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Development of iron–ascorbic acid microcapsules using Brewer's spent grain arabinoxylans as wall materials and study of their application as fortifiers in extruded corn products

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Fortification is a sustainable long-term strategy to address iron deficiency and anemia. Microencapsulation could be used to protect iron from interaction with other food components and increase its bioaccessibility. This study aimed to develop iron microcapsules using arabinoxylans (AXs) extracted from Brewer's spent grain as an encapsulating material and ascorbic acid (AA) as an absorption promoter for use as fortifiers in extruded corn products. Two levels of iron were studied (12.8 and 24.4 mg Fe per g solids), with AA (in an AA : Fe molar ratio of 1.5 : 1), keeping the iron : AX ratio constant at 1 : 20. The microcapsules were produced through spray drying. Subsequently, corn extrudates fortified with iron microcapsules or ferrous sulfate were produced, and the stability of the fortified samples stored at room temperature for one year was studied. Iron bioaccessibility from microcapsules and extruded corn products was determined after *in vitro* gastrointestinal digestion. Results indicated that ascorbic acid was partially protected from oxidation during the spray drying process (~53%). This allowed the microencapsulated iron to remain bioaccessible under the conditions of the gastrointestinal environment (~20%). The extruded corn product with the addition of microcapsules presented good iron bioaccessibility, which was higher than that of ferrous sulfate (~18 *vs.* 12%). However, the wall material failed to protect ascorbic acid from degradation during the thermal extrusion process. The products fortified with the microcapsules with the lowest iron level were more stable than the product fortified with ferrous sulfate. It was feasible to obtain an iron fortifier with good bioaccessibility using AXs as encapsulating agents.

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Sustainability spotlight

Brewer's spent grain (BSG) is the most abundant by-product generated by beer manufacturing, which can represent an interesting material for obtaining macromolecules with greater added value. We completed a bio-refinery process of BSG, obtaining proteins, arabinoxylans, polyphenols (ferulic acid) and microcrystalline cellulose, contributing to sustainable food production (goal 12). In this manuscript, we present a study of the application of arabinoxylans as a wall material for the encapsulation of iron. We used the iron microcapsules as fortifiers for corn extrudates. Cereals are staple food suitable as iron carriers to fight anemia, contributing to zero hunger (goal 2). The topics developed are related to the following: (i) novel and sustainable food resources and food ingredients. (ii) Food fortification. (iii) Circular economy approaches, including methods that enhance the value of food by-products and food waste (recovery and valorization).

1. Introduction

According to the World Health Organization (WHO), anemia is a major public health concern, mainly affecting young children (40% of children aged 6–59 months), menstruating women (30% of women aged 15–49 years), and pregnant women (37% of pregnant women), with dietary iron deficiency being among the leading causes. Fortification is a sustainable long-term strategy to address iron deficiency at the population level. There are different sources of iron fortification that vary in their bioavailability and reactivity with the food matrix. In general, the most reactive and bioavailable sources are those soluble in water.[1] Cereal-based foods are good candidates for fortification as they are staple in many communities around the world and can be handled in solid form to manufacture the iron fortified food.[2] The iron compounds recommended by the WHO to fortify cereals are ferrous sulphate, ferrous fumarate, ferric pyrophosphate and electrolytic iron. However, some compounds such as phytic acid can form insoluble complexes with the mineral, hindering its absorption. Conversely, non-heme iron absorption may be enhanced by simultaneous intake of ascorbic acid and animal protein.[3] A strategy used to protect iron from interaction with other food components and

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increase its release in a specific environment of the gastrointestinal tract is microencapsulation.[4] The capsule wall material creates a barrier between the iron and food matrix, preventing the interaction of iron with absorption inhibitors[5] and thus increasing its bioaccessibility.[3] In this regard, several researchers have studied iron encapsulation. Cian *et al.*[3] developed a microencapsulated iron fortifier with Brewer's spent grain (BSG) proteins, locust bean gum and maltodextrin, achieving iron bioaccessibility between 15.5% and 30.7%, depending on the formulation. Moreover, iron can undergo oxidation after release from the encapsulating matrix, which affects its solubility and therefore its bioavailability. A current and novel alternative to maintain iron in its ferrous state in the gastrointestinal environment is the use of wall materials with chelating ability such as: proteins, peptides or galactomannans.[6] Zhang *et al.*[7] fortified soybean flour using microencapsulated iron with alginate, caseinate and an emulsion of soybean oil and Span 80. These authors reported that the formation of lipid oxidation products such as carbonyl compounds, malondialdehyde (MDA), pentanal and hexanal was reduced during an accelerated stability test, improving oxidative stability and delaying sensory deterioration compared to soybean flour with un-encapsulated iron. Recently, Silva *et al.*[8] have encapsulated ferrous sulphate through complex coacervation using sodium alginate and soy protein isolate as wall materials. They reported an encapsulation efficiency higher than 90% and an iron bioaccessibility of 59%. When microcapsules were incorporated into cookies, no significant differences were observed in the color or texture of cookies compared with the control sample. Furthermore, Handayani *et al.*[9] fortified jelly foods with chitosan microparticles loaded with ferrous gluconate, ascorbic acid and folic acid. The presence of ascorbic acid and folic acid increased the iron bioaccessibility of microcapsules by sixfolds. Furthermore, the iron bioaccessibility from the fortified jelly foods increased more than 5 folds compared to that of the microparticles. Although various types of complex carbohydrates have been proposed as wall materials for encapsulating iron, there is no information on the use of arabinoxylans (AXs).

BSG is the main by-product of the brewing industry.[3] Arabinoxylans represent around 10% of this by-product. It is formed by a linear backbone of xyloses (X) linked by β(1–4) glycosidic bonds, with arabinose (A) residues linked to X by α(1–3), α(1–2), or both glycosidic bonds.[10,11] Galacturonic and glucuronic acids may also be present, and arabinose typically has ferulic acid attached at the O-5 position.[12,13]

Recently, it has been reported that AXs obtained from different cereal sources can be used as probiotic encapsulating materials.[14] Moreover, Heinen *et al.*[15] reported the use of BSG AX for calcium encapsulation, obtaining microcapsules with high calcium bioaccessibility (~37%). Moreover, they found that AX microcapsules provided fermentable fibers and phenolic compounds after *in vitro* colonic fermentation. Additionally, Heinen *et al.*[15] demonstrated that glucuronic acid present in the AX structure can interact with divalent cations such as calcium, and thus, its interaction with ferrous iron is likely. Therefore, the aims of this work are: (i) to develop iron microcapsules using the AX extracted from BSG as an encapsulating material and ascorbic acid as an absorption promoter, and (ii) to study the use of iron microcapsules as fortifiers for extruded corn products.

