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Egg albumin–folic acid nanocomplexes: Performance as a functional ingredient and biological activity

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ABSTRACT

The ability of egg white (EW) nanoparticles to bind folic acid (FA) and protect it through the gastrointestinal tract and the resulting properties of the mixtures as functional ingredient was investigated. Two kinds of EW nanoparticles (USN and TSN) were mixed with FA to generate nanocarriers (USF and TSF). The particle size distribution of USN remained unaltered after the binding of FA, while a little increase in particle size was observed for TSN. Zeta (ζ) potential and fluorescence intensity did not show any significant change after FA addition for both nanoparticles. The percentage of bound folic acid (% BFA) was 78.0 ± 9.1 and 79.7 ± 9.0, for USF and TSF, respectively. A slight formation of aggregates in the samples was observed after freeze-drying and redispersion of the nanocarriers, which was also confirmed by confocal laser scanning microscopy. Nanocarriers particle size did not change after adjusting the pH from 3 to 4, but strongly increased after adjusting it to 5, 6 or 7. The % BFA at pH 4 was similar to that at pH 3, but greatly decreased at pH 7. The bioavailability of FA for *Lactobacillus rhamnosus* was enhanced when the vitamin was incorporated in the form of digested nanocomplexes USF or TSF. The interaction of EW nanoparticles with FA has proven to be beneficial for the transport and release of FA after *in vitro* digestion.

1. Introduction

In recent years, the food industry has increasingly incorporated bioactive compounds to their manufactured products. These compounds are usually highly susceptible to environmental, processing and/or gastrointestinal conditions and therefore, encapsulation has been proposed as an approach for their effective protection (Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011). Food proteins can be used to prepare a wide range of matrices and multicomponent matrices in the form of hydrogel, micro- or nanoparticles, all of which can be tailored for specific applications in the development of innovative functional food products. The ability to control the particle size of proteinaceous materials is of primary importance not only for determining food properties such as taste, aroma, texture,

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and appearance, but also for determining the release rates of the carried bioactive compounds and ultimately how much is absorbed into the body and hence the overall efficacy of the compounds (Chen, Remondetto, & Subirade, 2006).

Folate, an important B-group vitamin, participates in many metabolic pathways such as DNA and RNA biosynthesis and amino acid interconversions. It is involved in essential functions of cell metabolism such as DNA replication, repair and methylation and synthesis of nucleotides, amino acids, and some vitamins. Mammalian cells cannot synthesize folate; therefore, an exogenous supply of this vitamin is necessary to prevent nutritional deficiency (Iyer & Tomar, 2009). Thus, the World Health Organization established the recommended daily amounts (RDA) of 400 dietary equivalents (DFE) for adults, 600 DFE for pregnant women, and 500 DFE for nursing mothers (World Health Organization, 2006). For this reason and due to the appearance of deficiencies in the general population, the FDA has recommended fortifying commercial flours with folic acid (FA) since 1998 (Food and Drug Administration, 1996). The folate form commercially used in supplements and fortified foods is the fully oxidized monoglutamate form, FA. In aqueous solution, FA is stable at 100 °C for 10 h in a pH range of 5.0–12.0 when protected from light, but becomes increasingly unstable as the pH decreases below 5.0 (Akhtar, Khan, & Ahmad, 1999; Paine-Wilson & Chen, 1979). Alkaline hydrolysis under aerobic conditions promotes oxidative cleavage of the FA molecule to yield *p*-aminobenzoylglutamic acid and pterine-6-carboxylic acid, whereas acid hydrolysis under aerobic conditions yields 6-methylpterin (Tannenbaum, Archer, & Young, 1985). FA is also sensitive to light exposure in aqueous solutions and is degraded to pterine-6-carboxylic acid and *p*-amino-benzoyl-L-glutamic acid; the rate of photolysis is gradually decreased on moving from the acid to the alkaline region (Akhtar et al., 1999; Akhtar, Khan, & Ahmad, 2003).

