Food Hydrocolloids 37 (2014) 86–92

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Preparation and characterization of ovalbumin and carboxymethyl cellulose conjugates via glycosylation

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ARTICLE INFO

Article history:
Received 29 July 2013
Accepted 28 October 2013

Keywords:
Ovalbumin
Carboxymethyl cellulose
Glycosylation

ABSTRACT

Protein and polysaccharide complex has aroused increasing interest in food applications. In this study, ovalbumin (OVA) and carboxymethyl cellulose (CMC) conjugates were prepared by glycosylation reaction and characterized by SDS-PAGE, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and circular dichroism (CD) analysis. The degree of grafting (DG) and browning index (OD) increased with reaction time and 8 d was selected as the optimized reaction time to equilibrium the glycosylation and unfavorable browning effect. The weight ratio (OVA: CMC) was optimized at 4:1 and pH was 7 or 8. The glycosylation grafting was proven by SDS-PAGE and FTIR. DSC showed that the thermal stability of OVA was improved after glycosylation reaction, which was positively related with molecular weight and the degree of substitution DS of CMC. From CD spectra, the secondary structure of OVA was affected by the grafted CMC with increasing β-sheet and random coil and decreasing α-helix and β-turns. The information obtained from this study can enrich the theoretical frame of protein and polysaccharide interaction, broaden the possible applications of food ingredients from egg source and polysaccharide, and provide the theory evidence on developing tailored egg powder.

1. Introduction

Proteins and polysaccharides are natural polymers that are widely used as functional ingredients in food, which can not only provide nutrition, but also can greatly affect the food sensory properties and physical chemical properties (McClements, 2006). Protein-polysaccharide complex is of great interests in scientific research and in various biological and industrial applications (Li, Shi, An, & Huang, 2012), which can enhance emulsion, solubility properties and broaden the range of pH values, compared with pure protein (Burova et al., 2007; Sato, Katayama, Sawabe, & Saeki, 2003; Wooster & Augustin, 2007). Proteins always have unique space conformation and their characteristics largely depend on their structure and multiple conformation. Therefore, the structure modification can lead to the enormous change of their properties. Their specific properties might be expressed in a particular conformation. Ovalbumin (OVA) is an important food ingredient with various structural functionalities including emulsifying property and foaming stability (Choi, Kim, Park, & Moon, 2005). Its structure and properties predominantly affect the functional properties of egg white protein (Sun, Hayakawa, & Izumori, 2004). Therefore, studying the physicochemical and structural changes of OVA–saccharide conjugates will help to further understanding of the structure and properties of Maillard reaction products of egg white. Numerous studies about the OVA–saccharide conjugates have been carried out, focusing on the improvement of solubility, heat stability, foaming property, emulsifying property and gelling property (Aoki et al., 1999; Kato, Minaki, & Kobayashi, 1993; Nakamura, Kato, & Kobayashi, 1992; Sánchez-Gimeno, Vercet, & López-Buesa, 2006). Many attempts have been made to convert proteins into useful products with better functional properties, through enzymatic and chemical modification (Al-Hakkak & Al-Hakkak, 2010). Some researchers attempted to synthesize high performance glycosylation-conjugates using different proteins, such as whey protein isolate, sodium caseinate, casein, egg proteins and others, and a wide range of polysaccharides (Akhtar & Dickinson, 2003, 2007; Al-Hakkak & Kavale, 2002; Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005; Fechner, Knoth, Scherze, & Muschiolik, 2007; Ibanoglu & Erçelebi, 2007; Li, Enomoto, Ohki, Ohtomo, & Aoki, 2005; Oliver, Melton, & Stanley, 2006).

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http://dx.doi.org/10.1016/j.foodhyd.2013.10.027

What's more, protein through glycosylation had been succeeded in delineating the pathways involved in archaeal N-glycosylation and post-translational protein modification, helping archaea against in some of the harshest environments on the planet (Eichler, 2013).