2. Materials and methods

2.1 Raw materials and Brewers' spent grain arabinoxylan concentrate

Brewer's spent grain (BSG) was supplied by Cervecería Santa Fe® (Santa Fe, Argentina). The BSG arabinoxylan concentrate (AX-c) was obtained by alkaline extraction, followed by ethanol precipitation according to Heinen *et al.*[15] The protein, ash, and total carbohydrate contents of AX-c in dry base were 3.0 ± 0.3, 16.2 ± 0.4, and 70.6 ± 1.9 g per 100 g, respectively. Moreover, the moisture content was 17.4 g per 100 g, and the arabinose/xylose ratio was 0.6 ± 0.1.

2.2 Microcapsule formulation and physicochemical characterization

Two iron levels were used in the microcapsule formulation: 0.7 (C1) and 1.4 (C2) g $FeSO_4$ per g solids, corresponding to iron levels of 12.8 and 24.4 mg Fe per g solids, respectively. The Fe : AX ratio was kept constant at 1 : 20 in all microcapsules. Ascorbic acid was added as an iron absorption promoter at an AA : Fe molar ratio of 1.5 : 1. Maltodextrin 15 DE (El Bahiense, Buenos Aires, Argentina) was added to make 10 g solids per 100 mL in the case of C1 formulation. The dispersions were spray dried using a laboratory spray dryer (Mini Spray Dryer Yamato ADL311S, Japan) according to the conditions proposed by Cian *et al.*[16] Briefly, the dispersions were fed to the main chamber (70 cm diameter) through a peristaltic pump and the feed rate was controlled by the rotation speed of the pump (3.1 mL min^{-1}). The drying air flow rate was 357 L h^{-1}, and the compressor air pressure was 6–8 bar. The inlet and outlet air temperatures were 180 ± 2 °C and 96 ± 8 °C, respectively. Two replicates were tested for each formula.

The moisture and protein contents of the microcapsules were determined by AOAC methods.[17] The total carbohydrate content of the microcapsules was determined according to DuBois *et al.*[18] The iron content of each microcapsule was determined by atomic absorption spectroscopy (PerkinElmer Analyst 300) after wet mineralization. The iron encapsulation efficiency (EE) was calculated as follows:

$$\text{EE } (\%) = \frac{\text{mg Fe in capsule}}{\text{mg Fe used in the formulation}} \times 100$$

The ascorbic acid content of the microcapsules was determined according to Van de Velde *et al.*[19] using a Shimadzu LC-20AT Series pump, with a Shimadzu SPDM20A diode array detector, equipped with a 250 × 4.6 mm i.d. reversed-phase column (Novapak C18, 5 μm; Gemini 110A C-18 Phenomenex column). Data were processed using the Shimadzu LC Solution® software (Shimadzu Co., Kyoto, Japan). The ascorbic acid content was expressed in g per 100 g solid.

The morphology and particle size of the microcapsules were assayed by scanning electron microscopy (SEM). The SEM images were acquired using a scanning electron microscope (SEM 505, Philips, The Netherlands) according to Cian *et al.*[16]

The zeta potential of the microcapsules was determined using a microelectrophoresis and dynamic light scattering instrument (Zetasizer Nano ZS90, Malvern Instruments Ltd, UK). The microcapsules were dispersed in distilled water at a concentration of 0.1 g per 100 mL, shaken and equilibrated for 1 h at room temperature. Finally, 1 mL of each sample was added to the measuring chamber. The determinations were performed in triplicate.

The Fourier transform infrared (FTIR) spectra of AX-c, C1, and C2 were obtained using an FTIR spectrometer (SHIMADZU, IR Prestige-21 model), under the conditions described by Cian *et al.*[16] Samples were analysed in duplicate.

2.3 Formulation and production of extrudates

Fortified extruded corn products were produced. Corn flour with a particle size < 590 μm was used. Samples were conditioned 1 h before extrusion by adjusting the moisture content to 14 g per 100 g. Four samples of 2 kg, namely, unfortified control (E0), sample fortified to 30 mg Fe per kg level using C1 (E1), sample fortified to 30 mg Fe per kg level using C2 (E2) and sample fortified to 30 mg Fe per kg level using $FeSO_4$ (E3), were prepared. Extrusion was carried out using a closed barrel twin-screw extruder (Jinan Kelid Machinery Co. Ltd) (China), having four independent temperature sectors with electric heaters and water circulation cooling to adjust the values in the range of 80 to 300 °C, a screw diameter of 30 mm, an *L*/*D* value of 20, a cylindrical die of 4.8 mm diameter. The screws were set at 40 Hz and the outlet temperature at 140 °C.

2.4 Characterization of extruded corn products

The expansion (ratio between the diameter of the extruded material and the diameter of the die) and specific volume (volume/weight dry base, mL g^{-1}) of the extruded corn products were determined. The microstructure of the extrudates was determined by scanning electron microscopy (SEM). The SEM images were acquired using a scanning electron microscope (SEM 505, Philips, The Netherlands). Iron and ascorbic acid contents of the extruded corn products were measured as indicated above. In addition, the bioaccessibility was determined as described in the following section.

