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Biopolymer nanoparticles designed for polyunsaturated fatty acid vehiculization: Protein–polysaccharide ratio study

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ABSTRACT

Information about the design of biopolymer nanoparticles (BNPs) for polyunsaturated fatty acid (PUFA) vehiculization is provided. Linoleic acid (LA) was used as a model PUFA. The binding ability of LA to β-lactoglobulin (BLG) was applied for obtaining BLG–LA complexes. BLG–LA complex formation was monitored by fluorimetry and it was observed that a moderate heat treatment (60 °C, 10 min) enhanced BLG–LA complexation. Obtaining BNPs involved the electrostatic deposition of high methoxyl pectin (HMP) onto the BLG–LA complex surface. The phase behavior of biopolymer systems was discussed at different Prot:HMP ratio ($R_{Prot:HMP}$, wt.%) levels (1:1–6:1). Absorbance at 600 nm, particle size, and ζ potential were analyzed at pH 4.0. At 1:1–2:1 $R_{Prot:HMP}$, BNPs showed appreciable turbidity, a nanometric diameter (337–364 nm), and a negative ζ potential. Finally, intrinsic and extrinsic fluorimetry was used for examining the HMP protective role at the LA binding site. At 2:1 $R_{Prot:HMP}$, HMP cover could promote significant LA protection in BNPs.

1. Introduction

Currently, one of the main challenges in the development of new functional foods is the incorporation of bioactive compounds into food matrices. Because of recognized properties of bioactive compounds, the new generation of food products promotes functional and nutritional requirements, producing additional effects on health. Effective bioactive compound incorporation into food matrices depends on their physicochemical properties and the existence of favoured molecular interactions with other food ingredients. Unfortunately, the most interesting bioactive compounds to be incorporated are very sensitive to the environmental conditions of processing and storage of foods (oxygen, UV radiation and temperature). Hence, in order to reach the desired effects, a strategy for incorporating bioactive additives is needed, e.g. through encapsulation technologies. In this sense, several encapsulation techniques for sensitive bioactive molecules have been developed, according to their physicochemical properties and environmental susceptibility (Augustin & Hemar, 2009; Joye, Davidov-Pardo, & McClements, 2014; Matalanis, Jones, & McClements, 2011).

One of the encapsulation strategies uses biopolymer nanoparticles (BNPs) as matrices or vehicles for the transport, protection and controlled release of bioactive compounds (Jones & McClements, 2011; Joye et al., 2014; Zimet & Livney, 2009). These particles can be obtained under aqueous medium conditions in which protein–polysaccharide self assembly take place (Jones, Decker, & McClements, 2009; Jones, Lesmes, Dubin, & McClements, 2010). Moreover, the employment of these biopolymer materials is possible due to their high availability, biocompatibility and biodegradability. Moreover, the small size of BNPs could have a lot of advantages over conventional encapsulation systems, such as higher stability to aggregation and gravitational separation, higher optical clarity and improved bioavailability (Joye et al., 2014; Lesmes & McClements, 2009). Nevertheless, the design of BNPs for encapsulation purposes involves the systematic study of the functional properties of individual biopolymers, the molecular interactions between biopolymers and the bioactive compounds, and the process variables that govern such interactions. Another important aspect in the design of BNPs, for bioactive compounds vehiculization in foods, is the knowledge of factors involved in the phase behavior and colloidal stability, such as particle size and electrical properties. It is well established that a lower particle size and higher electrical potential are required for a good colloidal

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stability in aqueous mediums (Jones et al., 2009, 2010; Lesmes & McClements, 2009; Ryan et al., 2011).

In this context, the aim of the present paper was to provide experimental information about the design of BNPs for polyunsaturated fatty acid (PUFA) vehiculization in acidic, aqueous systems. β-Lactoglobulin was used due to its recognized ability for binding lipophilic compounds (Kontopidis, Holt, & Sawyer, 2004). Linoleic acid (LA) and high methoxyl pectin (HMP) were employed as models of PUFA and anionic polysaccharides, respectively. The applied strategy consisted of: (i) obtaining BLG–LA complexes via BLG ability for binding LA, and (2) BNP production via HMP electrostatic deposition (attractive electrostatic interaction) onto the surface of pre-formed BLG–LA complexes. LA encapsulation using BNPs is justified due to their high environmental susceptibility in food matrices (Perez, Andermatten, Rubiolo, & Santiago, 2014; Sponton, Perez, Carrara, & Santiago, 2014, 2015a, 2015b). This strategy was reported by Zimet and Livney (2009), supporting the idea that a cover of polysaccharide on the surface of the protein-PUFA complexes could favour the protection of PUFA molecules, mainly against oxidation. However, here we propose a systematic study about the process variables, such as pH and protein–polysaccharide concentration ratio ($R_{Prot:HMP}$), for obtaining BNPs for PUFA vehiculization with special emphasis on their phase behavior and colloidal stability.

