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Preparation and characterization of iron-alginate beads with some types of iron used in supplementation and fortification strategies

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ABSTRACT

Our work aimed to develop and characterize different alginate beads with either non-heme iron or a blend of heme/non-heme iron. We chose non-heme iron salts such as ferrous sulfate (FS), ferrous ammonium sulfate (FAS), ferric citrate (FC), ferrous fumarate (FF), and ferrous bis-glycinate chelate (FCH) at different concentrations as a source of iron for our beads. We also chose spray-dried blood cells (SDBC) as a source of heme iron to be mixed with non-heme iron sources for the development of blend beads. FS, FAS and FC did not form beads by the traditional method of external ionic gelation, unlike FF, FCH and their blends with SDBC, which did form beads for every solution concentration. These beads were characterized by iron content, encapsulation efficiency (EE%), size, color, structure by FTIR, morphology, swelling studies and *in vitro* iron release studies. Blend beads showed a spherical shape, more homogeneous surface, high iron content (31.3 ± 1.4 to 61.1 ± 4.4 mg Fe/g dried beads) and high EE% (57.6 ± 7.7% to 78.5 ± 2.9%). Major structural interactions were of hydrophilic nature, for all beads. Under simulated gastric incubation conditions, blend beads showed higher stability and released less iron (11–13%) than FF and FCH-alginate beads (19–23%). Under simulated intestinal incubation conditions, all beads released their iron content over a 3 h period.

1. Introduction

Iron deficiency anemia continues to be the most prevalent nutritional deficiency in the world, affecting about 29% of population (World Health Organization, 2015). This deficiency usually results from insufficient dietary iron intake due to consumption of plant-based diets containing low levels of heme iron (Carpenter & Mahoney, 1992; Heath & Fairweather-Tait, 2002). Bioavailability of heme iron is greater than that from non-heme iron (Conrad & Umbreit, 2000). Nonetheless, fortification and supplementation strategies have been implemented and they have had an important beneficial effect. Yet iron deficiency remains very prevalent in developing countries (Galicia, Grajeda, & López de Romaña, 2016), and these strategies appear to have had little impact until recently (Lynch, 2005). Discontinuation of supplementation therapy or consumption of iron-fortified foods by patients is explained by some of the following reasons: organoleptic problems generating free iron in foods (Douglas, Rainey, Wong, Edmondson, & La Croix, 1981; Hurrell, 2002), decreased bioavailability due to interactions of iron with other components of the diet at the gastrointestinal level (Conrad & Umbreit, 2000), therapy oblivion, low tolerance to iron (Coplin, Schuette, Leichtmann, & Lashner, 1991), and gastrointestinal disorders (Hallberg, Ryttinger, & Solvell, 1966).

Encapsulation technology has brought improvements regarding several of the above mentioned problems. The main encapsulation method used for iron supplementation or fortification strategies is its entrapment in liposomes (Mehansho, 2006; Zimmermann, 2004). However, as liposomes are thermodynamically unstable they will aggregate, fuse, flocculate and precipitate during storage (Zuidam, 2012). In that regards, Kokkona, Kallinteri, Fatouros, and Antimisiaris (2000) state that when faced with intestinal-like conditions i.e. the presence of biliary salts and pancreatin enzymes, liposomes that are composed of lipids with low transition temperatures become highly unstable and release its contents. An alternative to liposomes, the gelation ionic method to form alginate beads has been widely studied in food but sparsely investigated to encapsulate iron forms for supplementation or fortification purposes (Al Gawhari, 2016; Perez-Moral, Gonzalez, & Parker, 2013; Valenzuela, Hernández, Morales, & Pizarro, 2016; Valenzuela,

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Hernández, Morales, Neira-Carrillo, & Pizarro, 2014). This encapsulation method could be promising as it has exhibited several desired properties in this regard, namely a high encapsulation efficiency for heme iron sources such as spray-dried blood cells (SDBC) (Valenzuela et al., 2014), low iron release in gastric conditions, and high iron release in the duodenal medium (Perez-Moral et al., 2013; Valenzuela et al., 2016).

Spray-dried erythrocytes constitute a good and safe source of heme iron (Toldrà, Elias, Parés, Saguer, & Carretero, 2004) that can be encapsulated by means of ionic gelation, though the iron content of these beads is very low (Valenzuela et al., 2014). This inconvenience however, could be improved upon by their mixture with non-heme iron sources. Among the main kinds of non-heme iron currently used for oral supplementation or fortification strategies we can find ferrous sulfate (which is recognized as the gold standard), other iron salts (such as ferrous gluconate, ferrous fumarate, ferrous lactate, ferric ammonium, ferric citrate), and protected iron in the form of Na-Fe-EDTA and ferrous bis-glycinate chelate (Hurrell, 1997; Mehansho, 2006). In the case of ferrous bis-glycinate chelate, this has been shown to have a higher bioavailability than ferrous sulfate and has also been widely used in oral fortification (Pineda & Ashmead, 2001).

