Whey protein retention in particles obtained by ionic gelation

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INTRODUCTION AND OBJECTIVE

One of the most popular procedure to produce microparticles is the ionic gelation using polysaccharides as sodium alginate or low methoxyl pectin and calcium ions since the microparticles have benefits of being non toxic and the polysaccharides available at low cost (Gombotz and Wee, 1998). Also microencapsulation of biological active materials as tissues, enzymes and cells using ionic gelation is commonly applied for immunoisolation (de Vos et al., 2006). Ionic gelled microparticles appeared to be porous and coating beads with additional materials can fill or cover the porous matrix to improve the stability of the gelled microparticles (Allan-Wojtas et al. 1999).

The electrostatic interaction between the biopolymer presents occurs when there is a balance between positive and negative charges as in the pH range where the pKa of the polysaccharide and the protein isoelectric point have similar charges but in opposite sign (Liu et al., 2010). The wey proteins are one alternative polycation and are composed mainly by β-lactoglobulin (Sgarbieri, 1996). The β-lactoglobulin are denatured with heat and can form aggregates and changes their functional properties.

The aim of this study was to evaluate preliminarily if the methodology of protein incorporation influences the final concentration of protein and if the thermal denaturation of whey protein exert some influence on the protein contents.

MATERIALS AND METHODS

Materials

For the production of the microparticles the following materials were used : whey protein concentrate – WPC (Lacprodan Lot LAC804U17601, Moisture : 6.86±0.12% ; protein 81.02±1.00 ; ash 2.77±0.06 ; lipids 16.21±0.5 (AOAC, 2006) ; low methoxyl amidated pectin – LMAP (CP Kelco, galacturonic acid (GA) 85.9% ± 1.9, esterification degree (DE) 34.1 % ±1.3 e amidation degree (DA) 5.5 % ± 0.4) and low methoxyl pectin - LMP (CP Kelco, galacturonic acid (GA) 92.6% ± 1.4, esterification degree (DE) 40,9 % ± 0,8 e amidation degree (DA) 0.3 % ± 0.01) (FAO, 2009) ; calcium chloride (Merck) ; soy bean oil (Lisa) with paprica oil resin 10 :1 (Citromax). The WPC was denatured at following conditions : 90ºC for 30 minutes.

Methods

Particles containing protein inside the matrix: the microparticles were prepared with pectin solution (2% w/w) emulsified with soy oil (2% in relation to dry matter) and whey protein solution (10-40% denatured and non denatured in dry basis), using an ultra-turrax homogenizer (14000 rpm/3 min) and atomized with a double fluid atomizer in a calcium chloride solution (2% w/w, pH 4.0) under magnetic stirring. The microparticles were allowed to stand for 30 min in the calcium chloride solution to complete gelation. The microparticles were washed in sieves (diameter 125µm) and freeze-dried.

Particles containing protein recovering the particles : the particles where produced as the same manner described above without proteins inside the emulsion. After 30 min in calcium chloride the microparticles were washed, sieved and dispersed in non denatured or denatured WPC solutions (4, 6 and 8% w/w, pH 4.0) under magnetic stirring for 30 min. The microparticles were washed, sieved (diameter 125µm) and freeze-dried. The microstructure of the moist and rehydrated microparticles (water pH 4.0) were observed using optical microscopy (OM) and the morphology was evaluated using freeze-dried particles by scanning electron microscopy (SEM).

RESULTS AND DISCUSSION

Influence of the protein thermal process on the protein retention inside the emulsion

It was found that there is a greater protein retention when WPC was in the denatured form (Table 1) and the highest protein levels were when 40% WPC was used in the emulsion. This bigger retention when the protein is denatured can be linked to protein aggregation due to denaturation at temperatures above 65ºC, being easier to encapsulate.

Table 1 : Protein content of microparticules (% dry basis) produced by ionic gelation with protein in emulsion.

%WPC in emulsion	ND	D
10	11.5 ± 2.0^{Ba}	13.0 ± 1.6^{Ca}
20	9.5 ± 2.3^{Bb}	18.7 ± 1.2^{Ba}
30	13.8 ± 0.4^{Bb}	21.4 ± 4.9^{Ba}
40	17.2 ± 1.6^{Ab}	31.2 ± 1.2^{Aa}

Means followed by the same letter (uppercase in the

columns and lowercase in the rows) do not differ according Tukey test (p>0.05). WPC – whey protein concentrate; ND – non denatured; D – denatured.

Influence of the protein thermal process in the protein adsorption on the microparticles

The highest levels of adsorbed protein were obtained when the protein was in a non-denatured form for the recover (Table 2).

Table 2 : Protein content of microparticules produced by ionic gelation and electrostatic interaction.

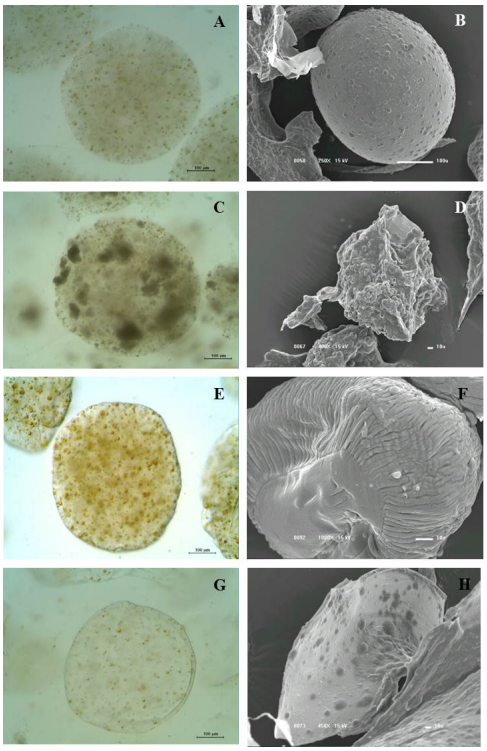
%WPC in solution	ND	D
4	50.5 ± 0.6^{Ba}	29.4 ± 2.4^{Bb}
6	60.6 ± 1.1^{Aa}	38.3 ± 1.3^{Ab}
8	58.4 ± 0.6^{Aa}	25.8 ± 1.7^{Bb}

Means followed by the same letter (uppercase in the columns and lowercase in the rows) do not differ according Tukey test (p>0.05). WPC – whey protein concentrate; ND – non denatured; D – denatured.

Microestruture and morphology

The particles morphology (Fig 1) shows that moist and freeze dried particles presented spherical shape. Dry capsules with non-denatured protein adsorbed by electrostatic interaction (F), observed in the SEM show a filamentous surface due to adsorbed protein. The protein aggregation, when added as a constituent of filling, can be observed by optical microscopy (C).

Figure 1: Microstructure and morphology of the microparticles



A and B – particle with 40% non-denatured WPC as a constituent of filling; C and D - particle with 40% denatured WPC as a constituent of filling; E and F – particle with protein (6% non-denatured WPC) adsorbed by electrostatic interaction; G and H - particle with protein (6% denatured WPC) adsorbed by electrostatic interaction.

CONCLUSIONS

The aggregation of the proteins after heat denaturation makes them closer, and could facilitate the encapsulation process when it was present as a constituent of the filling. This was not true when the protein was used as a coating material, decreasing the levels of proteins associated with the particles subjected to electrostatic interaction with the proteins.

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