International Journal of Biological Macromolecules 112 (2018) 399–404

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: https://www.journals.elsevier.com/ijbiomac

Controlled release and antioxidant activity of chitosan or its glucosamine water-soluble derivative microcapsules loaded with quercetin

Noelia L. Vanden Braber [a], Alejandro J. Paredes [b], Yanina E. Rossi [a], Carina Porporatto [a], Daniel A. Allemandi [b], Claudio D. Borsarelli [c,*], Silvia G. Correa [d], Mariana A. Montenegro [a,*]

[a] Centro de Investigaciones y Transferencia de Villa María (CITVM-CONICET), Universidad Nacional de Villa María, Campus Universitario, Arturo Jauretche 1555, Villa María, Córdoba, Argentina
[b] Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA-CONICET), Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba (UNC), Haya de la Torre y Medina Allende, Ciudad Universitaria, X5000HUA Córdoba, Argentina
[c] Instituto de Bionanotecnología del NOA, (INBIONATEC), Universidad Nacional de Santiago del Estero (UNSE), CONICET, RN9, Km 1125, G4206XCP Santiago del Estero, Argentina
[d] Centro de Investigación en Bioquímica Clínica e Inmunología (CIBICI- CONICET), Universidad Nacional de Córdoba (UNC), Córdoba, Argentina

ARTICLE INFO

Article history:
Received 5 October 2017
Received in revised form 29 December 2017
Accepted 13 January 2018
Available online xxxx

Keywords:
chitosan
microcapsules
crosslinking
controlled release
reactive oxygen species

ABSTRACT

The controlled release and antioxidant properties of the flavonoid quercetin (Qr) incorporated into crosslinked microcapsules using chitosan (Ch) or its derivative modified with glucosamine by Maillard reaction (GACh) as wall materials were evaluated. The microcapsules containing Qr (Qr-MC) were obtained by the spray-drying technique with high microencapsulation efficiency of Qr, and with spherical shape of average size of 2.0 ± 1.5 μm. Under gastrointestinal simulated conditions, the Qr-MC showed controlled release within few hours, being the release rate faster under gastric than intestinal conditions. The rate of release of Qr by GACh-MC was almost double than those made with Ch under gastric conditions, but the same release rate was observed for both Qr-MC under intestinal conditions. Efficient antioxidant activity of the Qr-MC against reactive oxygen species (ROS) including hydroxyl radical $HO^{\bullet}$, anion superoxide $O_2^{\bullet-}$ and singlet oxygen $^1O_2$ was observed, indicating that Ch biopolymers are also suitable functional coating materials for flavonoid microencapsulation, regarding the gastro resistance, antioxidant activity and controlled release properties that could increase the bioavailability of the flavonoid.

1. Introduction

Chitosan (Ch), a natural amino-polysaccharide biopolymer, has received much attention due to its unique biocompatible properties, e.g. nontoxic, immunostimulant, anticancer, antimutagenic, mucoadhesive and antimicrobial, yielding a large variety of applications in food industry, including the production of nutraceutical and functional foods [1–3]. Chemically, Ch is composed of glucosamine and N-acetylglucosamine and produced by the alkaline N-deacetylation of chitin, the major component of the shells of crustaceans such as crab, shrimp, and crawfish [4,5]. The physical properties of Ch depend on a number of parameters including molecular weight (MW), deacetilation degree (DD) and sequence of the amino and the acetamido groups [6]. However, the intrinsic insolubility of Ch at neutral or high pH limits often its application in aqueous media under those pH conditions [1,7,8]. Recently, it has been shown that aqueous solubility of Ch is improved by the covalent attaching through Maillard reaction (MR) of mono- or disaccharide residues such as glucosamine (GA), preserving the global properties of the polysaccharide [8–11], even enhancing its antioxidant properties under neutral pH conditions [12].