2.5 Iron bioaccessibility

To estimate the iron bioaccessibility from microcapsules and fortified expanded products, a simulated *in vitro* gastrointestinal digestion test was performed according to Drago *et al.*[20] Briefly, 10 g samples were dispersed in 100 mL of water and the pH was adjusted to 2.0 with 3 mol L^{-1} HCl. After addition of 0.8 mL of porcine gastric mucosa pepsin (P-7000) solution (16 g pepsin in 100 mL of 0.1 mol L^{-1} HCl), the samples were incubated at 37 °C for 2 h in a shaking water bath. At the end of digestion, dialysis bags (cut-off: 6–8 kDa) containing 10 mL of PIPES buffer were placed and incubated for 50 min in a shaking water bath at 37 °C. Then, 6.25 mL of porcine pancreas pancreatin (P-1750) solution (0.4 g of pancreatin in 100 mL of 0.1 mol L^{-1} $NaHCO_3$) was added to each sample and incubated for another 2 h. The contents of the bags corresponding to the dialysates and the digested samples were transferred to tubes, weighed and frozen at −20 °C until analysis. Iron bioaccessibility (IB%) was expressed as the dialyzable iron fraction relative to the iron content of the sample.

$$\text{IB } (\%) = \frac{\text{mg Fe dialyzate}}{\text{mg Fe sample}} \times 100$$

2.6 Stability test of extruded corn products

The stability of the extruded corn products against oxidation at room temperature was analyzed for 1 year. For this, the samples were dried to 6 g per 100 g moisture content, packed in individual polypropylene bags and stored at room temperature. The samples were taken at 0, 1, 2, 5, 8 and 12 months. The content of substances reactive to thiobarbituric acid (TBA) was determined according to Siu and Draper.[21] In addition, the samples were ground using a cyclone mill (Cyclotec UDY) with a 1 mm sieve and the color was measured using a colorimeter (Minolta CM-508d, Japan), based on the color space defined by the CIE (Commission Internationale de l'Eclairage) standard. The measurement conditions were Illuminant D65, observation angle 10°, and occluded specular component. The color difference (ΔE^*) between the extrudates was determined taking into account the sample at the initial time with respect to E0, and in the evolution over time, each sample was compared with respect to its initial value, according to the following equation:

$$\Delta E^* = \sqrt{\left\{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right\}}$$

2.7 Statistical analysis

Each determination was performed at least in duplicate. The results are expressed as mean ± standard deviation (SD). The Statgraphics Centurion XV 15.2.06 software was used for data analysis by one-way and multifactor ANOVA. Differences between samples were determined by the LSD test ($p < 0.05$).

3. Results and discussion

3.1 Physicochemical characterization of microcapsules

The EE of iron was 92.2 ± 0.8 and 98.2 ± 1.1% for C1 and C2, respectively. This result indicated very good performance during spray drying. In addition, a higher concentration of $FeSO_4$ increased the EE.

Fig. 1A and B show the scanning electron microscopic (SEM) images of C1 and C2, respectively. The microcapsules had a mean size (considering a normal distribution) of 5.13 and 4.80 μm for C1 and C2, respectively. These values were within the expected range for microcapsules produced by spray drying (1–50 μm).[22] In addition, the microcapsules were spherical in

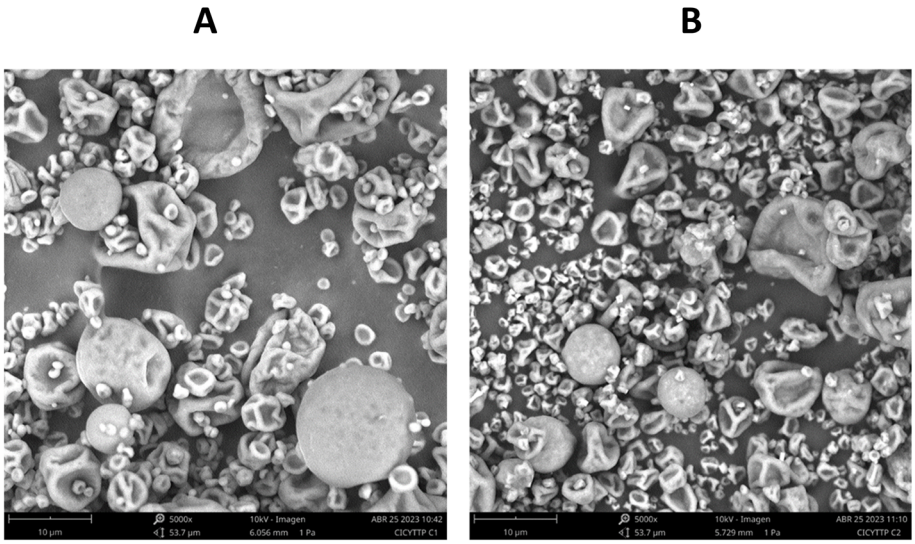

Fig. 1 Scanning electron microscopy (SEM) images (5000×) of microcapsules C1 (11 774 ± 104 mg Fe per kg) (A) and C2 (23 985 ± 256 mg Fe per kg) (B). Both microcapsules have an Fe : AX ratio of 1 : 20.

shape, with a smooth surface and some with concavities. This morphology is characteristic of products obtained by spray drying.[16] The mean size values obtained for C1 and C2 were similar to those reported by Kowalska *et al.*,[23] who obtained an average size of 11 μm for microcapsules formulated from rice-AX and honey by spray drying. Additionally, the C1 formulation resulted in larger, dented microcapsules that were able to maintain their shape. For C2, a smaller number of dented microcapsules were observed, whose diameters were lower than those obtained for C1. These characteristics could indicate that the wall thickness and the iron level used were adequate in both cases. However, it was observed that the microcapsule C2 was physically more agglomerated, with greater difficulty to spread, probably due to the absence of maltodextrin in its formulation.

The zeta potential of AX-c was negative in the pH range from 9.0 to 2.0 and tended to become zero as the pH decreased to 2.0 (Fig. 2A). The maximum zeta potential value of AX-c (−12.78 ± 0.01 mV) was observed in alkaline pH solutions. A similar profile has also been observed in other natural gums.[24] The negative charge of AX at high pH values could be attributed to glucuronic and ferulic acids and the proteins co-extracted with AX.[15] In the case of microcapsules, the higher the Fe content of the formulation, the lower the absolute value of the zeta potential, which could indicate the neutralization of the surface charge of the AX molecule with the addition of the ferrous ion. The Fe : AX ratio of the microcapsules was 1 : 20 for both formulations, but C2 was not formulated with maltodextrin; consequently, its iron content by weight was higher than that

