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Pectin-iron capsules: Novel system to stabilise and deliver lactic acid bacteria

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ABSTRACT

The delivery of probiotics has been a challenge because microorganisms must overcome harmful environments both at a technological and at a physiological level. Encapsulation is one of the strategies to protect microorganisms against both problems. This work aimed at encapsulating probiotic *Lactobacillus plantarum* CIDCA 83114 in pectin-iron beads obtained by ionotropic gelation, enabling bacteria safe delivery and providing a source of iron and fibre.

Microorganisms in the stationary phase were suspended in a 4% w/v pectin solution at pH 5, and the suspension, dripped into a 150 mM $FeSO_4$ solution. The beads were freeze-dried and stored for 60 days at 4 °C. The morphology of the beads was observed by scanning electron microscopy. Bacterial culturability was determined after freeze-drying, and after the exposure to simulated saliva, gastric and intestinal conditions. The iron and pectin releases were also investigated in the same digestive conditions.

Microorganisms were fully entrapped in smooth and spherical pectin-iron beads of *ca.* 1–2 mm diameter. Bacterial culturability did not decrease during storage. Encapsulation protected microorganisms against simulated digestive conditions, also enabling the complete release of iron and pectins in the gut.

The results obtained support the safe delivery of both probiotic bacteria and iron to the gut. As iron deficiency still continuous to be a worldwide problem, using iron-pectin beads could be an adequate strategy to functionalise food products, contributing to attain the recommended iron intake.

1. Introduction

Lactic acid bacteria and probiotics have an important role in the food and pharmaceutical industries, as they are extensively used as starters in the development of food and pharmaceutical products. To exert their action, viable bacteria must arrive at suitable levels to the colon. However, in their way to the colon, microorganisms are exposed to adverse conditions such as those of the gastro-intestinal environment (*i.e.*: low gastric pH, bile salts, digestive juices). For this reason, different strategies must be considered to protect them against these harmful conditions, microencapsulation being one of the most extended ones (Chávarri, Marañón I., & M. C., 2012).

Different polymer matrices have been used for bacterial encapsulation. Their main function is to provide a physical barrier to protect microorganisms during technological processes and storage, and also after ingestion (Kailasapathy, 2009). Most of the encapsulation technologies are based on the bacterial immobilization into matrices that remain intact for an appropriate period until their release in the colon (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012). Natural biopolymers, including dietary oligo and polysaccharides, are particularly interesting encapsulating materials because of their good biocompatibility, non-toxicity and controlled release properties (Coviello, Matricardi, Marianecci, & Alhaique, 2007). One of the most extended approach to obtain capsules is the ionotropic gelation method, in which cations form insoluble associates with carbohydrate chains resulting in the so-called “egg-box” complexes (Bourgeois, Gernet, Pradeau, Andremont, & Fattal, 2006). Alginate, majorly associated to calcium cations, has been largely used to encapsulate lactobacilli and bifidobacteria (Chaluvadi et al., 2012; Chen, Chen, & Kuo, 2007; Hotchkiss et al., 2008; Kent & Doherty, 2014; Klemmer, Korber, Low, & Nickerson, 2011; Nazzaro, Fratianni, Coppola, Sada, & Orlando, 2009; Zheng et al., 2017).

Pectins, linear chains of partially methyl-esterified (1 → 4)-linked α-d-galacturonic acid residues, are useful polysaccharides for ionotropic gelation, in particular, those of low degree of esterification (< 50%) (Sinha & Kumria, 2001). As pectins belong to dietary fiber, they are not hydrolyzed in the upper part of the gastro-intestinal tract, being degraded only by the colonic microflora. Therefore, pectin matrices have

