Food Hydrocolloids 84 (2018) 267–275

Contents lists available at ScienceDirect

Food Hydrocolloids

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

In vitro digestion of lactoferrin-glycomacropeptide nanohydrogels incorporating bioactive compounds: Effect of a chitosan coating

Ana I. Bourbon[a,b,*], Ana C. Pinheiro[a,c], Miguel A. Cerqueira[a,b], António A. Vicente[a]

[a] CEB, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] International Iberian Nanotechnology Laboratory, Department of Life Sciences, Av. Mestre José Veiga s/n, 4715-330 Braga, Portugal
[c] Instituto de Biologia Experimental e Tecnológica, Avenida da República, Quinta-do- Marquês, Estação Agronómica Nacional, Apartado 12, 2781-901 Oeiras, Portugal

ARTICLE INFO

Keywords:
Nanotechnology
Functional compounds
Curcumin
Caffeine

ABSTRACT

The behaviour of lactoferrin (Lf) – glycomacropeptide (GMP) nanohydrogels with and without a chitosan coating was evaluated during gastrointestinal digestion. The application of a chitosan coating allowed to reduce the protein degradation from 86% to 76% for Lf and from 71% to 53% for GMP.

Protein bioaccessibility results showed that in nanohydrogels with chitosan coating 23% of Lf and 40% of GMP remained intact until absorption. Based on these results, the bioaccessibility of two different bioactive compounds encapsulated in Lf-GMP nanohydrogels with chitosan coating was evaluated during gastrointestinal digestion. Curcumin was used as lipophilic model compound and caffeine as a hydrophilic model compound. Bioaccessibility of curcumin in coated nanohydrogels was 72% while the corresponding value for curcumin in free form only reached 66%. It was also observed that under simulated gastric and intestinal conditions, free curcumin lost around 68% of its antioxidant activity while when incorporated into nanohydrogels only 30% of this activity was lost. Results also showed that the bioaccessibility of caffeine encapsulated in coated nanohydrogels was 63% while caffeine in free form only reached 59%.

1. Introduction

The growing awareness of the relevance of food in health maintenance and the difficulties of changing eating habits, calls for an enrichment of foods with bioactive compounds. One of the challenges of food enrichment with such bioactive compounds is related to the poor solubility, in the case of hydrophobic compounds, in food matrices and their instability during digestion and consequent poor bioavailability. These challenges are promoting research efforts to find more effective delivery systems based on natural biopolymers (Livney, 2012). Protein nanohydrogels are promising systems to be used as carriers of bioactive compounds in food products (Martins et al., 2015). The high nutritional value, non-toxicity and ability of proteins to bind to hydrophobic and hydrophilic bioactive compounds make them a useful vehicle to help to incorporate such bioactives in foods (Wong, Camirand, Pavlath, Parris, & Friedman, 1996). However, during the gastric digestion step, proteins are denatured by environmental conditions (low pH and high ionic force) and hydrolyzed by enzymes (pepsin) (Yvon, Beucher, Scanff, Thirouin, & Pelissier, 1992). One of the strategies to improve the protein nanohydrogels stability during enzymatic digestion and the controlled release of active ingredients during gastric and intestinal digestion is the addition of a polysaccharide coating (Liu, Gao, & Yuan, 2014).

In our previous work, nanohydrogels composed by lactoferrin (Lf) and glycomacropeptide (GMP) were coated with a chitosan layer (Bourbon, Pinheiro, Cerqueira, & Vicente, 2016) and the stability of protein nanohydrogels with chitosan coating and the release properties of caffeine were studied during gastric digestion. The addition of a chitosan coating on Lf-GMP nanohydrogels had a positive effect on the stability of Lf-GMP nanohydrogels during gastric digestion, once the presence of nanohydrogels coated with chitosan was stable for longer periods of time. During the gastric digestion, the hydrolysis of proteins was slower in nanohydrogels coated with chitosan than in nanohydrogels alone. It was also observed that the release of caffeine was governed by Fick's diffusion and by relaxation of the matrix, for nanohydrogels with and without chitosan coating. However in nanohydrogels with the presence of a chitosan coating, the mass of caffeine released was lower, showing that the chitosan coating can be used to control the initial “burst release” of nanohydrogels without coating. Despite all these findings, more information is needed to understand the protein-based nanostructures digestion process and its impact on the release and uptake of encapsulated bioactive compounds under specific

