P-025

Production of microparticles using pectin and denatured whey protein

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INTRODUCTION

Alginate and low methoxyl pectin are often used as a gelled matrix to encapsulate molecules of biological significance (Mestdagh 1998). However, since gelled microcapsules appear to be porous, coating the beads with additional materials to cover the porous matrix of the capsules can improve the stability and increase the barrier properties of these microcapsules (Annan 2008, Gbassi, 2008). The aim of this research was to produce and characterize microparticles using low methoxyl amidated pectin and denatured whey protein associating ionotropic gelation and complex coacervation.

MATERIALS AND METHODS

The microcapsules were prepared with pectin solution (2% w/w, pH 4.0). Butter (2% w/w, 50 ^{o}C) was added, and an emulsion was obtained using an ultra-turrax homogenizer (14000 rpm/3 min) and atomized (double fluid atomizer) in a calcium chloride solution (2% w/v, pH 4.0) under magnetic stirring (320 rpm). The microparticles were allowed to stand for 30 min to complete gelation, and then dispersed in a denatured (30 min/90^{o}C) whey protein solution (WPC, 8% w/v, pH 4.0) for 30 min. After washing the microparticles were frozen (-18 ^{o}C) and freeze-dried.

The diameter of the moist microparticles was measured using water at pH 4.0. Dried microparticles were previously rehydrated (water at pH 4.0 for 15 min) and after, the particles were submitted to different pH/time combinations (pH 1.2 or 2.0 for 2 h; pH 1.2 or 2.0 for 2 h plus 5 h at pH 7.0) and the diameter measured again. The morphology was evaluated by scanning electron (SEM) and laser confocal microscopy (LCM, fluorescein 5-isothiocyanate and nile red). Three independent experiments were carried out and the values were presented as the means with the respective standard deviation.

RESULTS AND DISCUSSION

Under all the pH conditions, the average size of the rehydrated particles was smaller than that of the moist particles obtained at pH 4.0 (Table 1)

Table 1: Mean diameter and standard deviation of moist and rehydrated microparticles swelling at different pH (n = 3).

Particles Characteristics	pH (time conditions)	Mean diameter of microparticles (μm)
Moist	4.0	200.5 ± 91.6
Rehydrated	4.0 (15 min.)	110.8 ± 53.1
Rehydrated at pH 4.0 (15 min.) and submitted at	1.2 (2 h)	90.8 ± 48.1
	2.0 (2 h)	100.7 ± 52.5
	1.2 (2 h) + 7.0 (5 h)	121.0 ± 63.6
	2.0 (2 h) + 7.0 (5 h)	142.2 ± 65.2

Under more acid conditions, the average size decreased when compared to particles rehydrated at pH 4.0, as observed previously (Rosenberg 2004). Opposite effect was also observed before (Santipanichwong 2008). Nevertheless, the microparticles submitted to acid conditions followed by an increase in pH (1.2–7.0 or 2.0–7.0) showed larger average sizes than those rehydrated at pH 4.0. At values above the protein pI and the pectin pKa, negative charges will occur, resulting in electrostatic repulsion between both molecules and, as a consequence, greater swelling was observed at pH 7.0 than at pH 4.0 (Table 1)

Confocal laser scanning microscopy (Fig. 1) shows that a thin layer of denatured whey protein-FITC, represented by a green ring, was formed around the perimeter of the microparticle, as obtained before (Annan 2008).

It can be observed by scanning electron microscopy that the microparticles had a round flattened shape and were not completely smooth, showing no visible cracks or pores on the surface (Fig. 2A).

The overcoating produced by the denatured whey protein can be observed in Fig. 2B and amplified in Fig. 2C. Drying and maintaining the integrity of particles with high moisture content (97.0±0.2%) is a challenge, and it is possible that the butter added to the formulations was effective in increasing the resistance of the particles during drying.

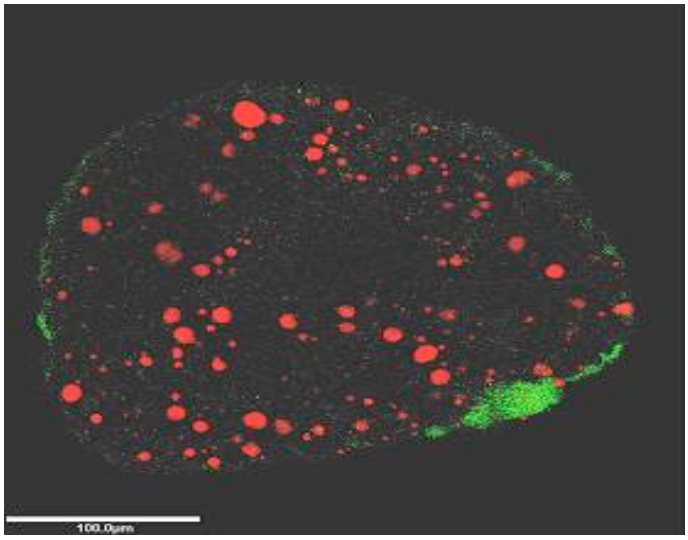

Figure 1: LCM micrographs. Microcapsules after rehydration followed by maintenance of the pH value at 2.0 (2 h) and 7.0 (5 h). Bars = 100µm.

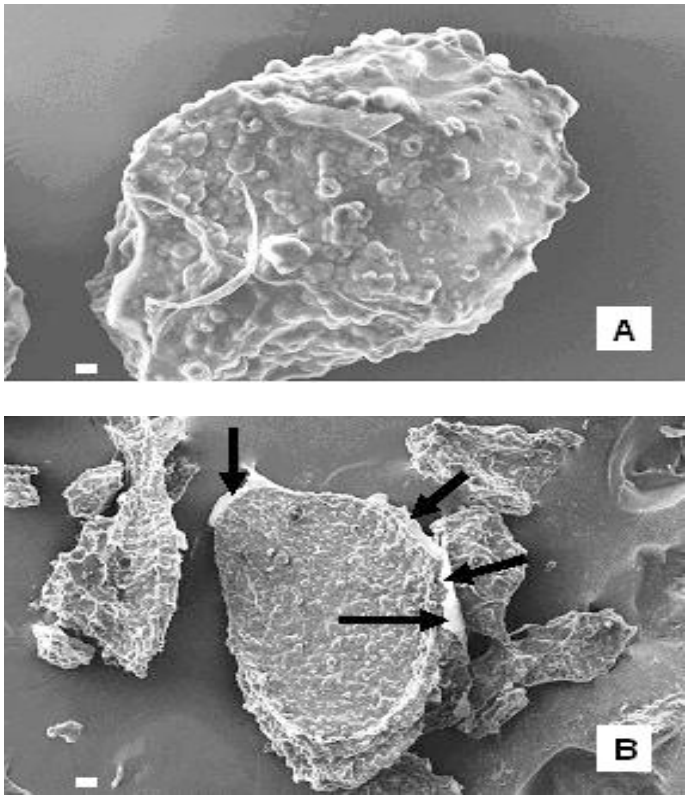

Figure 2: SEM micrographs microparticles A: whole microparticle. B, C: arrows show the WPC layer coating the particle. (A, C: bars, 10 µm; B: bar, 30 µm).

CONCLUSIONS

With the association of ionotropic gelation with Ca^{2+} and complex coacervation using low methoxyl amidated pectin and whey protein was possible to obtain microparticle with round flattened shape, without visible cracks or pores. Particles showed resistance during drying and maintained their integrity after swelling. The swelling behavior suggest that these microparticles could be highly favorable to protect the acid sensitive encapsulated material under gastric conditions and needs to be deeply investigated.

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