Food Hydrocolloids 74 (2018) 255–266

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Preparation and characterization of hsian-tsao gum and chitosan complex coacervates

Gang You [a, b], Xiao Ling Liu [a, **], Mou Ming Zhao [a, *]

[a] College of Light Industry and Food Engineering, Guangxi University, NO. 100, Daxue Road, Nanning 530004, Guangxi, China
[b] Guangxi Colleges and Universities Key Laboratory of Development and High-value Utilization of Beibu Gulf Seafood Resources, Qinzhou University, No. 12, Binhai Road, Qinzhou 535000, Guangxi, China

ARTICLE INFO

Article history:
Received 16 December 2016
Received in revised form
1 July 2017
Accepted 6 August 2017
Available online 11 August 2017

Keywords:
Hsian-tsao gum
Chitosan
Complex coacervates
Characterization

ABSTRACT

In this study, a novel formation of coacervates originated from hsian-tsao gum (HG) and chitosan (CS) was investigated. Preparation of coacervates was carried out through associative phase separation between CS (1 wt%) and HG (0–2 wt%) as a function of pH. Meanwhile, the coacervates formation mechanism and properties of formed coacervates were evaluated using zeta potentiometry, rheological analysis, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), thermogravimetry (TG), differential scanning calorimetry (DSC) and microstructure measurements. The results obtained suggested that interactions between CS and HG promoted coacervates formation by forming a new network through the electrostatic interaction between the $-NH_3^+$ groups of CS and $-COO^-$ groups of HG. The charge neutralization between HG and CS was further confirmed in dilute solution by zeta potentiometry. Particularly, with increasing HG concentration in mixed dispersions, the formed coacervates showed enhanced apparent viscosity with higher elasticity, which might be related with the coarsening phenomenon as indicated by optical measurements, and enhanced aggregated complexes-solvent interactions. The formed coacervates also showed a pH dependent viscoelasticity. Additionally, an increase in HG concentration could significantly change surface structure of formed coacervates, leading to the formation of large aggregates associated from small complexes. These findings suggested by adjusting HG concentration and pH of HG-CS mixture, a new type of food-grade coacervates with novel properties could be formed, showing a promising potential to be used in food industry.

1. Introduction

Biopolymers, for instance, polysaccharides or proteins, are the main nutritional components coexisted in formulated food products, contributing to their structure, stability, and texture (Schmitt et al., 2001). But when foods are processed, it is prone to inducing the interactions between biopolymers, including polysaccharide-polysaccharide interactions, polysaccharide-protein interactions and protein-protein interactions, which may influence their microstructure, texture, mechanical stability and, eventually appearance, flavor and taste (Semenova, 2007). Under certain conditions, biopolymers may possess different charges in one of three charge properties such as positive, negative and neutral charge. Electrostatic interactions between biopolymers induce associative or segregative phase separation, resulting from the attractive or repulsive interactions, respectively (Espinosa-Andrews, Báez-González, Cruz-Sosa, & Vernon-Carter, 2007). In generally, one of the three concentration-dependent behaviors could be observed when mixing two biopolymers in solution, i.e. miscibility, thermodynamic incompatibility and complex formation (Espinosa-Andrews, Sandoval-Castilla, Vázquez-Torres, Vernon-Carter, & Lobato-Calleros, 2010). In dilute solutions, the mixed biopolymers are miscible and stable resulting from the weaker inter-polymeric attractions and the dominated entropy of mixing in the system (Kruif & Tuinier, 2001). When increasing biopolymer concentrations over the critical concentration, the mixed biopolymers with the like charges induce repulsive forces at the molecular level and, therefore cause a segregative separation of the mixed system into two phases regarded as thermodynamic incompatibility. In contrast, the oppositely charged biopolymers

* Corresponding author.
** Corresponding author.
E-mail addresses: yougang8901@163.com (G. You), xiaolingliu@hotmail.com (X.L. Liu), zmmgxu@gxu.edu.cn (M.M. Zhao).

http://dx.doi.org/10.1016/j.foodhyd.2017.08.004
0268-005X/

interact associatively to form complex networks: (i) formation of a coacervate phase, if interactions are appropriate, and (ii) formation of a precipitate state, if interactions are strong (de Kruif, Weinbreck & de Vries, 2004; Espinosa-Andrews et al., 2010). The charge neutralization of the biopolymers reduces the repulsive forces and, subsequently, causes an associative separation of the solution in two phases known as complex coacervation. In essence, complex coacervation is induced by the attractive forces between oppositely charged polymers. Two theories about the spinodal decomposition and nucleation and growth mechanism could be used to explain the separation mechanism during complex coacervation. The former is characterized by long-range/small-amplitude concentration fluctuations, which generally exhibits an interconnected network, whereas the latter proceeds through initial short-range/high-amplitude concentration fluctuations, which ends up with spherical droplets dispersed in a continuous phase (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). Sanchez, Mekhloufi, and Renard (2006) demonstrated that the complex coacervation between β-lactoglobulin and acacia gum was a nucleation and growth process using small angle static light scattering. Yuan, Wan, Yang, and Yin (2014) observed that soluble complex and coacervate prepared in glycinin/chitosan or β-conglycinin/chitosan mixtures at specific pH, followed a nucleation and growth mechanism.