The essence of glycosylation reaction is to produce amino aldose or amine ketone sugar through condensation reaction between dissociative amino and reducing sugar for the formation of glycosylation protein (Sinay, 1991). Glycosylation reaction is the initial phase of the Maillard reaction belonging to the protein chemical modification, which does not need any chemical reagent and controlling reaction conditions for the formation of soluble protein-sugar graft copolymer by protein-sugar covalent bonding. Mainly,

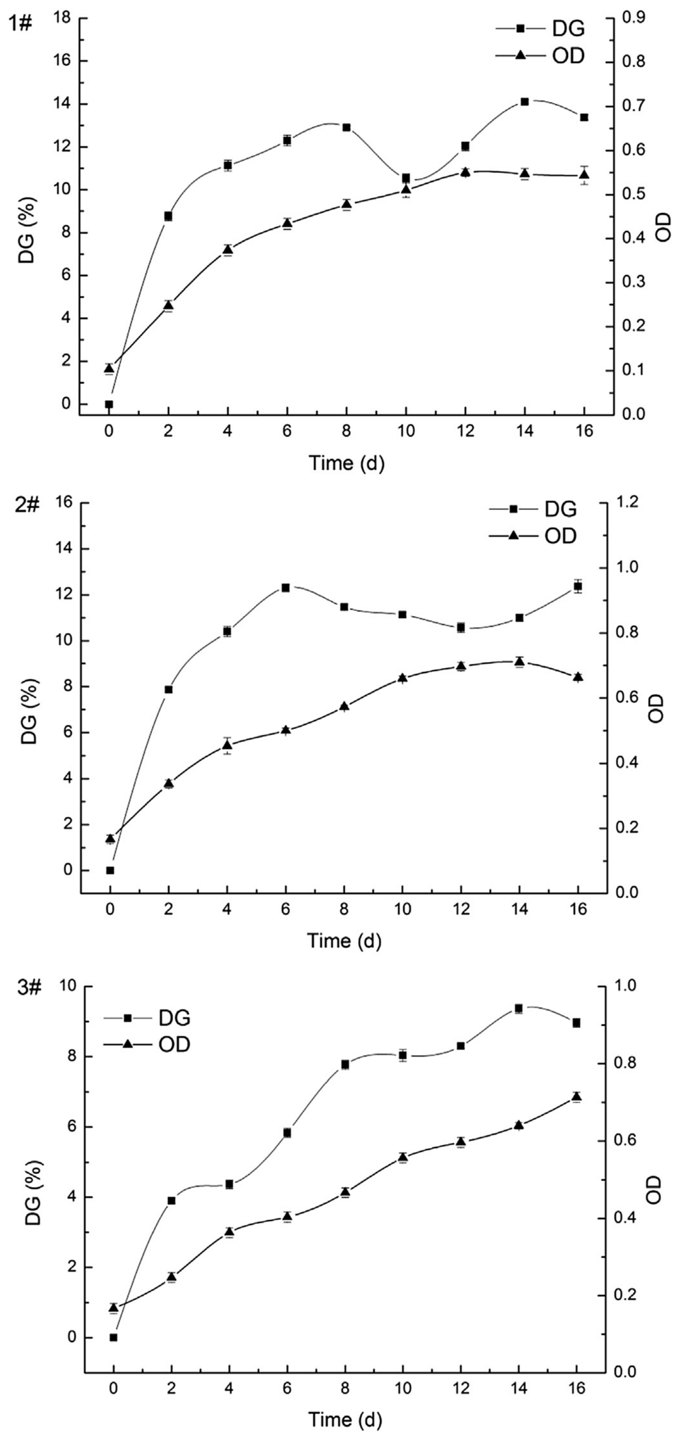

Fig. 1. Effect of incubation time on grafting degree (■) and browning (▲). Three kinds of CMC with different DS (0.69, 0.81, and 0.89) were selected to form CMC-OVA conjugates, which were named as 1#, 2# and 3#, respectively. The weight ratio of OVA-CMC was 1:1 and CMC-OVA conjugates were dissolved in phosphate buffer (pH 7.0). The DG and OD of conjugates were measured after incubation at 60 °C and 79% relative humidity for different incubation time.