On the other hand, the application of polyphenols in the preparation of functional foods and pharmaceutical formulations, especially including flavonoids such as quercetin (Qr) is of superior commercial interest, on account of their health benefits to humans [13]. Qr (3,3′,4′,5,7-pentahydroxyflavone) is widely distributed in the vegetal kingdom, and due to the large hydroxyl substitution onto the aromatic ring system presents remarkable free radical scavenging and metal cation chelating capacities, inhibition of the activity of oxidases, and also singlet molecular oxygen quenching ability [14–18]. Hence, Qr is a biocompatible molecule with potent antioxidant properties. However, the effectiveness of polyphenols depends on preserving the stability, bioactivity and bioavailability of the active ingredients [19]. At the

Abbreviations: Ch, Chitosan; ChMC, Chitosan Microcapsules; DD, Deacetylation Degree; GRAS, Generally Recognized as Safe; GACh, Glucosamine chitosan derivative; $HO^{\bullet}$, Hydroxyl Radical; MR, Maillard reaction; MC, Microcapsules; ME, Microencapsulation efficiency; MY, Microencapsulation yield; MW, Molecular Weight; % RS, Percent of Radical Scavenged; Qr, Quercetin; Qr-MC, Quercetin loaded Microcapsules; ROS, Reactive Oxygen Species; $^1O_2$, Singlet molecular oxygen; $O_2^{\bullet-}$, Superoxide Anion Radical.

* Corresponding authors.
E-mail addresses: cdborsarelli@gmail.com.ar (C.D. Borsarelli), mamontenegro03@yahoo.com.ar (M.A. Montenegro).

https://doi.org/10.1016/j.ijbiomac.2018.01.085
0141-8130/

same time, as opposed to quercetin glycosides, Qr is poorly absorbed in the intestine, being preferentially absorbed in the stomach, like occurs with others aglycone flavonoids [20].

Therefore, there is considerable interest on the development of formulations of Qr capable of improving its chemical and biological stability and its bioavailability. In this regard, microencapsulation using biocompatible polymers is an excellent tool to vehiculate bioactive compounds, and depending on the properties of the polymeric coating, it can be controlled the release of the core material in a specific site [19]. Among microencapsulation techniques, spray-drying is an economical, flexible, and continuous operation procedure widely used for the production of high quality microparticles in food and pharmaceutical industries and lab scale [21].

In the present study, the effectiveness of both Ch and GACh as coating material for the spray-drying microencapsulation of Qr was evaluated, with the purpose to design a gastro-resistant microcapsules, in order to enhance the bioavailability and antioxidant properties of the flavonoid in the whole gastrointestinal apparatus, since it is well-known that Ch is resistant to gastric digestion [3,22–24]. The morphology, release kinetic, and antioxidant activity against reactive oxygen species (ROS) including hydroxyl radical ($HO^{\bullet}$), anion superoxide ($O_2^{\bullet-}$) and singlet molecular oxygen ($^1O_2$) of both Qr loaded MC were also studied.

2. Materials and methods

2.1. Materials

Ch medium MW (583 kDa, 78% DD), Qr (aglycone, MW 302.24 Da), glucosamine hydrochloride (GAHC), nitrotetrazolium blue chloride (NBT), 2-Deoxy-D-ribose (DoR), hydroxylamine hydrochloride (HAHC), trichloroacetic acid (TCA), tris(bipyridine)ruthenium(II) dichloride ($Ru(bpy)_3Cl_2$), sodium tripolyphosphate (TPP) were from Sigma-Aldrich (MO). Ferric chloride ($FeCl_3$), monobasic potassium phosphate (KH_2PO_4), sodium chloride (NaCl), sodium hydroxide (NaOH), potassium chloride (KCl), sodium bicarbonate ($NaHCO_3$) and ascorbic acid, all analytical grade, were obtained from Cicarelli (Argentina). The thiobarbituric acid (TBA) and ethylenediaminetetraacetic (EDTA) were provided by Merck (Germany) and hydrogen peroxide (H_2O_2), hydrochloride acid (HCl), isopropanol and glacial acetic acid (CH_3COOH) by Anedra (Argentina). Aqueous solutions were prepared with ultrapure water.

2.2. Preparation of glucosamine chitosan derivative

GACh derivative was obtained by Maillard reaction (MR) between native Ch with GAHC as described by Vanden Braber et al. (2017) [12]. Briefly, 1% (w/v) Ch in 0.25 M CH_3COOH was mixed with 1% (w/v) GAHC and kept in an orbital shaker at 65 °C for 48 h at pH 3. Then the sample was centrifuged with a Sorval LST 16R centrifuge (Thermo Scientific, USA) at 5.000 rpm for 20 min. The supernatant was dialyzed against distilled water through a membrane with MW cutoff of 12–14 kDa (Sigma-Aldrich) for 96 h at 4 °C. Detailed procedures for their preparation and characterization were described in our previous paper [12].