To the best of our knowledge, there have been no studies about the encapsulation of blended heme iron sources with the main sources of non-heme iron that are currently used to prevent iron deficiency anemia. This blend could improve the bioavailability of the resulting formulation and therefore, we have aimed in this study to develop and characterize different alginate beads with either non-heme iron or blends of heme and non-heme iron sources.

2. Material and methods

2.1. Material

Sodium alginate (viscosity of 25.7 cps at 25 °C, 2 g/100 mL solution) was purchased from Sigma-Aldrich, USA, and was used as wall material.

Common supplementation and/or fortification iron salts were used as core material. Non-heme iron salts used were: ferrous sulfate heptahydrate (FS), ferrous ammonium sulfate (FAS), ferric citrate (FC), ferrous fumarate (FF) and ferrous bis-glycinate chelate (Ferrochel®) (FCH). These iron salts were purchased from Merck S.A. with the sole exception of FCH, which was purchased from Albion Laboratories Inc, Clearfield, Utah. Heme iron was sourced from bovine spray-dried blood cells (SDBC) and purchased from Licán Alimentos S.A. This multinational company follows the strictest international hygiene and quality standards (HACCP Codex Alimentarius, GMP, ISO 9001:2008-UKAS) when performing animal blood harvest procedures as well as when processing spray-dried blood cells, which are also subjected to thermal processing (140 °C on entry and 80–90 °C on exit) to reduce any microbial load present in them (Toldrà et al., 2004). This treatment along with the afore mentioned industry certifications allow for this product to be deemed a food ingredient that is safe for human consumption.

Total iron content was determined for all core materials through atomic absorption spectrophotometry techniques. Reagents were all of analytical grade and purchased from Merck S.A. Bile extract and pancreatin (trypsin, amylase, lipase, ribonuclease, and protease) were purchased from Sigma-Aldrich, USA.

2.2. Iron beads preparation

Control beads were prepared without including any iron form in them. Non-heme iron beads were prepared following a three-step procedure: suspending iron salts in a water solution, mixture with a gelification solution, and bead shaping and drying. A sodium alginate solution (2% w/v in deionized water) was chosen as solvent for suspending FS, FAS and FC iron salts at 0.05, 0.1, 0.5 and 1% w/v. On the other hand, FF and FCH salts were suspended at 1, 2 and 3% w/v. Afterwards, these suspensions were collected in a tuberculin syringe and dropped into a gelling solution made from calcium chloride in deionized water (5% w/v). Finally, beads were formed and then deposited in plastic boxes to be dried until reaching a constant weight at a temperature of 40 °C (≈10 h). Once dried, these beads were removed from their boxes and stored at environmental conditions.

As those suspensions based on FS, FAS and FC did not form beads, only FF and FCH were used to prepare blended non-heme/heme iron beads. The same process as outlined above was repeated with the sole difference that a blend of FF and FCH suspensions at 1, 2 and 3% w/v with SDBC at 10% w/v became the basis to prepare the blend beads.

2.3. Appearance and color

Beads appearance was captured with a Sony DSC-HX1 (Sony Corporation, Japan) digital camera, their color was measured and registered on triplicate according to the Hunter Lab color scale (N = 30 for each replicate) with a Konica-Minolta CR-300 (Konica Minolta Inc, Japan) colorimeter.

2.4. Iron content

Beads total iron content was determined through acid digestion (method 999.11) (AOAC, 1996) coupled with an atomic absorption spectrophotometer (GBC, 905AA, Australia). Spectrophotometric measurements were performed on triplicate validated against a standard curve assessed at λ = 248.3 nm, using a commercial iron standard, 1000 μg/mL (J.T. Baker, USA).

2.5. Encapsulation efficiency (EE%)

Following Valenzuela et al. (2014) method, EE% was determined calculating the difference between theoretical iron content of each suspension and the total iron content for beads according to Equation (1).

To determine theoretical iron content, we poured ten drops of each suspension into 10 mL of deionized water and mechanically stirred continuously to homogenize it. These homogenized solutions were then subject to atomic absorption spectrophotometry to measure their iron content.

On the other hand, we proceeded to determine total surface iron of 10 beads which were filtered through a Whatman (N°2) paper filter to remove the gelling solution, and then the cake was dispersed in 10 mL of deionized water. These dispersions were sonicated twice, 20 min each time, using an ultrasonic bath (Elmasonic E08.2011, Germany) and samples were then centrifuged at 2900xg for 10 min. The amount of surface iron released into solution was also quantified through atomic absorption spectrophotometry techniques.

$$EE = \frac{Fe_t - Fe_s}{Fe_t} \times 100 \quad (1)$$

where: EE is encapsulation efficiency, Fe_t is the encapsulated theoretical iron and Fe_s is the amount of total surface iron.