In recent years, a number of studies have shown that protein can interact with polysaccharide to form electrostatic complexes, which accurately controlled by vital parameters, i.e., pH, ionic strength, mix ratio, total biopolymers concentration, charge density (de Kruif, Weinbreck & de Vries, 2004). Protein–polysaccharide coacervates have gained wide applications in fat substitution (Ramírez-Santiago, Lobato-Calleros, Espinosa-Andrews, & Vernon-Carter, 2012), enzyme immobilization, flavour microencapsulation (Xiao, Yu, & Yang, 2011), and thickening and stabilizing food products (Sittikijyothin, Sampaio, & Gon Alves, 2010) as well as incorporating functional ingredients. However, compared to applied research of protein–polysaccharide systems, there are relatively scarce reports about ionic polysaccharide-polysaccharide interactions and its application. It was reported that negatively charged polysaccharide including mesquite gum (Ruíz-Ramos et al., 2006), gum Arabic (Espinosa-Andrews et al., 2007), xanthan (Argin-Soysal, Kofinas, & Lo, 2009) interacted with cationic chitosan to form electrostatic complexes. Especially, xanthan and chitosan complex coacervates were recognized as promising biocarrier, with relatively high enzymatic resistance, for targeted delivery and controlled release of encapsulated products (Chellat et al., 2000).

In this study, we focus on the characterization of complex coacervates formed by interactions between CS, a cationic polysaccharide and HG, an anionic polysaccharide, in mixed solutions. Chemically, chitosan, as a linear cationic polysaccharide at acidic pH values, is a (1, 4)-linked 2-amino-2-deoxy-β-D-glucan derived from fully or partially deacetylated chitin. Due to its biocompatibility and nontoxicity, chitosan has potential use in pharmaceutical/biomedical applications, and increasingly, food encapsulation (Van den Broek, Knoop, Kappen, & Boeriu, 2015), in addition to emulsion stabilization (Yuan et al., 2013) and microencapsulation (Kurukji, Norton, & Spyropoulos, 2016). Hsian-tsao gum (HG) is extracted from hsian-tsao (*Mesona procumbens Hemsl*), an annual herbal plant of Taiwan and south China, as well as other countries of southeast Asia (e.g. Indonesia, Vietnam and Burma), by sodium bicarbonate or sodium carbonate solutions (Lai, Chou, & Chao, 2001). Traditionally, this edible plant containing polysaccharide gum is processed into a herbal tea or as a functional food ingredient added in the starch solutions to produce jam-type dessert in China, because of its therapeutic effectiveness for calenture, hypertension, diuresis and joint pain (Lai & Chao, 2000; Lai & Lin, 2004). HG is an ionic heteroglycan, constituted mainly of mannose, rhamnose, galactose, glucose, arabinose, xylose and 40–55% of uronic acid depending on the cultivation conditions (Lai & Liao, 2002). HG possesses ionic nature mainly from the presence of uronic acid with carboxyl group (-COOH), which has a low pK_a contributing to negative charges in solution (Harnsilwat, Pongsawatmanit, & Mcclements, 2006). Many studies have shown that HG can synergistically interact with starch and form new edible films and fat replacements (Chen, Kuo, & Lai, 2009; Chen, Kuo, & Lai, 2013; Lai & Lin, 2004). Recently, Yang et al. (2015a, 2015b) successfully prepared new blend films by HG with soy protein isolate and by HG with casein, and demonstrated that both of the blend films were possessed of the better mechanical characteristic, less sensitive to moisture and stronger barrier properties than pure protein. As an approach to exploring its application in our lab, HG has been blended with CS to prepare complex coacervates, showing a promising application of microencapsulation in food formula. Nevertheless, the mechanism of complex coacervates formed through the interactions between HG and CS has not been investigated.

The objectives of this work were (i) to investigate the zeta potential of CS, HG and mixtures as a function of pH; (ii) to evaluate the rheological properties of complexes formed by the interactions between CS and HG as a function of HG concentration and pH; (iii) to characterize the structural, thermal and morphological properties and molecular interactions of complex coacervates.

2. Materials and methods

2.1. Materials

Hsian-tsao (*Mesona procumbens Hemsl*) gum (HG) powder (moisture 5.01 ± 1.01%) was supplied by Friendship Food Co. Ltd. (Meizhou, China). The powder was further purified according to the method reported by Lai and Chao (2000) and Lai et al. (2001). The purified HG powder composition was (g/100 g, dry basis): carbohydrate 61.19 ± 1.25, fat 0.23 ± 0.01, protein 2.16 ± 0.01, ash 27.26 ± 1.03. The differences between the values measured by other authors (Chen & Lai, 2008), could be explained by the different local growing conditions of hsian-tsao plant. Chitosan (CS) (medium molecular weight about 300 kDa, degree of deacetylation of 90%, moisture 5.15 ± 1.03%, ash content 0.5 ± 0.13%) was purchased from Yuanye Biotech Co. Ltd. (Shanghai, China). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were of analytical grade supplied by Guangzhou Chemical Reagent Factory (Guangzhou, China). Analytical grade acetic acid was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Preparation of HG and CS stock solutions

CS (2 wt%) and HG (4 wt%) stock solutions were prepared by dispersing chitosan powder in MilliQ-grade water with 1% (v/v) acetic acid solution and the HG powder in MilliQ-grade water (deionized water, R = 18.2 MΩ cm; Millipore Billerica, MA, USA), respectively. The solutions were gently stirred for 8 h at ambient temperature and stored over night at 4 °C for full hydration and then centrifuged (XL-100 K, Beckman instrument, USA) for 30 min at 6000 g to remove insoluble matter and air bubbles at ambient temperature.

2.3. Zeta-potential of stock dispersions

The zeta-potential values were determined using dynamic light scattering based Zetasizer Nano ZS90 equipment (Malvern Instruments, Worcestershire, UK) according to the method previously