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Functional properties of ovalbumin glycosylated with carboxymethyl cellulose of different substitution degree

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ABSTRACT

The glycation of hen ovalbumin (OVA) with carboxymethyl cellulose (CMC) was studied in an attempt to improve its functionality. OVA was dry-heated with CMC with three different degrees of substitution (0.69, 0.81, 0.89) at 60 °C and 79% relative humidity for 7 days. The emulsifying properties of OVA–CMC conjugates were strengthened remarkably with increasing substitution degree of CMC. The foam stability of OVA–CMC conjugates was improved strongly with the extension of incubation time while the foaming ability decreased. The rheological behaviors of all the three grafted products were similar. With the incubation time extension, it took more time for the formation of the protein gel network, and the thermal denaturation temperature of protein became higher. The glycation also produced OVA–CMC conjugates with higher surface hydrophobicity and lower content of lysine and arginine.

1. Introduction

Egg white protein (EW) is not only used as a good source of protein but also extensively utilized as a functional food material in food processing (Kato, Minaki, & Kobayashi, 1993; Matsudomi, Nakano, Soma, & Ochi, 2002). Much research has been conducted in order to improve the functional properties of EW proteins. Experimental data have proved that controlled denaturation of EW proteins can exert a beneficial impact on the applications of OVA in the food industry, through improving its emulsifying ability, heat stability, and gelation behavior (Aoki, Hiidome, Sugimoto, Ibrahim, & Kato, 2001; Campbell, Raikos, & Euston, 2003; Chang & Chen, 2000; Enomoto et al., 2009; Tsubokura, Fukuzaki, Noma, Igura, & Shimoda, 2009). Glycosylation is an effective method to improve the food-use functional properties of albumin. Numerous studies have confirmed that protein–polysaccharide conjugates show better functional ability than the native protein (French & Harper, 2003; Handa & Kuroda, 1999; Mu, Pan, Yao, & Jiang, 2006). OVA is rich in sulfur-containing amino acids, containing 15 methionine residues per molecule with one disulfide bond and four sulfhydryl groups. As the main constituent of EW proteins, OVA strongly affects the application of EW proteins (Nakamura & Kato, 2000; Sun & Hayakawa, 2002; Sun, Hayakawa, & Izumori, 2004). Therefore, improving the functional properties of OVA will help to further the utilization of EW in the industry.

Some research showed that the glycosylation of OVA with other polysaccharides is effective, but not economical, which is bound to limit the application of the OVA and polysaccharides conjugates in food industry. Therefore, the search for an economic and effective modification method is of significance. Carbomethoxy cellulose (CMC), one of the most important derivatives of cellulose, is a classical anionic polysaccharide that has been widely used as a stabilizer in food industry (Toğrul & Arslan, 2004a; Toğrul & Arslan, 2004b). CMC chains are linear β (1 → 4)-linked glucopyranose residues (Su, Huang, Yuan, Wang, & Li, 2010). The average degree of substitution (DS) of CMC is defined as the average number of carboxymethyl groups per repeating unit and is usually in the range of 0.4–1.5. CMC generally exists under the sodium salt form, a water-soluble product for DS > 0.5. A maximum degree of substitution of 1.5 is permitted, but more typically DS is in the range 0.6–0.95 for food applications. Importantly, it is a safe, low-cost anionic polysaccharide (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Diftis and Kiosseoglou grafted soy protein isolate with high, medium and low viscosity of the CMC at a temperature of 60 °C under a dry grafting reaction. The results showed that emulsifying activity of the graft-product is related to the molecular weight of the polysaccharide, the mass ratio of protein and polysaccharide and

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the reaction time (Diftis & Kiosseoglou, 2003). The products of CMC with proteins were also prepared by other means, such as heating treatments and electrosynthesis (Yu, Sabato, D'Aprano, & Lacroix, 2004; Zaleska, Tomasik, & Lii, 2002). Lii et al. prepared the complexes of CMC-OVA in different ratio by electrosynthesis at pH 7, 9, and 11 and characterized the products (Lii, Zaleska, & Tomasik, 2002). However, little information is available for the conjugates of OVA with CMC of different DS prepared by glycation, one of the most typical reactions in food process, which can improve the functional properties of food ingredients and render food with particular quality (Liu, Ru, & Ding, 2012).

In this study, the glycosylation reaction between OVA and CMC was carried out under controlled temperature and relative humidity. CMC with different DS (0.69, 0.81, 0.89) were chosen to study the influence of the CMC with different DS on the glycosylation. The emulsifying property, rheological property and surface hydrophobicity of OVA and CMC conjugates were characterized. The effects of glycosylation reaction on functional properties of conjugates were meanwhile investigated. Moreover, the amino acid composition of grafted OVA was determined.

2. Materials and methods

2.1. Materials

Ovalbumin (OVA, A5253, purity as 62–88%, molecular weight as 45 kDa) was purchased from Sigma. Sodium carboxymethyl cellulose (CMC, viscosity 300–800 mPs and 800–1200 mPs, viscosity average molecular weight 9.72E + 04, 2.35E + 05 and 6.34E + 05) was obtained from Sinopharm Chemical Reagent Co.Ltd. CMC FH6 was acquired from local market. CMC were purified by alcohol precipitation before use and their DS were 0.69, 0.81 and 0.89 respectively. Corn oil was purchased from local market and used without further purified. All other chemicals were of analytical grade.

