Food Hydrocolloids 45 (2015) 286–290

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Polymeric complexes obtained from the interaction of bovine serum albumin and κ-carrageenan

Clitor J.F. de Souza [a], Andresa V. Ramos [b], Paulo B.S. Câmara [b], Eliana S. Gulão [a], Marcos F. de Campos [b], Edwin E. Garcia-Rojas [a, b, *]

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

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

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form
20 October 2014
Accepted 24 October 2014
Available online 13 December 2014

Keywords:
Polymeric complex
κ-carrageenan
Milk proteins
Biopolymer
Bovine serum albumin

ABSTRACT

This study aimed to characterize and optimize the formation of polymeric complexes obtained from the interaction between bovine serum albumin (BSA) and κ-carrageenan, in different aqueous systems containing different pH values and various concentrations of polymer and NaCl. Response surface methodology was applied to optimize the process. The proposed polynomial model showed significant adjustment ($R^2 = 0.84$), and the optimal conditions for the formation of carrageenan-BSA polymeric complexes were verified to be 6.40 mg of κ-carrageenan, 0.58 mol/L NaCl, and pH 6.30. The rheological analysis showed that the polymeric complex had a less viscous character than carrageenan. Scanning electron microscopy and X-ray diffractometry showed that the complex had a more well-differentiated morphology compared to the original biopolymers.

1. Introduction

Carrageenan is an anionic polysaccharide found in marine algae of the class *Rhodophyceae*. One of the most important properties that differentiate it from other hydrocolloids is its unique ability to form complexes and interact with milk proteins under various conditions. Highly sulfated, carrageenan interacts with casein of milk through electrostatic interactions, which provide a high capacity for water absorption. This feature is widely exploited by the food industry to increase yields and reduce the costs of the final product. When the moisture content of food is drastically reduced during production or storage, soluble proteins and sensory characteristics are easily lost (Phillips & Williams, 2000). Thus, another advantage of carrageenan that should be emphasized is its ability to maintain the nutritional and sensory characteristics of food because the moisture content is not changed.

The complexes formed between carrageenan and protein also exhibit other technologically functional properties such as thickening, gelling, emulsifying, and stabilizing fats; freeze-thaw stability; and controlled texture, melting properties, and viscosity, which are useful in processing dairy products, canned meats, jellies, jams, light food, pasta, and others (Phillips & Williams, 2000; Ribeiro & Seravalli, 2007).

Milk proteins can be separated into casein and whey proteins, which have concentrations ranging from 30 to 36 g/L. Thus, approximately 20% of milk proteins are present in whey; α-lactalbumin and β-lactoglobulin are present in the greatest quantities, while immunoglobulins, serum albumin, and other protein components are present in lesser amounts. The rich composition of sulfur amino acids in whey proteins makes these proteins interesting food ingredients from a nutritional standpoint (Damodaram, Parkin, & Fennema, 2008). Human serum albumin and bovine serum albumin (BSA) are each composed of 582 amino acids arranged in a single polypeptide chain with 17 disulfide bonds, which provide stability to its tertiary structure. BSA has a p*I* around pH 4.8, and its molar mass is approximately 69 kDa (Antunes, 2003).

The interaction between proteins and polysaccharides can result in a phase separation that can be segregative or associative. In

* Corresponding author. Programa de Pós-graduação em Ciência e Tecnologia de Alimentos (PPGCTA), Universidade Federal Rural de Rio de Janeiro (UFRRJ), Rodovia BR 465, Km 7, CEP 23890-000 Seropédica, RJ, Brazil. Tel.: +55 24 2107 3563; fax: +55 24 3344 3019.
E-mail address: edwin@vm.uff.br (E.E. Garcia-Rojas).

http://dx.doi.org/10.1016/j.foodhyd.2014.10.023
0268-005X/

segregative phase separation, a two-phase system is formed, with one phase rich in polysaccharides and the other rich in proteins. In associative phase separation, the force of attraction between the positive charges of proteins and the negative charges of polysaccharides form a two-phase system, in which one phase is rich in solvent and the other is rich in polysaccharide-protein complex (Tolstoguzov, 1991; Weinbreck, Nieuwenhuijse, Robinjn, & Kruif, 2004). Studying the formation of polymeric complexes can help optimize the purification processes of proteins and the formation of microcapsules, biomaterials, and new food ingredients (Guzey & Mcclements, 2007; Malmsten, Bysell, & Hansson, 2010; Ould Eleya & Turgeon, 2000; Souza, Rojas, Melo, Gaspar, & Lins, 2013).

Thus, the objective of this work was to optimize the process of κ-carrageenan-BSA polymeric complex formation and evaluate the influence of pH and concentrations of κ-carrageenan and NaCl.

