P-070

Microparticles containing a high amount of protein

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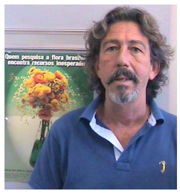


INTRODUCTION AND OBJECTIVES

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, 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 1999). Microparticles produced by ionic gelation and after covered with a protein layer have been studied aimed to produce gastro-resistant microparticles (Chen, 2005; Gbassi, 2008).

The aim of this research was to produce microparticles using ionic gelation (low methoxyl pectin or low methoxyl amidated pectin + calcium ions) and after recover the particles using electrostatic interaction with whey proteins. Charge density of the solutions were evaluated by zeta potential measurements and microparticles evaluated with respect to morphology, amount of bound protein, moisture and rehydration capacity after being freeze dried.

MATERIALS AND METHODS

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 as determined 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) as determined FAO (2009) ; calcium chloride (Merck) ; soy bean oil (Lisa) with paprica oil resin 10 :1 (Citromax).

The microparticles were prepared with pectin solution (2% w/w) emulsified with soy oil (2% in relation to dry matter) using an ultra-turrax homogenizer (14000 rpm/3 min) and atomized with a double fluid atomizer with compressed air (0,125 kgf/cm^2) 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 and dispersed in non denatured WPC solutions (4, 6, 8 and 12% w/w, pH 4.0) under magnetic stirring for 30 min. The microparticles were washed in sieves (diameter 125μm) and freeze-dried.

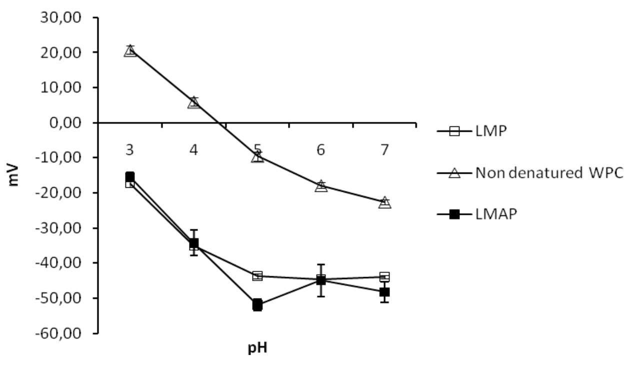
The microstructure of the moist and rehydrated microparticles (water pH 4.0) were observed using optical microscopy (OM) and the morphology was evaluated in freeze-dried particles by scanning electron microscopy (SEM).

The zeta potential of the solutions (0,5% w/w) - WPC, non amidated and amidated pectin was evaluated in the pH range of 3-7. The values were evaluated using a Zetasizer (Malvern, Worcestershire, UK).

RESULTS AND DISCUSSION

The conditions chosen to produce gelled microparticles worked well with LMAP. In the case of LMP only was possible to obtain particles when LMP concentration was adjusted to 3% (w/w) and the calcium concentration adjusted to 5% (w/w). Amidated low methoxyl pectins have some advantages compared to non amidated pectins as they need less calcium to gel and they are less sensitive to precipitate by high amounts of calcium (Racape et al., 1989).

Figure 1: Influence of the pH on the electrical charge (ζ potential) of the solutions (0,5% w/w) of pectins and WPC.



The zeta potential of the solutions of LMAP or LMP are similar in the measured pH range (3-7) and equal at pH 4 (Fig. 1) indicating that the electrostatic interaction between pectin and protein could occur regardless of the type of pectin. The isoelectric point of WPC solution was 4.5, close to that reported in the literature (Chen, 2005). Fig 1 indicates that the electrostatic interaction pectin-protein is favored at pH values below 4.5.

Unfortunately the charge density of the particles obtained by ionic gelation cannot be measured due to size/weight of them and the impossibility of keeping them in suspension during the measurement of zeta potential electrophorethic. The values of zeta potential of the solution are only an indicative that the pectin-protein association can occur, however, do not represent the real charge on the surface of the particles.

