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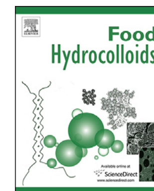

Alginate and pectin-based particles coated with globular proteins: Production, characterization and anti-oxidative properties

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ABSTRACT

The aim of this work was to produce and characterize particles obtained by ionic gelation with pectin or alginate and coated with egg white proteins, whey protein or a mixture of both; the ability of these particles to protect a model oil against lipid oxidation was also assessed. The zeta potentials of the protein solutions, the polysaccharide-oil emulsions and the particles were measured. The encapsulation efficiency, calcium content, size, solids, morphology and adsorbed protein of the particles were determined. High encapsulation efficiencies (91.70 and 95.50%) and average particle sizes (140 and 215 μm) for alginate and pectin particles, respectively, were obtained. Lower amounts of calcium were observed for particles of pectin (1.44 μmol Ca/mg of particle) than for particles of alginate (2.48 μmol Ca/mg of particle). After cross linking with calcium, the zeta potential of the particles was significantly lower than the zeta potential of their respective emulsions. More concentrated protein solutions resulted in increased amounts of adsorbed protein. The coated particles produced with alginate increased in size, whereas the coated particles produced with pectin decreased in size. The particles coated with protein were better able to protect the encapsulated oil against lipid oxidation than the uncoated particles. The highest protective capacity was observed for the particles made with pectin and coated with egg white proteins.
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1. Introduction

Alginates are polysaccharides extracted from brown algae composed of guluronic and mannuronic acids that form regions of mannuronic and guluronic blocks and regions of alternating sequences and quantities that vary with the type of algae from which the alginate is extracted (Martinsen, Skjåk-Braek, & Smidsrød, 1989). Pectins are structural components of cell walls that are mainly extracted from citrus byproducts. Pectin is an anionic polysaccharide composed primarily of galacturonic acid (α, 1–4) containing neutral sugars, such as arabinose, galactose, xylose and rhamnose (Lopes Da Silva & Rao, 2006).

Alginates and pectins can associate with divalent ions through ionic interactions to form gels. These gel matrices enable the encapsulation of hydrophobic compounds, such as polyunsaturated fatty acids (Yoo, Song, Chang, & Lee, 2006); however, because the surfaces of the particles forming these gels are porous (Smidsrod, 1974), gels exposed to high temperatures and to oxygen can undergo oxidation of long chain fatty acids.

Polyunsaturated fatty acids are susceptible to oxidative deterioration and the consequent production of unwanted odors. Thus, these oils must be protected to make them more stable during handling, processing and storage. The encapsulation of oils for nutritional, therapeutic and flavoring purposes has been reported in the literature; in particular, fish oil, tocopherol, wheat germ oil, lemon oil and citronella oil, among others, have been used as model oils encapsulated in calcium alginate particles (Chan, 2011).

Many studies have focused on improving the functionality of these encapsulating particles. In particular, combined techniques, such as ionic gelation and complexation with cationic polyelectrolytes (proteins), have been studied (Hérbrad, Moffart, Cardot, Subirade, & Beyssac, 2013).

Egg white proteins and whey protein concentrates have a globular structure. Ovalbumin, the main protein of egg whites, is a monomeric phosphoglycoprotein consisting of 385 amino acid residues with an internal disulfide bond and four free sulfhydryl

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groups. This protein has a molar mass of 46.0 kD and an isoelectric point (pI) of 4.8 (Oakenfull, Pearce, & Burley, 1997). Whey protein concentrates are mostly composed of β-lactoglobulin and α-lactalbumin, with molar mass of 18.50 and 14.50 kDa, respectively, and isoelectric points ranging from 4.4 to 5.2 (Damodaran, 2008).

The aim of this study was to test the hypothesis that the adsorption of a protein layer onto a particle produced by ionic gelation may improve the protection of encapsulated oil against lipid oxidation. The adsorption of a variety of proteins (egg white proteins (OVA), whey protein concentrate, (WPC), and a mixture of OVA:WPC, 1:1, w/w of protein) onto particles produced by ionic gelation with alginate or pectin was evaluated. The particles and the optimal conditions for the electrostatic adsorption of proteins onto the surfaces of the particles were characterized. A model oil containing polyunsaturated fatty acids was encapsulated, and the oxidative deterioration of this oil (i.e., the formation of peroxides) was monitored for four weeks at 45 °C.

2. Materials and methods

2.1. Materials

The following materials were used in this study: sodium alginate (ALG) (high molar mass, high content of guluronic acid, batch G4200301 MANUGEL DMB, FMC Biopolymer, Campinas, São Paulo, Brazil), citrus pectin (PEC) GENU® with an amidated low-methoxyl content (CP Kelco, Limeira-SP, Brazil, content of galacturonic acid (GA) 81.3 ± 1.2%, degree of esterification (DE) 30.4 ± 1.6% and degree of amidation (DA) 10.1 ± 1.0%, FAO (2009)), whey protein concentrates (WPC) (Lacprodan – batch: Lac804U17601, 76, Arla Foods Ingredients, Porteña, Cordoba province of Argentina, 80.53 ± 0.35% protein, 3.93 ± 0.04% ash, AOAC (2006), 7.58 ± 0.31% lipids, Bligh and Dyer (1959)), egg white proteins (OVA) (Salto's Alimentos Ltda, Salto, SP, Brazil, 90.48 ± 0.78% protein, 4.98 ± 0.12% ash, 0.44 ± 0.06% lipids, anhydrous calcium chloride (Dinâmica, Diadema - SP, Brazil, batch 36308), commercial sunflower oil, a model oil, 0.2 N hydrochloric acid (Merck, Germany), sodium hydroxide (Nuclear, Diadema SP, Brazil), sulfuric acid (Synth, Diadema – SP- Brazil) and deionized water. All reagents used were of analytical grade.

