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Development of Iron Alginate Beads by Ionotropic Gelation for Nutritional Applications

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Abstract

Iron deficiency is the most common micronutrient deficit worldwide that affects people in both developed and developing countries. Food fortification products have been one of the most popular ways to alleviate iron deficiency anemia but challenges remain due to the delicate balance between iron bioavailability and stability in foods. Ferrous iron (Fe^{2+}) compounds are generally well absorbed in humans but cause unacceptable changes when added to foods. Ferric iron $\left(Fe^{3+}\right)$ compounds, on the other hand, are less reactive in food but also tend to be less bioavailable. The binding of iron to biopolymers such as alginate may reduce the reactivity in foods while enhancing iron uptake in the body. Alginate is a biopolymer found in brown algae and is widely used in the food industry as thickening agent. When added to a solution containing divalent $\left(M^{2+}\right)$ or trivalent $\left(M^{3+}\right)$ metal ions, alginate spontaneously forms beads through ionotropic gelation. Here, we used ionotropic gelation to prepare iron alginate beads, and the effect of iron type $\left(Fe^{2+}\right.$ or $\left.Fe^{3+}\right)$ on bead structure, iron release, and stability in foods was studied. The iron alginate beads were characterized for their size (ImageJ software), iron content (atomic absorption spectrophotometry), and structural interactions (FTIR spectroscopy). The iron release was measured in simulated gastrointestinal conditions and reactivity of the iron beads was assessed in difficult-to-fortify foods. We demonstrate that the iron oxidation state greatly influenced the bead structure, with Fe^{3+} producing smaller beads. The Fe^{3+} alginate beads were less reactive in foods than Fe^{2+}-containing beads but had a slower iron release in simulated gastrointestinal conditions. The alginate beads contained a high iron content of 10-15 wt %. In conclusion, the properties of iron alginate beads can be tuned by the Fe^{2+}/Fe^{3+} content, making the iron alginate beads a promising approach to overcome the challenges with conventional food fortificants.

KEYWORDS: Iron deficiency, alginate, food fortification, reactivity

1 INTRODUCTION

Iron (Fe) deficiency is the most common dietary deficiency in the world, and it is a public health issue in both developed and developing countries. Almost all organisms require Fe as a trace metal. Iron plays key metabolic roles in humans, including binding oxygen to hemoglobin and serving as the catalytic core of numerous enzymes (e.g., cytochromes). The

median Fe intake of adults is approximately 12-18 mg/day, which is mostly utilized to create hemoglobin in erythrocytes (Food and Nutrition Board, 2001). Iron is also important for a variety of cellular and tissue processes, including respiration, mitochondrial function, energy production, cell division, and DNA repair (Cappellini et al., 2020). The absorption level in the small intestine regulates the body's Fe levels (Zimmermann and Hurrell, 2007). According to the World Health Organization (WHO), Fe deficiency causes anemia in up to 2 billion people worldwide, or around 30% of the population (World Health Organization, 2008). Iron deficiency anemia affects about two-thirds of children in developing countries, compared to 1-2% in developed countries (Parkin et al., 2016).

The two most common ways to meet the body's Fe requirements are to take supplements and to consume fortified foods. High-risk groups can benefit from Fe supplementation (e.g., pregnant women). However, if supplements are used too frequently and in large amounts, side effects such as diarrhea and vomiting might occur (Santiago, 2012). In recent years, food fortification products have been one of the most popular ways to alleviate Fe deficiency anemia (Cheng et al., 2020). Food fortification is the addition of essential nutrients to food to prevent or correct dietary deficiencies in affected populations (Huma et al., 2007). Compared to supplementation, Fe is added to the diet at much lower levels, making the Fe more tolerable to the body. However, food fortification with Fe is more challenging than fortification with other nutrients (like iodine in salt or vitamin A in cooking oil). Iron compounds generally contain Fe with two oxidation states, namely in ferrous $\left(Fe^{2+}\right)$ and ferric $\left(Fe^{3+}\right)$ forms. The most bioavailable Fe compounds like ferrous sulfate $\left(FeSO_4\right)$ are water-soluble, but these frequently react with other food components, resulting in off-flavors, color changes, and fat oxidation. Ferric iron compounds like ferric phosphate $\left(FePO_4\right)$, on the other hand, are more stable in foods, but are generally also poorly bioavailable (Zimmermann and Hurrell, 2007).