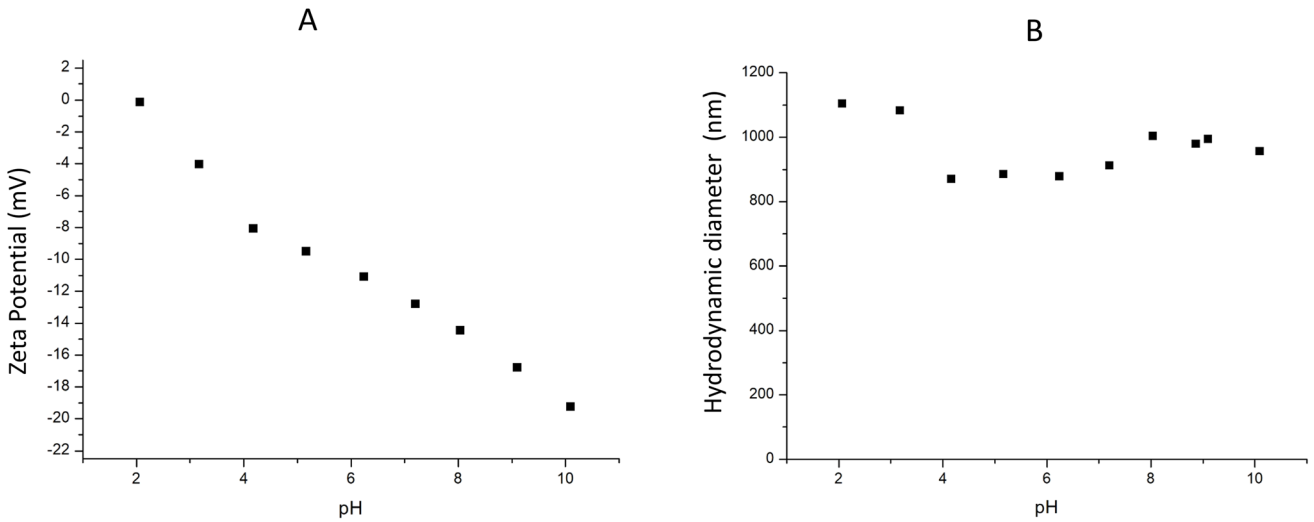

Fig. 2 Zeta potential of microcapsules C1 (11 774 ± 104 mg Fe per kg) (A) and C2 (23 985 ± 256 mg Fe per kg) (B). Both microcapsules have an Fe : AX ratio of 1 : 20.

found in C1 (23 985 ± 256 *vs.* 11 774 ± 104 mg Fe per kg capsule, respectively). Kaul *et al.*[25] encapsulated iron using potato starch and maltodextrin, obtaining microcapsules with a zeta potential value of 0.181–0.486 mV, for $FeSO_4$ concentrations ranging from 3 to 15%, although they did not report the pH of the system. The use of a hydrocolloid slightly charged in C1 and C2 made it possible to obtain microcapsules with a higher zeta potential value (−2.21 ± 0.01 mV and −1.74 ± 0.01 mV, respectively), which would imply greater colloidal stability than that reported by these authors.

The FTIR profile of AX-c and the microcapsules are shown in Fig. 3A and B. Li *et al.*[26] reported that the interactions between COO^- groups and Fe^{2+} can be detected by FTIR spectroscopy through the separation of the wavenumbers corresponding to the asymmetric (ν_{as}) and symmetric (ν_s) stretching vibrations of COO^- groups, reflecting the possibility of Fe^{2+} and COO^- groups bonding. If the wavenumber spacing ($\Delta\nu_{as-s}$) is higher than 200 cm^{-1}, the carboxylic group is bound to a single Fe atom (monodentate). However, the $\Delta\nu_{as-s}$ values lower than 200 cm^{-1} indicate chelating bonds.[27,28] Two peaks were observed at 1651 cm^{-1} and 1415 cm^{-1}, which are attributed to the asymmetric and symmetric stretching vibrations of the COO^- groups in the AX chains.[29] For C2, two asymmetric stretching vibrations ν_{as} were observed at 1789 and 1651 cm^{-1} and the symmetric stretching vibration ν_s at 1415 cm^{-1}. Thus, the wavenumber separations ($\Delta\nu_{as} - \nu_s$) for this microcapsule were 374 and 236 cm^{-1}. These results indicated that the carboxylic group only bound in a monodentate mode between C2 molecules. As for C2, the C1 FTIR spectrum showed an asymmetric peak only at 1651 cm^{-1}, which was consistent with monodentate bonding. According to these results, the AX present in C1 and C2 interacts with Fe in a monodentate manner, with no chelating interactions being observed.

3.2 Study of the fortification of extruded corn products

Regarding the characteristics of the extruded corn products, expansion and specific volume (SV) are shown in Table 1. In general, there were no significant differences in terms of SV and expansion between the samples with the different fortifiers and the control ($p > 0.05$), except for the expansion of E3. These values correspond to samples extruded under operating conditions that allow obtaining very good expansion and degree of cooking of the starchy material. In this regard, Sahu *et al.*[30] reported expansion values ranging from 2.02 to 3.39 for corn and soy protein extrusion. Moreover, Bajaj and Singhal[31] found that the expansion and the specific volume of rice extruded corn products with added guar gum or xanthan gum were in the ranges of 3.19–3.22 and 3.84–4.54 $cm^3\ g^{-1}$, respectively. Therefore, the addition of C1 and C2 to extruded corn products did not affect the expansion and specific volume of the final products. Fig. 4 shows the scanning electron microscopic (SEM) images of E0 and E2. Similar polygonal particles with rough surfaces and transversal alveolar sections were observed for

Table 1 Characteristics of unfortified expanded products (E0) and those fortified with the microcapsules C1 (E1) and C2 (E2) and ferrous sulfate (E3)[a]

Samples	Specific volume ($cm^3\ g^{-1}$)	Expansion
E0	8.39 ± 0.50	3.46 ± 0.25[a]
E1	8.17 ± 0.31	3.48 ± 0.10[a]
E2	8.23 ± 0.21	3.40 ± 0.13[a]
E3	8.08 ± 0.43	3.61 ± 0.20[b]

[a] X ± SD. Different letters in a column indicate significant differences between samples ($p < 0.05$).