* Corresponding author. International Iberian Nanotechnology Laboratory, Department of Life Sciences, Av. Mestre José Veiga s/n, 4715-330 Braga, Portugal.
E-mail address: isabelbourbon@gmail.com (A.I. Bourbon).

https://doi.org/10.1016/j.foodhyd.2018.06.015
Received 15 February 2018; Received in revised form 8 May 2018; Accepted 11 June 2018

gastrointestinal conditions. *In vitro* digestion models have recently gained much attention as a tool for understanding the basic physiochemical processes that occur during the digestion and the release of encapsulated compounds (Ahmed, Li, McClements, & Xiao, 2012). To exert a health benefit, bioactive compounds need to withstand food processing, be released from the food matrix after ingestion and be bioaccessible in the gastrointestinal tract, undergo metabolism and reach the target tissue of action (Rein et al., 2013). Due to the complexity of gastric and intestinal digestion and the many factors affecting their transition during digestion (e.g. pH, ionic strength, enzymes), unravelling the factors affecting the bioaccessibility of bioactive compounds is still a challenge. In fact, the assessment of the bioaccessibility of a health bioactive compounds is important for the understanding of the relationship between food and nutrition (Rein et al., 2013).

In the present study, a dynamic *in vitro* digestion model was used to evaluate the stability and bioaccessibility of protein-based nanohydrogels coated with a chitosan layer and to evaluate the bioaccessibility of two bioactive compounds when loaded in these coated nanohydrogels. Curcumin, as a lipophilic model compound and caffeine as a hydrophilic model compound were encapsulated in Lf-GMP nanohydrogels coated with a chitosan layer and their bioaccessibility, was evaluated during *in vitro* digestion and compared with free solutions of curcumin and caffeine. The digestion process was carried out in a dynamic model that mimics the environmental (temperature, pH, ionic strength) and physical conditions (peristaltic and intestinal movements) of the gastrointestinal tract. The knowledge obtained from this study will be relevant to better design our future foods, containing new delivery systems for enhancing the bioaccessibility of health-promoting compounds.

2. Materials and methods

2.1. Materials

Purified lactoferrin (Lf) powder was obtained from DMV International (USA) and it is composed by 96% protein, 0.5% ash, 3.5% moisture and an iron content is around 120 ppm (composition expressed as a dry weight percentage). Commercial glycomacropeptide (GMP) was kindly offered by Davisco Food International, INC. (Le Sueur, USA) and its reported composition is: 82.5% protein, 1% fat, 7% ash and 7% moisture. Chitosan of low molecular weight (molecular weight ranging between 50 and 100 kDa and with a deacetylation degree ≥ 95%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Lactic acid (90%) was obtained from Acros Organics (Geel, Belgium). Curcumin was purchased from Sigma-Aldrich (St. Louis) and caffeine was purchased from VWR (USA). All the samples were dissolved in deionized water purified to a resistance of 15 MΩ (Millipore, France).

2.2. Encapsulation of bioactive compounds in Lf-GMP nanohydrogels coated with chitosan layer

Lf-GMP nanohydrogels were prepared as described in Bourbon et al. (2016). Briefly, 0.02% (w/w) of Lf and 0.02% (w/w) of GMP were dissolved separately, in deionized water purified at 25 °C. The pH values of biopolymer solutions were separately adjusted to 5.0, with 0.1 mol.L^{-1} of hydrochloric acid. Lf aqueous solution was added dropwise into GMP aqueous solution with gentle stirring until the final molar ratio of volume (MR) 1:7 of Lf to GMP. At this stage, bioactive compounds were added to the mixture.

