Food Research International 49 (2012) 560–566

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Production and characterization of microparticles containing pectin and whey proteins

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ARTICLE INFO

Article history:
Received 10 May 2012
Accepted 21 July 2012

Keywords:
Microencapsulation
Ionic gelation
Electrostatic interaction
Low methoxyl amidated pectin
Whey protein concentrate

ABSTRACT

The objective of this study was to produce and evaluate pectin particles obtained by ionic gelation and coated with whey protein without heat treatment and heat treated. The zeta potential of pectin solutions, protein solutions, mixtures of pectin solution and protein solutions and mixtures of particles of pectin and whey protein solutions were evaluated to identify the pH and best pectin particles:whey protein solution ratio to allow for the electrostatic interaction between particles and protein solutions. The effect of different concentrations of proteins in solution on the protein adsorption on the surface of pectin particles was assessed. The morphology of the particles was determined and also the mean size at different pH levels. The protein solubility of moist particles subjected to *in vitro* gastrointestinal conditions (pH, temperature and enzymes) was evaluated. The negative charge remaining on the surface of pectin particles permitted protein adsorption by electrostatic interaction, and higher adsorption was observed for pectin particles covered with whey protein without heat treatment (49.2% - dry basis) compared to particles covered with whey heat treated (27.6% - dry basis) when 4% proteins in solution were used. The proteins adsorbed on the surface of the particles were highly solubilized under simulated conditions of the gastric tract at a pH of 1.2 (>40%). However, when the initial pH was 3.0, solubilities were limited, reaching 5.6% for pectin particles covered with whey protein without heat treatment and 18.6% for those coated with whey heat treated. The particles coated with protein desintegrated, when the pH was changed to 7.0 in the presence of pancreatin.

1. Introduction

Microparticles are mainly used to protect encapsulated materials from adverse environmental conditions and allow for the controlled release of functional substances, such as natural antioxidants, essential fatty acids, enzymes and probiotic microorganisms (Annan, Borza, & Hansen, 2008; Zimet & Livney, 2009). Particles that are resistant to gastric conditions are increasingly being studied in an attempt to understand the mechanism of active release in their action site (Kosaraju, 2005; Pawar, Pawar, & Patel, 2011).

Several methods and materials have been investigated for the production of microparticles, including ionic gelation using natural polysaccharides and calcium ions (Krasaekoopt, Bhandari, & Deeth, 2004). Ionic gelation does not require high temperatures or organic solvents (Patil, Kamalapur, Marapur, & Kadam, 2010), and can encapsulate emulsions containing hydrophilic or hydrophobic compounds (McClements, 2005); however, the gel matrix is porous (Burey, Bhandari, Howes, & Gidley, 2008; Picot & Lacroix, 2004).

During the ionic gelation not all carboxylic groups interact with calcium ions allowing a surplus of negative charges on the surface of the gel particles and enabling them to interact with a polyelectrolyte of opposite charge, forming a protective layer on the particle surface (De Vos, De Haan, Kamps, Faas, & Kitano, 2007). The electrostatic interaction depends on several factors, including the polysaccharide:protein ratio, pH, ionic strength and polyelectrolyte concentration (Ye, 2008). The protein adsorption on solid surfaces was recently reviewed (Rabe, Verdes, & Seeger, 2011).

Pectin is a polysaccharide composed of a linear chain of galacturonic acid units joined by α-1.4 links, interspersed by rhamnose units linked by α-1.2, in which the carboxylic groups of the galacturonic acid may be esterified by methyl groups (Thakur, Singh, & Handa, 1997). The pK_a of pectin is approximately 2.9 (Ralet, Dronnet, Buchholt, & Thibault, 2001).

Whey proteins are a versatile nutritional source. The major whey protein is β-lactoglubulin (β-Lg), a globular protein (MW 18.400 Da) mainly

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0963-9969
http://dx.doi.org/10.1016/j.foodres.2012.07.041

responsible for the irreversible process of whey thermal denaturation (Schmitt et al., 2001). β-lactoglobulin has an isoelectric point of approximately 4.6 (Jones & McClements, 2010).

