Carbohydrate Polymers 109 (2014) 64–70

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Phase behavior of ovalbumin and carboxymethylcellulose composite system

Wei Jia[a,b,1], Bing Cui[a,b,1], Ting Ye[a,b], Liufeng Lin[a,b], Hao Zheng[a,b], Xiangxing Yan[a,b], Yan Li[a,b], Ling Wang[a,b], Shilin Liu[a,b], Bin Li[a,b,*]

[a] *Key Laboratory of Environment Correlative Dietology, Huazhong Agricultural University, Ministry of Education, China*
[b] *College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China*

ARTICLE INFO

Article history:
Received 13 November 2013
Received in revised form 3 March 2014
Accepted 11 March 2014
Available online 24 March 2014

Keywords:
Ovalbumin
Sodium carboxymethylcellulose
Phase behavior
Protein–polysaccharide interactions

ABSTRACT

The phase behavior, rheological properties and microstructure of ovalbumin and carboxymethylcellulose (OVA–CMC) conjugates were studied and the influence parameters were investigated. The results showed that the phase behavior of OVA–CMC conjugates was related to pH and concentration of CMC and NaCl. When pH was over 5.0, discrete phase separation occurred in the mixture system, which indicated that OVA and CMC were thermodynamic incompatible. The mixture system turned into uniform stable emulsion system when pH reduced below 5.0. The addition of NaCl can improve the stability of composite system against pH sensitivity. CLSM and particle size distribution and ultraviolet spectrum analysis results confirm that emptying interactions play a leading role in the separation system.

1. Introduction

Egg white proteins are widely used as a functional food material in food processing (Huang et al., 2012). Ovalbumin (OVA) is the major protein component of egg white and the most widely used model protein. It is an important food ingredient which possesses structural functionality including emulsifying property and foaming stability (Choi, Kim, Park, & Moon, 2005; Song et al.,2013). It is applied as foodstuffs because of not only their functional properties, but also high nutritive value. Its structure and properties predominantly affect the functional properties of egg white protein (Huang et al., 2012; Liu et al.,2014; Sun, Hayakawa, & Izumori, 2004).

The interaction between protein and polysaccharide was a hot scientific topic and the frontier in the field. The protein–hydrocolloid interactions in bulk solutions and at interfaces have an important influence on the stability properties of food dispersions (Doublier, Garnier, Renard, & Sanchez, 2000; Ercelebi & Ibanoğlu, 2007). The mixture of protein–hydrocolloid in an aqueous solution can exhibit one of the three different equilibrium situations: (a) miscibility, (b) thermodynamic incompatibility or (c) complex coacervation (or complexation) (De Kruif & Tuinier, 2001; Martinez, Baeza, Millan, & Pilosof, 2005). Both incompatibility and coacervation (or complexation) appear at high concentrations, which is depended on whether the protein–hydrocolloid interaction is a net repulsion or a net attraction, respectively (Dickinson, 2003). Phase-separated networks are formed by incompatible biopolymers where interactions between the different polymers are repulsive when the two types of polymers show different appetency toward the solvent (Doublier et al., 2000; Grinberg & Tolstoguzov, 1997; Van den Berg, van Vliet, van der Linden, van Boekel, & van de Velde, 2007). Phase-separated structures are most likely the outcome of gelation (Spotti, Santiago, Rubiolo, & Carrara, 2012; Tavares, Monteiro, Moreno, & Lopes da Silva, 2005).

Numerous studies about the OVA-saccharide conjugates have been carried out, focusing on the improvement of gelling property (Sánchez-Gimeno, Vercet, & López-Buesa, 2006) and foaming property, solubility, emulsifying property heat stability (Aoki et al., 1999; Kato, Minaki, & Kobayashi, 1993; Nakamura, Kato, & Kobayashi, 1992). Many researchers have investigated that the improvement of functionalities is due to using coexisting protein and polysaccharide (Babiker & Kato, 1998; Dickinson, 2003; Kim, Choi, Shin, & Moon, 2003). Scientific understanding of biological macromolecules hybrid system phase behavior can help to design and control the microstructure of the product. Phase behavior theory and its influence on product structure motivate many scientists study enthusiasm.

---

[*] Corresponding author at: College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China. Tel.: +86 13296597469; fax: +86 27 87282966.
E-mail address: libinfood@126.com (B. Li).

[1] Wei Jia and Bing Cui contributed equally to this work as co-first authors.

http://dx.doi.org/10.1016/j.carbpol.2014.03.026

Carbomethoxy cellulose (CMC), one of the most important derivatives of cellulose, is a typical anionic polysaccharide that has been widely utilized as a stabilizer in food (Toğrul & Arslan, 2004a, 2004b). CMC is one of the natural water-soluble cellulose derivatives that do not have harmful effects on human health (Su, Huang, Yuan, Wang, & Li, 2010). CMC is applied as a highly effective additive to improve the processing properties of products in fields of application varying from cosmetics, pharmaceuticals and foodstuffs (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Ye, 2008;Zhu et al.,2013)

In view of the poor research of interaction between OVA and the ionic polysaccharide, the relatively low price anionic polysaccharide sodium carboxymethylcellulose (CMC) was chosen to explore the influence that the physical blend and glycosylation modification of OVA and CMC on the structure and functional properties of products. So as to further reveal the mechanism of interaction between OVA with CMC. The study enriched the theoretical frame of interaction between protein and polysaccharide and provided fundamental resources for intensive study; at the same time, it also offered guidance on usage of mixture of food ingredients from egg source and polysaccharide.