A maximum encapsulation efficiency of 95.12 ± 1.4 and 90.02 ± 2.1% was obtained when 0.082 mg mL^{-1} of curcumin and 0.03 mg mL^{-1} of caffeine was encapsulated in Lf-GMP nanohydrogels (Sanguansri & Augustin, 2006). Briefly, curcumin solution (0.082 mg mL^{-1}) or caffeine solution (0.03 mg mL^{-1}) was added to the mixture solution of Lf (0.02% (w/w), pH 5.0) and GMP (0.02% (w/w), pH 5.0) with a molar ratio of 1:7 of Lf to GMP at 25 °C. After gentle stirring for 30 min, the mixture of Lf-GMP with bioactive compounds was subsequently heated at 80 °C for 20 min in a water bath (closed system) to obtain a homogeneously dispersed nanohydrogel solution. The chitosan was assembled on the Lf-GMP nanohydrogels by the layer-by-layer (LbL) deposition technique. Nanohydrogels with bioactive compounds encapsulated were added to a chitosan solution (1 mg mL^{-1}, pH 3, dissolved in 1% of lactic acid) at volume ratios (VR) of 0.1 of Lf-GMP nanohydrogels to chitosan, with constant stirring of 200 rpm during 15 min, creating the nanohydrogels with a coating (Bourbon et al., 2016).

2.3. In vitro digestion

2.3.1. Gastrointestinal model

A dynamic gastrointestinal model system was used in the *in vitro* digestion experiments. This model simulates the main events that occur during digestion and consists of four compartments simulating the stomach, duodenum, jejunum and ileum. Each compartment consists in two connected glass reactors with a flexible wall inside and water is pumped around the flexible walls to maintain the temperature at 37 °C and to enable the simulation of the peristaltic movements (by the alternate compression and relaxation of the flexible walls). The changes in water pressure are achieved by peristaltic pumps which alter the flow direction according to the time-controlling devices connected to them. The compartments are connected by silicone tubes and, at a predefined time, a constant volume of chyme is transferred. All compartments are equipped with pH electrodes and pH values are controlled by the secretion of HCl (1 mol.L^{-1}) into the stomach and $NaHCO_3$ (1 mol.L^{-1}) into the intestinal compartments. The gastric and intestinal secretions are added via syringe pumps at pre-set flow rates. The jejunum and ileum compartments are connected with hollow-fibre devices (SpectrumLabs Minikros®, M20S-100-01P, USA) to absorb digestion products and water from the chyme and to modify electrolyte and bile salts concentration of the chyme (Reis et al., 2008).

2.3.2. Experimental conditions

In vitro digestion was performed as described by other authors (Reis et al., 2008) with some modifications. A volume of 60 mL of nanohydrogels with chitosan coating (with and without bioactive compounds encapsulated) was introduced into the dynamic gastrointestinal system (gastric compartment) and the experiment was run for a total of 5 h, simulating average physiological conditions of GI tract by the continuous addition of gastric, duodenum, jejunal and ileal secretions. The gastric secretion consisted of pepsin and lipase in a gastric electrolyte solution (NaCl 4.8 g.L^{-1}, KCl 2.2 g.L^{-1}, $CaCl_2$ 0.22 g.L^{-1} and $NaHCO_3$ 1.5 g.L^{-1}), secreted at a flow rate of 0.33 mL min^{-1}. The pH was controlled to follow a predetermined curve (from 4.8 at $t = 0$ to 1.7 at $t = 120$ min) by secreting HCl (1 mol.L^{-1}).

The duodenal secretion consisted of a mixture of 4% (w/v) porcine bile extract, 7% (w/v) pancreatin solution and small intestinal electrolyte solution (SIES) (NaCl 5 g.L^{-1}, KCl 0.6 g.L^{-1}, $CaCl_2$ 0.25 g.L^{-1}) secreted at a flow rate of 0.66 mL min^{-1}. The jejunal secretion fluid consisted of SIES containing 10% (v/v) porcine bile extract solution at a flow rate of 2.13 mL min^{-1}. The ileal secretion fluid consisted of SIES at a flow rate of 2.0 mL min^{-1}. The pH of the different parts of small intestine was controlled by the addition of 1 mol.L^{-1} $NaHCO_3$ solution to set-points of 6.5, 6.8 and 7.2 for simulated duodenum, jejunum and ileum, respectively. During *in vitro* digestion, samples were collected directly from the lumen of the different compartments, from the jejunal and ileal filtrates and from the ileal delivery. The jejunal and ileal filtrates were used to determine the bioavailable fraction of bioactive compounds. The samples were tested in the dynamic gastrointestinal model at least in triplicate.

2.4. Size distribution

Nanohydrogels with and without chitosan coating were characterized in