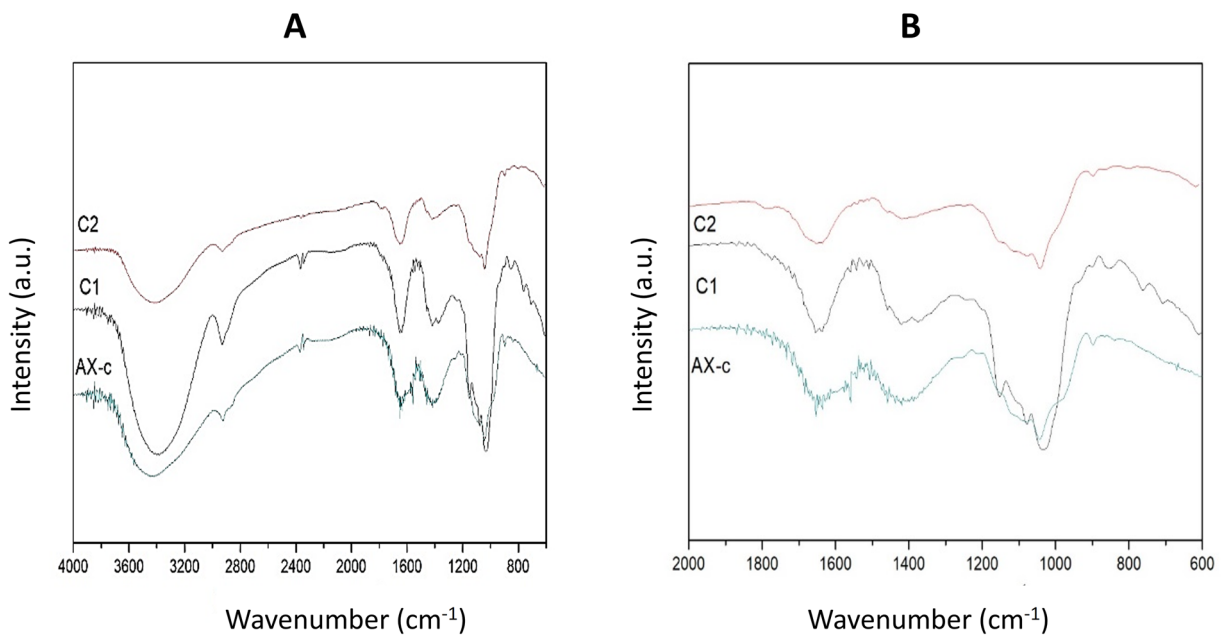

Fig. 3 FTIR spectra of AX-c and the different microcapsules in the range of 4000–800 cm^{-1} (A) and 2000–600 cm^{-1} (B).

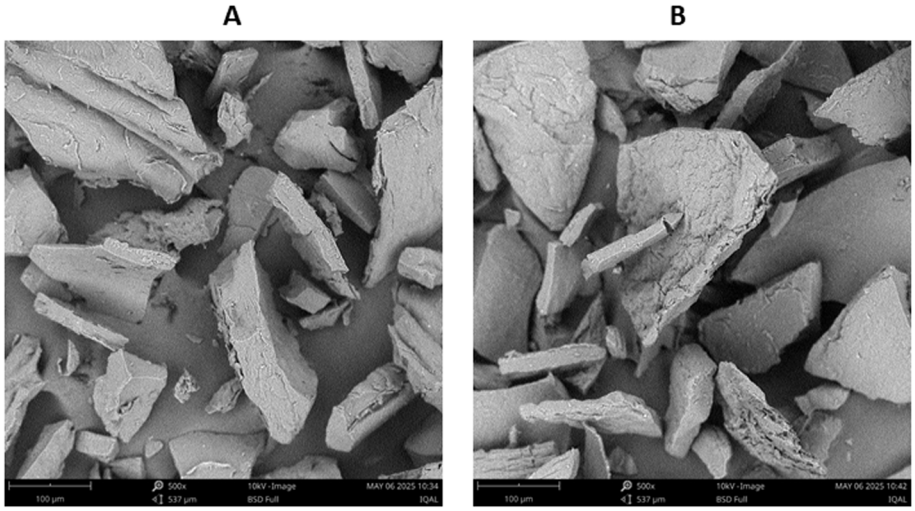

Fig. 4 Scanning electron microscopy (SEM) images (500×) of the un-fortified expanded corn product E0 (A) and expanded corn product fortified with C2 (23 985 ± 256 mg Fe per kg) E2 (B).

both samples, indicating that the addition of C1 and C2 to extruded corn products did not affect the microstructure.

The ascorbic acid contents in C1, C2, E1 and E2 are shown in Table 2. The microcapsules were formulated to a molar AA : Fe ratio of 1.5 : 1. However, during spray drying, a loss of 46–48% of ascorbic acid occurred, resulting in an AA : Fe molar ratio of ~0.9 : 1. The AA is very sensitive to temperature and oxidation in the presence of divalent cations such as iron. In turn, its oxidation increases as water activity decreases during spray drying. All of these factors contributed to the loss of AA during drying.[32] According to Teucher *et al.*,[32] for foods with low to medium levels of inhibitors, the addition of AA in an AA : Fe molar ratio of 2 : 1 is required. To promote the iron absorption in the presence of high levels of inhibitors, it is necessary to add AA in a molar ratio higher than 4 : 1, which can be impractical. In fact, the amount of AA to be added in the microcapsule formulation was excessive and decreased the iron content of the fortifier. However, in the extruded food, a good retention of AA was not achieved, possibly due to the thermo-sensitivity of AA and the unfavourable conditions of the extrusion process related to high temperatures (140 °C) and shear stresses.[33] Encapsulation with AX-c may probably protect AA during less drastic thermal processes than extrusion.

3.3 Iron bioaccessibility of microcapsules

Iron bioaccessibility (IB%) was estimated from the dialyzability of this mineral. The IB% values were similar between the microcapsules (19.60 ± 0.78 and 20.13 ± 1.04% for C1 and C2, respectively). These values can be considered high and are due to the fact that the formulation included ascorbic acid (AA), which is a promoter of iron absorption.[3] AA exerts its effect both through its reducing and complexing capacity, keeping iron in its ferrous form, which is more soluble, and forming soluble AA–Fe^{2+} and AA–Fe^{3+} complexes.[34] Iron absorption largely depends on its chemical form (ferrous/ferric) and its speciation.[34] Ferric iron is precipitated in solutions with a pH higher than 3.0, while most ferrous iron remains soluble at a pH lower than 5.0. At the level of the gastrointestinal tract, iron must first be solubilized in the stomach and complexed with other components present in food that keep it soluble at the absorption site (duodenum). However, complexing compounds can be enhancers or inhibitors of iron absorption, depending on their solubility.[5] Therefore, dietary composition is one of the main factors influencing the absorption of non-heme iron.[35] These results are similar to those obtained by Cian *et al.*[3] who developed iron–AA microcapsules with BSG peptides and locus beam gum. These authors reported an IB% between 15.5 and 30.7%, depending on the formulation of the wall material and the AA : Fe ratio used. As mentioned above, AA keeps iron in its ferrous state and acts as a chelating agent, promoting iron absorption.[36]

Table 2 Ascorbic acid content and retention[a]

Samples	mg TAA per 100 g	Retention (%)	AA : Fe molar ratio
C1	3490.0 ± 75.7	54.16 ± 1.17	0.94 ± 0.02
C2	6823.7 ± 280.4	52.00 ± 2.14	0.90 ± 0.04
E1	1.69 ± 0.03	11.70 ± 0.21	0.11 ± 0.01
E2	1.74 ± 0.07	12.32 ± 0.51	0.11 ± 0.01

[a] $X \pm$ SD. TAA: total ascorbic acid. AA: ascorbic acid.

