Alginate-based microparticles coated with whey protein: gastrointestinal resistance.

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INTRODUCTION AND OBJETIVES

The production of microparticles using ionic gelation with alginate and calcium ions does not require high temperatures or organic solvents (Patil 2010), however the gel matrix is porous (Picot 2004). During ionic gelation not all carboxylic groups interact with calcium ions allowing supplies of negative charges on the surface of the microparticles, enabling them to interact with a polyelectrolyte of opposite charge through electrostatic interactions (De Vos 2007).

The aim of this research was to produce microparticles using ionic gelation and after recover the microparticles with a layer of whey proteins. Initially, the zeta potential of protein and polysacaride solutions were determinated using different pH range. The microparticles were characterized with respect to the adsorbed protein, solid matter content and average size. Recovered microparticles were subjected to gastrointestinal in vitro simulation measuring the amount of protein released during the simulation.

MATERIALS AND METHODS

Sodium alginate, ALG (Manugel DMB, lot G4200301), whey protein concentrate, WPC (Lacprodan - Arla Foods Ingredients, lot 80 4U17601, Porteña, CO, Argentine), commercial sunflower oil, pepsin, pancreatin and porcine mucin (Sigma Aldrich, St. Louis, MO, USA) were used. Microparticles were produced from an emulsion (oil:alginate, 0.825:1) containing 2% of alginate (w/w) adjusted to pH 4.0. The emulsion (14,000 rpm, 3 min, Ultra-Turrax, IKA, Germany) was atomized using a double fluid atomizer (∅=1 mm, air pressure 0.125 kgf/cm2, atomization speed 555 mL/h and height of 12 cm between the atomizer and the calcium chloride solution 2% (w/w), pH 4.0). After atomization, the microparticles were maintained during 30 min in the calcium chloride solution, removed, washed with water-milli-Q, pH 4.0. Microparticles were transferred to WPC solution (8%, pH 4.0) and maintained inside protein solution during 30 min, and after, washed with water pH 4.0. Zeta potential (solutions at 0.2% (w/w), Zetasizer model Nano-Z, Malvern, Malvern Instruments, Worcestershire, WR, UK), morphology (optical microscope, lens 12.5 and 25x and optics of 1 and 1.25x, Jenaval, Germany) and mean sizes (d_{50}, Mastersizer 2000, Hydro 2000 S sampling unit, Malvern, Worcestershire, WR, UK) were measured. Moist microparticles were submitted to gastro intestinal in vitro simulation (Sultana 2000; Mozzi 2009).

RESULTS AND DISCUSSION

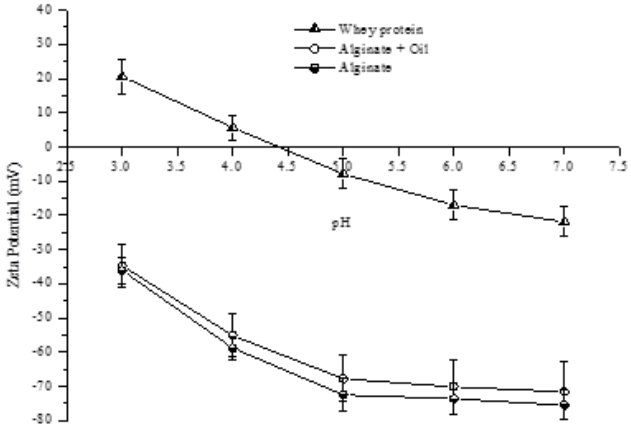

Figure 1: Zeta potential of biopolimers

As expected, the zeta potential values of polysaccharide solution presented a negative value throughout the pH range evaluated (3.0 - 7.0). Zeta potential of emulsion (polysaccharide + sunflower oil) did not present significant differences compared to polysaccharide solution. Also as expected, the zeta values below the isoelectric point (IP) of protein (4.5, Figure 1) were positive and above IP, negative. Considering the zeta potential values obtained, pH 4.0 was chosen to produce the protein adsorption. When diluted solutions (0.2%) were combined zeta potential equal to zero was obtained at ratio 1:14 (v/v), polysaccharide:protein.