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been widely used to deliver drugs whose target is the colon (Assifaoui, Chambin, & Cayot, 2011; Das & Ng, 2010; Das, Ng, & Ho, 2010; Dhalleine et al., 2011; El-Gibaly, 2002; Liu, Fishman, Kost, & Hicks, 2003; Munjeri, Collett, & Fell, 1997; Murata, Miyashita, Kofuji, Miyamoto, & Kawashima, 2004). In spite of the advantages arising from their capacity to deliver compounds to the gut, pectins have been scarcely used for probiotic encapsulation. In this regard, Gerez, Font de Valdez, Gigante, and Grosso (2012) combined ionotropic gelation and complex coacervation techniques to obtain microcapsules of pectin covered with whey proteins, which improve the survival rate of *Lactobacillus rhamnosus* to low pH and bile. Oliveira et al. (2007) used pectins and casein followed by spray-drying, to encapsulate *Lactobacillus acidophilus and Bifidobacterium lactis.* Sandoval-Castilla, Lobato-Calleros, García-Galindo, Alvarez-Ramírez, and Vernon Carter (2010) used pectins together with alginate to entrap and protect *Lactobacillus casei* when incorporated in yogurts, and upon subsequent exposure to simulated gastro-intestinal conditions. In turn, Nualkaekul, Cook, Khutoryanskiy, and Charalampopoulos (2013) compared the efficiency of alginate and pectin beads for improving the survival of *Lactobacillus plantarum* and *Bifidobacterium longum* in pomegranate and cranberry juices during storage.

In using pectin ionotropic gelation, divalent cations (*i.e.:* calcium and zinc) have been majorly employed as cross-linking agents, but up to our knowledge, no attempts to use iron in that function have been performed hereto. Iron deficiency is the most common nutritional deficit worldwide, and represents a public health problem in both industrialized and non-industrialized countries. To overcome it, food fortification with iron has been a commonly used strategy all over the world (Uauy, Hertrampf, & Reddy, 2002). Ferrous salts are generally used for iron fortification (*i.e.:* sulphate, gluconate, fumarate, succinate) (Allen, de Benoist, Dary, & Hurrell, 2006). Considering the divalent character of ferrous cation, ionotropic gelation with pectin could be an interesting and not yet explored strategy to provide a mechanical barrier to protect lactic acid bacteria from the gastro-intestinal conditions, also enhancing the iron consumption. For this reason, the goal of this work was to encapsulate *Lactobacillus plantarum* CIDCA 83114 using pectin-iron capsules obtained by ionotropic gelation. *L. plantarum* CIDCA 83114 is an especially interesting strain because of its inhibitory properties against *E. coli* O157:H7, *Shigella* and *Salmonella* (Hugo, Kakisu, De Antoni, & Pérez, 2008; Kakisu, Abraham, Tironi Farinati, Ibarra, & De Antoni, 2013; Kakisu, Bolla, Abraham, de Urraza, & De Antoni, 2013). Bacterial culturability was assessed immediately after encapsulation and freeze-drying, after the exposure to gastro-intestinal conditions, and at regular intervals for up to 60 days of storage at 4 °C. In parallel, the iron and pectin release was investigated in saliva, gastric and intestinal conditions, thus fulfilling a study that supports the safe delivery of both probiotic bacteria and iron in the gut.

2. Materials and methods

2.1. Bacterial strain and growth conditions

L. plantarum CIDCA 83114 was isolated from kefir grains (Garrote, Abraham, & De Antoni, 2001) and maintained frozen at −80 °C in 120 g/L non-fat milk solids (Difco, MA, USA). Microorganisms were grown in MRS broth (de Man, Rogosa, & Sharpe, 1960) at 37 °C in aerobic conditions. Cultures in the stationary phase (~2×10^8 CFU/mL) were harvested by centrifugation at 6000 rpm for 5 min and the pellets, used for encapsulation.

2.2. Encapsulation

Pectin from citrus peel (galacturonic acid ≥ 74.0%, Sigma Aldrich, Buenos Aires, Argentina) was prepared in 0.060 M acetic acid-sodium acetate (Sigma Aldrich, Buenos Aires, Argentina) pH 5.0, at 4% w/v. The bacterial pellets obtained in Section 2.1 were suspended in the pectin solution. The obtained suspension was dripped into a 150 mM $FeSO_4$ solution using a 0.3 mm needle (~10 µL/drop) under continuous agitation for 30 min. The capsules were harvested by filtration through a stainless steel mesh of 0.10 mm, and rinsed three times with distilled water. Then, the capsules were frozen at −80 °C and freeze-dried for 48 h on a Heto FD4 equipment (Heto Lab Equipment, Denmark) operating with the condenser at −45 °C at a chamber pressure of 0.04 mbar. The obtained samples were stored for 60 days at 4 °C.

