Food Hydrocolloids xxx (2016) 1–8

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Interpolymeric complexing between egg white proteins and xanthan gum: Effect of salt and protein/polysaccharide ratio

Clitor J.F. Souza [a], Edwin E. Garcia-Rojas [a, b, *]

[a] Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos (PPGCTA), Universidade Federal Rural do Rio de Janeiro (UFRRJ), Rodovia BR 465 Km 7, Seropédica, RJ, CEP 23890-000, Brazil

[b] Laboratório de Engenharia e Tecnologia Agroindustrial (LETA), Universidade Federal Fluminense (UFF), Av. dos Trabalhadores N° 420, Volta Redonda, RJ, CEP 27255-125, Brazil

ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form
18 November 2016
Accepted 21 November 2016
Available online xxx

Keywords:
Lysozyme
Ovalbumin
Interpolymeric complex
Rheology
Electrostatic interactions

ABSTRACT

The aim of this work was to characterize the complexes lysozyme (Lyso)/xanthan gum (XG) and ovalbumin (Ova)/xanthan gum in terms of their physicochemical properties and to study the influence of salt and protein/polysaccharide ratio on rheological properties. As the protein/polysaccharide ratio was increased from 1:1 to 10:1, critical structure events shifted to higher pHs, resulting in the formation of insoluble complexes even at pH 12.0. Lyso/XG complex formed a stronger gel-like complex with smaller loss tangents compared with the Ova/XG complex; however, those gels showed a slight tendency to breakdown under higher frequency variation when compared with Ova/XG complex. The correlation between rheological data, the effect of ionic strength and FTIR analysis suggests that although an electrostatic interaction is the main mechanism of interaction between biopolymers, non-electrostatic interactions also plays a role in the strength of the interaction. Egg white protein/XG complexes represent a great technological to formulate functional hydrogels.

1. Introduction

Proteins and polysaccharides are present together in many food systems. Both contribute to the structure, texture, and stability of food. Interestingly, when complexes are formed, their functional properties are potentially better than those of the proteins and polysaccharides alone (Burgess, 1990; Dickinson, 2003). Protein/polysaccharide coacervation in an aqueous dispersion is often accompanied by either segregative or associative phase separation, depending mainly on the electrical charges on the biopolymers. Several parameters have been shown to exert influence on electrostatic interactions, such as pH, biopolymer ratios, charge density, size and shape of the biopolymers, ionic strength, and ion type (Kizilay, Kayitmazer, & Dubin, 2011; Schmitt & Turgeon, 2011; Souza, Garcia-Rojas, Melo, Gaspar, & Lins, 2013; Souza & Garcia-Rojas, 2015).

The process of coacervation between a protein and anionic polysaccharide primarily correspond with the presence of co-soluble biopolymers in the solution (pH > pH_c). The critical pH value (pH_c) is traditionally defined as the onset of an increase in scattered light intensity associated with primary intramolecular soluble complexes (weak interactions) (Fanny Weinbreck, de Vries, Schrooyen, & de Kruif, 2003). When the pH is reduced, soluble intramolecular complexes start to aggregate into insoluble protein/polysaccharide complexes at a second critical pH ($pH_{\varphi 1}$). The $pH_{\varphi 1}$ is traditionally defined as a sharp increase in turbidity (Jones & McClements, 2010; Fanny; Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2004). It is worth noting that the maximum scattered light intensity often coincides with the pH_{φ} (Kaibara, Okazaki, Bohidar, & Dubin, 2000; Fanny Weinbreck et al., 2003). Other authors have stated that this pH is the pH_{max} (Large insoluble complex) (Chai, Lee, & Huang, 2014; Kaibara et al., 2000). Eventually, when the pH decreases below the pK_a of a polysaccharide, the turbidity returns to the baseline (co-soluble biopolymers), and a third critical point ($pH_{\varphi 2}$) is reached (Turgeon, Schmitt, & Sanchez, 2007). The anionic groups on polysaccharide molecules lose their charge and promote dissociation of the protein/polysaccharide complex.