2. Experimental

2.1. Materials

Ovalbumin (OVA) and nile blue chloride were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Sodium carboxymethylcellulose (CMC, viscosity 300–800 mPs, viscosity average molecular weight 9.72E + 04), disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, sodium hydroxide and hydrochloric acid were purchased from Sino harm Chemical Reagent Co., Ltd. (China). CMC were purified by alcohol precipitation before use and DS is 0.69.

2.2. Preparation of OVA/CMC conjugates

The bulk OVA and CMC solutions were prepared by dissolving a certain amount of OVA and CMC powder into 50 mM phosphate buffer at room temperature. The OVA–CMC mixture systems were obtained by adding CMC solution into OVA solution. The concentration of OVA was fixed at 1 wt% and CMC concentration was varied concentration from 0 to 0.5 wt%. Each sample was kept stirring for 1.5 h at room temperature after mixing.

2.3. Phase diagram

The phase diagram was set up by transforming pH, NaCl and CMC concentration. pH of all the solutions were adjusted from 2.5 to 7 with 1 M HCl and NaOH. 0–500 mM NaCl were added into the mixture system. The OVA–CMC mixture systems were stored overnight at the room temperature before measurement. The phase diagram was draw out through the macroscopic observation.

2.4. Confocal laser scanning microscope (CLSM)

The microstructure of OVA–CMC conjugates was observed by CLSM (LSM 510 META, Zeiss, Germany) equipped with a He/Ne mixed gas laser. Nile blue chloride was used to dye OVA. A drop of nile blue chloride was added into the fresh OVA solutions or OVA–CMC mixture and then mixed well by vortex mixer. An aliquot of sample was then placed on a microscope slide, covered by a cover slip. A 40× objective lens was used to capture confocal images. Digital image files were acquired in 1024 × 1024 pixel resolution.

2.5. Rheological properties

Rheological properties were evaluated on a stress-controlled AR2000ex rheometer (The United States TA instrument Co., New Castle DE, USA) with parallel plates ($d = 40$ mm) at 25 °C. The sample was dumped on the bottom of frustum of a cone, and lowered the fixture. Determination was carried out with steady shear experiment and selected the flow pattern and determination of shear rate range was 1–300 s^{-1}.

2.6. Determination of average particle size

The droplet size distribution of the OVA–CMC conjugates systems was measured using a laser light scattering instrument (Malvern Instruments, Nano-ZS, Worcestershire, UK). Each sample was diluted under the same ion strength and pH of phosphate buffer before the test on the volume ratio of 1:10. Each sample was analyzed in triplicate.

2.7. UV–Vis analysis of OVA–CMC conjugates

The UV–Vis analysis of OVA–CMC conjugates was performed using a spectrophotometer (UV-1700, Japan). The conjugate was centrifuged for 20 min at 15,000 r/min. Model reaction conjugates with thirty-fold dilution by phosphate buffer was placed in quartz cuvette, and the sample was scanned from 220 to 400 nm.

2.8. Transmission electron microscopy (TEM)

For the analysis of the OVA–CMC conjugates by JEM-2010FEF (NEC, Japan), a droplet of a dilute suspension of OVA–CMC composite solution was deposited on a carbon-coated grid and allowed to dry (Dolphen & Thiravetyan, 2011). The accelerating voltage was 200 kv.

3. Results and discussion

3.1. Influence of pH, CMC and ion concentration on the phase behavior of OVA–CMC composite system

The phase behavior of OVA–CMC mixture system was studied by varying pH, NaCl and CMC concentration. The resulting phase diagram and visual images were shown in Fig. 1. (Overall, three kinds of phenomenon were observed, which were precipitation, phase separation with lower turbidity and relatively transparent upper phase and milky white uniform phase.)

Fig. 1a displayed the influence of pH on the phase behavior of OVA–CMC mixture systems. When pH was over 5, discrete phase separation obviously occurred by forming the epinephelos lower layer and relatively transparent upper layer. This is because OVA and CMC have the same charge and they may occur depletion interaction. It could also be observed that the phase separation was independent on the concentration of CMC. The height of lower phase increased with higher CMC concentration. The results were consistent with the previous study by Blonk et al. (Blonk, van Eendenburg, Koning, Weisenborn, & Winkel, 1995). Protein macromolecules are easy to form relatively compact lower phase at low concentration, presenting the formation of network structure. Part of polysaccharides embed in the network structure. Along with the increase of the concentration of polysaccharide, height of the lower phase increases.

When pH was less than 5, there might be electrostatic interactions between CMC and OVA molecules. Because OVA is positively charged when pH is lower than the isoelectric point of OVA (pI 4.6), which prompted the intermolecular electrostatic interaction between OVA and anionic polysaccharide CMC.