Bacterial plate counts were carried out on encapsulated and non-encapsulated microorganisms. To plate count fresh microorganisms (controls), 100 µL bacterial suspensions were diluted in 1 mL phosphate saline buffer (PBS) (K_2HPO_4 0.144 g/L; NaCl 9.00 g/L; Na_2HPO_4 0.795 g/L, pH 7). Plate counts after encapsulation and during storage, were carried out by solubilizing 10 beads (representing about 100 µL of fresh culture) in 1 mL PBS under continuous stirring. Bacterial suspensions arising from both encapsulated and non-encapsulated microorganisms were serially diluted, plated on MRS agar, and incubated at 37 °C for 48 h in aerobic conditions.

2.3. Scanning electronic microscopy (SEM)

The beads obtained in Section 2.2 were mounted on metal stubs with double sided adhesive tape, coated with gold using a sputter coater (Polaron Thermo VGScientific, East Grinstead, Sussex, UK) under vacuum and 18 mA at room temperature (Martin-Dejardin et al., 2013). The beads' morphology was observed by Scanning Electron Microscopy, focusing both on the surface aspect and on the inner structure of the capsules. The samples were examined using an environmental scanning electron microscope (ESEM) (FEI La B6, Eindhoven, Netherlands) at 14 kV accelerating voltage with an electron detector for low vacuum conditions. For examination of the inner structure, the beads were cut in half with a steel blade.

2.4. In vitro digestion

Experiments were carried out on the freeze-dried beads, using non-encapsulated microorganisms as controls. The test was divided into three stages: saliva (10 mg/mL α-amylase from *Bacillus subtilis* in PBS, pH 6.8) (Sigma Aldrich, St. Louis, MO, USA), gastric digestion (3 g/L porcine pepsin, 125 mM NaCl, 7 mM KCl, 45 mM $NaHCO_3$, pH 2.5) (Sigma Aldrich, St. Louis, MO, USA) and intestinal digestion (1 mg/mL pancreatin, 1.5 mg/mL bile salts, 22 mM NaCl, 3.2 mM KCl, 7.6 mM $NaHCO_3$, pH 8.0) (Sigma Aldrich, St. Louis, MO, USA) (adapted from Grimoud et al., 2010). In brief, 10 beads (weighting about 3.5 mg, and representing about 100 µL of fresh culture) were suspended in 1 mL of human total saliva for 2 minutes. Saliva was thoroughly removed and then, 1 mL of simulated gastric juice was added to the sedimented beads. Samples were incubated for 1.5 hours at 37 °C under continuous gentle shaking (50 rpm, MaxQ 4000, Thermo Scientific, USA). Afterwards, the gastric juice was thoroughly removed and 1 mL simulated intestinal juice was added to the beads and incubated for 3 hours at 37 °C, under continuous gentle shaking (50 rpm, MaxQ 4000, Thermo Scientific, USA). For non-encapsulated microorganisms, 100 µL of fresh cultures were centrifuged, and the pellets suspended in the different gastro-intestinal fluids, following a similar protocol than that used for the encapsulated bacteria.

To determine bacterial release after each digestion stage, aliquots of 1 mL saliva, gastric and intestinal fluids were diluted in PBS and plated on MRS agar for bacterial enumeration, as described in Section 2.2.

2.5. Quantification of pectin and iron

The whole content of iron cations [Fe(II) and Fe(III)] and pectins in the capsules (both loaded with bacteria and empty -controls-) was assessed by quantifying the free analytes after complete disruption of 10 beads in 1 mL PBS. In addition, both quantifications were carried out

after each step of digestion on the simulated digestive fluids (saliva, gastric and intestinal juices) (see Section 2.4).

Free iron cations were determined by using the selective chelator 2,2′-bipyridyl (Sigma Aldrich, Buenos Aires, Argentina) (adapted from Moss and Mellon (1942), Heaney and Davison (1977), Elmagirbi, Sulistyarti, and Atikah (2012)). In brief, 0.01 mL of 0.045 M ascorbic acid (Sigma Aldrich, Buenos Aires, Argentina) were added to 0.02 mL of samples. After 5 min, 0.770 mL of a 0.1 M HCl and 0.1 M $NaC_2H_3O_2$ solution, were added to each tube. Then, 0.2 mL of 2,2′-bipyridyl reagent were added, samples were vortexed and stayed for 15 min. The absorbance of the red complexes was read at 521 nm. A blank tube in which samples were replaced by milli Q water was also prepared. Free ferrous ion was determined by using the same chelator in the absence of ascorbic acid.

