Proceedings of the Nutrition Society (2018), **77 (OCE2)**, E45 doi:10.1017/S0029665118000393

Spring Conference 2018, 26–27 March 2018, Nutrient-nutrient interaction

Iron uptake by Caco-2 cells from a Brazilian natural plant extract loaded into chitosan/pectin nano- and micro-particles

V.B.V. Maciel[1,3], C.M.P. Yoshida[2], C. Boesch[3], F.M. Goycoolea[3] and R.A. Carvalho[1]
[1]*Faculty of Animal Science and Food Engineering, University of São Paulo, 13635900 São Paulo, Brazil,* [2]*Department of Exact and Earth Science, Federal University of São Paulo, 09913030 São Paulo, Brazil and* [3]*School of Food Science and Nutrition, University of Leeds, LS2 9JT, UK.*

Pereskia aculeata Miller, known in Brazil as ora-pro-nobis (OPN) is a non-conventional edible plant and considered of high nutritional value, in particular due to its high iron and calcium content[(1)]. Considering the low availability of plant iron, there is great interest in the development of improved iron delivery systems. The aim of this study was to develop an alternative carrier system loaded with OPN extract into chitosan/pectin particle formulations and to evaluate cellular iron availability. Oppositely charged, chitosan (CS) and pectin (PT) are expected to interact via polyelectrolyte electrostatic self-assembly, resulting in a very strong intermolecular interaction and highly ordered orientation of the rigid-chain polymers[(2)]. The resultant particles of the self-assembly process should efficiently encapsulate the OPN iron.

CS/PT/OPN nano- and micro-particles were prepared at two different polycation/polyanion molar ratios, n^+/n^- 0.25 (excess of pectin) and 5.00 (excess of chitosan), and total charge of 1.0×10^{-6} M. We evaluated the iron association efficiency, cytotoxicity by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and iron uptake as intracellular ferritin protein synthesis indicating cellular iron absorption in the Caco-2 cell model[(3)]. Ferritin concentration in cell lysates was measured using ELISA. Nano- and micro-particles were also produced using $FeSO_4$ as a positive control for iron uptake experiments. Statistically significant differences between the different treatments were assessed by ANOVA and Tukey comparison test ($p < 0.05$).

As shown in Table 1, increasing the chitosan concentration in the CS/PT/OPN and CS/PT/FeSO4 nano- and micro-particles, improved iron incorporation significantly and was generally higher in $FeSO_4$ formulations. Increasing the CS ratio in OPN particles also led to higher ferritin levels, contrasting to high CS $FeSO_4$ particles which showed higher ferritin levels with lower CS/PT ratio. Cytotoxicity tests performed with nano- and micro-particles loaded iron from OPN extract presented high cell viability (80.77 ± 1.49 to 99.08 ± 3.62 %).

Table 1. Iron association efficiency and Caco-2 cell iron uptake from particles, OPN extract and $FeSO_4$ solution.

Samples	Iron association efficiency (%)	Ferritin (ng/mg cell protein)
CS/PT/OPN 0.25	33.99 ± 2.54^{d}	3.53 ± 0.21^{g}
CS/PT/OPN 5.00	59.56 ± 3.33^{b}	6.81 ± 0.36^{f}
CS/PT/$FeSO_4$ 0.25	45.78 ± 2.87^{c}	28.18 ± 1.97^{d}
CS/PT/$FeSO_4$ 5.00	63.21 ± 4.69^{a}	16.41 ± 1.45^{e}
OPN extract[#]	–	174.96 ± 15.44^{c}
$FeSO_4$ solution[#]	–	508.79 ± 26.43^{a}
Negative control	–	0.89 ± 0.01^{h}

Results are mean with SE of three independent experiments. Different letters differ statistically ($p < 0.05$) by Tukey's test. [#]Amount of iron: 1522 ng/mL.

The higher efficiency of iron association to CS/PT particles with higher positive charge is only reflected in higher cellular ferritin levels in the OPN group. Differences to $FeSO_4$ may relate to the differing iron type and iron release patterns, thereby impacting on iron absorption in Caco-2 cells. Further studies are required to better understand mechanisms of iron binding and release using novel designed delivery systems.

This work was financed by the São Paulo Research Foundation (Brazil).

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