Table 1: Amount of protein adsorbed, dry matter content (%) and main size as a function of the protein content in solution (%)

% Protein in solution	% Protein adsorbed	% Dry matter	Mean size (d_{50}, µm)
0	0	5.82±0.05	135.18±3.76
8	47.3±0.3	14.63±0.23	163.36±2.98

A high amount of adsorbed protein was obtained when 8% of protein solution was used. The mean size of the microparticles increased after protein adsortion compared to the size of microparticles obtained after ionic gelation.

At pH 2.0 after gastric evaluation only 11.5% of the total amount of adsorbed protein was solubilised showing that whey protein was resistant to pepsin hydrolysis as observed before (Kitabatake 1998). After gastrointestinal evaluation, the amount of solubilised protein increased to 82.6 % of the total amount of adsorbed protein.

Table 2: Solubility of adsorbed protein (%) after gastric and gastrointestinal evaluation

Gastric assay (GA) (pH 2, pepsin, 2h, 37 °C)	Intestinal assay (IA) (GA + pH 7 + pancreatin, 5h, 37 °C)
11.55 ± 1.13	82.59 ± 5.31

Figure 2 shows that alginate-based microparticles were not solubilised after gastrointestinal in vitro evaluation. Opposite effect was obtained when low methoxy amidated pectin microparticles recovered with whey proteins were submitted to gastrointestinal in vitro evaluation (Souza 2012).

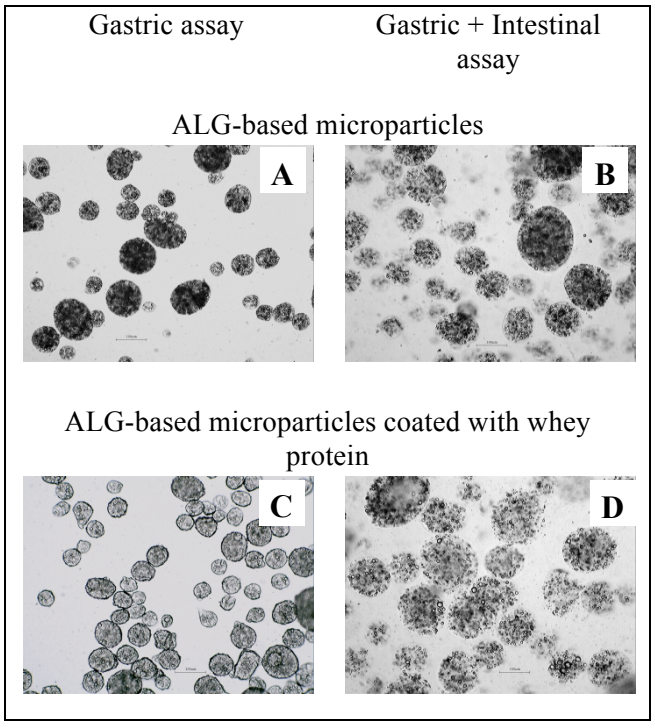

Figure 2: Morphology of microparticles produced using ionic gelation, coated and uncoated protein during sequential exposure to GA and IA.

CONCLUSIONS

A high amount of protein adsorption was still possible through electrostatic interaction after produce microparticles using ionic gelation. Also a high amount of oil can be carried inside the gelled microparticles open the opportunity to encapsulate hydrophobic or hydrophilic core material besides to produce microparticles with a high amount of bounded protein.

REFERENCES

- De Vos P. et al. (2007) Zeta-potentials of alginate-PLL capsules: A predictive measure for biocompatibility? Journal of Biomedical Materials Research Part A 80A 813-819.
- Kitabatake N. et al. (1998) Digestibility of bovine milk whey protein and β-lactoglobulin in vitro and in vivo. Journal of Agricultural and Food Chemistry, 46, 4917–4923.
- Mozzi F. et al. (2009) Functionality of exopolysaccharides produced by lactic acid bacteria in an in vitro gastric system. Journal of Applied Microbiology 107(1) 56-64.
- Patil J. S. et al. (2010) Ionotropic gelation and polyelectrolyte complexation: the novel techniques to design hydrogel particulate sustained, modulated drug delivery system: a review. Digest Journal of Nanomaterials and Biostructures, 5 241-248.
- Picot A. et al. (2004) Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. International Dairy Journal 14 505-515.
- Souza F. N. et al. (2012) Production and characterization of microparticles containing pectin and whey proteins. Food Research International 49 560-566.
- Sultana K. et al. (2000) Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. International Journal of Food Microbiology 62 47–55.

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