2. Materials and methods

2.1. Materials

β-Lactoglobulin (BLG) was provided by Davisco Food International (USA) and its chemical composition was (wt.%): 90.82% protein, 0.20% fat, 1.90% ash, 4.80% moisture and 2.28% other. Linoleic acid (LA, cis, cis-9, 12-octadecadienoic acid) was purchased from Sigma (USA). LA was kept under a N_2 atmosphere at −18 °C according to manufacturer advice. High-methoxyl pectin (HMP) was kindly supplied by Cargill (Argentina). HMP was obtained as a mixture extracted from citrus peels and apple pomace, and had the following characteristics (data supplied by Cargill): 68.0 ± 2.0% degree of esterification (DE) and composition (wt.%): 87.0% carbohydrate, 11.0% moisture and 2.0% ash (Na^+ 480 mg/100 g and K^+ 160 mg/100 g, Ca^{+2} 200 mg/100 g, Mg^{+2} 30 mg/100 g and Fe^{+2} 2 mg/100 g). Aqueous medium pH modifier, glucono-δ-lactone (GDL), was purchased in Sigma (USA). The fluorescence probe, 1-anilino-8-naphtalenesulphonic acid (ANS), was purchased in Fluka Chemie (Switzerland).

2.2. Obtaining of BLG–LA complexes

BLG dispersion was prepared at 0.16 wt.% in MilliQ ultrapure water, and the pH adjusted to 7.0 using 0.1 M HCl or NaOH. This stock dispersion was stirred for 1 h at room temperature and, subsequently, it was filtered through a glass microfiber pre-filter and cellulose ester filter of 0.22 and 0.45 μm pore size (Millipore, USA). Filtration was done in order to eliminate possible protein aggregates. The BLG stock dispersion was diluted at 0.08 wt.%. Additionally, a stock ethanolic solution of 4.0 M LA was prepared. BLG–LA complex production was carried out by the addition of 38 μl of the LA solution into 2 ml of the BLG diluted dispersion. This experimental condition (based on previous studies) was adequate for producing the binding site saturation on BLG (Perez et al., 2014). The ethanol concentration did not exceed 2.0%, therefore, it was assumed that no protein structural modification was produced (Sponton et al., 2014). After LA addition, tubes were vigorously stirred for 2 min in a vortex. It was assumed that ethanol dissipates in the aqueous medium, and the LA molecule binds to BLG producing BLG–LA complexes (Zimet & Livney, 2009). BLG–LA complex production was monitored by means of fluorimetry using a spectrofluorimeter (Hitachi 2000, Japan) equipped with a 1-cm pathlength quartz cell. Excitation and emission wavelengths were 295 and 332 nm, respectively. Fluorescence values were expressed in terms of relative fluorescence intensity (RFI), being RFI = F/F_0, where F is the fluorescence intensity of the BLG–LA complex and F_0 corresponds to the fluorescence intensity of pure BLG (Perez et al., 2014; Sponton et al., 2014). Usually, addition of LA to the BLG dispersion cause an increase in RFI, which would correspond to an increase in BLG–LA complex amount in aqueous solutions (Frapin, Dufour, & Haertle, 1993). Furthermore, the effect of temperature on RFI of BLG–LA complexes was evaluated in order to observe if heat treatment could modify the protein–ligand binding properties. For this, dispersions containing BLG–LA complexes were heated in a thermostatic bath (Dalvo instruments, BTMP model) in the range of 25 and 90 °C for 5 and 10 min. All experiments were done in triplicate.

2.3. Formation of biopolymer particles

2.3.1. Experimental

Biopolymer particles for LA vehiculization were obtained by HMP electrostatic deposition onto the BLG–LA complex surface in acidic pH. In this work, the effect of different levels of protein–HMP concentration ratio ($R_{Prot:HMP}$, wt.%) on the formation of biopolymer particles was evaluated. The $R_{Prot:HMP}$ levels comprised between 1:1 and 6:1 (wt.%) For this, BLG–LA complexes and HMP dispersions were prepared in MilliQ ultrapure water at pH 7.0 and appropriate volumes of these dispersions were mixed. The HMP dispersion was previously heated to 70 °C for 15 min, promoting adequate polysaccharide hydration. In all systems the BLG concentration was maintained constant at 0.08 wt.% (protein concentration used for BLG–LA complex production). Biopolymer particles formation at the different $R_{Prot:HMP}$ levels were evaluated by decreasing pH which promote the attractive electrostatic interaction between BLG–LA complex cationic groups and HMP anionic groups. Decrease in aqueous medium pH was conducted as will be mentioned below.

2.3.2. Phase behavior diagrams

The phase behavior of biopolymer systems was evaluated according the method described by Fioramonti, Perez, Aríngoli., Rubiolo., and Santiago (2014). In order to decrease the aqueous medium pH, GDL was added to mixed dispersions containing BLG–LA complexes and HMP. The GDL concentration used was 0.35 wt.%. From these mixed dispersions, temporal evolution of transmittance (T%) and pH values were determined. Transmittance measurements were performed in a Turbiscan MA 2000 (France). The variation of aqueous medium pH was automatically measured using a Sper Scientific pH meter (USA). Evolution of pH values were registered by means of instrument specific software. With the T% and pH dynamic profiles, curves of T% vs pH were constructed. From these curves, transition pH values (pH_c and pH_ϕ) were calculated as the intersections of the tangents between the inflection points of T% vs pH curves. In order to examine the phase behavior of the biopolymer mixed systems, pH vs $R_{Prot:HMP}$ phase diagrams were constructed from pH_c and pH_ϕ values (Weinbreck, de Vries, Schrooyen, & de Kruif, 2003). All measurements were performed in triplicate at 25 °C.

2.3.3. Absorption and fluorescence spectroscopy

In order to evaluate the molecular characteristics of the biopolymer particles for LA vehiculization, absorption and fluorescence (intrinsic and extrinsic) spectroscopy was applied. In these experiments, biopolymer particles were obtained by decreasing