Many researchers have focused on the study of the interaction between FA and proteins in order to generate complexes for different applications (Bourassa, Hasni, & Tajmir-Riahi, 2011; Jha & Kishore, 2011; Pérez, David-Birman, Kesselman, Levi-Tal, & Lesmes, 2014), for target delivery of nanoencapsulated drugs (Teng, Luo, Wang, Zhang, & Wang, 2013; Zhao et al., 2010) and to provide FA protection against photodegradation (Liang, Zhang, Zhou, & Subirade, 2013), among other purposes. However, the interaction between FA with egg white (EW) proteins has never been investigated yet.

High intensity ultrasound (HIUS) technology has been effective in controlling particle size of different biopolymers. In a previous research, EW nanoparticles have been developed by application of HIUS or thermosonication (Arzeni, Pérez, & Pilosof, 2015). By applying HIUS without heating for 20 min to an EW solution at pH 3, a monomodal population of 220 nm of diameter was obtained as evaluated by dynamic light scattering. On the other hand, when applying thermosonication at 85 °C for 20 min to an EW solution at pH 3 it was possible to obtain a bimodal distribution, with particles of 295 and 70 nm of diameter, the last one being the main population of the distribution. These two kinds of nanoparticles would have the potential to act as food grade carriers for FA. Therefore, the objective of this work was to assess the ability of EW nanoparticles to bind FA and protect it through the gastrointestinal tract and to evaluate the resulting properties of the mixtures as functional ingredients.

2. Materials and methods

2.1. *Preparation of EW and FA solutions*

EW powder was provided by Ovoprot International S.A. (Buenos Aires, Argentina) and was used as starting material. The moisture content was 5.66% (wet basis) and the pH was 7.34, as specified by the manufacturer. The protein content (total basis) of the powder was 88.93 ± 1.18% (N × 6.25) (AOAC, 1980). A thorough characterization of reconstituted EW (10% (w/w) solutions) was carried out in a previous research (Arzeni et al., 2012). Solutions at 5% (w/w) were prepared with double distilled water. Sodium azide (0.02% w/w) was added in order to prevent microbial growth, except for the determinations described in Sections 2.5 and 2.6. Solutions were centrifuged for 1 h at 12,857 × *g* and 20 °C (Centrifuge 5804 R, Eppendorf, Hamburg, Germany). The supernatant was used for the determinations and the pH was adjusted to 3 with 1M HCl.

FA powder (DSM Nutritional Products Argentina S.A., Buenos Aires, purity: 99.5%, dry basis) was kindly provided by Laboratorios Bagó S.A. (La Plata, Argentina). FA solutions at 1% (w/w) were prepared daily for all the experiments. The pH was adjusted to 7 with NaOH 1N (for mixtures) or to 3 with HCl 1N (control). The solutions were covered with aluminum foil to prevent exposure to light.

2.2. *Design of EW nanocarriers*

EW nanoparticles were designed according to a previous work (Arzeni et al., 2015). Briefly, EW solutions (5% (w/w), pH 3 and 7) were sonicated for 5, 10, 15 and 20 min using an ultrasonic processor Vibra Cell Sonics, model VCX 750 (Newtown, CT, USA) with a maximum net power output of 750 W at a frequency of 20 kHz and an amplitude of 20% (maximum amplitude 40%, 228 μm). Temperature was kept constant at 0.5 °C to dissipate the heat produced during sonication in order to evaluate the effect of HIUS alone or, at 80 and 85 °C, for thermosonication treatment. In order to generate EW nanocarriers for FA, an aliquot of 100 μl of FA solution (1% (w/w), pH 7) was added to 4 ml of EW nanoparticles, with continuous stirring for 5 min. The ratio of FA:EW nanoparticles was 1:200 (v/v). The mixtures were covered with aluminium foil and prepared daily for each experiment.

2.3. *Particle size and zeta potential determinations*

Particle size was measured by dynamic light scattering (DLS) with a Zetasizer Nano-Zs analyser from Malvern Instruments (Worcestershire, UK) as described elsewhere (Martínez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2009). The samples were diluted at 0.1% (w/w) in double distilled water and measured without any further filtering. In the present work, the mean particle size of each peak is reported in intensity and the plot of the distribution is reported in volume, in order to visualize the relative contribution of each population.

Measurements of zeta potential (ζ) were made with the same analyzer at a fixed angle of 17°. The solutions were diluted at 0.01% (w/w) with double distilled water and placed into special folded capillary cells (DTS1060C, Malvern Instruments,