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Investigation of pectin/starch hydrogel as a carrier for oral delivery of probiotic bacteria

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ABSTRACT

The present study highlights the fabrication of novel food-grade hydrogel particles based on pectin and starch for probiotic colon delivery. *Lactobacillus plantarum* ATCC:13643 (*L. plantarum*) cells were encapsulated in pectin/starch hydrogels by extrusion method. Four batches were formulated with different ratios of starch/pectin solutions. Optical and scanning electron microscopy obviously showed the random distribution of *L. plantarum* throughout the hydrogel network. The viability of encapsulated cells in simulated gastric fluid (SGF) and bile salt solution was significantly higher when compared to nonencapsulated cells. Results demonstrated that encapsulated cells in pectin/starch hydrogels were resistant against adverse conditions of the gastro–intestinal tract and bile salt solution compared to non-encapsulated cells. After sequential exposure to simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) for 2 h almost complete death of free cells was observed however the numbers of surviving cells were 5.15 and 6.67 Log CFU/g for pectin and pectin/starch hydrogel, respectively.

1. Introduction

The fabrication of functional probiotic-based products aiming to improve health in the human body is the subject of extensive investigation among the scientific and industrial community [1,2]. Probiotics are live microbial dietary supplements that have been postulated to play a significant role in maintaining health because of the multitude beneficial effects they exert on the host system when ingested in adequate amounts [3,4]. The oral intake of probiotics has demonstrated potential in clinical trials for the alleviation of several clinical disorders such as diarrhea, lactose intolerance, and certain allergies. Probiotics are also effective in enhancing the bioavailability of zinc, calcium, copper, iron, manganese, phosphorus and modulation of the immune [5]. Nevertheless, ingested probiotics must survive while passing through the stomach and reach the intestine in large quantities in order to incur beneficial influence on host health and physiology [6]. According to the Food and Agriculture Organization-World Health Organization (FAO-WHO), the minimum level of probiotics should be 10^7 CFU/g to achieve desired therapeutic outcomes [7,8]. However the main challenge to the survival of ingested microorganisms is the harsh condition of acidity in the gastrointestinal tract of humans. Besides, environmental factors such as oxygen, heat, and humidity during drying and storage process can cause lipid oxidation, cell wall deterioration, or changes in the cell membrane [9,10]. Therefore, technologies that ensures probiotic viability is required for both economical and health reasons. Microencapsulation technique has been extensively used for entrapment of probiotic cells into carrier matrices [11]. Microencapsulation can enhance protection of cells against harsh gastrointestinal conditions, facilitate the gradual cell release in the intestine and increase the stability and viability of probiotic cells in different heat/moisture conditions during processing and storage time [12,13]. Carbohydrate polymers including chitosan, alginate, *k*-carrageenan, starch, gelatin and pectin have been tremendously utilized as promising encapsulation matrices for probiotic formulations [14,15]. Starch is a cheap, abundant and edible polysaccharide with fantastic properties such as biodegradability, biocompatibility and cost-effectiveness and has a long history as an excipient in drug formulations [16]. Starch is applied in the food industry as gel formers, colloidal emulsifiers and thickening agents [17]. For instance Kanmani et al. reported the successful microencapsulation of *lactobacillus plantarum* GG ATCC 53103, *lactobacillus reuteri* ATCC 55730 and *lactobacillus acidophilus* DSM 20079 in pullulan/starch edible films [18]. Iyer et al.

