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Iron-entrapped niosomes and their potential application for yogurt fortification

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ABSTRACT

Several formulations were tested for iron-entrapped niosomes with high stability and encapsulation efficiency. A modified ethanol injection method was selected for niosomes preparation.

Niosomes formulated with Glycerol monooleate, Polyglyceryl-3 dioleate or Sorbitan monooleate as food-grade surfactants and dodecanol as membrane stabilizer showed iron encapsulation efficiencies in the 72–84% range.

Niosomes stability against simulated gastric fluid and simulated intestinal fluid was evaluated by measuring the entrapment efficiency and oxidation of iron.

The best formulations were selected for preparation of iron-fortified yogurts. Sensory, rheological and stability properties of the control yogurt were only slightly affected by iron-entrapped niosomes. These results demonstrated that yogurt could be a suitable vehicle for the iron-entrapped niosomes formulated in this work.

1. Introduction

Iron is an essential nutrient in the human diet and its deficiency has adverse effects on health. Iron is a component of several proteins, including enzymes and hemoglobin, which are essential for the transport of oxygen to tissues (Wang & Pantopoulos, 2011).

Iron homeostasis must be regulated strictly to maintain a stable and functional environment in the body. It is necessary to keep the balance between iron absorption and iron release.

Iron deficiency can result from inadequate iron intake, low iron absorption, and increased iron demand or loss. Children, teenagers, and women of childbearing age are especially prone to develop iron-deficiency anemia (Denic & Agarwal, 2007). Currently, oral iron supplements, such as ferrous sulphate, ferrous gluconate, and ferrous fumarate, are commonly used to treat iron deficiency-related diseases. However, oral iron supplements often are absorbed ineffectively and potentially can cause adverse effects in the gastrointestinal tract, primarily due to the oxidative toxicity of ferrous iron (Cancelo-Hidalgo et al., 2012).

Iron-enriched functional foods could play an important role in the prevention of iron deficiency, when the contribution of dietary iron is insufficient, especially in high-risk groups (Derbyshire, Brennan, Li, & Bokhari, 2010).

Fortification of dairy products has always been considered one of the best approaches to prevent iron-deficiency disorders and their properties depend on both the type of mineral source and the amount of added compound (Gaucheron, 2000). Two principal off-flavours are exhibited by fortified yogurt: oxidized flavour and metallic flavour, which are due to the catalytic role of iron and the presence of iron salts, respectively (Jackson & Lee, 1991). However,

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fortified yogurt by casein-chelated iron or by whey protein-chelated iron showed similar organoleptic properties with respect to unfortified yogurt used as control (El-Kholy, Osman, Gouda, & Ghareeb, 2011; Hekmat & McMahon, 1997; Jackson & Lee, 1991). Recently, preparation of whey protein-iron/zinc complexes has been standardized on the basis of maximum mineral binding ability of proteins (Shilpashree, Arora, & Sharma, 2016).

New strategies have been developed to avoid direct contact of iron compounds with food such as the technology called "GrowthPlus" (Mehansho, 2006), iron liposome encapsulation (Ding, Xia, Hayat, & Zhang, 2009; Ding et al., 2011; Xia & Xu, 2005; Xu et al., 2014; Yuan et al., 2013), and iron microencapsulation (Gupta, Chawla, & Arora, 2015; Gupta, Chawla, Arora, Tomar, & Singh, 2015; Kim, Ahn, Seok, & Kwak, 2003; Nkhata, Ustunol, & Menevseoglu, 2015).

Encapsulation of iron in vesicles and other systems not only helps to reduce organoleptic problems of iron-fortified foods, but it also protects iron from oxidation caused by external agents, enhancing its bioavailability and intestinal absorption (Zimmermann, 2004). In addition, lipid oxidation is reduced using encapsulated iron, as it was demonstrated by comparison with non-encapsulated iron fortified yogurt (Gahruie, Eskandari, Mesbahi, & Hanifpour, 2015).

Niosomes are a novel type of nanovesicles formed by the self-assembly of non-ionic surfactants in aqueous media resulting in closed bilayer structures (Uchegbu & Vyas, 1998). These vesicles can entrap both hydrophilic and lipophilic compounds leading to a large number of applications in food, pharmaceutical and cosmetic industries. Hydrophilic compounds are entrapped in the aqueous compartments between the bilayers while the lipophilic components are preferentially located within the surfactant bilayer (Devaraj et al., 2002). Niosomes show clear advantages with respect to the phospholipid compounds typically used in liposomes, because of their lower cost and higher stability during storage (Kopermsub, Mayen, & Warin, 2011; Marianecci et al., 2014).