2.3. Spray-drying microencapsulation of Qr

0.5% (w/v) Ch and GACh solutions in 0.5% (v/v) CH_3COOH were used as encapsulant material. Qr was added into encapsulant solutions in a proportion of 5% (w/w) of the biopolymers, under continuous stirring. The mixture was homogenized at 18.000 rpm for 5 min protected from light using an Ultraturrax homogenizer (IKA T18, Germany). Then, under constant stirring, 1% (w/v) TPP was added as ionic crosslinker agent in a ratio of 5% of encapsulant material solution volume. MC loaded with Qr (Qr-MC) were obtained by spray-drying process in a BÜCHI Mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Switzerland), equipped with a two-fluid nozzle with a cap pinhole diameter of 0.5 mm. The working conditions such as inlet temperature, liquid flow, aspiration rate and compressed spray air flow (represented as the volume of the drying air input) were set at 130 °C, 4 mL/min, 100% and 1.05 m^3/h, respectively. Empty MC were prepared in the same conditions without adding Qr in the mixture to be spray-dried. The powders obtained after spray-drying were stored at room temperature in desiccator containing silica pearls to avoid moisture.

2.4. Qr microencapsulation efficiency and yield

To quantify the total content of microencapsulated Qr ($[Qr]_T$), an weighted amount of Qr-MC was dissolved in 0.1 N HCl in isopropanol: water (1:1). This solution was sonicated during 2 h to ensure complete release of the flavonoid. Instead, for the quantification of the adsorbed content of Qr onto the MC surface ($[Qr]_S$), an isopropanol:water mixture (1:1) was used to disperse a known amount of Qr-MC and the supernatant was immediately separated by centrifugation (500 rpm). In both cases, the flavonoid content was quantified by registering the absorbance at 366 nm (absorption maximum for Qr) using a Specord S600 UV–Vis diode array spectrophotometer (Analytik Jena, Germany), after subtracting the background absorbance and/or scattering of a same concentration MC solution without Qr. A calibration curve made with Qr solutions in water at different working pH was used. The microencapsulation efficiency (ME), defined as the percentage of Qr molecules inside the microcapsule in relation to its total (inside + surface) concentration, was calculated with Eq. (1) [25].

$$ME(\%) = 100 \times ([Qr]_T - [Qr]_S)/[Qr]_T \quad (1)$$

In turns, the microencapsulation process yield (MY) was calculated according to Eq. (2),

$$MT(\%) = 100 \times (m_{MC}/m_T) \quad (2)$$

where m_T and m_{MC} are the mass of solids obtained before and after spray-dried encapsulation.

2.5. Morphology and size of Qr-MC

Morphology and size distribution of the spray-dried powders were evaluated by scanning electronic microscopy (SEM) with a ZEISS SIGMA VP Field Emission Scanning Electron Microscope (FE-SEM) (ZEISS, Germany), using an acceleration voltage of 5 kV. The MC were fixed in stubs containing a double-faced adhesive metallic tape and coated with gold in a CED 010 vacuum evaporator (Balzers Union, Liechtenstein). Size distribution of spray-dried powders was evaluated with the ImageJ 2014 software (Rasban, National Institute of Health, USA).

2.6. Qr release in simulated gastrointestinal digestion conditions

The in vitro Qr release kinetic patterns were studied during 7 h using an orbital shaker at a rotational speed of 150 rpm and at 37 °C [23]. Two different digestion stages were performed: firstly, 0.05 g of Qr-MC were mixed with ultrapure water and placed into dialysis membrane with MW cutoff of 12–14 kDa (Sigma-Aldrich) and disposed in 60 mL of 125 mM NaCl, 7 mM KCl, 45 mM $NaHCO_3$, 0.1 N HCl at pH 1.2 used to simulate the gastric fluid conditions during 2.5 h. In the second stage, the same dialysis membrane was disposed in 60 mL of 50 mM KH_2PO_4, 22.4 mM NaOH at pH 6.8 during 4.5 h in order to simulate intestinal fluid conditions. At the indicated time points, samples were collected and the volume replaced with fresh medium to maintain the same conditions. Collected samples were analyzed spectrophotometrically at 366 nm to determine Qr content using calibration curves as described in sub-section 2.4. In addition, the stability of Qr in