2. Materials and methods

2.1. Materials

BSA and κ-carrageenan were purchased from Sigma Aldrich (St. Louis, MO, USA). Chemical reagents of analytical grade and deionized water were used in all experiments.

2.2. Formation of polymeric complexes

The κ-carrageenan was added in distilled water at a concentration of 0.5% w/w, using an analytical balance (Tecnal, B-TEC−210ª, Piracicaba, Brazil). After this procedure, the solution was stirred for approximately 10 min using an Ultra-Turrax (IKA, T10 Basic, Germany). To a beacker containing 15 mL of water, 100 mg of BSA and the respective concentrations of NaCl and κ-carrageenan were added. Next, the pH of the solutions was adjusted and then the samples were shaken for 2 h at 25 °C using an orbital shaker (Tecnal, TE 420, Brazil). In order to ensure the formation of the polymeric complex, the samples were kept refrigerated for 24 h prior to subsequent centrifugation (Cientec, TC-6000, Brazil) at 6000 rpm for 30 min (Souza et al., 2013). After centrifugation, an aliquot of 4 mL of the supernatant was taken for determination of the transmittance at 400 nm by using a spectrophotometer (Biochrom, Libra S12, England). The turbidity was obtained according to Eq. (1) (Chollakup, Smitthipong, Eisenbach, & Tirrel, 2010), in which the turbidity (T) is defined by

$$T = -\ln\left(\frac{I}{I_0}\right) \tag{1}$$

where I_0 is the intensity of incident light and I is the intensity of light that passes through the sample volume.

2.3. Experimental design

In order to determine the influence of each factor analyzed on the formation of the polymeric complex, as well as to identify the parameters of maximum polymeric complex formation, an experimental design was used (Table 1). Response surface methodology was applied to optimize the process. The optimum surface region was obtained by applying the maximum inclination path (Box, Hunter, & Hunter, 1978; Myers & Montgomery, 1995), comprising a 2^3 factorial design with levels +1 and −1, six replicates at the center point (zero level), and six axial points (−1.68 and +1.68), resulting in twenty experiments. The results of the experimental design were fit with a second-order polynomial equation by a multiple regression technique. The quadratic equation used to predict the optimal point is as follows in Eq. (2):

$$Y_1 = \beta_0 + \beta_1 X_1 + \beta_{11} X_1^2 + \beta_2 X_2 + \beta_{22} X_2^2 + \beta_3 X_3 + \beta_{33} X_3^3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon \tag{2}$$

where Y_1 is the dependent variable (turbidity); β_0 is the model constant; β_1, β_2, and β_3 are the model coefficients; and ε is the error. They represent the linear, quadratic, and interaction effects of the variables.

The experimental data were analyzed using the statistical package SAS® Version 9.2 (SAS, 2008). The reliability of the obtained polynomial model equation was evaluated by the coefficient of determination (R^2), and the statistical difference between the results was calculated by analysis of variance at a significance level of 0.05.

2.4. Characterization of the polymeric complex

A sample was prepared in duplicate using the concentrations of reagents and pH conditions found at the optimal formation point of the complex. The precipitates were centrifuged and freeze-dried (Enterprise I, Terroni, Brazil).

2.5. Scanning electron microscopy

The lyophilized samples were analyzed with the aid of a scanning electron microscope (Evo Ma 10, Zeiss, Germany), which was operated in the secondary electron mode with an accelerating voltage of 20 kV.

2.6. X-ray diffraction

The internal structure of the lyophilized polymeric complex was characterized using an X-ray diffractometer (XRD 6000, Shimadzu, Japan) to evaluate the crystallinity of the particles. The analysis was performed using cokα radiation (λ = 1.7889 Å), scan angle of 2θ range of 15–60°, with a steep angle of 2°/min^{-1} and working conditions of 40 kV and 30 mA.

2.7. Rheological analysis

The rheological behavior of the κ-carrageenan (1% w/w) and polymeric complex (1% w/w) formed was measured using a rotational rheometer (Haake™ Mars III, Thermo Scientific Inc., Germany) with cone and plate attachments (60 mm, angle: 1°) and a gap of 0.025 mm between the elements. The storage (G′) and dissipation (G″) modulus were measured while the frequency varied from 0.01 to 10 Hz. All samples were analyzed at 25 °C. To classify the fluid behavior, flow and viscosity curves were generated. The consistency index, k (Pa·s^n), and the flow behavior index, n, were estimated by applying the Ostwald-de Waele model.

Table 1
Variables and levels of experimental design for the formation of interpolymeric complex.

Variables	Symbol	Levels				
		−1.68	−1	0	1	1.68
pH	X_1	3.0	4.4	6.5	8.6	10.0
NaCl (mol/L)	X_2	0	0.2	0.5	0.8	1
κ-carrageenan (mg)	X_3	2.5	7.06	13.75	20.44	25