2.2. Zeta potential of the solutions/emulsions

The zeta potentials of the protein solutions (SOL_{OVA}, $SOL_{OVA:WPC}$ (1:1) and SOL_{WPC}), the polysaccharides (SOL_{ALG} and SOL_{PEC}) and the polysaccharide emulsions with sunflower oil were determined. Solutions of OVA, the OVA:WPC mixture, WPC, PEC and ALG were prepared at a concentration of 0.2% (w/w) and stirred overnight; the zeta potential of these solutions was determined while varying the pH from 3.0 to 7.0 at room temperature. Before the readings, the pH of the solutions was manually adjusted using hydrochloric acid (HCl, 0.1 N) or sodium hydroxide (NaOH, 0.5 N). Solutions (2%) of pectin and alginate were emulsified with 1.65% (w/w) sunflower oil in a Turrax stirrer at 14,000 rpm for 3 min (IKA, Works do Brazil, RJ). Emulsions of alginate and pectin were diluted to a concentration of 0.2% (v/v), and the surface charges of these compounds were measured while varying the pH from 3.0 to 7.0 at room temperature. Measurements were performed with a Zetasizer Nano-Z device (Malvern, Worcestershire, U.K.). All analyses were performed in triplicate.

2.2.1. Zeta potential of the particles produced by ionic gelation

The zeta potentials of the particles produced by ionic gelation were determined using a SurPASS Electrokinetic Analyzer (Anton Paar GmbH, Austria) equipped with a cylindrical cell. The streaming potential of the particles was measured with Ag/AgCl electrodes. For each measurement, approximately 0.5 mL of moist particles was transferred to the glass cylinder in the measuring cell and washed with deionized water. An electrolyte of 1 mmol/L KCl solution was used. The zeta potential was obtained from the measured streaming potential using the Smoluchowski equation according to the procedure described by Xie et al. (2009) with the aid of Anton Paar in Austria. All measurements were performed in triplicate.

2.3. Production of alginate and pectin particles

The emulsions were prepared with solutions of alginate or pectin (2%, w/w solution) and sunflower oil or model oil (1.65% w/w solution) at 25 °C using the Turrax homogenizer at 14,000 rpm for 3 min (IKA, Works do Brasil, RJ). The emulsion was atomized to a solution of calcium chloride (2% w/w) with the pH adjusted to 4.0, using a double fluid atomizer measuring 1 mm in diameter with a distance of 12 cm between the atomizer tip and the calcium chloride solution at an air pressure of 0.185 kgf/cm^2 and an atomization speed of 555 mL/h, according to Souza et al. 2012. During atomization, the emulsion remained under constant agitation at 25 °C for the $EMUL_{PEC}$ and at 50 °C for the $EMUL_{ALG}$. After atomization, the particles were maintained in the calcium chloride solution for 30 min (hardening time). Subsequently, the particles were separated and washed with deionized water (pH 4.0) using steel mesh sieves (ϕ 53 μm). Three independent batches of particles were produced. A fraction of the moist particles was frozen and lyophilized (Mod. 501, Edwards Pirani, Crawley, West Sussex, UK) at −40 °C and 0.1 mmHg with a total cycle time of 48 h; the dried particles were packaged in flasks with lids and refrigerated.

2.4. Encapsulation efficiency of alginate and pectin particles

To determine the amount of total oil encapsulated by the particles, sodium citrate was added at a concentration of 3% (w/w) to 5 g of moist particles to release the oil. The amount of oil released was quantified according to the method of Bligh and Dyer (1959). The encapsulation efficiency was determined by the relationship:

$$EE\left(\%\right) = \frac{\text{Total oil of the particles (g)/total solids (g)}}{\text{Initial oil (g)/total solids (g)}} \times 100$$

2.5. Calcium content of the alginate and pectin particles

The amount of calcium in cross linked particles was determined using a standard calcium solution (1000 μg/mL, SCP Science, batch S120221015, Quebec, CA) and an atomic absorption spectrophotometer (Analytik Jena AG-NOVAA300, JENA, GERMANY) in the absorption mode with an air-acetylene flame detector. Samples of moist particles (400 mg) were dissolved in 25 mL of a 3% sodium citrate solution. Preliminary tests showed that the sodium citrate solution does not interfere with the calcium analysis. Each measurement was performed in triplicate.

2.6. Coating of alginate and pectin particles with OVA, OVA:WPC and WPC proteins

Moist pectin or alginate particles (40–50 g) were transferred to a 200 mL protein solution (SOL_{OVA}, $SOL_{OVA:WPC}$ (1:1, w/w of protein) and SOL_{WPC}) adjusted to pH 4.0. The protein solutions had concentrations of 2, 4, 6 or 8% (w/w). The particles were maintained in the protein-containing solutions for 30 min under constant

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