Production of $FeSO_4$ microparticles using pea protein concentrate as wall material

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INTRODUCTION

Iron deficiency affects more people than any other nutrient deficiency, constituting a public health condition of epidemic proportions that can reduce the work capacity of individuals and entire populations, bringing serious economic and health consequences. In infants and young children, diets with inadequate levels of iron impair physical growth, mental development, and learning capacity. It is the only nutrient deficiency which is also significantly prevalent in industrialized countries (WHO, 2007). Despite repeated public commitments and availability of various forms of iron supplements, rates of anemia remain high. A major reason for this lack of success has been poor adherence to supplement consumption. Distribution of micronutrient powder is becoming a preferred strategy for addressing micronutrient deficiencies (Ip et al, 2009). In response, different formulations are being developed.

Pierucci et al developed (2006) and Pereira et al (2009) improved a new microencapsulation system for ascorbic acid and tocopherol (2007) using pea protein concentrate (PPC) as coat protector. There is great interest in the encapsulation area of edible substances to find new alternatives of natural, low cost and biocompatible materials. Legume seeds proteins seems to attend these specificities and have gained increasing attention with regard to their use as functional agents in food development (Pedrosa et al, 2000) since they have functional properties like emulsification, film formation and gelation (Rangel et al 2003, Choi et al 2002). Making use of these globulins attributes, we used here, for the first time, a legume protein isolate (PPC) as encapsulating matrix for iron. Therefore, the aim of this work was to produce bioavailable microparticles of $FeSO_4$ for supplementing diet purpose using PPC as coating agent.

MATERIAL AND METHODS

Production of microparticles - PPC was gently given by Labonathus Laboratory (Brazil). Fe-PPC microparticles were obtained by a spray-drying process using PPC gelified dispersion as coat material and an aqueous solution (30mM) of $FeSO_4$ as core material. PPC was submitted to the same process as Fe-PPC, intending to be further used as a control. The drying process was performed in a mini spray dryer Buchi B-290 at stable process conditions: inlet air temperature at 180 ± 2ºC and outlet at 90 ºC; air pressure at 80 lb; vacuum at -50 mbar; nozzle of 0,3 mm; and feed of 0,8L h^{-1}.

Iron, protein and moisture contents - Moisture content of microparticles was determined by desiccation in an oven at 105 ºC until constant weight, according to AOAC (1990). Analyses of the iron content of the solution were done by standard atomic absorption spectrometry technique using a Perkin-Elmer 3300 spectrometer - Perkin-Elmer, Shelton, CT (Remondetto et al, 2004). Protein percentage was estimated according to Lowry et al (1951).

Microparticles morphology – Morphology of microparticles was examined by scanning electron microscopy (SEM). The dried particles were directly deposited on a carbon conductive tape on aluminum stubs and sputtered with thin gold layer (Balzer Union, FL-9496).Samples were observed in a JEOL 5310, operated at 15 kV.

Particle size – The SEM images were transferred to the Image J software (version 1.28u, National Institutes of Health, Washington, DC, USA) for measurement of microparticles diameter.

Release studies - the release profile of microparticles was studied through dissolution tests in modified USP 27 (2007) Apparatus 1, assembling membrane diffusion technique. Samples were transferred to a dialysis bag and immersed into 0.1L of a solution that simulated gastric fluid (pH 1.2) and enteric fluid (pH 6.8) (USP 27 2007). Temperature was kept at 37 ºC ± 0.5 ºC.

Gastric Acid Medium Preparation - All chemicals and enzymes were obtained from Sigma Chemical Co., St. Louis, MO. The simulated gastric fluid consisted of 0.2 g of sodium chloride, 0.7 mL of 37% hydrochloric acid, and of double-distilled water to complete 0.1L. The final pH was 1.2. Dissolutions were carried out with or without pepsin (0.32 g, 3200 units/mg of protein) to evaluate digestion by acid and enzyme separately. Dissolution of the different microparticles was followed for 6 h.

Intestinal Alkaline Medium Preparation - The simulated intestinal fluid consisted of 0.68 g of monobasic potassium phosphate dissolved in 25 mL of double-distilled water and added to 19 mL of 0.2 N sodium hydroxide and 40 mL of double-distilled water. Dissolutions were carried out with or without pancreatin (1.0 g, activity equivalent to USP specifications) added to this mixture. The pH was adjusted to 7.5 using 0.2 N sodium hydroxide, and the final volume was then brought to 0.1L with double-distilled water. Dissolution of the different microparticles was followed for 6h.

In Vitro Gastrointestinal Assays -The following protocol was applied to simulate the succession of pH conditions and enzymatic activities encountered after ingestion (Remondetto et al, 2004). The microparticles were subjected to gastric conditions with pepsin (pH to 1.2) for 30 min, and the pH was then adjusted to 7.5 with 1 N sodium hydroxide and monobasic potassium phosphate. Pancreatin was then added as described above to transform the simulated gastric conditions into intestinal conditions. Microparticles dissolution was followed for 6 h with periodic sample withdrawal.

Culture Cell Preparation - All cell culture media and reagents were obtained from Gibco. Caco-2 cells were grown at 37 °C in an atmosphere of 5% CO_2-95% air at constant humidity in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum, 1% nonessential amino acids, and 1% streptomycin/penicillin. The cells were routinely propagated in tissue culture flasks (75 cm^2) and harvested at 80-90% confluence by treatment with 0.25% trypsin in 1 mM EDTA, thoroughly washed, and replaced in supplemented growth medium.

