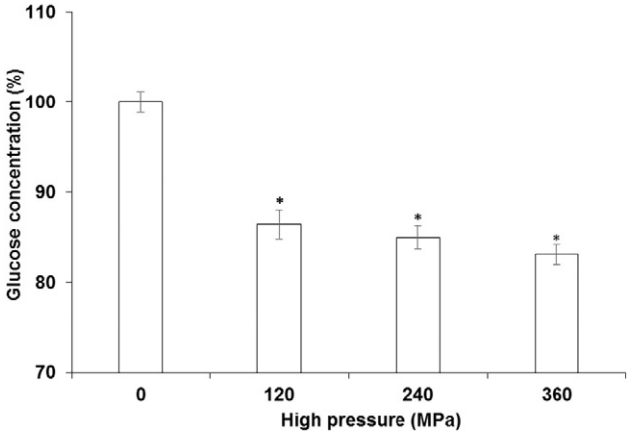

Fig. 1. Changes of glucose concentrations after mixing with BSA, and treated at 0 (control), 120, 240 and 360 MPa for 4 h. The * means significant difference comparing with the control.

2.2. Preparation of Maillard reaction products

Briefly, 100 mg BSA was mixed with 100 mg glucose, and dissolved in 20 mL deionized water (di-water). Each sample solution was hermetical in polyethylene bags for ultrahigh pressure treatment.

A UHPF2750Mpa ultrahigh pressure system (Baotou Kefa high pressure technology LLC, Baotou, China) was used for ultrahigh pressure treatment. Pressure treatment was carried out at 120, 240 and 360 MPa, respectively. The pressure was raised to the objective pressure at a rate of 2.5 MPa/s, with 4-h incubation at 60 °C. Control (0 MPa) were incubated at temperature of 60 °C and air pressure in a water bath for 4 h. All samples were cooled to 4 °C by iced water immediately after ultrahigh pressure treatments, and kept at 4 °C before further analyses.

2.3. Measurement of intermediate products and browning

The intermediate products and browning of BSA-glucose conjugates were measured at 294 and 420 nm by a UV-1810 spectrophotometer (Puxi, Beijing, China).

2.4. Glucose measurement

All the sample solutions were filtered through a Millex-HN nylon clarification kit of 0.45 μm pore size (Millipore, Bedford, MA), and analyzed using an HPLC-Refractive Index Detector (RID) system. The system consisted of a Sugar-pak1 6.5 × 300 mm Ion Exchange Chromatography (Waters Co., Milford, MA), a Waters 600 pump and a Waters 2414 refractive index detector (Waters Co., Milford, MA). The injection volume was 20 μL, and the mobile phase was a 50 mg/L EDTA-Ca water solution at a flow rate of 0.5 mL/min and column temperature of 90 °C.

2.5. Electrophoresis

The SDS-polyacrylamide gel electrophoresis (SDS-PAGE) fabricated by 4% stacking gel and 10% running gel was used for electrophoresis analysis by a Mini-Protein II electrophoresis apparatus (Bio-Rad Laboratories, Richmond, CA). The 5 μL sample was separated by SDS-PAGE at 15 mA. After separation, protein bands were stained by Coomassie Brillant Blue R-250 (0.2%) in 25% methanol and 10% acetic acid. Destaining was carried out using methanol-acetic acid (4:1, *v*/v) mixture.

2.6. Dialysis

Forty millilitres of sample solution was dialyzed by a dialysis tube (33 × 21 mm, width × diameter, 12.4 kDa of molecular weight cutoff) in 1.5 L di-water at 4 °C. The di-water was replaced each 6 h for 3 days. After dialysis, all samples were freeze-dried and stored at 4 °C before circular dichroism (CD) analysis.

2.7. Far ultraviolet (UV)-CD analysis

The secondary structure of BSA after conjugating with glucose was detected by a Jasco J-720 CD spectropolarimeter (Jasco, Easton, MD). The far-UV CD spectra was carried out at a sample concentration of 0.1 mg/mL, and CD spectra were recorded in the wavelength ranges of 190–250 nm at 25 °C in a 0.1-cm path-length cell.

2.8. Statistical analysis

All experiments were carried out at least twice. Means and standard deviations were calculated for each treatment. Analysis of variance

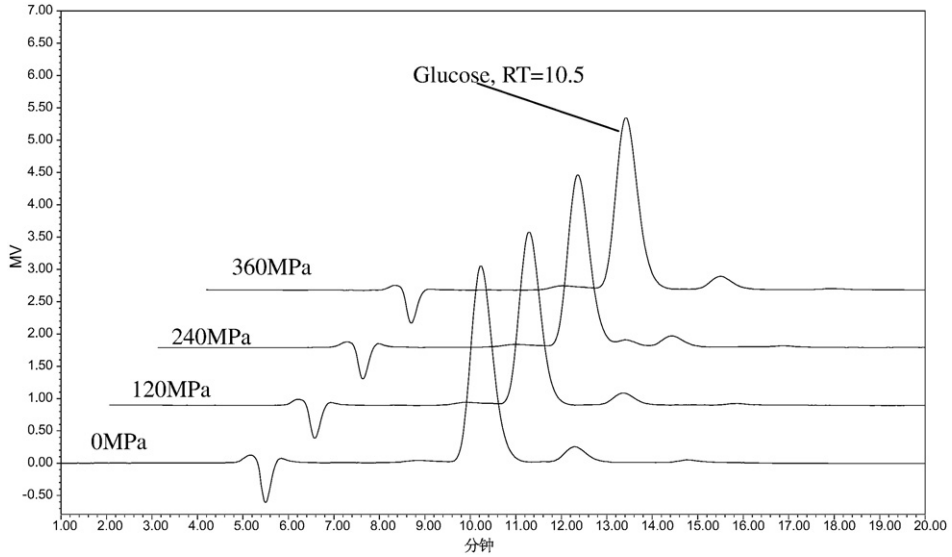

Fig. 2. Changes in the content of glucose (without BSA) as affected by UP treatment.