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Protein adsorption onto alginate-pectin microparticles and films produced by ionic gelation

Karla Córdova Aguilar [a], Fernando Tello [b], Andrea C.K. Bierhalz [c], Ma.G. Garnica Romo [d], Héctor E. Martínez Flores [e], Carlos R.F. Grosso [b,*]

[a] Programa Institucional de Maestría en Ciencias Biológicas de la Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico
[b] Department of Foods and Nutrition, Faculty of Food Engineering, University of Campinas, Campinas, São Paulo 13083-862, Brazil
[c] School of Chemical Engineering, University of Campinas, Campinas, São Paulo 13083-852, Brazil
[d] Facultad de Ingeniería Civil, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico
[e] Facultad de Químico Farmacobiología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico

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ABSTRACT

Microparticles and films containing sunflower oil were produced by ionic gelation using a 1:1 alginate:pectin mixture and were electrostatically coated with whey and egg white proteins. Emulsions of the polysaccharide mixture and the protein solutions were evaluated in terms of their zeta potentials. The microparticles were characterized based on their mean size, size distribution, moisture content, calcium content, adsorbed protein content, encapsulation efficiency and morphology. The films were characterized with respect to their thickness, moisture content, calcium content, adsorbed protein content, mechanical properties, water vapor permeability and morphology. High encapsulation efficiency (87.6% at pH 3.5 and 90.8% at pH 3.75) was obtained for the microparticles produced by ionic gelation. The calcium content after ionic gelation was significantly higher in the films (on average, 3.0 μmol/mg db) than in the microparticles (on average, 1.62 μmol/mg db). For the microparticles, an increase in the protein content in solution yielded an increase in the protein content adsorbed, independent of the type of protein used. When 4% protein in solution was used, protein adsorption onto the microparticles (59.2% for whey protein and 45.5% for egg white protein) was significantly higher than that onto the films (25.3% for whey protein and 24.1% for egg white protein) likely due to the smaller amount of calcium present on the microparticles and the larger surface area of the particles relative to that of the films. Although the process of producing films by ionic gelation and later coating them with proteins was straightforward, homogeneous drying of the films was difficult.

1. Introduction

External ionic gelation is a simple process used to obtain gels in which, an anionic polysaccharide solution is dripped over an ionic solution at appropriate concentrations, making it possible to obtain gels of different shapes and sizes that form three-dimensional structures with a high water content (Gombotz and Wee, 1998; Smrdel et al., 2008).

Among polysaccharides, alginate, pectin or a mixture of the two may be used to produce matrices in the form of microparticles or films. The cation concentration, the ionic strength and the pH determine the kinetics of gel formation as well the volume and stability of gel beads (Mestdagh and Axelos, 1998).

Alginate has a linear structure and a high molar mass and comprises two types of uronic acids, β-D mannuronic acid (M) and α-L guluronic acid (G), with either homopolymeric or heteropolymeric blocks in which the G units form crosslinks with divalent ions, to produce "egg-box" model gels (Grant et al., 1973). Alginate has pK_a values between 3.20 and 3.38 (Martinsen et al., 1992).

The characteristic structure of pectin is a linear chain composed of α – (1 → 4)-D-galacturonic acids that are partially esterified with methoxyl groups and neutral sugars, such as galactose, glucose, rhamnose, arabinose and xylose. Pectin has a pK_a value of 2.9 (Ralet et al., 2001).

Although ionic gelation is a simple and mild technique, the gels produced are porous, which can accelerate oxygen permeation through the matrix or allow for the release of active compounds with low molar mass that are inserted into the gels (Sezer and Akbuga, 1999). The size of the pores varied greatly, ranging from

* Corresponding author at: Depan, Fea, Unicamp. Tel.: +55 19 3521 4079.
E-mail address: grosso@fea.unicamp.br (C.R.F. Grosso).

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5 to 200 nm (Mestdagh and Axelos, 1998; Smidsrod, 1974). To circumvent this limitation and improve the functionality of the gels in film or microparticle form, the literature proposes a mixing with other biopolymers (Devi and Kakati, 2013) or coating with a layer of polyelectrolytes with the opposite charge by electrostatic complexation (Gbassi et al., 2011; Hébrard et al., 2010).

Complexes between proteins and polysaccharides can form spontaneously in an aqueous solution due to the electrostatic interactions between groups with opposite charges (Tolstoguzov, 2003). The formation and stability of the complexes are affected by the pH, ionic strength, protein: polysaccharide ratio, and biopolymer charge density as well as production conditions such as temperature, shearing and agitation time (Ye, 2008). The formation of protein and polysaccharide complexes generally occurs between the pK_a values of the anionic groups of the polysaccharide and the isoelectric point (pI) of the protein (De Kruif et al., 2004).

Coating pectin and alginate microparticles with polycations (such as chitosan and whey proteins) has been observed to result in the formation of a membrane on the microparticleś surface which retard the release of the active encapsulated compounds (Humblet-Hua et al., 2011). The electrostatic interactions in the microparticles can be controlled by varying the charge of the biopolymer surface. Recently, the adsorption of whey proteins onto alginate or pectin microparticles was investigated (Gbassi et al., 2011).

Egg white and whey proteins are two sources of globular proteins used in food products. The major egg white protein is ovalbumin (54%, 44.5 kDa) with a reported isoelectric point of 4.8 (Oakenfull et al., 1997). Whey proteins contain a mixture of β-lactoglobulin (82%, 18.5 KDa) and α-lactalbumin (15%, 14.5 KDa) with isoelectric points ranging from 4.4 to 5.2 (Damodaran, 2008).