* Corresponding author. Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos (PPGCTA), Universidade Federal Rural do Rio de Janeiro (UFRRJ), Rodovia BR 465 Km 7, Seropédica, RJ, CEP 23890-000, Brazil.
E-mail addresses: clitorrj@gmail.com (C.J.F. Souza), edwin@vm.uff.br (E.E. Garcia-Rojas).

http://dx.doi.org/10.1016/j.foodhyd.2016.11.032
0268-005X/

Xanthan gum is an anionic extracellular polysaccharide secreted by the micro-organism *Xanthomonas campestris*. It is widely used in the food industry as a stabilizer (over wide pH and temperature ranges) and thickener due to its specific physical (viscosity, pseudoplasticity) and chemical (soluble in cold water and resistant to enzymatic degradation) properties. The primary structure of xanthan gum is a linear (1–4) linked β-D-glucose backbone with a trisaccharide side chain on every other glucose at C-3, containing a glucuronic acid residue linked (1–4) to a terminal mannose unit and (1–2) to a second mannose that connects to the backbone (Monsanto, 2009).

Chicken egg ovalbumin is the major egg white protein synthesized in hens' oviducts. It accounts for 54% of the total egg white protein (Stadelman & Cotterill, 1995). The molecular weight of ovalbumin is 45 kDa, and the protein consists of 385 amino acid residues. Ovalbumin is an important food ingredient with structural functionality, including emulsifying properties and foam stability (Stadelman et al., 1995). Lysozyme is a ubiquitous enzyme that is present in almost all secreted body fluids and plays an important role in natural defense mechanisms. The most plentiful source of lysozyme is in hen egg white albumin, which contains approximately 3.4% of the total egg white protein. The molecular weight of lysozyme is 14.4 kDa, and this enzyme consists of 129 amino acid residues. Its isoelectric point is 10.7. In the food industry, lysozyme is a major bacteriolytic protein (Stadelman et al., 1995).

The present study aimed to elucidate the influence of protein, pH, ionic strength, and ratio of lysozyme or ovalbumin/xanthan gum in the kinetics of the formation of interpolymeric complexes, as well the rheology properties of the complexes.

2. Materials and methods

2.1. Materials

Lysozyme (Lyso; purity > 90%), ovalbumin (Ova; purity > 90%), and xanthan gum (XG) were obtained from Sigma Chemicals (St. Louis, USA). Sodium chloride (NaCl, purity > 99%), hydrochloric acid (HCl, 0.5 mol/L), and sodium hydroxide (NaOH, 0.5 mol/L) were purchased from VETEC® Ltda, (Rio de Janeiro, Brazil). The water used was ultrapure with a conductivity of 0.05 μS/cm (Gehaka, Master P&D, Brazil). Stock solutions of xanthan gum (0.1% w/w), ovalbumin (0.1–1% w/w), and lysozyme (0.1–1% w/w) were prepared by gently stirring the powders in deionized water for 6 h at room temperature (25 °C). After complete dissolution of biopolymers, the pH of solutions was adjusted to pH 12.0 using 0.5 mol/L NaOH and 0.25 mol/L HCl.

2.2. Formation of complexes

2.2.1. Preparation of complexes

For the preparation of systems, the concentration of XG was fixed at 0.1 wt%, and stock solutions were used to prepare five ratios of protein:XG (1:1, 2:1, 3:1, 5:1, 10:1). To determine the effect of NaCl on complex formation, Lyso:XG or Ova:XG complexes were formed in five concentrations of NaCl (0.01, 0.05, 0.1, 0.2, 0.4 mol/L). The protein:XG mixtures were previously stirred and adjusted to pH 12 for turbidimetric measurements.