Ionotropic gelation, in which polymeric beads are formed by binding to divalent and trivalent cations, is a promising method to avoid undesired organoleptic effects of iron in fortified foods. Natural biopolymers are particularly appealing for this purpose due to their high biocompatibility, low toxicity, and controlled release qualities (Bourgeois et al., 2006). Several of the above-mentioned issues with Fe have been improved because of encapsulation technology. Various materials, including alginate, have been suggested as suitable encapsulants (Won et al., 2005).

Alginate is a naturally occurring anionic polysaccharide extracted from the cell walls of brown algae. The linear polymer chain of alginate consists of guluronic (G) and mannuronic acid (M) unis that form blocks depending on their composition (i.e., M blocks, G blocks, and MG blocks containing alternating M and G residues) (Lee and Mooney, 2012). In the presence of divalent or trivalent cations, alginate can form a gel through ionotropic gelation (Figure 1). The dropwise addition of an alginate solution to a crosslinking bath containing such crosslinking ions results in the spontaneous formation of alginate beads. Most commonly, calcium $\left(Ca^{2+}\right)$ is used as crosslinking ion, but also $Ba^{2+}, Cu^{2+}, Sr^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Al^{3+}$, and Fe^{3+} have been used (Hu et al., 2021).

Previous studies have developed Fe-loaded alginate beads for nutritional applications. Valenzuela et al. (2016) prepared Ca-alginate beads loaded with heme Fe (from spray dried blood cells). The Fe release in gastric conditions (pH 2) was low, but greatly increased in simulated intestinal fluid (pH 6). However, the beads had very low Fe contents (<0.1 wt%).

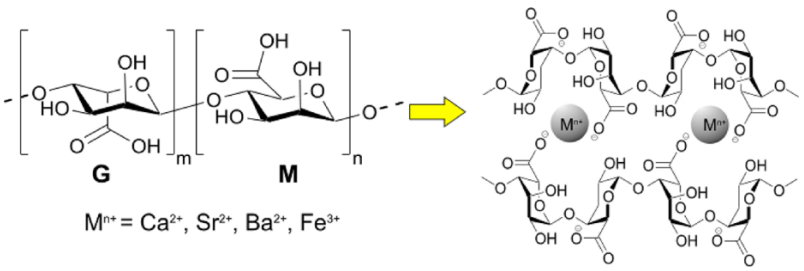

Figure 1: *Structure of alginate and schematic representation of crosslinking of alginate by M^{n+} metal cations. Here, G and M represent the guluronic acid and mannuronic acid units, respectively, of alginate. Reprinted with permission from Narayanan et al. (2012). Copyright 2012 American Chemical Society.*

Perez-Moral et al. (2013) studied the effects of alginate composition (M/G ratio), bead formation route, and Fe type (different Fe^{2+} and Fe^{3+} salts) on bead formation and bead Fe content. The authors observed that Fe^{2+} salts were best incorporated into the beads when the Fe^{2+} salt was dissolved in the Ca^{2+} crosslinking bath, whereas Fe^{3+} salts were best incorporated when pre-formed Ca alginate beads were immersed in a Fe^{3+} salt solution. The Fe-loaded beads gradually released iron under simulated gastrointestinal conditions, demonstrating their promise for nutritional applications. Similar results showing the promise of Fe-loaded alginate beads were shown by others (Katuwavila et al., 2016; Churio et al., 2018).

