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Scale-up production of vitamin loaded heteroprotein coacervates and their protective property

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ABSTRACT

The production of biocarriers for the protection and controlled delivery of bioactives is of great interest to develop functional foods. The ability of two whey proteins (WP), Beta-Lactoglobulin (BLG) and Lactoferrin (LF), to efficiently entrap vitamin B9 by complex coacervation has been reported at laboratory scale. In the present work, we report on the scaling-up production of B9 loaded BLG-LF coacervates (B9-WP coacervates). Complex coacervation was performed at bench scale, using commercial protein solutions. Under optimized conditions, B9-WP coacervates were produced by static mixing at a flow rate of 300 mL/min. Bench scale efficiency similar to that found for laboratory scale was reached with a coacervation yield of 65% and the B9 entrapment of 98%, demonstrating an efficient scaling-up. B9-WP coacervates showed good protection property for B9 during storage treatments, confirming the efficiency of this type of biocarrier for the development of natural functional foods.

1. Introduction

A current trend in the development of new food products is to add bioactives molecules in their formulation to provide specific nutritional and health benefits (vitamins, minerals, essential fatty acids) and/or added value (new flavors). The protection and controlled delivery of such molecules is a key factor to guarantee the final quality and properties of the resulting functional food products. As a result, the food industry have been investigating more and more controlled release technologies, with a special emphasis on microencapsulation technologies (Gouin, 2004). As one of the microencapsulation technologies, the complex coacervation of two biopolymers to form a biocarrier for third molecules is of great interest.

Complex coacervation is a liquid-liquid phase separation of a colloidal system occurring between two oppositely charged biopolymers through electrostatic interactions. It results in the formation of two phases: one is the polymer-poor continuous phase and the other is the polymer-rich dense phase, named coacervate. The concept behind the formation of biocarriers by complex coacervation is to first dispersed the bioactive in one of the initial biopolymer solution and then use the phase separation to newly form the coacervate phase containing the bioactive ingredient (Sánchez et al., 2016). The formation of biocarriers by coacervation presents numerous advantages for the protection of high-value and labile functional ingredients. It only requires mild preparation conditions with no use of neither organic solvent, drastic temperature nor high pressure which is of interest to reduce the industrial cost of biocarriers formation (Yan and Zhang, 2014). Coacervation also offers high shell integrity, high encapsulation efficiency and good controlled-release properties (Gouin, 2004). Moreover, this method has the advantage that a specialized equipment is not required to complete the coacervation process. Hence, it appears as potentially scalable to the industrial production of biocarriers (Shewan and Stokes, 2013). Numerous biopolymers systems have been studied to use complex coacervation as a technology to form biocarriers. Most of them studied the interactions between proteins and polysaccharides (Doublier et al., 2000; Schmitt and Turgeon, 2011) to form biocarriers for specific core bioactives such as essential oils or lipophilic vitamins (Matalanis et al., 2011), flavors (Koupantsis et al., 2014; Xiao et al., 2014) or probiotics (de Vos et al., 2010) for example. Nevertheless, few studies deal with the complex coacervation of a two proteins systems.

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Proteins are major nutrients for a balanced diet and contribute to the final structure, texture and stability of foods (Doublier et al., 2000). They are generally recognized as safe (GRAS) by the food industry and present excellent functional properties (Chen et al., 2006). Thus, they stand as interesting biomaterials for biocarriers. Among food proteins, whey proteins display interesting properties as biomaterials (Tavares et al., 2014). They are also a natural by-product from the dairy industry. Thus, it is relevant to seek enhanced functionalities for them, creating benefit to the sector. Diverse strategies have been investigated to exploit whey proteins as biocarriers for bioactive molecules, such as the formation of simple complexes with ligands, the formation of gel networks, or their complex coacervation through electrostatic interactions (Bouhallab and Croguennec, 2014; Diarrassouba et al., 2015; Schmitt et al., 2009).

The potentiality of using the complex coacervation of two whey proteins (WP), the lactoferrin (LF) and the beta-lactoglobulin (BLG), to form a biocarrier for a bioactive, the vitamin B9 (B9), was previously investigated (Chapeau et al., 2016). This work had established the proof of concept that LF and BLG can spontaneously co-assemble in presence of B9 by complex coacervation. The complex coacervation of WP with B9 occurred preferentially for mixing solutions of medium proteins concentrations (10–15 g/L) and lead to the formation of spherical supramolecular co-assemblies from 8 to 15 μm, appearing as small droplets suspended in solution. These small droplets tend to coalesce into a dense phase called *the B9-WP coacervate*. This dense phase can be obtained by the centrifugation of the coacervated solution of B9-WP. The B9-WP cocervates recovered after centrifugation is highly concentrated in whey proteins (≈320 g/L) and B9 (4–16 mg B9/g proteins) (Chapeau et al., 2016). Other encapsulation technologies such as the nano-spraydring or the electrospraying of whey proteins showed similar biocarrier efficiency for B9 (Pérez-Masiá et al., 2015). Thus, it was concluded that B9-WP coacervate demonstrated good potential as biocarrier for B9. Nevertheless, this work was carried out at a laboratory scale (μL). For a possible industrial application of B9-WP coacervate, the scale-up production to bench scale (L) has to be considered. However, coacervation is known to be a process highly dependent of the biomaterials system involved and each coacervation system operates under a unique set of conditions (Yan and Zhang, 2014). Moreover, the scale-up of the complex coacervation process is often achieved empirically, leading to possible trial and errors setup and may involve expense of cost and resources (Lemetter et al., 2009; Paul et al., 2004). That is why the specific conditions of complex coacervation of such proteins systems have to be specifically investigated, especially when industrial applications are considered to maximize process yield and minimize loss and batch-to-batch variations (Thies, 2007).