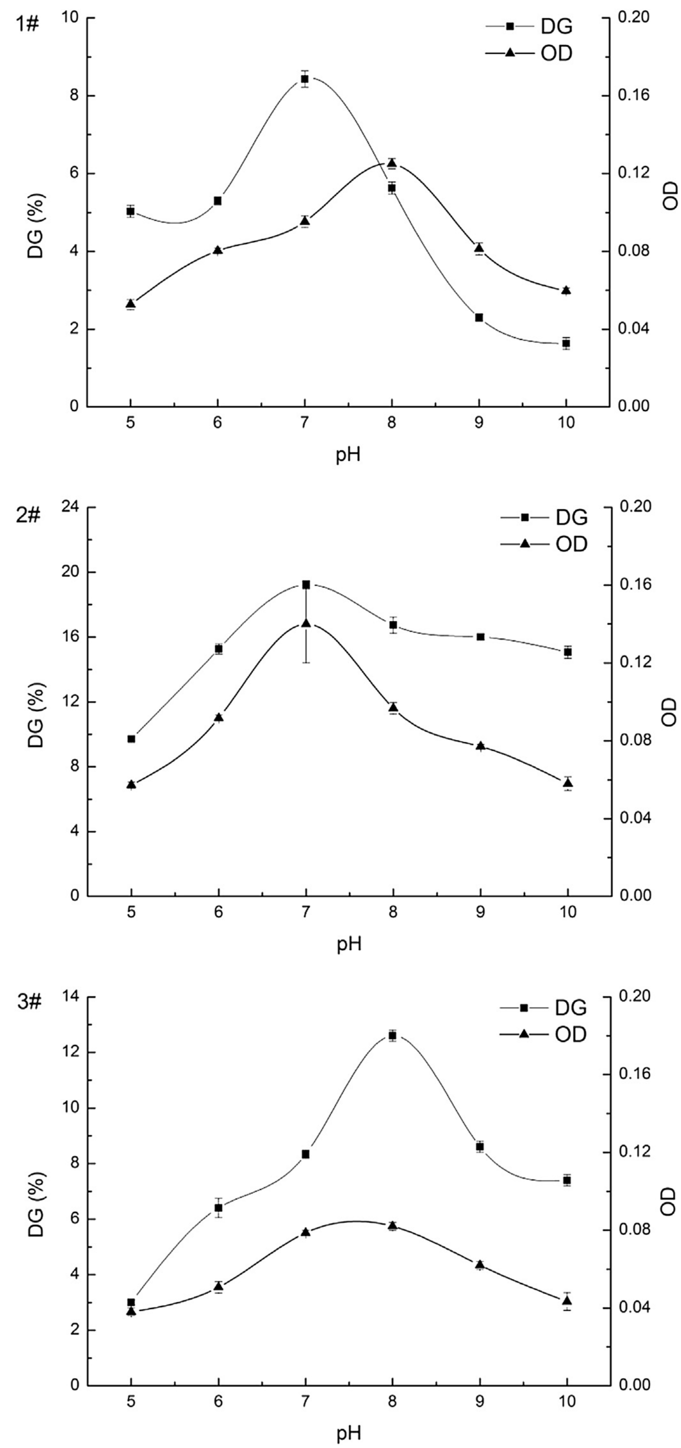

Fig. 2. Effect of different pH value on grafting degree (■) and browning (▲). Three kinds of CMC with different DS (0.69, 0.81, and 0.89) were selected to form CMC-OVA conjugates which were named as 1#, 2# and 3#, respectively, after incubation at 60 °C and 79% relative humidity for 8 d. The weight ratio of OVA-CMC was 1:1 and CMC-OVA conjugates were distilled in different pH phosphate buffers.