Nevertheless, these previous studies have prepared Fe-loaded alginate beads that were crosslinked by Ca^{2+} ions. The presence of Ca^{2+}, however, is undesired because Ca^{2+} may inhibit the duodenal Fe uptake, reducing the Fe bioavailability (Lynch, 2000). It would therefore be greatly preferred to load alginate beads with Fe by directly binding the Fe^{2+} or Fe^{3+} to alginate. Using Fe as alginate crosslinking ion would also ensure a high Fe loading in the beads, reducing the amount of material needed to fortify the foods. Previous reports have discussed the preparation of both Fe^{2+}-alginates (Hernández et al., 2010; Quadrado and Fajardo, 2017; Hu et al., 2021) and Fe^{3+}-alginates (Sreeram et al., 2004; Quadrado and Fajardo, 2017; Hu et al., 2021; Massana Roquero et al., 2022), although for applications other than nutritional ones.

In food fortificants, Fe^{2+} compounds like $FeSO_4$ are generally more bioavailable but also more reactive than Fe^{3+} compounds like $FePO_4$. Whether these properties are also present in alginates has, to the best of our knowledge, not yet been investigated. The aim of this research project was to prepare Fe alginate beads by direct ionotropic gelation of alginate with Fe^{2+} or Fe^{3+}. The effect of the Fe oxidation state $\left(Fe^{2+}\text{ or }Fe^{3+}\right)$ on bead structure, total Fe content, potential Fe bioavailability, and stability in difficult-to-fortify foods was assessed.

2 MATERIALS AND METHODS

2.1 Preparation of Fe-alginate beads

The Fe-alginate beads were prepared by ionotropic gelation (Knijnenburg et al., 2021). A 2% sodium alginate solution was prepared by dissolving 0.5 g sodium alginate in 25 mL DI water. Using a syringe with needle (0.8 mm diameter), the alginate solution was manually dropped into 50 mL crosslinking solution that consisted of either $0.15M FeSO_4 \cdot 7H_2O$ solution

(to prepare Fe^{2+}-Alg beads) or 0.15M$FeCl_3$ solution (to prepare Fe^{3+}-Alg beads). After the bead formation was complete, the beads were left to stabilize for 1 hour in the crosslinking solution and then separated by filtration. The fresh beads were washed 3x with DI water and dried in an oven overnight at 40°C. The dry beads were stored in zip lock bags at room temperature.

2.2 Physicochemical characterization of alginate beads

The average diameter of the fresh and dry beads was measured using ImageJ software (version 1.53k) from optical photographs of the samples. For each sample, at least 50 beads were counted and the mean diameter with standard deviation is reported.

The structural interactions within the beads were studied by Fourier transform infrared (FTIR) spectroscopy. The FTIR spectra were measured on a Bruker Alpha II ATR-FTIR in the range of 4000− 400 cm^{-1} with 32 scans at a resolution of 2 cm^{-1} (Kniinenburg et al., 2021).

The total Fe content of the beads was measured in triplicate by immersing 50mg sample in 25 mL of 2.5MHCl. The solutions were left to stand overnight, and the dissolved Fe concentrations were measured by atomic absorption spectroscopy (AAS, Perkin Elmer PinAAcle 900 F) with external calibration (Hilty et al., 2010).

2.3 Simulated iron release in gastrointestinal conditions

The Fe release from the beads was measured in triplicate according to the standardized method (Swain et al., 2003; Lynch et al., 2007). Here, 25mg of each sample was added to a 250 mL Erlenmeyer flask, to which 125 mL of a prewarmed solution of 0.1MHCl(pH1.00 ± 0.05) was added. The flasks were closed and kept shaking at 130rpm and 37°C (NB-205, HandyLAB System, N-BIOTEK). After 30, 60,90 , and 120 min, a 2 mL sample was taken and filtered through a 0.22μm nylon syringe filter. The filtrate was diluted in 0.1MHCl and the dissolved Fe concentrations were measured by AAS with external calibration.