Pectins were determined by using m-hydroxydiphenyl (Sigma Aldrich, Buenos Aires, Argentina) for analysis of uronic acids (Ibarz, Pagán, Tribaldo, & Pagán, 2006; Kintner & van Buren, 1982). In brief, 1.25 mL of sulfuric acid/sodium tetraborate solution (0.0125M sodium tetraborate; 36.8N sulfuric acid) were added to 0.25 mL of samples and cooled in an ice bath. Samples were vortexed and heated at 80 °C for 6 min. After cooling in an ice bath, 0.025 mL of m-hydroxydiphenol reagent (0.15% w/v m-hydroxydiphenol in 0.5% w/v NaOH) were added to each tube. For each sample, absorbance at 520 nm was read at 15 minutes. A blank tube in which the sample was replaced by milli Q water was also prepared.

2.6. Statistical analysis

All the assays were conducted in triplicate and in three independent assays. Average values were used for data analysis. Analysis of variance (ANOVA) was carried out for all the assays, using the statistical program InfoStat 2008 (Infostat Group/FCA. National University of Cordoba. Ed. Brujas, Cordoba, Argentina). Comparison of means was tested using Tukey methods, and if $p < .05$, the difference was considered statistically significant.

3. Results

Pectin-iron beads were spherical, with a diameter within 1 and 2 mm (Fig. 1). Their external surface was rather smooth (Fig. 1A) and the inner structure showed a compact texture (Fig. 1BI), with regularly observable holes (Fig. 1BII). Pectin-iron beads containing microorganisms presented clear images of entrapped bacteria homogeneously distributed on the external surface of the beads (Fig. 1C). The inside of the beads exhibited a compact texture with holes (Fig. 1DI and II), also with groups of microorganisms embedded in the gelled iron-pectin material (Fig. 1DIII). The culturability of the encapsulated microorganisms did not show any significant decrease after 60 days of storage at 4 °C ($p > .05$) (Fig. 2).

The efficiency of pectin-iron complexes to protect microorganisms from gastro-intestinal conditions was assessed by plate counting beads exposed to such conditions, using non-encapsulated bacteria as controls (Fig. 3). The culturability of both encapsulated and non-encapsulated microorganisms was above 7.30 log CFU/mL. Non-encapsulated microorganisms showed no significant decrease of culturability when exposed to saliva. Gastric conditions led to a noticeable decrease of the log CFU/mL, and no additional decrease was observed when exposed to intestinal conditions ($p > .05$) (white bars in Fig. 3). In turn, the culturability of encapsulated microorganisms exposed to saliva significantly decreased ($p < .05$) (grey bars in Fig. 3). Microorganisms were released in gastric and intestinal conditions, accounting 6.45 ± 0.21 log CFU/mL at the end of the digestive process. The beads stored at 4 °C were also exposed to simulated saliva and gastro-intestinal conditions, showing no significant differences with regard to those obtained at time equal to zero (data not shown).

As the goal of this work was focused not only on bacterial stability but also on iron delivery, the integrity of the beads was determined by quantifying iron and pectins' release after exposing them to simulated saliva and gastro-intestinal conditions (Fig. 4A). It is important to point out that in preliminary assays, carried out without the addition of ascorbic acid to the complexation reaction of iron with 2,2′-bipyridyl (selective chelator of ferrous cations), no iron could be detected. In other words, the coloured complexes with iron were only observed when ascorbic acid was incorporated to the reaction medium, indicating that the concentration of ferrous cation was below the detection limit of the method.

Once ascorbic acid was incorporated, the total iron content of the beads (which was only ferric) was 2.06 ± 0.00 mM. A low concentration of iron was released in saliva (0.12 ± 0.04 mM). The major release of iron occurred after exposure to gastric and intestinal conditions (0.79 ± 0.06 and 1.14 ± 0.04 mM, respectively), which altogether accounted the whole concentration of iron in the beads. Iron was also quantified in empty beads in the same conditions (controls), showing not significant differences with respect to the counterpart loaded beads in all conditions except when exposed to intestinal conditions, in which the iron release was 0.77 ± 0.02 mM (*ca.* 68% lower than for beads containing bacteria) (data not shown).