Particle production using whey proteins (native or denatured) associated with polysaccharides, including pectin and alginate, has been extensively studied. Rosenberg and Lee (2004) investigated the production of microparticles containing high whey protein content covered by a layer of alginate. Recently, β-lactoglobulin and beet pectin or β-lactoglobulin, alginate and chitosan were used in the production of nanoparticles for various applications (Li & McClements, 2011; Santipanichwong, Suphantharika, Weiss, & Mcclements, 2008). These studies utilized the thermal denaturation of the protein for the initial production of micro- or nano- particles, which can result in the loss of core material susceptible to heat (Chen & Subirade, 2006).

As an alternative, whey protein and alginate particles can also be produced via denaturation prior to the production of these particles (Beaulieu, Savoie, Paquin, & Subirade, 2002). Mixtures of whey protein, alginate and pectin were used to produce particles through a transacylation reaction (Guérin, Vuillemard, & Subirade, 2003). Recently, alginate or pectin particles obtained by ionic gelation were coated with whey proteins without thermal denaturation for the encapsulation and gastric protection of probiotic microorganisms (Gbassi, Vandamme, Yolou, & Marchioni, 2011; Gerez, de Valdez, Gigante, & Grosso, 2012). The authors however, did not assess whether different amount of protein could be adsorbed and also the effect of adsorbed amounts on the protection against gastric conditions.

The aim of this research was to produce and characterize pectin particles (Pec_{PART}) obtained by ionic gelation and coat them with whey protein without heat treatment (Pec_{PART}–WPC_{WHT}) and heat treated (Pec_{PART}–WPC_{HT}). Initially, the zeta potential of pectin solutions (Pec_{SOL}) and WPC solutions (WPC_{SOL}) and different ratios of mixtures of Pec_{SOL}:WPC_{SOL} were evaluated. The study were repeated for the same ratios but using Pec_{PART}:WPC_{SOL}. The effect of different concentrations of protein solutions on protein adsorption on the surface of Pec_{PART} was assessed, as well the morphology of the moist or dry particles. The average size of the moist coated particles at different pH levels was also assessed. The protein solubility of moist particles subjected to *in vitro* gastrointestinal conditions (pH, temperature and enzyme) was evaluated.

2. Materials and methods

2.1. Materials

The GENU® low methoxyl amidated pectin (CPKelco, Limeira, S.P., Brazil) containing 81.3 ± 1.2% galacturonic acid, 30.4 ± 1.6% degree of esterification and 10.4 ± 1.0% degree of amidation (FAO, 2009) was used to create pectin solutions. Whey protein concentrate (Lactoprodan, Arla Food Ingredients, lot: 80 4A12701, Portenâ, CO, Argentine) containing 73.3 ± 0.9% protein and 6.0 ± 0.1% moisture (AOAC, 2006) was used to prepare the protein solutions. Anhydrous calcium chloride (lot: 36308, Dinamica, Diadema, S.P., Brazil) common commercial unsalted butter (Aviação, São Sebastião do Paraiso, M.G., Brazil), swine pepsin, swine pancreatin and mucin (Sigma Aldrich, St. Louis, M.O., USA) were also used. Distilled and deionized water and other reagents of analytical grade were used.

2.1.1. Zeta potential of Pec_{SOL} and WPC_{SOL}

Pec_{SOL} at 2% (w/w), solutions of whey protein whithout heat treatment (WPC_{WHT}) and whey protein heat treated (WPC_{HT}) (80 °C/15 min) at 4% (w/w) protein were prepared and maintained under agitation overnight. The solutions were diluted to a final concentration of 0.2% (v/v) and the zeta potential was measured at pH intervals from 3.0 to 7.0. After dilutions, the solutions were remained under agitation for 4 h at room temperature. The analyses were carried out in triplicate. All of the zeta potential determinations were performed using the Zetasizer model Nano-Z (Malvern Instruments, Malvern, Worcestershire, WR, UK).

