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Protein-polysaccharide associative phase separation applied to obtain a linoleic acid dried ingredient

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ABSTRACT

This paper gives experimental information about the application of protein-polysaccharide associative phase separation to produce a dried ingredient (powder) of linoleic acid (LA). Powder production process consisted in: (i) LA binding to a well-characterized ovalbumin nanosized heat-induced aggregate (OVAn) to form LA-OVAn complexes in solution, (ii) polysaccharides (PS) addition to promote the associative phase separation of LA-OVAn complexes, and (iii) freeze-drying of the precipitated phase in order to obtain LA-OVAn-PS powders. For this, OVAn and anionic PS (gum Arabic -GA- and high methoxyl pectin -HMP-) mixed systems were studied at different OVAn-PS concentration ratio ($R_{OVAn:PS}$) and aqueous medium pH by means of a complementary techniques set: optical density at 400 nm (as a measure of turbidity), zeta potential and biopolymer phase composition determination. Biopolymer associative phase separation process was described in terms of OVAn and PS separation yield (Y_{OVAn} and Y_{PS}, respectively) and PS content necessary to precipitate OVAn (PS_p). Y_{OVAn} and PS_p results suggest that $R_{OVAn:PS}$ 1:1 and 2:1 for OVAn-GA and OVAn-HMP systems, respectively, and pH 3.0 were the most suitable conditions to obtain LA-OVAn-PS freeze-dried powders. Powders water dispersibility and LA oxidative stability were evaluated over 13 days (expressed as non-deteriorated LA percent -LA_{ND}-). Results revealed that water dispersion behavior of LA-OVAn-HMP powder was better than LA-OVAn-GA; besides it has the highest LA_{ND} (~80%) at the 13 day. These experimental findings highlighted that OVAn-PS associative phase separation was a convenient strategy, besides this information could be relevant to produce a LA functional ingredient.

1. Introduction

Currently, the encapsulation of polyunsatured fatty acids (PUFAs) employing biopolymer nanoparticles constitutes an interesting topic in food science and technology field. Protein-polysaccharide electrostatic complexes have been proposed as encapsulating matrices due to their relatively easy production and their advantages on PUFAs molecular preservation (Santiago & Castro, 2016; Semenova, 2016). In these supramolecular structures, protein is the vehicle in which PUFA is attached forming an inclusion complex (Joye & McClements, 2014), whereas polysaccharide (PS) is electrostatically deposited onto the surface of the protein-PUFA nanocomplex, conferring to the ligand additional protection against injurious factors, e.g. oxygen, UV radiation, etc. (Perez, Sponton, Andermatten, Rubiolo, & Santiago, 2015; Zimet & Livney, 2009). Several proteins were assayed as PUFAs vehicles (and other lipophilic bioactive compounds), e.g. β-lactoglobulin (Perez, Andermatten, Rubiolo, & Santiago, 2014; Zimet & Livney, 2009), β-conglicinin (David, Zagury, & Livney, 2015), α-lactalbumin (Kehoe & Brodkorb, 2014), zein, gliadin (Joye, Davidov-Pardo, Ludescher, & McClements, 2015) and ovalbumin (OVA) and their nanosized heat-induced aggregates (OVAn) (Sponton, Perez, Carrara, & Santiago, 2015a,b). Recently, OVA and OVAn were evaluated as nanocarrier systems to vehiculize linoleic acid (LA), which was taken as a model PUFA. Data strongly highlighted the increased LA binding ability of OVAn due to a greater exposition of hydrophobic residues promoted by thermal denaturation (Sponton et al., 2015a,b). Furthermore, Sponton Perez, Carrara and Santiago (2016) pointed out the appropriate conditions to produce LA-OVAn nanocomplexes, considering stoichiometric, kinetic and thermodynamic aspects.

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Nevertheless, in order to contribute to the knowledge about how is the vehiculization process of PUFAs, a study about OVAn-PS interactions in aqueous solution could be required. Hence, in this work, the interactions among OVAn with two PS commonly used in the food sector (gum arabic, GA, and high methoxyl pectin, HMP) is proposed.

Gum Arabic is an arabinogalactan-type polysaccharide exuded from the African tree *Acacia senegal*. It is composed by six carbohydrate moieties. The polysaccharides units are linked to a common polypeptide chain forming a “wattle blossom”-type structure. GA is negatively charged at pH > 2.2, whereas below this pH carboxyl group dissociation is suppressed (Weinbreck, de Vries, Schrooyen, & de Kruif, 2003).

Pectins are linear polysaccharides with negative charges due to galacturonic acid dissociation (Wicker, Kim, Kim, Thirkield, & Lin, 2014; de Jong & van de Velde, 2007). Pectins are divided in two groups according to the degree of methyl esterification (DE) of carboxylic groups. Pectins with DE > 50% are called high methoxyl pectins (HMP), whereas low methoxyl pectins (LMP) have DE < 50% (Ptaszek et al., 2015). The carboxylic groups along the backbone have a pKa ~3.6 (Humblet-Hua, Scheltens, van der Linden, & Sagis, 2011).