Iron Intracellular Absorption - Caco-2 cells seeded at 7 x 10^4 cells/cm^2 in tissue culture flasks (75 cm^2) were used in iron intracellular absorption experiments after 20 days of culture. Each flask was washed carefully with PBS, and 5 mL of gastrointestinal dissolution filtrate(0.45 µm) was added. Flasks of washed cells without dissolution filtrate were treated as reference samples. The cells were then incubated for 2 h and washed again with PBS, and 5 mL of DMEM supplemented with 10% fetal calf serum was added before the culture flasks were returned to the incubator for 22 h. Cells were then harvested in 2 mL of PBS buffer and iron was quantified by ET-AAS (Zeiner et al, 2005).

RESULTS AND DISCUSSION

Microparticle production - Fe-PPC microparticles were obtained by spray-drying the feed suspension composed of gelified PPC and $FeSO_4$ aqueous solution. Protein, iron and moisture contents were stabilished (table 1) as described in methods. Fe-PPC powder showed 2,5% of elemental iron. Retention of elemental iron was about 103% ± 2,13. It was determined as a percentage based on the elemental iron content per total solids of feed suspensions and the elemental iron content per total solids of the microparticles. Based in these results, 280 mg of Fe-PPC microparticles provide approximatelly the amount equivalent to the dietary reference intake (DRI) value for iron (infant, 1-3 years) (Trumbo et al, 2001). This amount can be easily included into a meal or added into industrialized foods.

	PPC	Fe-PPC
moisture	5.01% ± 0.13	5.17% ± 0.09
protein	82.46%	81.82%
elemental iron	ND	2.5%
mean diameter size (μm)	4.78 ± 4.20	3.25 ± 2.28

Table 1: protein, iron and moisture contents; (ND – not detected) .

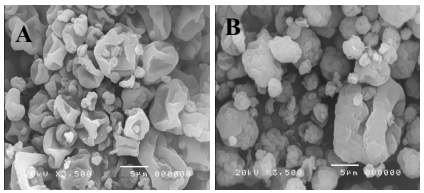

Figure 1: SEM of PPC microparticles (A) or Fe-PPC microparticles (B) showing spherical morphology and the rough surface.

Microparticles morphology - Characterization of overall morphology was based on analysis of the outer surface and geometry of the microparticles by SEM (figure 1). They showed spherical morphology and rough surface. Mean particle sizes were determined by analysis in Image J software and results are shown in table 1. External appearance of microparticles was modified by the addition of $FeSO_4$.

Iron delivery under gastrointestinal conditions – Aiming to have an overview of the release kinetics of iron from microcapsules along the gastrointestinal (GI) tract, an in vitro digestion simulation is going to be performed. Fe-PPC microparticles susceptibility to GI conditions will therefore be studied using a two-step proteolysis at 37 °C, including pepsin predigestion at pH 1.2 for 30 min to simulate gastric conditions, followed by pancreatin digestion at pH 7.5 for 6 h to simulate intestinal conditions.

Caco-2 cells iron absorption from Fe-PPC microparticles – Since Caco-2 cell monolayers have similarly behavior to human intestinal mucosa, these were used for iron absorption analysis. Intracellular iron concentrations obtained with the exposure to Fe-PPC microparticles was about 30 ppb, while it was not detected in the control condition (PPC microparticles) of this study.

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CONCLUSION

The preliminary results showed here encourage the usage of PPC as matrix for iron microencapsulation. This kind of material seems to be useful since it presents relative low cost and it is edible. PPC presented good capacity to retain the core by means of spray drying process. Furthermore, iron microencapsulated by this methodology was capable to be absorbed by Caco-2 cells. To our knowledge, it is the first time that a legume protein concentrate is used as wall material for iron microencapsulation.

BIBLIOGRAPHY

Ip H. et al (2009) *Improved adherence and anaemia cure rates with flexible administration of micronutrient Sprinkles: a new public health approach to anaemia control*. European Journal of Clinical Nutrition 63(2) 165-72

Lowry DH. et al (1951) *Protein measurement with the folin phenol reagent*. Journal of Biological Chemistry 193(1) 265-275

Pedrosa C. et al (2000) Selective neoglycosylation increases the structural stability of vicilin, the 7S storage globulin from pea seeds. Archives of Biochemistry and Biophysics 382(2) 203-210

Pereira HVR. et al (2009) *Legumes seeds protein isolates in the production of ascorbic acid microparticles.* Food Research International 42(1) 115-121

Pierucci AP. et al (2006) *New microencapsulation system for ascorbic acid using pea protein concentrate as coat protector.* Journal of Microencapsulation 23(6) 654-62

Pierucci AP. et al (2007) *Comparison of alpha-tocopherol microparticles produced with different wall materials: pea protein a new interesting alternative.* Journal of Microencapsulation 24(3) 201-13

Remondetto GE. et al (2004) *Iron availability from whey protein hydrogels: an in vitro study*. Journal of Agricultural and Food Chemistry 52(26) 8137-8143

Trumbo P. et al (2001) *Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc.* Journal of the American Dietetic Association 101(3) 294-301

WHO/UNICEF (2001) *Iron Deficiency Anemia: Assessment,Prevention and Control. A Guide for Programme Managers.*WHO/UNICEF: Geneva.

Zeiner M. et al (2005) *Determination of Iron in Caco-2 cells by ET-AAS.* Analytical Bioanalytical Chemistry 382(1): 239–242