Food Hydrocolloids xxx (2017) 1–10

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Oat protein-shellac beads: Superior protection and delivery carriers for sensitive bioactive compounds

Chen Yang [a], Yixiang Wang [a], Lei Lu [b, c], Larry Unsworth [b, c], Le Luo Guan [a], Lingyun Chen [a, *]

[a] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada
[b] Department of Chemical & Materials Engineering, University of Alberta, Edmonton, AB, T6G 2P5, Canada
[c] National Institute for Nanotechnology, 11421 Saskatchewan Drive, Edmonton, AB, T6G 2M9, Canada

ARTICLE INFO

Article history:
Received 17 June 2017
Received in revised form
5 November 2017
Accepted 12 November 2017
Available online xxx

Keywords:
Oat protein
Shellac
Controlled release
Encapsulation
Bioactive compounds

ABSTRACT

The purpose of this work was to better understand the interactions between oat protein (OPI) and shellac to form oat protein-shellac based gels at near neutral pH as carrier to protect and deliver sensitive bioactive compounds. There were moderate interactions between OPI and shellac with a binding constant (K_a) of 2.088 × 10^3 M^{-1} via hydrophobic interactions and hydrogen bonding as revealed by Isothermal Titration Calorimeter (ITC) and Fourier Transform Infrared Spectroscopy (FTIR). Such interactions allowed the formation of a gel-like mixture with good compatibility that only one endothermic peak and T_g value for OPI-shellac mixture as demonstrated by differential scanning calorimetry (DSC) analysis. Novel core (OPI)-shell (OPI-shellac mixture) beads were then developed by a cold-gelation method at near neural pH and ambient temperature. The optimized samples possessed a homogeneous, smooth and integrated shell structure. This structure could effectively restrict the swelling of the shell and prevent premature diffusion of the contained riboflavin. Also, this structure could efficiently protect the survival of *L. acidophilus* as 85.5% and the activity of amylase as 80.0% in the harsh environment of simulated gastric fluids after 1 h. When transferred to a simulated intestinal tract, riboflavin and *L. acidophilus* were sustainably released. Since the beads are easily prepared using a simple extrusion method at near neutral pH and ambient temperature, they are excellent candidates for natural delivery systems for sensitive bioactive compounds in the food and biomedical industries.

1. Introduction

Many bioactive compounds, such as bioactive peptides and probiotics, confer health benefits such as antioxidant, antidiabetic, antihypertensive, antitumor, and antimicrobial effects (Sanjukta & Rai, 2016; Ambalam, Raman, Purama, & Doble, 2016). Oral administration is by far the most convenient way to deliver bioactive compounds, because of easy handling, high patient compliance, and low cost of production, especially when routine administration is necessary (Bysell, Månsson, Hansson, & Malmsten, 2011). However, the oral route is restricted for sensitive compounds due to the proteolytic activity in both stomach and intestine and low pH of the stomach. These harsh conditions can inactive probiotics, destabilize and degrade proteins, thus lead to loss of biological activities (Vermonden, Censi, & Hennink, 2012). Lipid and synthetic polymer based delivery systems that protect and transport therapeutic proteins and peptides to reserve their functions and release at physical target have been developed for the biomedical and pharmaceutical sectors (Nolan, Reyes, Debord, Garcia, & Lyon, 2005; Niu, Conejos-Sánchez, Griggin, O'Driscoll, & Alonso, 2016). Synthetic polymers are not generally recognized as safe for daily consumption, while some of lipid-based delivery systems need surfactants and complex formulation techniques (Langer & Peppas, 2003; Pouton & Porter, 2008). Natural polymer-based encapsulation materials have the potential to be used in biotechnology (cells and enzymes) and food (flavors and probiotics) due to their excellent biocompatibility and biodegradability (Langer & Peppas, 2003; de Geest, De Koker, Sukhorukov, Kreft, Parak, Skirtach, & et al, 2009). Food proteins are generally recognized as safe (GRAS) and biocompatible and biodegradable materials with good gelling and emulsifying capacities, which allow them to entrap

* Corresponding author.
E-mail address: lingyun1@ualberta.ca (L. Chen).

https://doi.org/10.1016/j.foodhyd.2017.11.017
0268-005X/

both water- and lipid-soluble bioactive molecules (Chen & Subirade, 2008; Dickinson, 2003; Wang, Bamdad, Song, & Chen, 2012).

