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Chitosan nanoparticles loaded with 2,5-dihydroxybenzoic acid and protocatechuic acid: Properties and digestion

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ABSTRACT

Research efforts on the production of chitosan nanoparticles (NP) as delivery systems of bioactive compounds such as polyphenols have been made along the last decade. Nevertheless, the effect of the phenolic compound structure in the production of these NP was never evaluated so far. Low and high molecular weights chitosan (LMWC and HMWC) NP loaded with the phenolic acids, protocatechuic (PA) and 2,5-dihydroxybenzoic acids (2,5-DHBA) were produced by ionic gelation. Antioxidant activities were determined by ORAC assay. Physical and thermal properties were evaluated by dynamic light scattering (DLS) and differential scanning calorimetry (DSC), respectively. Stability and release of phenolic acids during simulation of gastrointestinal tract (GIT) conditions were also assessed. Nanoparticles sizes ranged from 300 to 600 nm and maintained stable during storage at 4 °C during 30 d. Antioxidant activities of the phenolic acids decreased when loaded in the NP. High molecular weight chitosan NP adsorbed higher energy and melted at lower temperatures than LMWC NP. Nanoparticles produced with HMWC released higher phenolic acids % at GIT simulated conditions and with slight increases in their sizes. The most proper systems for delivery of PA and 2,5-DHBA were found to be LMWC and HMWC NP, respectively. These NP could be used to as functional food ingredients or as models for production of phenolic acids-rich extracts NP for future incorporation in food matrices.

1. Introduction

Nowadays, there is a rising attention in the application of nanotechnology in food industry, namely in the production of nanoparticles (NP) as new functional food ingredients and as a way to improve the bioactive compounds functionality with proved benefits to human health. These new structures can protect the bioactive compounds during food processing and storage, and during passage by the gastrointestinal tract (GIT) (Ha et al., 2013; Zou et al., 2012). The polymeric NP are colloidal carriers with sizes ranging from 10 to 1000 nm. Great part of the studies use chitosan as entrapment material for NP production and bioactive/drug compounds controlled delivery, protection from degradation and oxidation, production of nanocomposites, among other applications (Agrawal et al., 2010; Dehnad et al., 2014; Huh and Kwon, 2011). Chitosan is an abundant natural biopolymer obtained from chitin deacetylation, and acquired from crustaceans shells or as a natural component of specific fungi genus (Mucoraceae) (Agrawal et al., 2010; Dash et al., 2011). This polymer is polycationic with constant acidity (pKa = 6.5) and insoluble at neutral pH (Agrawal et al., 2010). Chitosan is claimed as non-toxic, biologically compatible polymer, non-immunogenic and highly antimicrobial (Kean and Thanou, 2010; Mukhopadhyay et al., 2012). The difference in the relative proportions of N-acetyl-D-glucosamine and D-glucosamine residues, provide specific structural changes, which give rise to chitosans that are distinguished on the basis of their degree of deacetylation (DD) and molecular weight. The polymers have different molecular weights (50–2000 kDa), viscosity and DD (40–98%). Commercial chitosans are sold as high and low molecular weight chitosans, and characterized containing between 20 and 190 kDa with DD > 75% and between 190 and 375 kDa with DD > 75%, respectively.

On the other hand, there is a huge interest in encapsulation of phenolic acids or their extracts, due to its high biological activity, namely on the antioxidant capacity found in this large group of compounds present in plants (Mohammadi et al., 2015a, 2015b).

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The encapsulation of these compounds, especially the ones with hydrophobic character is justified by their higher level of reactivity, chemical susceptibility to the harsh conditions of the GIT, low oral bioavailability and intestinal absorption (Tang et al., 2013). They must be enough time in the intestinal lumen to adhere to cell apical surface and then, be transcytosed by intestinal cells.

The phenolic compounds used in this study were protocatechuic acid (PA) and the 2,5-dihydroxybenzoic acid (2,5-DHBA) (also known as gentisic acid). Protocatechuic acid is the major metabolite of antioxidant phenolic compounds found in green tea, and has been described to possess several bioactivities such as antimicrobial, anticancer, anti-ulcerogenic, anti-ageing (Kakkar and Bais, 2014). In addition, 2,5-DHBA has been characterized as antimutagenic, anti-inflammatory and antimicrobial (Boaventura et al., 2013). Is widely present in regular foods, including cereals such as wheat and rye, actinidia (e.g., kiwi) fruit, aloe vera, mushrooms as well as other sources (Juurlink et al., 2014). Besides their variety in terms of natural sources, these two phenolic acids are different in terms of their chemical structure, in what concerns the number of −OH groups which are 3 for PA and 2 for 2,5-DHBA.