Finally, the quantification of pectins showed a similar behaviour than that of iron (Fig. 4B). Indeed, low concentrations of pectins were released in saliva, and the major release occurred after exposure to the intestinal solution (Fig. 4B). Contrarily to what was observed for iron release, the empty beads (controls) showed a pectin concentration that was about 50% higher than that of the beads loaded with bacteria (data not shown).

4. Discussion

Delivery of probiotics has been a challenge because microorganisms must overcome harmful environments both at a technological and at a physiological level. Furthermore, microorganisms resistant to technological processes are not always resistant to digestive conditions (*i.e.:* low pH of the stomach, bile, digestive juices) and viceversa. Encapsulation is one of the strategies to protect microorganisms against both problems. This work aimed at going beyond the simple protection of probiotic microorganisms, by designing pectin-iron beads able to release iron and pectins in the gut.

L. plantarum CIDCA 83114 was embedded both in the outer and inner side of majorly regular beads (Fig. 1). The holes observed in the inner surface were probably due to eventual shrinkage and water evaporation during the freeze-drying process (Martin-Dejardin et al., 2013). The complete coating of microorganisms into iron-pectin beads stabilised them during storage, as no significant decrease of culturability was observed after 60 days at 4 °C (Fig. 2). Besides its probiotic properties (Hugo et al., 2008; Kakisu, Abraham, et al., 2013; Kakisu, Bolla, et al., 2013), *L. plantarum* CIDCA 83114 has demonstrated certain properties compatible with the incorporation into functional foods. In this regard, it can be entrapped into carboxymethylcellulose films (Romano et al., 2014), which can be subsequently used to functionalise apple snacks (Tavera-Quiroz et al., 2015). Moreover, spray-drying encapsulation in milk or whey permeate is an efficient strategy to stabilise this strain (Golowczyc, Silva, Teixeira, De Antoni, & Abraham, 2011; Golowczyc et al., 2013). In this context, the size and shape of the obtained beads loaded with bacteria (Fig. 1), together with their stability after 60 during storage support their use as functional ingredients in the formulation of functional foods (for example, incorporated into yogurts).

As the target of probiotic strains is the gut, the stability and proper release of microorganisms during the passage through the gastro-intestinal tract is very important. Although non-encapsulated bacteria overcame adequately both the freeze-drying process and the exposure to saliva, gastric conditions led to a noticeable decrease of culturability (Fig. 3). In previous works, it was reported that fermented media