2.1.2. Zeta potential of Pec_{SOL}:WPC_{SOL}

Mixtures of Pec_{SOL}:WPC_{SOL} were prepared using Pec_{SOL} and WPC_{WHT} or WPC_{HT} solutions at concentrations of 0.2% (w/w) . The solutions were remained under magnetic agitation for 12 h for complete dissolution, and the pH was adjusted to 4.0 before mixing. An emulsion was prepared after polysaccharide dissolution (2% butter, w/w of solution) and mixture with WPC_{SOL} at different ratios. Mixtures were prepared in the following volumetric relationships of Pec_{SOL}:WPC_{SOL} (WPC_{WHT} or WPC_{HT}): 1:1, 1:2.3, 1:4, 1:6, 1:9, 1:10 and 1:12. After preparation, the mixtures were remained at rest for 4 h and to determine the zeta potential of the mixtures, samples were manually agitated before analysis. The ratios were prepared in triplicate, and each ratio was measured in duplicate. The zeta potential values represent the mean of six measured values.

2.1.3. Pec_{PART}:WPC_{SOL} mixtures

The particles were suspended in water at a pH of 4.0 so that the Pec_{SOL} equivalent at a concentration of 0.2% (w/w) was obtained. Volumetric mixtures of Pec_{PART}:WPC_{SOL} (WPC_{WHT} or WPC_{HT}) using the same ratios as above (Section 2.1.2.) were prepared as duplicates.

2.2. Production of the microparticles

The emulsion used was prepared with an aqueous solution of 2% pectin (w/w of solution), pH of 4.0 and butter (2% w/w of solution) and maintained at 50 °C using a Turrax at 14 000 rpm/3 min (IKA, R.J., Brazil). The emulsion was atomized on a 2% (w/v) calcium chloride solution, at pH 4.0 using a double fluid atomizer with diameter of 1 mm, height of 12 cm between the atomizer and calcium chloride solution, an air pressure of 0.125 kgf/cm^2 and an atomization speed of 555 mL/h. After atomization, the microparticles were maintained in the calcium chloride solution for 30 min (hardening time) and washed in a sieve (diameter of 125 μm) with deionized water, pH, adjusted to 4.0. The particles obtained by ionic gelation were then transferred to WPC_{SOL} (WPC_{WHT} or WPC_{HT}) at different concentrations: 2, 4, 6, 8 and 12% (w/w) with the pH adjusted to 4.0 and maintained for 30 additional min, under agitation. The particles were washed with deionized water at pH 4.0 to remove the proteins that were not adsorbed on the particle surface. Three productions of particles were made, and for each one, the adsorbed protein and moisture content were determined in triplicate (AOAC, 2006). The nitrogen content of the particles without protein was determined and used to correct the total nitrogen content of the particles containing adsorbed protein. A portion of the moist particles was frozen and freeze dried (Mod. 501, Edwards Pirani, Crawley, West Sussex, UK) at an initial temperature of −40 °C, a pressure of 0.1 mmHg, a final temperature of 25 °/2 h, a total cycle time of 48 h and then, was kept under refrigeration.

2.2.1. Morphology and mean size of the microparticles

The morphology of the microparticles coated with whey proteins, moist or rehydrated, were observed in a JENAVAL optical microscope using lenses of 12.5 and 25 x and optics of 1 and 25 x. Images were captured using the software EDN-2 – Microscopy Image Processing System. The freeze-dried microparticles were observed using a scanning electron microscope (SEM). The samples were fixed in stubs double-sided copper tape and coated with a thin gold layer (180 s and a current of 40 mA) using a Baltzer evaporator (Baltec SCD50, Liechtenstein). The SEM (model JMS – T300 Jeol, Tokyo, Japan) was utilized at 15 and 20 kV.

The mean sizes (d 0.5) of the moist and rehydrated (after freeze drying) microparticles were measured in a Mastersizer 2000 and Hydro 2000 S sampling unit (Malvern, Worcestershire, WR, UK). Water was used as a suspending medium and a sample amount was added to produce a sufficient obscuration of 3-20%, as suggested by the manufacturer.