Thus, the aim of the present study was the development of model carrier systems of two phenolic acids with different structures, PA and 2,5-DHBA. These were produced with LMWC and HMWC and characterized for their physical, antioxidant and thermal properties. Afterwards their antioxidant activity and stability during a simulated pH gastrointestinal tract were also evaluated.

2. Material and methods

2.1. Preparation of NP

The concentrations of chitosan and phenolic compounds used in the production of the NP were the following: 0.2% (m/v) low molecular weight chitosan (LMWC), 0.4% (m/v) high molecular weight chitosan (HMWC), 0.3% (m/v) PA (LMWC_PA and HMWC_PA), and 0.3% (m/v) DHBA (LMWC_DHBA and HMWC_DHBA). All compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). Production followed the ionic gelation method described by da Silva et al. (2014), but with some modifications. Chitosan solutions were first dissolved in acetic acid (where the concentration of acetic acid was 1.75 times higher than chitosan concentration). Hence, acetic acid at 3.5 mg/mL was used for LMWC at 0.2% (m/v) and 7 mg/mL for 0.4% of HMWC. pH solution was adjusted to the physiologic value of 5.8 with NaOH 1 M. Afterwards, the phenolic compounds were dissolved in ultra pure water. Sodium tripolyphosphate (TPP) (Sigma-Aldrich) was prepared at different concentrations according the chitosan MW at a proportion [chitosan:TPP] of 7:1. Four mL of chitosan solution were placed under gentle stirring and 1 mL of phenolic compound was slowly added drop wise, followed by 2 mL of TPP also drop wise, at room temperature. Nanoparticles controls without phenolic compound were also produced by the same method. At the end, the pH of the final NP solutions loaded with phenolic compounds and controls without phenolic compounds were measured and then stored at 4 °C.

2.2. Determination of the physical properties

Physical properties such as particle size (PS), polydispersity index (PI) and zeta potential (ZP) were evaluated using the NP solutions, by dynamic light scattering (DLS) technique with a ZetaPALS, Zeta Potential Analyzer (Holtsville, NY, USA). The parameters were measured at the initial production time of NP and also after 1 month of storage time at 4 °C. All assays were performed in triplicate.

2.3. Determination of NP entrapment efficiencies

The NP suspensions were filtrated by a centrifugal filter units with a cut-off of 3 K (Amicon® Ultra-4, Millipore), and then centrifugated at 37,732 g, 25 °C, for 1 h. Samples supernatants were subject to analyses by HPLC method (all assays were done in triplicate). The entrapment efficiency (EE%) was determined as follows:

$$EE\% = \frac{Total\ amount\ of\ polyphenol - Total\ amount\ of\ polphenols\ in\ supernatant}{Total\ amount\ of\ polyphenols}$$

2.4. Determination of antioxidant activity

The antioxidant activity of loaded phenolic compounds NP was determined by oxygen radical absorbance capacity (ORAC), a fluorometric method that measures *in vitro* the antioxidant activity through fluorescein (Sigma, Steinheim, Germany) oxidation by the peroxilos radicals *in situ* by thermal decomposition from AAPH (Aldrich, Steinheim, Germany). This method measures the ability of oxygen radical absorption through the method developed by Ou et al. (2002) and adapted by Dávalos et al. (2004) using a microplate fluorescence reader. Twenty microliters of solution (NP and phenolic compounds) or sodium phosphate buffer (0.1 M, pH 7) in case of control, were mixed with 120 μL of fluorescein and preincubated for 10 min at 40 °C. After this step, 60 μL of AAPH was rapidly added in a polystyrene black microplate (Nunc, Denmark) and incubated at 40 °C until a total of 137 min was completed (with the 10 min included). The measurement was performed in a fluorometer FluoSTAR OPTIMA (BMG LABTECH GmbH, Offenburg, Germany) using a wavelength of excitation at 485 nm and the emission one at 520 nm, with a Fluostar Control version 1.32 R2 software. All analyses were performed in triplicate.

2.5. Determination of thermal properties

The NP thermal properties were evaluated by differential scanning calorimetry (DSC) using a DSC-60 (Shimadzu, Maryland, DC, USA). Nanoparticle suspensions were concentrated by centrifugation at 37,732 g, 25 °C, 1 h and 3 mg of the pellet were placed in an aluminium pans hermetically sealed. The thermal behaviour was determined in the range 20–200 °C, at a heating rate of 10 °C/min. Optimal melting temperatures and enthalpies were calculated by the equipment software (ta60 version 2.10, DSC software, Shimadzu). The mixtures of the phenolic compounds and TPP compound at the two different concentrations used (0.29 and 058 mg/mL) were also tested, to evaluate the effect of these compounds in the thermal properties of the NP. All NP samples were tested as well as the controls (without phenolic compounds) and raw compounds used in the NP production. All assays were performed in quadruplicate.