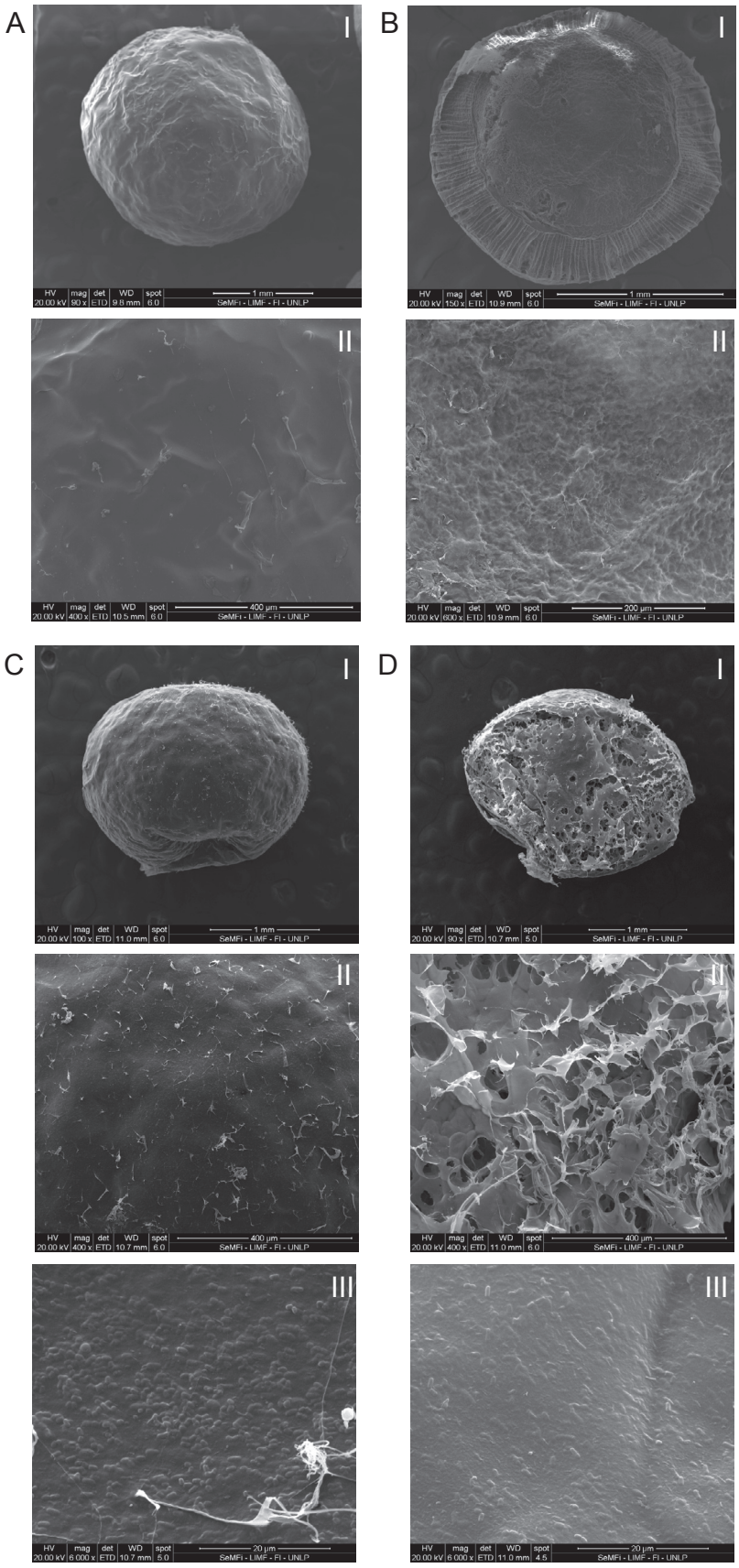

Fig. 1. Scanning electron microscope pictures of: (A) External surface of iron-pectin beads. I and II denote different scales. (B) Inner structure of iron-pectin beads. I and II denote different scales. (C) External view of iron-pectin beads with entrapped *L. plantarum* CIDCA 83114. I, II and III denote different scales. (D) Inner structure of iron-pectin beads with entrapped *L. plantarum* CIDCA 83114. I, II and III denote different scales.

including this strain must be neutralized before dehydration to preclude bacterial damage or death (Golowczyc et al., 2013). This behaviour is consistent with the sensitivity of this strain to low pH observed in this work (Fig. 3). Capsules containing microorganisms were firstly fully disrupted in PBS to determine the bacterial concentration before the digestive process, which was 7.30 ± 0.43 log CFU/mL (Fig. 3). The low

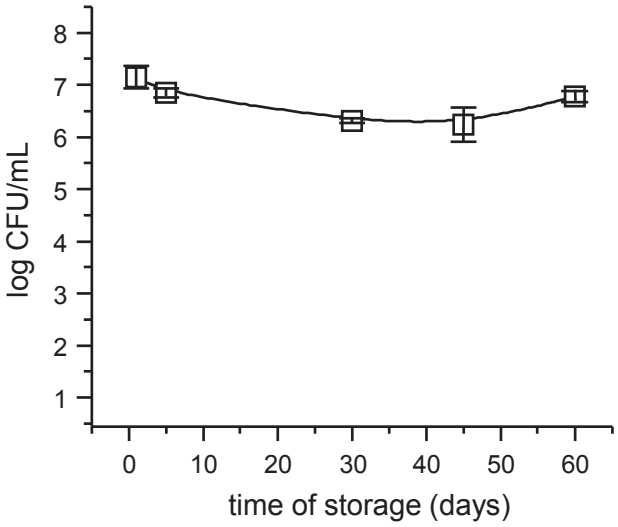

Fig. 2. Culturability of encapsulated *L. plantarum* CIDCA 83114 after freeze-drying and storage at 4 °C. Culturability before freeze-drying: $N_0 = 8.32 \pm 0.03$.