2.2. Preparation of OVA–CMC sodium conjugation

OVA (1.0 g) was dissolved in 100 mL phosphate buffer (0.05 mM, pH7.0). CMC powder (0.25 g) was added into OVA solution, mixed uniformly and then the mixture was freeze-dried. The lyophilized mixture was spread over a dryer with potassium bromide-saturated solutions at the bottom and incubated at 60 °C at 79% relative humidity. The samples were collected at 0, 2, 4, 6, 8, 10, 12 and 14 days. The resultant Maillard products (OVA–CMC conjugates) were kept at −20 °C before use. The CMC-OVA conjugates with DS of CMC 0.69, 0.81 and 0.89 were named as 1#, 2# and 3#, respectively.

2.3. Measurement of emulsifying properties

The emulsifying properties were determined by the method of Pearce and Kinsella (1978). The emulsion was prepared by homogenizing 10 ml of corn oil in 30 ml 0.1% w/v sample aqueous solution, using a homogenizer (T25, IKA) at 6000 rpm for 1 min at

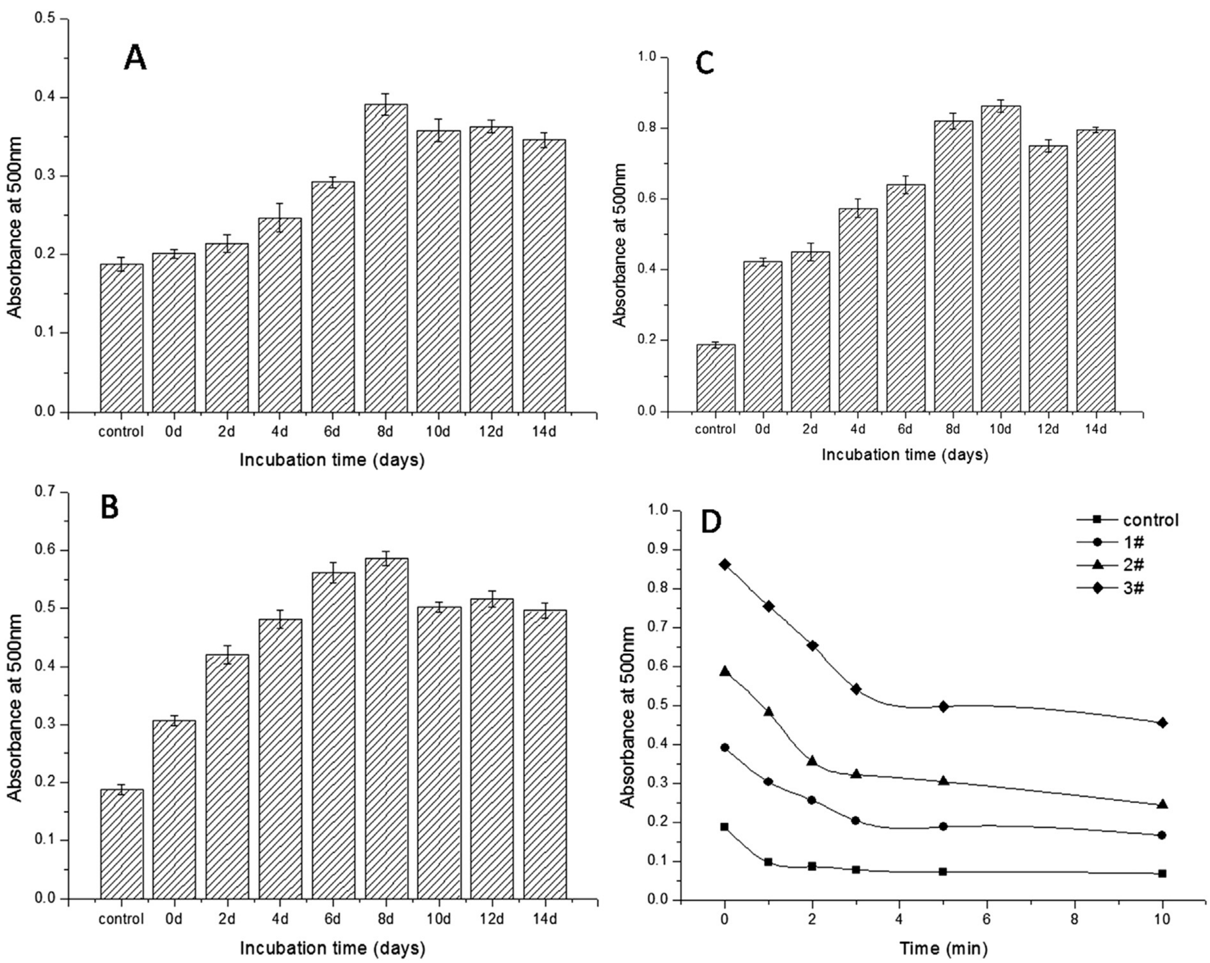

Fig. 1. Emulsifying activity of OVA and the conjugates of OVA–CMC at different incubation time in 0.1 M phosphate buffer, pH 7.4, in oil in water emulsion (O/W = 1/3); 0.1% OVA or conjugates of OVA with CMC with DS 0.69 (A), 0.81 (B) and 0.89 (C) conjugates and the emulsion stability of OVA and OVA–CMC conjugates in above emulsion (D).