2.2.2. Turbidimetric measurements

pH-dependent turbidity was measured at a wavelength of 400 nm using a spectrophotometer (Biochrom mod. LIBRA S12, England) calibrated with ultrapure water to 100% transmittance (T). Turbidity was defined as 100-%T. With the aid of a magnetic stirrer (Nova Tecnica, NT 101, Brazil) and pH meter (Tecnopon, mPA-210, Brazil), the pH values of the solutions were adjusted (12.0–1.0) with 0.5 mol/L HCl. Measurements of complexes and solutions with biopolymers isolates were performed at room temperature (25 °C ± 1). Each sample was measured four times at 1-min intervals.

2.3. Zeta - potential

A Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK) was used to determine zeta (ζ)-potentials. Lysozyme, ovalbumin and xanthan gum stock solutions and Lyso:XG or Ova:XG complexes formed in 0.01 mol/L NaCl were diluted to 0.01% w/w and transferred to an MPT-2 autotitrator (Malvern Instruments, Worcestershire, UK). The pH was titrated using 0.5 mol/L NaOH, 0.25 mol/L HCl, and 0.025 mol/L HCl solutions. The pH was varied from 12.0 to 1.0 by 0.3-unit increments with a confidence interval of ±0.1 unit. Each experiment was performed three times. Sample readings were performed in triplicate at 25 °C.

2.4. Characterization of the complexes

2.4.1. Rheological measurements

The rheological properties of protein/polysaccharide complex were determined using a rotational rheometer Haake™ Mars III Rotational Rheometer (Thermo Scientific Inc., Alemanha) with cone and plate attachments (20 mm, angle: 1°) and a gap of 0.025 mm between the elements. The ratios of Lys:Ca and Ova:Ca complex with different concentrations of NaCl were formed. After 24 h, the samples were centrifuged at 5 °C (OrtoAlresa, Digicen 21R, Spain) at 6000 rpm for 30 min. Before analysis, all complexes were placed in the rheometer plate (10 min) with a sample cover TM-IN-H (Thermo Scientific Inc., Alemanha) to stabilize the temperature and prevent water evaporation during analysis. Strain sweep tests were carried out to determine the linear viscoelastic range (0.1–100%). The storage modulus (G'), the dissipation modulus (G''), and the apparent viscosity (η^*) were measured. The frequency was varied from 0.1 to 100 rad/s. All samples were analyzed at 25 °C with four independent repetitions.

2.4.2. FTIR

Stock solutions of proteins, XG and protein/polysaccharide complexes were freeze-dried. The samples were placed into an FTIR GX System (Perkin-Elmer, Shelton, CT, USA) coupled to an ATR DuraSample II accessory. All spectra were an average of 16 scans from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1}.

3. Results and discussion

3.1. Effect of pH and ratio on protein/XG complexes

The biopolymer ratio is a critical parameter for controlling the charge balance in the mixed systems (Turgeon et al., 2007; Ye, 2008). Development of the critical pH transition points (pH_c, pH_φ, and pH_{max}) as a function of the biopolymer mixing ratio was performed by turbidimetric analysis during acid titration. The results are presented in Fig. 1.

As seen in Fig. 1A, four phases (I, II, III, and IV) were obtained by changing the turbidity of the Ova-XG mixtures with different ratios. The mixture turbidity remained almost constant at a pH above the pH_c, demonstrating that electrostatic repulsive forces prevented the formation of complexes and that the turbidity remained at the baseline (phase I). With further acidification from a neutral pH, soluble complexes (pH_c) characterized by weak electrostatic interactions were formed between the protein and polysaccharide (phase II) (Jones et al., 2010). Upon further continuous pH

Please cite this article in press as: Souza, C. J. F., & Garcia-Rojas, E. E., Interpolymeric complexing between egg white proteins and xanthan gum: Effect of salt and protein/polysaccharide ratio, *Food Hydrocolloids* (2016), http://dx.doi.org/10.1016/j.foodhyd.2016.11.032