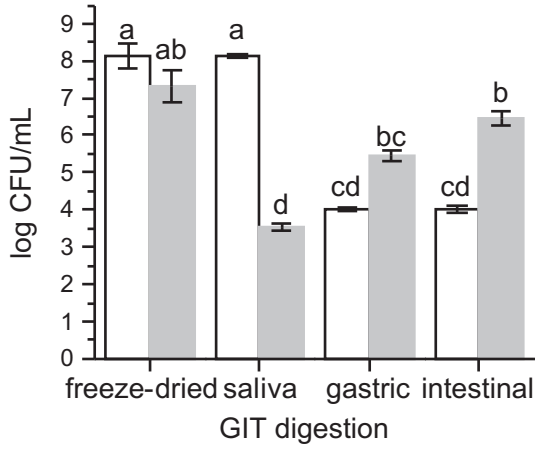

Fig. 3. Culturability of *L. plantarum* CIDCA 83114 after freeze-drying and exposure to saliva, gastric and intestinal conditions. White bars: non-encapsulated microorganisms, grey bars: encapsulated microorganisms. Different letters indicate significant differences ($p < .05$).

culturability observed after the contact of capsules with saliva indicates that capsules were majorly intact, thus denoting their stability. The beads were fully disrupted once arrived to the intestinal conditions, releasing 6.45 ± 0.21 log CFU/mL, a value slightly lower than that obtained for the capsules disrupted in PBS before exposure to simulated digestive conditions (Fig. 3). Considering that plate counts on encapsulated bacteria were carried out on 10 capsules disrupted in PBS (arising from 100 µL of fresh culture, whose weight was about 3.5 mg - ~0.35 mg/capsule-, see Section 2.4), the bacterial concentration was 6.30 log CFU/capsule (2.0×10^6 CFU/capsule) at the moment of being ingested and 5.45 log CFU/capsule (2.83×10^5 CFU/capsule) at the end of the digestive process. According to recommendations of international organisms like EFSA or FDA, the benefits of probiotic consumption can be attained when probiotic containing products have at least 7 log CFU of viable microorganisms per gram at the moment of being consumed (Aquilina et al., 2013; Hill et al., 2014; Phuapaiboon, Leenanon, & Levin, 2013; Tripathi & Giri, 2014). Hence, the consumption of just 5 beads would fulfil the recommended values at the moment of being ingested, and the consumption 35 would warrant these concentrations, also in the gut. Hence, it can be concluded that encapsulation was very important to protect bacteria from the gastric and intestinal conditions (Fig. 3).

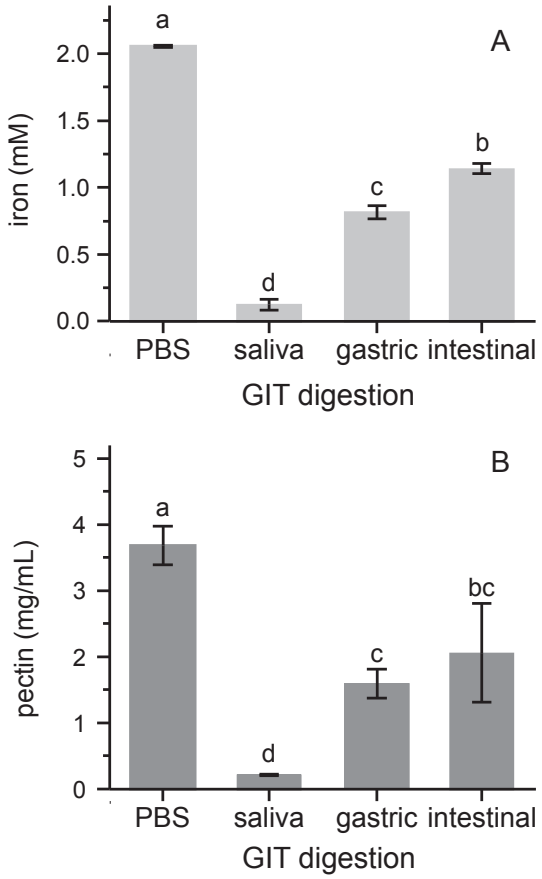

Fig. 4. A. Iron release after exposure to saliva, gastric and intestinal conditions. B. Pectin release in the same conditions. Different letters indicate significant differences ($p < .05$).