Table 3 shows the bioaccessibility and potential iron contribution of the fortified extruded corn products. The IB% of the fortified extrudates depended on the fortifier used, being higher in the case of microcapsules C1. As observed for C2, the higher iron content in the formulation of the microcapsules probably affected their dialyzability. Both products fortified with the microcapsules presented better iron bioaccessibility than the sample with ferrous sulphate, probably due to the presence of residual AA and a possible monodentate effect of the wall material that helps to maintain iron in a soluble state.

Among ferrous preparations, $FeSO_4$ remains the standard and established treatment for iron deficiency given its acceptable tolerability, high effectiveness and low cost.[37] However,

Table 3 Iron bioaccessibility (IB) and potential iron contribution of expanded products[a]

Fortified extruded products	IB (%)	Iron potential supply[b] (mg)
E1 (C1)	19.01 ± 0.28[a]	0.76 ± 0.02[a]
E2 (C2)	17.10 ± 0.32[b]	0.68 ± 0.01[b]
E3 ($FeSO_4$)	11.68 ± 0.23[c]	0.47 ± 0.01[c]

[a] $X \pm$ SD. Different letters in a column indicate significant differences between samples ($p < 0.05$). [b] For a 100 g portion of extrudate.

iron bioaccessibility is often affected by absorption-inhibiting factors present in the diet, which exert effects on the solubility of this iron form. Jaiswal and Lakshmi[38] developed a complementary food mix (CFM) using wheat, decorticated chickpea, oilseed flours and skimmed milk powder. Phytic acid was reduced by activating endogenous phytases at pH 5.2 and 45 °C with subsequent inactivation by heat treatment. Moreover, this product was fortified with different iron salts (iron and sodium EDTA and ferrous fumarate). Iron bioaccessibility (measured by the dialysis and simulated gastrointestinal digestion method) was 15.7% in CFM fortified with ferrous fumarate and 17.2% for the sample fortified with NaFeEDTA. These latter results, achieved with a highly bioavailable fortifier, are similar to those obtained in extrudates fortified with iron microcapsules.

These results have been obtained using an *in vitro* simulated gastrointestinal digestion methodology that takes into account the physicochemical factors that occur in the gastrointestinal lumen. However, *in vivo* studies have shown that oligosaccharides with a prebiotic effect can increase iron bioaccessibility. In this regard, Lynch *et al.*[39] have reported a prebiotic activity of AX fractions from BSG, which increased the level of *Lactobacillus*, *Bifidobacteria* and the production of acetate and propionate. Thus, in an *in vivo* study, an improvement in the results obtained from IB% could be seen.

3.4 Stability of fortified extruded corn products

In order to study the stability of fortified products, an assay was performed to measure oxidative rancidity by determining thiobarbituric acid reactive substances (TBARS). Table 4 shows the results obtained in the samples packaged in conventional snack containers and stored at room temperature for one year. The multifactor ANOVA showed that both factors (time and fortified type) and their interaction were significant ($p < 0.0000$). By analysing the effect of time, it can be seen that oxidative processes began after 150 days (Table 5). The average value for the initial day was 5.15 ± 0.35 nmol MDA per g sample. At the last day of storage (360 days later), it increased to 10.5–14.7 nmol MDA per g sample (average 12.08 nmol MDA per g), depending on the sample. However, by analysing the effect of the sample, the control sample (E0) was found to have the lowest average MDA content (5.95 nmol MDA per g), and the sample fortified with C2 (E2) had the highest one (7.54 nmol MDA per g) (Table 5).

Table 5 Multiple range tests for MDA[a]

By time	Media LS	Homogeneous groups
30	4.83	a
60	4.88	a
0	5.15	a
150	5.88	b
240	7.29	c
360	12.08	d

By sample	Media LS	Homogeneous groups
E0	5.95	a
E3	6.56	b
E1	6.69	b
E2	7.54	c

[a] Method: 95.0% LSD.

Table 6 shows the colour difference (ΔE^*) of the samples. Both factors, time and fortified type, were significant for the ΔE^* value (p-value = 0.0000). In addition, it is observed that ΔE^* was lower for the un-fortified sample (E0, ~3.71) and higher for the extrudates fortified with C1 (E1, ~6.65) and $FeSO_4$ (E3, ~6.34). However, the overall colour difference was similar up to 150 days of storage (~3.92) and then increased for 240 (~7.91) and 360 days (~12.20) (Table 7).

Table 4 Thiobarbituric acid-reactive substance (TBARS) content of extruded products fortified with different iron sources[a]

	TBARS (nmol MDA per g sample)			
Time (days)	E0	E1	E2	E3
0	4.79 ± 0.27[ab]	4.92 ± 0.39[a]	5.52 ± 0.14[a]	5.36 ± 0.60[b]
30	3.56 ± 0.48[a]	5.21 ± 0.95[a]	6.68 ± 0.13[b]	3.87 ± 0.70[a]
60	3.99 ± 0.44[a]	5.69 ± 0.54[a]	5.31 ± 0.42[a]	4.55 ± 0.01[ab]
150	5.97 ± 0.83[bc]	6.02 ± 0.60[a]	5.73 ± 0.29[a]	5.81 ± 0.49[bc]
240	6.87 ± 0.69[c]	7.84 ± 0.77[b]	7.08 ± 0.32[b]	7.14 ± 0.67[c]
360	10.51 ± 0.56[d]	10.49 ± 0.46[c]	14.67 ± 0.22[c]	12.66 ± 0.52[d]

[a] $X \pm$ SD. Time 0 corresponds to the day of packaging carried out one day after extrusion; E0: unfortified sample; E1: sample fortified with C1; E2: sample fortified with C2; E3: sample fortified with un-encapsulated $FeSO_4$. Different letters in a column indicate significant differences between samples ($p < 0.05$).