Table 1: Protein (%, d.b.) and moisture contents (% d.b.) of microparticules produced using ionic gelation and electrostatic interaction.

WPC	LMAP[1]		LMP[2]	
	Protein	Moisture	Protein	Moisture
4	67.18±1.15Aa	91.09±0.45Ab	54.15±0.84Bb	93.45±0.37Aa
6	71.20±1.87Aa	89.69±0.34Ba	59.40±0.99Ab	90.92±0.88Ba
8	69.01±1.35Aa	89.20±0.28Bb	54.90±3.71Bb	94.02±0.55Aa
12	67.20±1.46Aa	89.27±0.50Ba	58.87±0.71Ab	91.26±0.77Ba

Means followed by the same letter (uppercase in the columns and lowercase in the rows) do not differ according to the Tukey test (p>0.05). LMP – Low methoxyl pectin ; [2] LMAP – Low methoxyl amidated pectin. d.b : dry basis.

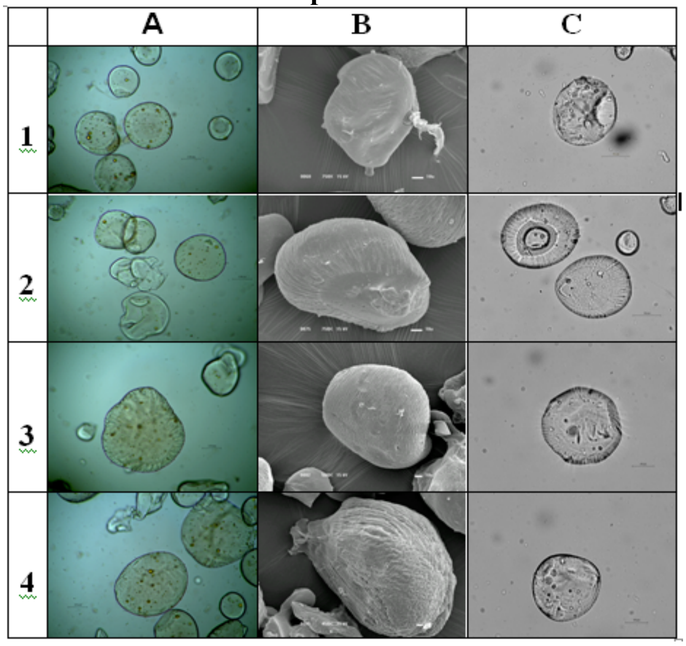
Apart of the type of pectin, high amounts of WPC were electrostatically bound to the surface of the particles obtained by ionic gelation (table 1). Values ranging from 54.1% to 71.2% of protein (dry basis) were obtained when WPC solution were varied from 4 to 12%. The particles also had a high moisture content, characteristic of the particles produced by ionic gelation. In the used conditions, particles produced with LMAP showed higher amount of over coating protein as compared to the particles produced with LMP.

The particles morphology (Fig 2) shows that moist, freeze dried and rehydrated particles had spherical shape with different sizes due to the double fluid atomizer used in the manufacture of the gelling ion particles. Dry capsules observed in the SEM show a filamentous surface due to adsorbed protein. The inclusion of emulsified oil in the polysaccharide solution appears have reinforced the particles helping to maintain its integrity during drying and rehydration, maintaining the spherical shape like the moist particles immediately after their production.

CONCLUSIONS

The ionic gelation associated with electrostatic interaction made possible the production of particles with high amount of protein associated with high moisture contets. Besides, protein overcoating can provide a greater protection for the particles, they have a high biological value considering the amino acid composition of WPC. Considering the good inclusion of emulsified oil, the particles can carry hydrophilic or hydrophobic compounds. Also the process of production is extremely mild done at aqueous system and room temperature.

Figure 2: Microstructure and morphology of the microparticles



Particles A – moist (OM); B – dried (SEM) and C – rehydrated (OM). Number 1 – 4% WPC; 2 – 6% WPC; 3 – 8% WPC and 4 – 12% WPC.

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