In this work, the materials constituting the beads were selected on the basis of their nutritional properties. Pectins take part of the soluble fibre and their consumption stimulates the development of bifidobacteria in the colon and prevents constipation, colon diverticulosis, carcinoma of the large bowel and stomach, type 2-diabetes, metabolic syndrome and cardiovascular disease (Anderson et al., 2009). Therefore, besides protecting microorganisms from gastro-intestinal conditions, pectin-iron capsules also constitute a source of nutritional soluble fibre. On the other hand, and considering that the iron deficiency is a problem worldwide, its administration in the shape of beads obtained by ionic gelation of pectin results an interesting way to warrant the safe arrival of iron to the gut. The results obtained for iron (Fig. 3A) and pectins' (Fig. 3B) release showed the whole disruption of the beads in the gut, following a similar pattern than that obtained for microorganisms. It is interesting to note that the higher concentration of iron observed for beads loaded with bacteria (with regard to the empty ones) could be ascribed to the fact that beads were fully disrupted in the intestinal fluid and as the bacterial surface is negatively charged (Gómez-Zavaglia, Kociubinski, Pérez, Disalvo, & De Antoni, 2002), part of iron could be adsorbed to the bacterial surface. The opposite behaviour was observed for pectin concentration, and could be explained considering that the inner part of the beads loaded with bacteria (Fig. 1DI and II) showed a compact texture with holes, explaining a lower density for pectin in the beads.

Iron dietary intake recommendations are given by the US Food and Nutrition Board (FNB) and may vary within 3–20 mg/day depending on age and physiological stages (*i.e.:* newborns, children, women in reproductive age, postmenopausal women, pregnancy) (Allen et al., 2006). Considering that the concentration of iron in the beads was 2.06 ± 0.01 mM (Fig. 4A), and each bead was prepared with ~10 µL fresh cultures suspended in pectins (100 µL for 10 capsules), it can be

calculated that each bead contained 0.206 μmol iron (~11.5 μg). This indicates that the consumption of ~500 beads would provide an intake of 5.75 mg iron, from which about 60% would be released in the gut (according to the information plotted in Fig. 4A). Considering the worldwide problematic of achieving the daily intake recommendations of iron, the consumption of the capsules designed in this work could be an alternative source of iron, suitable for being incorporated as functional ingredient not only because of their probiotic content (as mentioned above) but also for their iron content. Another advantage is that the presence of iron complexed with pectins in saliva (a low concentration of iron was released in saliva -Fig. 4A-), prevents the metallic taste of iron containing supplements.

Another point to be remarked is that 2,2′-bipyridyl only forms complexes with ferrous cations (Elmagirbi et al., 2012). Therefore, the ascorbic acid added as a first step of the colorimetric determination of iron leads to the reduction of all the iron cations present in the reaction medium. No iron could be detected in preliminary assays carried out without the addition of ascorbic acid (data not shown). Total iron could only be determined when ascorbic acid was incorporated. This indicates that all the iron present in the beads occurred as ferric cation. Ferric cation is far less soluble than ferrous one [solubility products at pH 7.0 (intestinal medium): Fe(III) (10^{-18} mol/L) *vs* Fe(II) (0.1 mol/L)] (Andrews, Robinson, & Rodriguez-Quinones, 2003). For this reason, iron is usually provided as ferrous sulphate. In the last years, iron chelates (*i.e.*, NaFeEDTA or ferrous bisglycinate) have been reported as promising alternatives to ferrous sulphate for food fortification because they are more stable when added to foods and are less affected by iron absorption inhibitors (Bovell-Benjamin, Allen, Frankel, & Guinard, 1999; Hurrell, 2002), as observed when added to curry powder, fish sauce, or soy sauce (Yeung, Glahn, & Miller, 2005). In this work, the complexation of iron with pectins precluded the precipitation of ferric cation at intestinal pH, which is an advantage not originally conceived, also providing soluble fibre as further healthy nutrient.

5. Conclusions

The main achievement of this work was the development of a single product enabling the double administration of iron and probiotic microorganisms. The pectin-iron beads appear as interesting ingredients for the double functionalization of food products with probiotics and iron, being also a source of fibre, thus representing an important contribution for the food and/or pharmaceutical industries.

As iron deficiency still continuous to be a worldwide problem, food fortification is an effective long-term approach to improve the iron status of populations. Using iron-pectin beads could be an adequate strategy to functionalise food products, contributing to attain the recommended iron intake.

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Competing interests

The authors declare that they have no competing interests.

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