Table 6 Color difference values (ΔE^*) of expanded products fortified with different iron sources over time[a]

	ΔE^*			
Days	E0	E1	E2	E3
0	—	5.37 ± 0.79	2.90 ± 0.41	4.92 ± 0.50
30	1.17 ± 0.25	3.30 ± 0.68	2.14 ± 0.10	1.86 ± 0.20
60	3.97 ± 0.24	3.25 ± 0.24	1.34 ± 0.68	2.57 ± 0.56
150	1.47 ± 0.18	4.40 ± 0.07	1.69 ± 0.02	7.03 ± 0.52
240	4.37 ± 0.61	9.24 ± 0.55	8.76 ± 0.24	8.86 ± 0.31
360	9.32 ± 0.59	14.16 ± 0.43	12.55 ± 0.93	12.71 ± 0.67

[a] $X \pm$ SD. ΔE initial: relative to E0 (un-fortified sample); ΔE 30, 60, 150, 240 and 360 days: calculated with the color parameter values of the corresponding sample at day = 0. Time 0 corresponds to the day of packaging carried out one day after extrusion; E0: unfortified sample; E1: sample fortified with C1; E2: sample fortified with C2; E3: sample fortified with un-encapsulated $FeSO_4$.

Table 7 Multiple range tests for ΔE^*[a]

By time	Media LS	Homogeneous groups
30	1.89941	a
60	2.78279	ab
0	3.7898	b
150	3.91781	b
240	7.90461	c
360	12.1997	d
By sample	**Media LS**	**Homogeneous groups**
E0	3.70921	a
E2	4.95937	b
E3	6.34287	c
E1	6.65126	c

[a] Method: 95.0% LSD.

4. Conclusions

It was possible to obtain an iron fortifier with good bioaccessibility (~20%) using AX-c from Brewer's spent grain as an encapsulating agent. The wall material managed to encapsulate iron and ascorbic acid and partially protected (~53%) this complex from oxidation due to spray drying, keeping the iron bioaccessible under ambient conditions of the gastrointestinal tract. When applied to a mass consumption food (precooked cereal matrix), it resulted in a fortified product with good iron bioaccessibility (~18%). However, good protection of ascorbic acid against the thermal extrusion process was not achieved. It would be appropriate to evaluate the stability of this compound in another thermal process less drastic than extrusion. However, the results of the evaluation of oxidative stability over time indicated that the extrudates fortified with formulation C1 were slightly more stable than those fortified with $FeSO_4$, reaching the same TBARS values than the sample without iron addition. Further studies should be conducted to study the deterioration kinetics under accelerated conditions and the shelf-life prediction of extrudates fortified with different iron sources.

Data availability

Data for this article, including an excel file, are available at **http://hdl.handle.net/11336/266009**.

Author contributions

G. D. Heinen: writing – original draft, validation, methodology, investigation, formal analysis. R. E. Cian: writing – review & editing. S. R. Drago: writing – review & editing, supervision, funding acquisition, data curation, conceptualization.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1 A. Kumari and A. K. Chauhan, *J. Food Sci. Technol.*, 2022, **59**(9), 3319–3335.
2 R. Blanco-Rojo and M. P. Vaquero, Iron bioavailability from food fortification to precision nutrition. A review, *Innovative Food Sci. Emerging Technol.*, 2019, **51**, 126–138.
3 R. E. Cian, J. L. Proaño, P. R. Salgado, A. N. Mauri and S. R. Drago, High iron bioaccessibility from co-microencapsulated iron/ascorbic acid using chelating polypeptides from Brewers' spent grain protein as wall material, *LWT*, 2021, **139**, 110579.
4 S. Gharibzahedi and S. Jafari, The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation, *Trends Food Sci. Technol.*, 2017, **62**, 119–132.
5 E. Piskin, D. Cianciosi, S. Gulec, M. Tomas and E. Capanoglu, Iron absorption: factors, limitations, and improvement methods, *ACS Omega*, 2022, **7**(24), 20441–20456.
6 M. Caetano-Silva, F. Netto, M. Bertoldo-Pacheco, A. Alegría and A. Cilla, Peptide-metal complexes: Obtention and role in increasing bioavailability and decreasing the pro-oxidant effect of minerals, *Crit. Rev. Food Sci. Nutr.*, 2020, **61**(9), 1470–1489.
7 K. Zhang, X. Yao, Y. Gao, N. Liu, J. Qiu, S. Li, X. Liu and Y. He, Improvement on storage stability of iron-fortified soybean