2.4 Stability in difficult-to-fortify foods

To study the stability of the beads in food, color changes were measured in strawberry yoghurt (a model matrix for difficult-to-fortify foods) as described by Hilty et al. (2009). The yoghurt (Dutchie strawberry yoghurt, 77.2% yoghurt, 22.8% strawberries in concentrated syrup) was homogenized with a handheld homogenizer and then sieved to filter out any seeds. The yoghurt was separated into 70 g portions, to which the Fe-loaded beads were added at a fortification level of 10mgFe/100 g yoghurt. A blank (unfortified) sample was prepared in each experiment. The yoghurt samples were kept stirring at room temperature for 120 min, after which the yoghurts were poured into a glass Petri dish (diameter 90 mm), and the color of the food matrices was measured with a colorimeter (WR10 FRU). For each sample three measurements were taken, and the average values of L, a, and b were recorded (Hunter (L^*a^* b) scale). The color difference from the unfortified sample was calculated from Eq. (1):

$$\Delta Eab^* = \Delta L^*2 + \Delta a^*2 + \Delta b^*2$$

Here, ΔL^*, Δa^* and Δb^* are the differences between the sample and the blank (unfortified) food matrix. Optical photographs were taken for comparison.

3 RESULTS AND DISCUSSION

In this research, alginate beads were made with 2 different crosslinking agents, namely ferrous sulfate ($FeSO_4$) and ferric chloride ($FeCl_3$), to produce Fe^{2+} - Alg and Fe^{3+} - Alg beads, respectively. Figure 2 presents optical photographs of the as-prepared (wet) Fe^{2+}-Alg and Fe^{3+}-Alg beads. The Fe^{2+}-Alg beads were white, whereas the Fe^{3+}-Alg beads were orange, which was ascribed to the color of the ferric chloride complex from the crosslinking solution.

The size of the wet and dry beads was measured as presented in Figure 3a. The $Fe^{3+} - Alg$ beads had a larger size than the Fe^{2+}-Alg beads in wet conditions, but in dry conditions, it was the opposite. The smaller size of the dry beads suggested that Fe^{3+} - Alg had a denser structure than Fe^{2+} - Alg, likely because Fe^{3+} has three positive charges that could bind to alginate, whereas Fe^{2+} only has two positive charges.

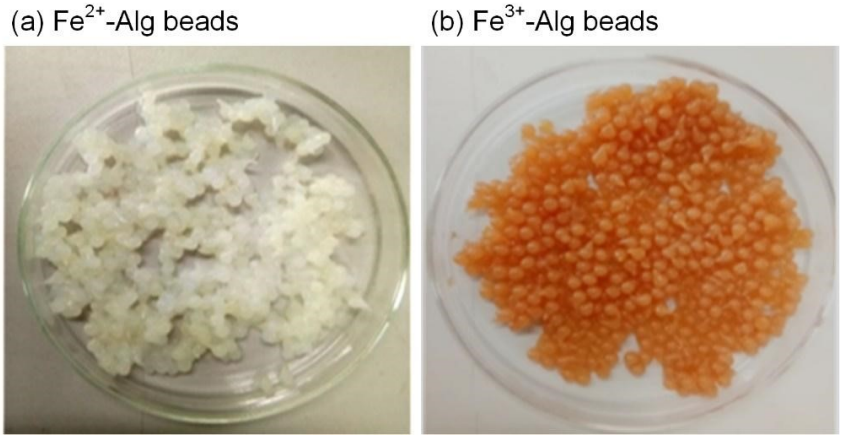

Figure 2: *Photographs of (a) wet* Fe^{2+}*-Alg beads and (b) wet* Fe^{3+}*-Alg beads.*

Both $Fe^{2+} - Alg$ and Fe^{3+}-Alg contained equal Fe contents of approximately 140mgFe/g beads (Figure 3 b), which means that 14wt% of the beads consisted of Fe. These Fe contents are comparable to state-of-the-art Fe compounds used in the food industry (Hurrell, 2002), and much higher than the Feloaded alginate beads of Valenzuela et al. (2016).