The preparation of microparticles via external ionic gelation has been widely studied and demonstrated to involve a high ease of production (De Vos et al., 2010; Sriamornsak and Kennedy, 2011). However, considering that the external ionic gelation process is very rapid and non-homogeneous, optimization of the film production process remains a challenge.

The objectives of this study were to produce microparticles containing sunflower oil by ionic gelation using a mixture of alginate and pectin (1:1, w/w). The microparticles were covered with egg white and whey proteins in solution (1.7%, 3.0% and 4.0% protein in solution). The zeta potentials of the proteins and polysaccharide mixture solutions/emulsions were then evaluated at various pH values. The microparticles were characterized in terms of their morphology, size, size distribution and quantity of adsorbed protein. Subsequently, films (F_P) were produced from the alginate-pectin mixture by ionic gelation. Then, the moist films were covered with proteins at the highest levels of protein adsorptions obtained with the microparticles. The amounts of calcium bound to the microparticles and to the films produced by ionic gelation were determined. The mechanical properties (tensile strength and elongation at break), morphology, permeability to water vapor and quantity of adsorbed protein of the films were evaluated.

2. Materials and methods

2.1. Materials

High molecular weight sodium alginate (ALG) with a high guluronic acid content (lot G3512301 MANUGEL DMB; FMC Biopolymer, Campinas, São Paulo, Brazil, 0.5 ± 0.0% moisture content, AOAC, 2006), citrus pectin (PEC) GENU® with a low amidation and low methoxyl content [CP Kelco, Limeira, São Paulo, Brazil; 81.3 ± 1.2% galacturonic acid content, 30.4 ± 1.6% degree of esterification and 10.1 ± 1.0% degree of amidation, determined according to FAO (2009) and 0.4 ± 0.0% moisture content, AOAC, 2006], whey protein concentrate (WPC) [Lacprodan – lot Lac804U17601, 76, Arla Foods Ingredients, Porteña, Province of Córdoba, Argentina; 7.2 ± 0.3% moisture content, 80.5 ± 0.3% protein content and 3.9 ± 0.0% ash content, determined according to AOAC (2006), and 7.6 ± 0.3% lipid content, determined according to Bligh and Dyer (1959)], egg white proteins (OVA) (Salto's Alimentos LTDA, Distrito Industrial, Parque do Lago – Salto, São Paulo, Brazil; 6.7 ± 0.5% moisture content, 90.5 ± 0.8% protein content, 0.4 ± 0.1% lipid content and 5.0 ± 0.1% ash content), anhydrous calcium chloride (Dinâmica, Diadema – SP, Brazil), sodium hydroxide (Nuclear, Diadema – SP, Brazil), hydrochloric acid and glycerol (Merck, São Paulo – SP, Brazil) and sulfuric acid (Synth, Diadema – SP, Brazil), commercial sunflower oil, and all other reagents used were of analytical grade. All aqueous solution/emulsions were prepared with destilled and deionized water.

2.2. Zeta potential of the biopolymers

The zeta potential was determined for protein solutions (OVA, OVA + Glycerol, WPC and WPC + Glycerol) and for alginate-pectin mixture solution and emulsion, at concentrations of 0.2% (w/w). To prepare the polysaccharide mixture solution, pectin and alginate were weighed as dry powders and mixed, deionized water added to adjust the volume. All solutions were stirred overnight at room temperature before measurements. The alginate-pectin mixture solutions (2% w/w, moist weight basis) were emulsified with 1.0% (w/w, moist weight basis) sunflower oil, at room temperature, in a Turrax agitator (IKA, Works do Brasil, Rio de Janeiro – RJ, Brazil) at 14,000 rpm for 3 min. The resulting emulsion was diluted to a concentration of 0.2% (v/v), and their zeta potentials were measured, at varying the pH values ranging from 3.0 to 7.0, at room temperature. Before the readings, the pH of the solutions/emulsions was adjusted manually with HCl (0.1 N) or NaOH (0.1 N). The measurements were performed using a Zetasizer Nano-Z (Malvern, Worcestershire, U.K.). At least five measurements were recorded at each pH.

2.3. Production of microparticles

The emulsion (alginate-pectin-sunflower oil) was sprayed over a 2% (w/w) calcium chloride solution (pH adjusted to 3.5 or 3.75) at room temperature using a double fluid atomizer (ϕ, 1 mm) with a distance of 12 cm between the tip of the atomizer and the surface of the calcium chloride solution, an air pressure of 0.125 kgf/cm^2 and a spraying rate of 555 mL/h. During spraying, the emulsion was constantly agitated at room temperature. After spraying, the microparticles were kept in calcium chloride solution for 30 min. Next, the microparticles were washed three times with deionized water (pH 3.5 or 3.75) and separated by sieves (ϕ 125 μm). The microparticles obtained by ionic gelation were transferred to egg white (pH 3.5) and whey (pH 3.75) protein solutions at concentrations of 1.7%, 3% and 4% (w/w), using ~50 g of moist particles and 200 mL of protein solution. The microparticles were kept in the protein solutions for 30 min at room temperature under agitation (500 rpm). Then, the microparticles were washed three times with deionized water (pH 3.5 or 3.75) and separated by sieves (ϕ 125 μm) to remove any protein that was not adsorbed onto the microparticles. Three sets of microparticles were produced independently for each evaluated concentration. A fraction of the moist microparticles was frozen and lyophilized (Mod. 501, Edwards Pirani, Crawley, West Sussex, UK) at −40 °C and 0.1 mmHg pressure for a total cycle time of 48 h. The dry microparticles were packed into flasks with lids and kept refrigerated. The protein and dry material contents of the microparticles were determined according to the AOAC (2006) guidelines using a conversion factors