The goal of this work was to formulate and produce niosomes containing ferrous sulphate for yogurt fortification without compromising its taste, appearance, and stability. Several formulations were tested to prepare iron-entrapped niosomes with high stability and encapsulation efficiency. Stability against simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were also measured. The best formulations were selected for subsequent preparation of iron-fortified yogurts. Textural and rheological properties of these yogurts were analysed and compared to a regular yogurt as control.

2. Materials and methods

2.1. Materials

Ferrous sulphate heptahydrate, citric acid anhydrous, disodium phosphate, hydrochloric acid (370 mL/L), sodium acetate trihydrate, sodium hydroxide and potassium phosphate monobasic were supplied by Panreac (Madrid, Spain). Ascorbic acid was purchased from J.T. Baker, Avantor (Center Valley, USA). Sorbitan monooleate (Span 80, S80) (hydrophilic-lipophilic balance = 4.3), lauryl alcohol or 1-dodecanol (D), absolute ethanol, hydroxylamine hydrochloride (990 g/kg) were supplied by Sigma-Aldrich (Saint Louis, USA). Polyglyceryl-3 Dioleate (Peceol, P) (hydrophilic-lipophilic balance = 1) and Glycerol monooleate, (Plurol Oleique, PO) (hydrophilic-lipophilic balance = 3) were a gift from Gattefossé (Nanterre, France). HPLC-grade methanol was obtained from Sigma-Aldrich (Saint Louis, USA). 1,10-phenanthroline monohydrate and sodium chloride were purchased from Merck (Darmstadt, Germany). BiPro whey protein isolate was supplied by Davisco Foods International, Inc. (Le Sueur, USA), Deionized water was used in all experiments.

2.2. Niosomes preparation

Iron-containing niosomes were prepared by a modified ethanol injection method (EIM). The conventional ethanol injection method, first described in 1973 (Batzri & Korn, 1973), offers advantages such as simplicity, absence of potentially harmful chemicals, and suitability for scaling-up (Pham, Jaafar-Maalej, Charcosset, & Fessi, 2012; Wagner, Vorauer-Uhl, Kreismayr, & Katinger, 2002).

The aqueous phase was a solution of ferrous sulphate, citric acid, ascorbic acid and disodium phosphate. The relative amounts of each compound were 300 mol/L of ferrous sulphate, 100 mol/L of ascorbic acid, and 200 mol/L of citric acid. The total iron concentration was 0.4 g/L.

The organic phase was prepared by dissolving appropriate weighed amounts of surfactants (P, PO, S80) and membrane stabilizer (D) at the same concentration into absolute ethanol. The surfactant concentration was always 2.4 g/L and therefore the total concentration of niosome membrane components was 4.8 g/L.

Then, this solution was injected, with a syringe pump (KD Scientific, Holliston, USA) at a flow of 130 mL/h, into the aqueous phase at 40 °C, stirring at 5000 rpm with a homogenizer (Silent-Crusher M, rotor model 22G, Heidolph, Schwabach, Germany). Although spontaneous niosomes formation occurred as soon as the organic solution was in contact with the aqueous phase (Pham et al., 2012), a vigorous agitation provided narrower size distributions. Once niosomes were formed, ethanol was removed in a rotary evaporator, Büchi R205 (Sigma-Aldrich, Saint Louis, USA), operating at low pressure (4500 Pa).

2.3. Niosomes size and zeta potential measurements

Mean (Z-Average) size and polydispersity index (PDI) of niosomes were determined via Dynamic Light Scattering using a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK). Three independent samples were taken from each formulation, and each sample was measured three times at room temperature without dilution.

Three independent samples were taken from each formulation and measured three times at room temperature to determine the zeta potential. Each of them was diluted until a concentration of 100 mL/L before measuring vesicle electrophoretic mobility by means of the M3-PALS (Phase Analysis Light Scattering) technique. High absolute values of zeta potential indicate electrostatic repulsion between vesicles, which results in high stability of the niosomal system.

2.4. Niosomes morphology

Morphological analysis of niosomes was carried out by Negative Staining Transmission Electron Microscopy (NS-TEM), using a JEOL-2000 Ex II TEM (Tokyo, Japan). A drop of the niosomal formulation was placed on a carbon-coated copper grid, and the sample excess was removed with filter paper. Then, a drop of 20 g/L phosphotungstic acid solution was applied to the carbon grid and left to stand for 2 min. Once the excess staining agent was removed with filter paper, the sample was air-dried and the thin film of stained niosomes was observed with the transmission electron microscope.

2.5. Niosomes stability

The stability of niosomes was determined by measuring