In terms of aqueous medium pH, different macromolecular interactions between protein and anionic PS could take place. In general, at pH > pI > pKa, mutual repulsion between protein and PS is due to the negative charges of both biopolymer. This behavior is known as cosolubility (Davidov-Pardo, Joye, & McClements, 2015; de Kruif, Weinbreck, & De Vries, 2004). When pH is around pI, association between protein cationic groups and PS anionic groups promotes electrostatic complexes formation. In general, the repulsion between PS residual negative charges promotes soluble complexes (Perez et al., 2015). These two kinds of interactions (cosolubility and soluble complexes) result in colloidal stable systems showing only one phase. However, at pKa < pH < pI, complex coacervation could occur due to the strong electrostatic attraction, and the complete neutralization of biopolymer charges (Weinbreck et al., 2003; Perez et al., 2014). This condition drives to associative phase separation, being one phase rich in both biopolymers and the other rich in solvent (Davidov-Pardo et al., 2015; Niu et al., 2014). Moreover, when pH is reduced too far, the coacervates precipitate, because they become closely packed together. Lastly, when pH < pKa cosolubility condition is newly reached due to the loss of charges of the polysaccharides (Davidov-Pardo et al., 2015). Associative phase separation involves both complex coacervation and precipitation. It is important to highlight that not only electrostatic interactions are involved in all these interactions. Hydrogen bonding, van der Waals and hydrophobic forces can also occur, depending on the biopolymer conformation and aqueous medium variables, e.g. pH and ionic strength (Fioramonti, Perez, Aringoli, Rubiolo, & Santiago, 2014; McClements, 2006).

On the other hand, in relation to biopolymer associative interactions, it is important to note that the strategy to produce biopolymer nanoparticles through protein-polysaccharide complexes is generally used at diluted biopolymer solution. Hence, it seem to be more appropriate, e.g., for PUFAs enriched beverages formulation (Zimet & Livney, 2009), limiting applications to others food matrices. The production and applications of nanoparticles in industry is still incipient, so an interesting alternative to increase their usage could be the production of biopolymer nanoparticles under powder form. However, this fact leads to the challenge of removing great water contents. In this sense, a strategy involving protein-polysaccharide associative phase separation could be adequate to concentrate biopolymer nanoparticles solutions, and consequently, to reduce energy consumption on the drying process. At the same time, PS addition could confer a protective effect on PUFAs-protein nanocomplexes (Zimet & Livney, 2009).

In this framework, the aim of this paper was to study the experimental conditions in which an associative phase separation process of OVAn-PS mixtures could take place in order to concentrate LA-OVAn nanocomplex solutions and to obtain a LA dried ingredient (powder). The associative phase separation process was evaluated in terms of: (i) two anionic PS, gum arabic (GA) and high methoxyl pectin (HMP), (ii) OVAn:PS concentration ratio ($R_{OVAn:PS}$) and (iii) aqueous medium pH. After this screening, the more appropriate conditions to promote associative phase separation were chosen and LA-OVAn-PS freeze-dried powders were obtained. In order to probe the success of the associative phase separation process, the dispersibility in water and oxidative stability of the freeze-dried powders were analyzed over 13 days. It is important to remark that there are not studies about protein-polysaccharide associative phase separation process applied to encapsulate PUFAs under powder form. Therefore, the present paper will give relevant information about technological aspects that could be useful to produce dried ingredients based on PUFAs.

2. Materials and methods

2.1. Materials

OVA (product A5503, purity 98% according to agarose gel electrophoresis) and LA samples were purchased from Sigma (USA). LA was kept under a N_2 atmosphere at −18 °C according to manufacturer advice. GA (*Acacia senegal*) was kindly supplied by Centro Enológico Rivadavia S.A. (Argentina). Its composition in dry basis (wt. %) was: 89.5% fiber, 4.5% sugar, 2.9% protein, 3.1% ash, and 0.05% fat. HMP (DE: 66–70%) was kindly supplied by Cargill™ (Argentina). Its composition in dry basis (wt. %) was: 74.7% fiber, 20.9% sugar, 2.2% protein, 2.2% ash and 0% fat. Deionized water (conductivity < 0.056 μS/cm) was used.

The solutions of Lowry Method to determine protein were (Hartree, 1972):

Solution A (1 g sodium-potassium tartrate, 50 g sodium carbonate and 250 mL 1 N NaOH diluted at 500 mL).

Solution B (2 g sodium-potassium tartrate, 1 g CuSO4·7H2O, 10 mL 1 N NaOH, and 90 mL water).

Solution C (1 volume of Foulin-Ciocalteu reagent diluted with 15 vol of water)

2.2. Ovalbumin nanoparticles production

OVAn was obtained by heat treatment (85 °C, 5 min) of 10 g/L OVA dispersion, at pH 7.5 and 50 mM NaCl, according to a previous work (Sponton et al., 2015a). It is important to remark that OVAn showed a monomodal particle size distribution (determined by dynamic light scattering, DLS) with a peak at 79 nm, and 0.208 polydispersity index (PdI), as it was reported in Sponton et al. (2015a).

2.3. Protein-polysaccharide interaction

In order to know the experimental conditions in which associative phase separation occur, a study of the macromolecular interactions in solution between OVAn and PS (GA and HMP) was firstly conducted. Results derived from this study were subsequently applied to concentrate LA-OVAn-PS particles solution. For this, OVAn dispersion (10 g/L) was diluted at 2 g/L with 50 mM pH 7.0 potassium phosphate buffer. Furthermore, GA and HMP stock solutions were prepared dispersing the proper PS amount in