The FTIR spectra (Figure 4) present the chemical interactions between Fe^{2+}/Fe^{3+} and alginate. Both materials display broad absorbance peaks at $2500 - 3700\ cm^{-1}$ that were ascribed to $O - H$ vibrations of hydroxyl groups in alginate (Papageorgiou et al., 2010). For Fe^{2+} - Alg, the main peaks were found at $975\ cm - 1$ (C-O stretching) and $1064\ cm - 1$ (O-C-O stretching), and the peaks at $1411\ cm^{-1}$ (COO symmetric stretching), and $1635\ cm - 1$ (COO asymmetric stretching) provided evidence for the interaction of Fe^{2+} with alginate (Quadrado and Fajardo, 2017). In Fe^{3+} - Alg, the COO asymmetric stretching peak was found at a lower wavenumber of $1572\ cm^{-1}$, and the symmetric COO stretching peak was found at $1433\ cm^{-1}$ (Dong et al., 2011). The differences in these peak positions for $Fe^{2+} - Alg$ and $Fe^{3+} - Alg$ suggested that Fe^{2+} and Fe^{3+} were bound to the carboxylate groups of alginate through different binding modes (Papageorgiou et al., 2010; Menakbi et al., 2016). For Fe^{3+}-Alg also a new peak appeared at $1737\ cm^{-1}$, which was ascribed to C-O stretching of free COOH groups (Valentin et al., 2005), suggesting that not all the carboxylic acid groups were bound to Fe^{3+}.

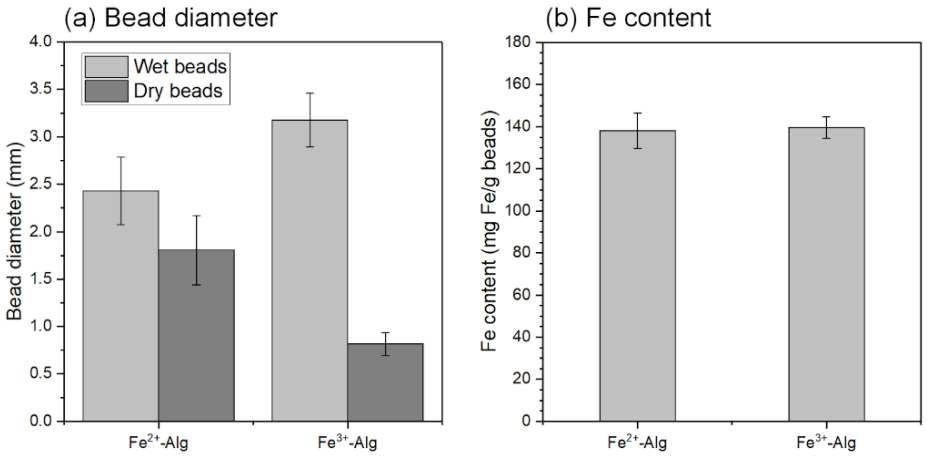

Figure 3: *(a) Average diameter and and (b)* Fe *content of* Fe^{2+} - Alg *and* Fe^{3+}*-Alg beads*

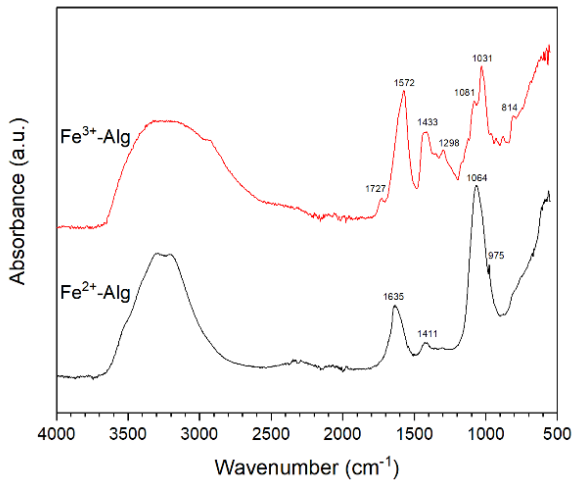

Figure 4: *FTIR spectra of the* Fe^{2+}*-Alg and* Fe^{3+}*-Alg beads. Approximate peak positions are indicated above each peak.*

The Fe release was measured in 0.1MHCl(pH1.0) as in vitro indicator for the in vivo Fe bioavailability (Figure 5). Whereas the Fe release from Fe^{3+} - Alg was gradual and reached 80% after 120 min, the Fe^{2+}-Alg readily released over 85% of its Fe within the first 30 min and was 100% released after 120 min. This suggests that the Fe from Fe^{2+}-Alg may be more bioavailable than that of Fe^{3+}-Alg. A possible explanation for this might be the weaker binding of Fe^{2+} with alginate compared to Fe^{3+}, making it easier for Fe^{2+} to be released than Fe^{3+}. This is in agreement with the smaller bead size for Fe^{3+} – Alg. (Figure 3a) and the different binding modes evidenced by FTIR spectroscopy (Figure 4).

