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Pectin-non-starch nanofibers biocomposites as novel gastrointestinal-resistant prebiotics

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ABSTRACT

Incorporation of nanofibers of chitin (NC), lignocellulose (NLC) and bacterial cellulose (BNC) in pectin was studied to improve prebiotic activity and gastrointestinal resistance of the pectin-nanofibers biocomposites for protection of probiotics under simulated gastrointestinal conditions. The biocomposites were prepared using various compositions of pectin and nanofibers, which were designed using D-optimal mixture method. The incorporation of the nanofibers in pectin led to a slow degradation of the pectin-nanofibers biocomposites in contrast to their rapid swelling. AFM analysis indicated the homogenous distribution of interconnected nanofibers network structure in the pectin-nanofibers biocomposite. FTIR spectra demonstrated fabrication of the biocomposites based on the inter- and intra-molecular hydrogen bonding and ionic interaction of pectin-Ca^{2+}. XRD patterns revealed the amorphous structures of the biocomposites as compared to the crystalline structures of the nanofibers. Among the compositions, the optimal compositions were as follows: 60% pectin + 40% NC, 50% pectin + 50% NLC and 60% pectin + 40% BNC, where the prebiotic score, probiotic survival under simulated gastric and intestinal conditions were optimum. The optimal biocomposite pectin-NC exhibited the highest survival of the entrapped probiotic bacteria under simulated gastric (97.7%) and intestinal (95.8%) conditions when compared with the corresponding to free cells (76.2 and 73.4%).

1. Introduction

The administration of probiotics is both viable and sufficiently numerous in the intestinal tract which is associated with passage through the gastrointestinal conditions. It is necessary to support health-promoting claims attributed to the beneficial effects of probiotics on the human gut flora. Designing entrapment biocomposites for the protection of probiotics with the aim of providing a successful delivery is an emerging method to reduce cell death during GI passage. Majority of this technology depends on properties of the matrix ingredients. Various polymeric biocomposites fabricated using prebiotic polysaccharides cannot only protect probiotics against harsh conditions by retaining biocomposites structure before degradation, disintegration or dissolution but also increase their growth in the intestine [1–3].

Among the polymers used for encapsulation, pectin as an aqueous soluble prebiotic, has gained so much attention in the food and pharmaceutical industries for its excellent biocompatibility, biodegradability, pH sensitivity, nontoxicity, therapeutic properties, cost-effectiveness and ability to increase in strength of the mucoadhesion on large intestinal mucosa [4,5]. The matrix made of pectin composited with biopolymers is suitable for use as a probiotic delivery vehicle since resistance of pectin-based delivery vehicles against gastric and pancreatic enzymes preserves the entrapped cells passage through the GI tract and it is selectively fermented by colonic bacteria [5,6].

N-Acetyl-glucosamine units as derivatives of glucose are linked together by β (1–4) linkages to make an aminopolysaccharide called chitin with the structure being analogous to cellulose [7]. Chitin is biocompatible and non-toxic for use in a variety of human applications [8]. By passing through the GI tract, chitin did not change in weight and shape whereas some of the polysaccharides such as chitosan and starch did [9]. In nature, chitin is considered as an insoluble component of many chitin–polymer biocomposites which increases hydrogen bonds between adjacent chains of polymers leading to greater strength [7]. The increasing interest in the use of chitin as a source of nanofibers is due to the intractable structure and inherent nature of supporting cell attachment [8,10,11]. Hence, nanochitin can be used in the fabrication of nanocomposites for encapsulation in the functional foods industry.

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Lignocellulose as an insoluble, non-digestible fiber fraction, mainly including cellulose, hemicellulose and lignin, is less considered as a prebiotic source which plays an important role in bacterial population and fermentation in the gut. Although phenolic compounds of lignocellulose can have antimicrobial effects, a low level (1.25%) of them improves the growth of probiotic bacteria and reduces *Escherichia coli* population in the gut [12]. In addition, lignocellulose is a bulky fiber with high water-binding capacity that can increase stomach distension and thereby enhance satiation. In vitro studies indicate that only little amounts (0–5%) of lignocellulose are fermented by the human gut [13]. The use of nanolignocellulose as a nanofiber can modify composite properties which are attributed to the high surface of nanofibers and the hydrogen bonding between lignocellulose and other ingredients of the composite [14,15]. Hence, nanofibers of lignocellulose can be used for fabrication of entrapment biocomposites due to the simplicity of the manufacturing process, low cost, light weight and properties which make them suitable for this application [16].

Cellulose as an important biopolymer and the most abundant renewable resource is used for a variety of applications due to its biocompatibility, availability, biodegradability and sustainable production potential. Intrinsic properties, such as nanoscale dimension, high surface area, unique morphology and mechanical strength contribute to expansion of the application fields of nanocellulosic materials as components in fabrication of nanocomposites [17]. Recently, a new entrapment matrix obtained by the incorporation of pectin with bacterial cellulose nanofibers was developed. This bionanocomposite showed an increase in survival rate of probiotics in the GI conditions [3]. The nanofibers show a promising potential for solving some of the problems associated with the probiotics protection in harsh conditions. Due to their nano-scale diameters, the nanofibers are advantageous in applications where resistant surface of the entrapment matrix is needed to promote the residence time of probiotic cells in the GI environment to enhance the viability [4].

