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Physicochemical study of mixed systems composed by bovine caseinate and the galactomannan from *Gleditsia amorphoides*

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ABSTRACT

Model systems formed by sodium caseinate (NaCAS) and espina corona gum (ECG) were studied. There was no evidence of attractive interactions between NaCAS and ECG macromolecules. Aqueous mixtures of NaCAS and ECG phase-separate segregatively over a wide range of concentrations. According to the images obtained by confocal laser scanning microscopy, NaCAS particles form larger protein aggregates when ECG is present in the system. An increase in the hydrodynamic diameter of NaCAS particles, as a result of ECG addition, was also observed by light scattering in diluted systems. A depletion–flocculation phenomenon, in which ECG is excluded from NaCAS surface, is proposed to occur in the concentrated mixed systems, resulting in NaCAS aggregation. ECG raises the viscosity of NaCAS dispersions without affecting the Newtonian flow behaviour of NaCAS. These results contribute to improve the knowledge of a barely-studied hydrocolloid which may be useful in the development of innovative food systems.

1. Introduction

Protein-polysaccharide mixtures are widely used in the food industry in order to impart desirable texture and sensory characteristics (Dickinson, 1998; Mohajer, Rezaei, & Hosseini, 2017). Understanding the behaviour of the systems composed either by milk proteins or hydrocolloids is of great importance, since the functional properties of milk proteins are usually affected by polysaccharides (Matignon et al., 2014; Mende et al., 2013; Rosa-Sibakov et al., 2016).

Due to their physicochemical and functional properties, sodium caseinates (NaCAS) are useful ingredients added to different food products as emulsifiers, gelling, texturizing and water and fat binding agents (Gaucheron, Le Graet, Boyaval, & Piot, 1997; Perrechil, Braga, & Cunha, 2009).

Vegetable galactomannans are a group of environment-friendly polysaccharides obtained from seeds of some Leguminosae tree and consist of chains of mannose residues with randomly attached galactose units as side-chains (Tavares, Monteiro, Moreno, & Da Silva, 2005). Many plant gums (e.g. carrageenan, pectin, starch, guar and sodium carboxyl methyl cellulose) are broadly used in food systems as thickeners, gelling and suspending agents, texture modifiers, emulsifiers and emulsion stabilizers (Balaghi, Mohammadifar, & Zargaraan, 2010; Dickinson, 1989; Saha & Bhattacharya, 2010). Mixed systems composed of food proteins and many galactomannans like guar gum, locust bean gum and tara gum, among others, have been extensively studied (Tavares & Da Silva, 2003).

Espina corona gum (ECG), the polysaccharide used in this work, is a galactomannan extracted from the seeds of *Gleditsia amorphoides*, a leguminous tree (Pavón, Lazzaroni, Sabbag, & Rozycki, 2014) and its chemical composition was described by Cerezo (1965). The molecular weight of ECG is 1390 Da and the galactose/manose ratio in ECG is 2.5 (Perduca et al., 2013), similar to that of guar gum, one of the galactomananns of the utmost commercial importance. It is a non-gelling biopolymer that may be used as a food thickener or stabilizer. In fact, the mechanical and microstructural properties of milk whey protein/ECG mixed gels were studied (Spotti, Santiago, Rubiolo, & Carrara, 2012) and a recent investigation reported the addition of ECG in cholesterol-reduced probiotic

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yoghurts (Pavón et al., 2014). However, the applications of this galactomannan have not yet been well studied (Albuquerque et al., 2014) and its effects on aqueous suspensions of NaCAS have not been reported so far.

The aim of this work was to study, from a physicochemical point of view, a model system containing NaCAS and ECG in order to explore future applications of this galactomannan in food products.

2. Materials and methods

2.1. Materials

Bovine sodium caseinate (NaCAS) and 1-anilino-8-naphtalene-sulfonate (ANS) were purchased from Sigma–Aldrich (Steinheim, Germany). ECG was gently donated by Idea Supply Argentina S.A. (Chaco, Argentina), and was used without further purification.

2.2. Preparation of the stock solutions

NaCAS powder was dissolved in distilled water in order to obtain a 10 wt.% NaCAS solution. A 1 wt.% ECG stock solution was prepared by dispersing ECG powder in distilled water at room temperature and was kept in stirring until complete solubilization of the polysaccharide. Diluted systems were prepared for spectroscopic techniques to ensure the correct dissolution of both biopolymers and to avoid an inner filter effect. A 1% w/v NaCAS and a 0.5% w/v ECG stock solutions were prepared. A small amount of sodium azide, purchased from Mallinckrodt Chemical (St. Louis, USA) was added to protein and polysaccharide suspensions in order to inhibit microbial development.