powder by microencapsulation, *J. Food Sci.*, 2021, **86**(3), 996–1003.
8 M. S. F. da Silva, B. S. T. Barbosa and E. E. Garcia-Rojas, Sodium alginate/soy protein isolate microcapsules enriched with iron sulphate for fortifying cookies, *Int. J. Food Sci. Technol.*, 2025, **60**(1), vvae052.
9 N. A. Handayani, K. Mulia, S. Kartohardjono and E. A. Krisanti, Fortifying jelly foods with microencapsulated anti-anaemic compounds, ferrous gluconate, ascorbic acid and folic acid, *J. Food Sci. Technol.*, 2023, **60**(1), 147–159.
10 A. L. Mc Carthy, Y. C. O'Callaghan, C. O. Piggott, R. J. FitzGerald and N. M. O'Brien, Brewers' spent grain; bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: a review, *Proc. Nutr. Soc.*, 2013, **72**(1), 117–125.
11 K. M. Lynch, E. J. Steffen and E. K. Arendt, Brewers' spent grain: a review with an emphasis on food and health, *J. Inst. Brew.*, 2016, **122**(4), 553–568.
12 M. Sajib, P. Falck, R. R. Sardari, S. Mathew, C. Grey, E. N. Karlsson and P. Adlercreutz, Valorization of Brewer's spent grain to prebiotic oligosaccharide: Production, xylanase catalyzed hydrolysis, in-vitro evaluation with probiotic strains and in a batch human fecal fermentation model, *J. Biotechnol.*, 2018, **268**, 61–70.
13 A. Morales-Ortega, E. Carvajal-Millan, Y. López-Franco, A. Rascón-Chu, J. Lizardi-Mendoza, P. Torres-Chavez and A. Campa-Mada, Characterization of water extractable arabinoxylans from spring wheat flour: rheological properties and microstructure, *Molecules*, 2013, **18**(7), 8417–8428.
14 H. Liu, M. Xie and S. Nie, Recent trends and applications of polysaccharides for microencapsulation of probiotics, *Food Front.*, 2020, **1**(1), 45–59.
15 G. D. Heinen, A. G. Garzón, R. E. Cian and S. R. Drago, Gastrointestinal and colonic bioaccessibility of calcium and ferulic acid from microcapsules made with brewer spent grain arabinoxylans, *Int. J. Biol. Macromol.*, 2025, **292**, 139237.
16 R. E. Cian, A. Campos-Soldini, L. Chel-Guerrero, S. R. Drago and D. Betancur-Ancona, Bioactive Phaseolus lunatus peptides release from maltodextrin/gum arabic microcapsules obtained by spray drying after simulated gastrointestinal digestion, *Int. J. Food Sci. Technol.*, 2019, **54**(6), 2002–2009.
17 Association of Official Analytical Chemists (AOAC), *Official Methods of Analysis*, ed. W. Horwitz, Washington, DC, 16th edn, 1995.
18 M. DuBois, K. A. Gilles, J. K. Hamilton, P. T. Rebers and F. Smith, Colorimetric method for determination of sugars and related substances, *Anal. Chem.*, 1956, **28**(3), 350–356.
19 F. Van de Velde, M. E. Pirovani, M. S. Cámara, D. R. Güemes and C. M. Bernardi, Optimization and validation of a UV–HPLC method for vitamin C determination in strawberries (Fragaria ananassa Duch.), using experimental designs, *Food Anal. Methods*, 2012, **5**, 1097–1104.
20 S. R. Drago, M. Binaghi and M. E. Valencia, Effect of gastric digestion pH on iron, zinc, and calcium dialyzability from preterm and term starting infant formulas, *J. Food Sci.*, 2005, **70**(2), S107–S112.
21 G. M. Siu and H. H. Draper, A survey of the malonaldehyde content of retail meats and fish, *J. Food Sci.*, 1978, **43**(4), 1147–1149.
22 A. Nesterenko, I. Alric, F. Silvestre and V. Durrieu, Vegetable proteins in microencapsulation: A review of recent interventions and their effectiveness, *Ind. Crops Prod.*, 2013, **42**, 469–479.
23 G. Kowalska, J. Rosicka-Kaczmarek, K. Miśkiewicz, M. Wiktorska, D. Gumul, M. Orczykowska and K. Dędek, Influence of rye bran heteropolysaccharides on the physicochemical and antioxidant properties of honeydew honey microcapsules, *Food Bioprod. Process.*, 2021, **130**, 171–181.
24 J. A. Barbosa, M. S. Abdelsadig, B. R. Conway and H. A. Merchant, Using zeta potential to study the ionisation behaviour of polymers employed in modified-release dosage forms and estimating their pK_a, *Int. J. Pharm.: X*, 2019, **1**, 100024.
25 S. Kaul, K. Kaur, N. Mehta, S. S. Dhaliwal and J. F. Kennedy, Characterization and optimization of spray dried iron and zinc nanoencapsules based on potato starch and maltodextrin, *Carbohydr. Polym.*, 2012, **282**, 119107.
26 J. Li, Z. Lu, Z. Chen, C. Li, Y. Du, C. Chen, L. Wang and P. Yu, Preparation and characterization of pH-responsive microgel using arabinoxylan from wheat bran for BSA delivery, *Food Chem.*, 2021, **342**, 128220.
27 X. Hu, J. Guo, Y. Wang and C. Liu, Synthesis, infrared spectra, thermal analyses and structural studies of half-sandwich Fe(III)/Fe(II) complex containing pyridine-2,6-dicarboxylate and 1,10-phenanthroline, *Spectrochim. Acta, Part A*, 2009, **74**(1), 48–51.
28 H. L. Gao, B. Ding, L. Yi, P. Cheng, D. Z. Liao, S. P. Yan and Z. H. Jiang, Syntheses and structures of 1D and 2D coordination polymers derived from pyridine-2,4,6-tricarboxylic acid, *Inorg. Chem. Commun.*, 2005, **8**(2), 151–154.
29 G. D. Manrique and F. M. Lajolo, FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit, *Postharvest Biol. Technol.*, 2002, **25**(1), 99–107.
30 C. Sahu, S. Patel and A. K. Tripathi, Effect of extrusion parameters on physical and functional quality of soy protein enriched maize based extruded snack, *Appl. Food Res.*, 2022, **2**(1), 100072.
31 S. R. Bajaj and R. S. Singhal, Effect of extrusion processing and hydrocolloids on the stability of added vitamin B12 and physico-functional properties of the fortified puffed extrudates, *LWT*, 2019, **101**, 32–39.
32 B. Teucher, M. Olivares and H. Cori, Enhancers of iron absorption: ascorbic acid and other organic acids, *Int. J. Vitam. Nutr. Res.*, 2004, **74**(6), 403–419.
33 M. N. Riaz, M. Asif and R. Ali, Stability of vitamins during extrusion, *Crit. Rev. Food Sci. Nutr.*, 2009, **49**(4), 361–368.

34 T. Ems, K. St Lucia and M. R. Huecker, Biochemistry, iron absorption, *StatPearls*, StatPearls Publishing, Treasure Island, FL, 2023, PMID: 28846259.
35 P. A. Sharp, Intestinal iron absorption: regulation by dietary & systemic factors, *Int. J. Vitam. Nutr. Res.*, 2010, **80**(4–5), 231–242.
36 R. Hurrell and I. Egli, Iron bioavailability and dietary reference values, *Am. J. Clin. Nutr.*, 2010, **91**(5), 1461–1467.
37 P. Santiago, Ferrous versus ferric oral iron formulations for the treatment of iron deficiency: a clinical overview, *Sci. World J.*, 2012, **2012**(1), 846824.
38 A. Jaiswal and A. J. Lakshmi, Maximising the bioaccessibility of iron and zinc of a complementary food mix through multiple strategies, *Food Chem.*, 2022, **372**, 131286.
39 K. M. Lynch, C. R. Strain, C. Johnson, D. Patangia, C. Stanton, F. Koc, J. Gil-Martinez, P. O'Riordan, A. W. Sahin, R. P. Ross and E. K. Arendt, Extraction and characterization of arabinoxylan from brewers spent grain and investigation of microbiome modulation potential, *Eur. J. Nutr.*, 2021, **60**(8), 4393–4411.