The aim of this study was to investigate the scale-up production of B9-WP coacervates from laboratory scale (μL) to bench scale (L) using batch and continuous mixing systems. Both systems allowed obtaining encapsulation yields for B9 up to 98%, similarly to yields obtained at the μL scale. Coacervation yields of whey proteins varied from 55% for the batch system (similar at μL scale) and up to 65% for the continuous system, which is higher than the yield obtained at μL scale. Finally, the protective effect of B9-WP coacervates against B9 degradation was shown for two types of food storage treatments, freezing and freeze-drying.

2. Materials and methods

2.1. Materials

Vitamin B9 (B9) was purchased from Sigma Aldrich (Folic acid, purity > 97%, Sigma Aldrich, St. Louis, MO, USA). Lactoferrin (LF) from bovine milk, (purity 90% and iron saturation level of 10%–20%) was purchased from the Fonterra Cooperative Group, New Zealand. LF powder was used without further modification. Beta-Lactoglobulin (BLG) powder was obtained from a confidential industrial source. Its composition (w/w) was: protein 93.5%, moisture 4% and ash < 1.8%. Protein purity was determined by reversed-phase HPLC and no proteins other than BLG were detected.

2.2. Stock solutions

B9 stock solution was prepared by solubilizing the vitamin powder in milliQ water and then adjusted to pH 5.5 using 1 M HCl solution. B9 solution was centrifuged at 28,000 g for 30 min at room temperature (centrifuge Hereaus Biofuge primo, KENDRO Laboratory products, Courtaboeuf, France) to remove the insoluble fraction of B9. The exact vitamin B9 concentration was determined by absorbance at 283 nm (spectrometer UVmc^2, Safas, Monaco) using 25.1 L g^{-1} cm^{-1} as extinction coefficient.

Before use, BLG was purified. BLG powder was dispersed in deionized water (45 g/L), adjusted to pH 4.6 with 1 M HCl and kept at 30 °C for 5 min in order to precipitate non-native forms of BLG. The dispersion was centrifuged at 20,000 g for 10 min at room temperature (Heraeus Biofuge Primo, Thermo Scientific, Waltham, MA, USA). BLG suspension was then freeze-dried and stored at -20 °C until use. Then, LF and BLG stock solutions were prepared by solubilizing the protein powders in milliQ water adjusted to pH 5.5 using 1 M HCl. The protein solutions were filtered through a 0.45 mm and a 0.2 mm membrane (cat. no. 4612, Pall Corporation, Ann Arbor, MI, USA). The exact proteins concentrations were determined by absorbance at 280 nm (spectrometer UVmc^2, Safas, Monaco) using 1.47 L g^{-1} cm^{-1} and 0.96 L g^{-1} cm^{-1} as extinction coefficients for LF and BLG respectively. B9, LF, and BLG stock solutions were stored at 4 °C and protected from UV light radiation.

2.3. Production of B9-whey protein coacervates

The production diagram of B9-whey proteins (B9-WP) coacervates is depicted in Fig. 1A. First, a solution of B9-LF complex was prepared by blending B9, LF and milliQ water, to have final concentrations of 4.15 g/L for LF and 0.11 g/L for B9. The B9-LF complex solution was let to equilibrate by itself for 10 min at 20 °C. Then, BLG solution was added to the B9-LF complex solution to reach a final BLG concentration of 9.15 g/L, in order to induce complex coacervation. Addition of BLG was performed under either batch or continuous mixing conditions. The coacervated phase recovered by centrifugation at 38,000 g for 45 min at 20 °C (Centrifuge Avanti J-26S XP, Beckman Coulter Inc., Brea, CA 92821 USA), was weighed and kept in the dark at 4 °C.

Batch mixing by mechanic stirring was performed by stirring the solution in a vessel containing a propeller pale of 2.5 cm diameter, with 3 blades (Fig. 1B). The propeller was set in rotational motion by the mean of an electric motor set at the desired stirring speed, during 2 min. Unstirred solution was also equilibrated during 2 min prior centrifugation.

Continuous mixing by static stirring was made using a static mixer (Fig. 1C) constituted by a series of 10 static mixer units of 0.4 × 0.6 cm diameter, set in-line, for a total length of 6 cm. The input pipes for B9-LF and BLG solutions were of 0.4 cm diameter and 30 cm length. The output pipes for B9-WP coacervates solution was of 0.2 cm diameter. According to preliminary tests to set the system, permanent regime of the static mixer was achieved after 20 s. Aliquots were withdrawn over time for further analyses.