2.3. Fluorescence spectroscopy

2.3.1. Intrinsic fluorescence spectra

Fluorescence emission spectra of NaCAS (0.01% w/v) were obtained at 35 °C, exciting at 280 nm, at increasing concentrations of ECG (0–0.3% w/v). The scanning rate was 10 nm/min and data acquisition was performed every 0.1 nm with a 0.1 nm slit from 300 to 400 nm in an Amico Browman spectrofluorometer Series 2000 using a thermostatized quartz cell of 1 cm path length. The inner filter effect, caused by the absorption of the incident light or the emitted light, was corrected in all fluorescence intensity (FI) measurements (Lakowicz, 2007).

2.3.2. Quenching of the intrinsic fluorescence

The fluorescence quenching of NaCAS tryptophan residues, when excited at 280 nm, was carried out using 4 M acrylamide, which is a collisional quencher. Protein concentration was fixed at 0.01% w/v, while ECG concentration ranged from 0 to 0.3% w/v. Emission intensities were recorded at 340 nm in the absence of acrylamide (FI_0) and after the addition of successive aliquots of 10 μL of quencher solution (FI).

2.3.3. Surface hydrophobicity

Surface hydrophobicity (S_0) was estimated according to the method described by Kato and Nakai (1980), using the ammonium salt of the fluorescent probe 1-anilino-8-naphtalene-sulfonate (ANS). FI of samples containing 4 mM ANS and different concentrations of ECG/NaCAS mixtures were determined. Measurements were carried out at 35 °C using the excitation and emission wavelength set at 380 and 484 nm, respectively. The initial slope of FI vs. protein concentration plot was used to estimate NaCAS surface hydrophobicity.

2.4. Zeta potential and hydrodynamic diameter measurements

Zeta potential and the hydrodynamic diameter (HD) of NaCAS were determined at 35 °C in a Nano Particle Analyzer Horiba SZ-100. Protein concentration was fixed at 0.01% w/v whereas ECG concentration varied over the range of 0–0.03% w/v. Each solution was filtered through a glass microfiber paper with a cut-off of 1.6 μm, in order to avoid powders in suspensions. Such diluted systems were prepared in order to avoid interferences with the dynamic light scattering technique (Anema & Klostermeyer, 1996).

2.5. Confocal scanning laser microscopy

Immediately after mixing and after a 24-h incubation at 35 °C, the effect of ECG on 3% NaCAS dispersions was observed using confocal scanning laser microscopy (CSLM). Rhodamine B (Sigma–Aldrich, St. Louis, USA) was added to those systems at a final concentration of 0.1 mg L^{-1}. Each sample (100 μL) was placed in a compartment of LAB-TEK II cells. Representative images were captured using a confocal microscopy (Nikon Eclipse TE-2000-E, Japan), with an objective of 60× (oil immersion lens), a magnification of 5× and a numerical aperture of 1.4. Digital images were acquired with a pixel resolution of 1024 × 1024.

2.6. Evaluation of the biopolymer concentration ranges for phase separation

Samples were prepared by carefully mixing weighed amounts of ECG and NaCAS stock solutions in order to obtain systems with different biopolymer composition (NaCAS and ECG concentrations ranged from 1 to 9 wt.% and between 0.1 and 0.9 wt.%, respectively). The systems were mixed in vortex and were placed under controlled temperature at 35 °C. The occurrence of phase separation was verified after 24 h of incubation, according to Hidalgo et al. (2015).

2.7. Viscosity measurement

The rheological measurements of NaCAS, NaCAS/ECG and ECG systems were performed at 35 °C using a viscometer Brookfield LVDV-II$^+$CP (USA) in steady shear with a cone-plate geometry (diameter 48 mm, angle 0.8°).

2.8. Statistical analysis

The determinations were made at least in triplicate. The significance of the effect of ECG on each parameter was determined by means of *t*-test.

3. Results

3.1. Spectroscopic studies of the NaCAS/ECG diluted mixed systems

Fluorimetric techniques allow the assessment of the global structure of proteins and therefore to determine the effect of ECG on NaCAS conformation in solution.

3.1.1. Intrinsic fluorescence spectra

The emission spectra of NaCAS at increasing ECG concentrations are shown in Fig. 1. It is to be noted that Trp fluorescence contributes the most to protein spectra due not only to its higher quantum yield but also to the energy transfer from Phe to Tyr and from Tyr to Trp. The FI is usually related to the solvent exposition of this fluorescent amino acid. There was a progressive increase in the FI values as the