The aim of this work was to fabricate new entrapment biocomposites from pectin composited with prebiotic nanofibers for probiotic delivery into the GI tract. Biocomposites of pectin-nanochitin (pec-NC), pectin-nanolignocellulose (pec-NLC) and pectin-bacterial nanocellulose (pec-BNC) were developed to entrap probiotic *Bacillus coagulans*. These composites were evaluated as prebiotic biocomposites and examined during exposure to simulated gastric and intestinal fluids. Their optimal compositions were determined using D-optimal mixture design to obtain optimum prebiotic activity and optimum survival in gastric and intestinal conditions, simultaneously. To the best of our knowledge, these produced biocomposites addressed a new approach in the preservation of probiotics in functional foods industry, and there is still no report in the literature related to the probiotics.

2. Materials and methods

2.1. Materials

Probiotic strain *Bacillus coagulans* IBRC-M 10807 and enteric strain *Escherichia coli* IBRC-M 10208 were obtained from Iranian Biological Research Center (IBRC). Pectin was extracted from citrus peel and dried using previously established method [18]. Bacterial nanocellulose (BNC) with the average fibril diameter of 50 nm, nanochitin (NC) with the average fibril diameter of 30 nm, and nanolignocellulose (NLC) with the average fibril diameter of 65 nm were purchased from Nano Novin Polymer Co. (Sari, Iran). Pepsin from porcine gastric mucosa (0.7 FIP-U/mg), pancreatin from porcine pancreas (350 FIP-U/g Protease, 6000 FIP-U/g Lipase, 7500 FIP-U/g Amylase), and calcium chloride were acquired from Merck (Darmstadt, Germany). Bile salts were supplied from Sigma–Aldrich (St. Louis, MO, USA). TSB was purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK). All chemicals were used as received without any further purification.

Table 1
Mixtures of pectin and nanofibers for fabrication of the entrapment biocomposites.

Mixture	Pectin (X_1) (g/g biocomposite)	Nanofiber (X_2) (g/g biocomposite)
1	0.9	0.1
2	0.8	0.2
3	0.7	0.3
4	0.6	0.4
5	0.5	0.5

2.2. Preparation of mixtures

The various compositions of pectin with NC/NLC/BNC for fabrication of entrapment biocomposites were designed using D-optimal mixture design and given in Table 1. As concentrations showed in Table 1, the ingredients were suspended in deionized water and mixed together at 60 °C to produce polysaccharide mixtures with the final concentration of 1% (w/v). The mixtures were agitated at 500 rpm for 60 min. Then, they were autoclaved at 121 °C for 20 min.

2.3. Entrapment of probiotic

B. coagulans was grown overnight in TSB medium at 37 °C. The cells were centrifuged at 6000 rpm for 10 min, then washed with sterile saline solution (0.5%, w/v) and resuspended in the same solution. The sterilized mixture prepared (Section 2.2), was mixed with the cell suspension at a ratio of 1:1 (v/v). In order to obtain the probiotic entrapped in the biocomposite, the synbiotic (probiotic cells + polysaccharide mixture) suspension was added dropwise to 5% $CaCl_2$ solution (crosslinking agent), then kept for 1 h at 4 °C to form and harden the biocomposite. The synbiotic biocomposite was centrifuged at 12,000 rpm for 10 min and then dried using freeze-dryer for 48 h.

2.4. Preparation of simulated gastric and intestinal fluids

Gastric and intestinal fluids were simulated as stated previously [19]. In brief, simulated gastric fluid (SGF) was prepared by suspending pepsin in sterile saline (0.5%, w/v) to a final concentration of 3 g/L and pH was adjusted to 2.00 using 1N HCl. Simulated intestinal fluid (SIF) was prepared by suspending pancreatin USP in sterile saline to a final concentration of 1 g/L and adding bile salts (4.5%, w/v) and pH was adjusted to 8.00 using sterile 1 N NaOH.

2.5. Characterization of biocomposite

2.5.1. Scanning electron microscopy (SEM)

The surface morphology of the samples was identified using the scanning electron microscopy (SEM) (XL30, Philips, Netherlands). The samples were coated with gold using the sputtering technique to improve the conductivity of the samples. The dried samples were fractured and observed at beam energy of 20.0 kV.

2.5.2. Atomic force microscopy (AFM)

The surface morphology of the entrapment biocomposite was analyzed in air at ambient temperature, using the contact mode of AFM (Veeco, Autoprobe CP research, USA) with a 5 × 5 μm scan size at a resolution <0.1 nm. Two statistical parameters of roughness,