Apart from the potential Fe bioavailability, the reactivity in food after Fe addition is something that must be considered. Here, strawberry yoghurt was used as model compound for difficult-to-fortify foods. The color changes (ΔE) with respect to an unfortified sample were measured after 120 min, and a lower ΔE value indicates a higher stability. Figure 6 demon-

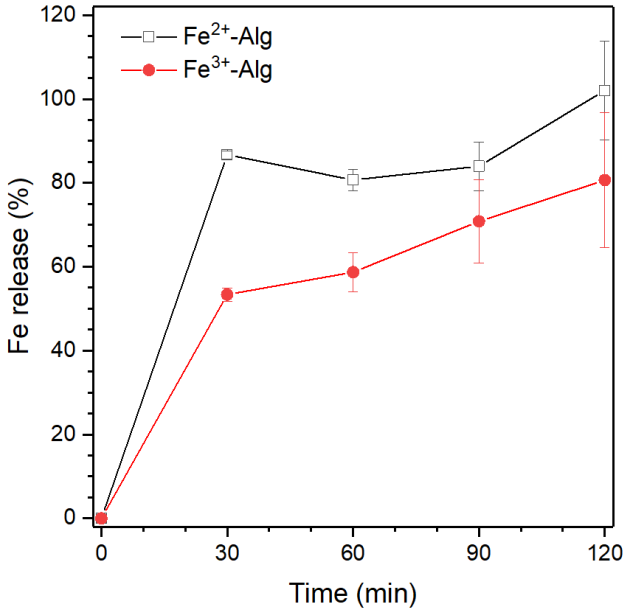

Figure 5: *Iron release in simulated gastrointestinal conditions (pH 1.0).*

strates that Fe^{3+}-Alg beads ($\Delta E = 4.1$) were less reactive than $Fe^{2+} - Alg(\Delta E = 5.4)$. This can also be seen from the photograph that shows the stronger color difference of the food containing Fe^{2+}-Alg relative to the blank sample.

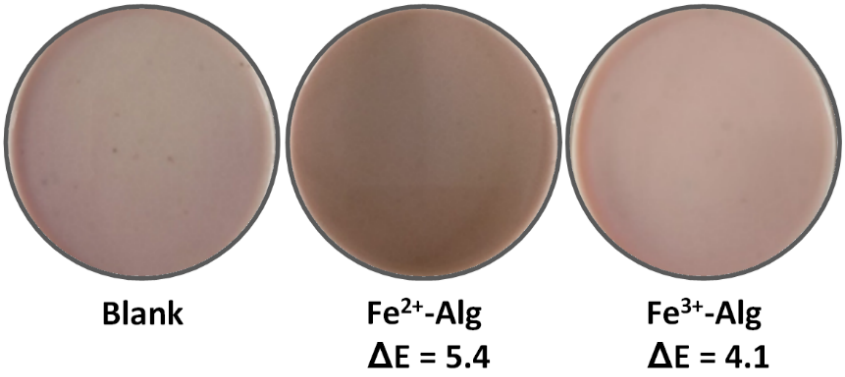

Figure 6: *Color changes of the beads in strawberry yoghurt compared to a blank (unfortified) sample.*

4 CONCLUSIONS

This work presents the synthesis of Fe alginate beads using ferrous and ferric iron to produce Fe^{2+}. Alg and Fe^{3+}-Alg, respectively. The beads had a high Fe content of 14wt%. The Fe^{3+}-Alg beads were less reactive in foods than Fe^{2+}-Alg, but had a slower Fe release in simulated gastrointestinal conditions, suggesting a higher Fe bioavailability from Fe^{2+}-Alg. In conclusion, the properties of Fe alginate beads can be tuned by the Fe^{2+} / Fe^{3+} content, making

the Fe alginate beads a promising approach to overcome the challenges with conventional food fortificants.

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