Oat has been gaining momentum mainly due to a growing public awareness of the health benefits of β-glucan, the soluble fiber component that can reduce blood cholesterol and regulate blood glucose levels (Zhu, Du, & Xu, 2016). Oat protein is the major by-product component after β-glucan extraction and isolation, and potential research is required to develop its full value. Our previous research using cold-set gelation method revealed that oat protein has an excellent gelling property (Yang, Wang, & Chen, 2017). This method requires a heating step during which proteins are denatured and then polymerized into soluble aggregates. This is followed by a cooling step and then the addition of Ca^{2+} or glucono-δ-lactone (GDL), resulting in the formation of a network of soluble aggregates at ambient temperature. Thus, cold-set gels provide an opportunity to carry and protect heat labile sensitive bioactive compounds. Unlike many other globular proteins (e.g. soy protein and whey protein) which normally form filamentous or globular gels, oat protein gels form polymer-like percolating network structures with better mechanical strength. The cold-set oat protein gels can initially resist the low pH environment such as harsh gastric conditions (pH 2), because the percolating structure acts as a "cage" to retain biomolecules. This network with abundant cross-linking points is able to decrease the digestibility and thus the permeability of the gel (Yang et al., 2017). However, this protection capacity quickly decreases when the gel is exposed to harsh gastric conditions for more than 1 h. To improve this gel system as a carrier of bioactive compounds, an improved controlled release dynamic with a prolonged protective capacity is required.

Oat protein-shellac combination gels are proposed here as a solution to increase the resistance to gastric low pH while maintaining capacity to release bioactive compounds in the intestinal environment. Shellac is a natural and biodegradable resin of insect origin and composed of mixture of polyhydroxy polycarboxylic esters, lactones, and anhydrides, with the main acid components of aleuritic and terpenic acids. It is insoluble in acidic to neutral aqueous media and thus acid resistant (Bellan, Pearsall, Cropek, & Langer, 2012; Xue & Zhang, 2008). Shellac can interact with hydrophilic polymers based on non-covalent interactions, due to the presence of a large number of carboxylic and hydroxyl groups and a large negative charge (Patel et al., 2013). For example, negatively charged shellac can interact with type A gelatin through electrostatic interactions when pH value is lower than 8. In previous work, shellac was used to encapsulate flavonolignan and silibinin, and the encapsulated silibinin was stable to acidic pH due to the pH dependent solubility of shellac (Patel, Heussen, Hazekamp, & Velikov, 2011). Encapsulation and pH-triggered release of yeast cells from shellac-based microcapsules cross-linked with Ca^{2+} have also been reported (Hamad, Stoyanov, & Paunov, 2012). However, the existence of functional groups may induce polymerization that cause poor mechanical properties and instability of shellac which have limited the use of shellac alone in applications (Limmatvapirat, Limmatvapirat, Putipipatkhachorn, Nuntanid, & Luangtana-anan, 2007). More recently, Patel et al. (2013) generated gelatin-shellac microcapsules to encapsulate epigallocatechin gallate (EGCG), silibinin, and curcumin (Patel et al., 2013). In this work, the gelatin-shellac mixture was precipitated in 0.1 M HCl (pH 1.0) to generate microcapsules. However, such a low pH was not suitable for the encapsulation of sensitive molecules, such as peptide, enzymes, and probiotics. It is possible to achieve oat protein-shellac gels at a neutral pH through the addition of $CaCl_2$, since both of them can be cross-linked by Ca^{2+}. In addition, based on a fundamental understanding of interactions between shellac and other hydrophilic polymers, the amino groups of oat protein and the carboxyl and hydroxyl groups of shellac may interact with each other. Therefore, oat protein-shellac combination gels made at neutral or near neutral pH, can potentially more resist to gastric condition which allow better protection of bioactive compound in the gastric tract for subsequent controlled intestinal release.

This research represents a proof of concept of a novel natural biopolymer delivery system for bioactive compounds for food and biomedical applications. Specifically, experiments were directed to better understand the interactions between oat protein and shellac and to develop oat protein-shellac based gels at neutral pH. These experiments tested delivery systems capable of protecting and releasing representative bioactive compounds, including a vitamin, an enzyme, and a probiotic.

2. Materials and methods

2.1. Materials

Naked oat grains (*Avena nuda*) (crude protein content 17.2%, w/w) were purchased from Wedge Farms Ltd., Manitoba, Canada. Oat protein isolate (OPI, M_w: 237 kDa) was extracted from defatted oat flour using alkaline solution according to our previous work (Nieto-Nieto, Wang, Ozimek, & Chen, 2014). The protein content of OPI was 85.07 ± 2.4% (w/w, dry weight basis) as determined by the Leco nitrogen analyzer (FP-428, Leco Corporation, St Joseph, MI, USA) and a nitrogen to crude protein conversion factor of 5.83 was used, while other components could be soluble fiber, starch, lipid, and ash, among others. Shellac (M_w: ~1000 Da), pepsin (from porcine gastric mucosa, 424 units mg^{-1}), pancreatin (from porcine pancreas, 200 USP units mg^{-1}), amylase (700 units mg^{-1}) and amylase activity kit were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada). *Lactobacillus acidophilus* (ATCC4536) was obtained from American Type Culture Collection (ATCC, MD, USA). Other chemicals used in the experiment were all analytical grade and from Fisher Scientific (Whitby, ON, Canada). Milli-Q water was used in all experiments.

2.2. Preparation of OPI, shellac solutions and their mixtures

OPI (10%, w/v) suspension was obtained by dispersing dry protein powders into Milli-Q water and stirring overnight at 20 °C. The suspension was then adjusted to pH 8 using 1 M NaOH and sealed tightly in a glass vial. The denatured OPI solution was obtained by heating the above suspension at 115 °C (above denaturation temperature) in oil bath for 15 min. Shellac (20%, w/v) was dispersed in Milli-Q water and adjusted to pH 8 using 1 M NaOH with agitation at 50 °C to ensure complete dissolution. Mixtures with various OPI to shellac ratios were prepared by mixing these two neat solutions under continuous stirring at 1000 rpm and 20 °C, and coded as OS-0, OS-1, OS-2, OS-3, OS-4, and OS-5, corresponding to shellac contents (based on total solid weight) of 0, 18, 46, 67, 82, and 100 w/w, respectively (Table 1).

2.3. Characterization of OPI-shellac mixtures

The dynamic rheological behavior of the OPI-shellac mixtures was characterized by a DHR-3 rheometer (TA Instruments, New Castle, DE, USA). Parallel plate geometry with a gap of 1 mm was used to measure the dynamic viscoelastic parameters including complex viscosity, shear storage modulus G' and loss modulus G". The value of the strain amplitude for all samples was set as 0.5%, which was within a linear viscoelastic regime. A frequency sweep was subsequently conducted as a function of angular frequency (ω) from 0.1 to 100 rad s^{-1} at 25 °C.

The thermodynamics of the interactions of OPI with shellac was

Please cite this article in press as: Yang, C., et al., Oat protein-shellac beads: Superior protection and delivery carriers for sensitive bioactive compounds, *Food Hydrocolloids* (2017), https://doi.org/10.1016/j.foodhyd.2017.11.017