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demonstrated that chitosan-coated alginate-starch microcapsules can provide noteworthy release for *lactobacillus* casei in ex vivo porcine gastrointestinal contents [19]. Native starches are almost completely degraded by pancreatic enzymes after oral ingestion promoting the absorption from the small intestine into the systemic circulation. To address this challenge, starch has been combined with other biomaterials [20]. Herein, starch was incorporated into pectin by the extrusion method. Pectin is an important heteropolysaccharide found in the primary cell walls of terrestrial plants. It is an essential ingredient in the initial growth and in the ripening process and has been widely explored as the matrix for oral drug delivery formulations [21]. Pectin is resistant to gastric or intestinal enzymes such as protease and amylase, which are active in the upper gastrointestinal tract. Additionally it can be easily digested by pectinases generated by the colonic microflora. This significant advantage makes pectin a promising drug vehicle for colon-specific drug delivery purposes [22]. As a high value functional ingredient in foods, pectin has gained fundamental attention for probiotic encapsulation in food products because of possessing striking characteristics such as low cost, nontoxicity and biocompatibility. It has been employed in a variety of applications in food industry as a gelling agent especially in jams and jellies [23]. In a research designed by Sun et al. it was observed that soy protein isolate–high methoxy pectin microcapsules can enhance the viability of *lactobacillus delbrueckii* strains by 3 log units compared with the control [24]. To the best of our knowledge no binary mixture of pectin/starch has been reported yet for probiotic delivery. The current study therefore deals with the development of novel pectin/starch hydrogel to improve the survival of probiotic in simulated gastrointestinal, bile salt solution and storage condition.

2. Experimental

2.1. Materials and microorganisms

Pectin as a potassium salt (28% degree of esterification) was purchased from Sigma Aldrich Co. (USA). Corn starch was purchased from Glucosan Co. (Gazvin, Iran). *L. plantarum* ATCC:13643 was purchased from Pastor Institute of Iran. de Man, Rogosa and Sharp (MRS) agar and MRS broth were purchased from Merck (Darmstadt, Germany). All other chemicals were analytical grade and used without any purification. *L. plantarum* ATCC:13643 was aerobically cultured in MRS media broth at 37 °C for 24 h. The culture was then harvested by centrifugation at 4200 × *g* for 20 min at 4 °C, washed with phosphate buffer saline solution (PBS, pH 7.4, 50 mM KH_2PO_4) and collected by centrifugation as above. The washed bacterial cells were re-suspended in 5 mL of sterile saline. The cell suspension of probiotic bacteria was divided into two parts: one part was used for encapsulation and the other as free cells for control.

2.2. Production of pectin/starch hydrogels

Pectin/starch hydrogels were prepared by external gelation method at various pectin/starch ratios as shown in Table 1. Pectin solution of different concentrations (2, 1.5, 1 and 0.5 wt%) were prepared in distilled water at ambient temperature. Starch solutions of different concentrations (0, 0.5, 1 and 1.5 wt%) were prepared separately by dissolving starch in distilled water at 70 °C. Two solutions were mixed together and the temperature was adjusted at 70 °C. The suspensions

were injected dropwise through a 5 mL sterile syringe (AVA LUERLOCK, LS20280 Tehran, Iran) into crosslinking solution (200 mL of 0.2 M $CaCl_2$) and stirred slowly for 60 min. The Ca^{+2} ions can interact electrostatically with carboxylate groups on pectin backbone, leading to the formation of hydrogel. The resultant hydrogels were collected and purified in excess distilled water and dried at room temperature. The as-prepared hydrogels were coded as Pectin, Pectin/starch1, Pectin/starch2 and Pectin/starch3 as shown in Table 1.

2.3. Water uptake measurements

To study water absorbency, two different sets of experiments were performed which include dynamic swelling measurement at simulated intestinal fluid (SIF) (phosphate buffer solution (PBS, pH 7.4), 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4, 1 L H_2O) and simulated gastric fluid (SGF) (hydrochloric acid buffer solution (HBS, pH 1.2), 0.15 M HCl, 0.05 M KCl) and kinetic swelling measurement in distilled water. For swelling measurements the dry native hydrogels (~0.2 g) were placed in media and kept at a constant temperature, 20 ± 0.5 °C. Swollen samples were periodically removed, weighted and the water absorbency was measured according to Eq. (1):

$$WA(g/g) = \frac{W_s - W_d}{W_d} \quad (1)$$

Where, W_s and W_d are the weights of the samples swollen in solutions and in dry state, respectively. The all swelling data were repeated three times and the mean values were shown in graphs (mean ± S.D. n = 3).

2.4. Microencapsulation of L. plantarum in pectin/starch hydrogel

The extrusion method was utilized for encapsulation of *L. plantarum* cells into pectin/starch mixture. Un-encapsulated cells suspended in sterile saline were used as the control. In the undertaken protocol, the resultant hydrogel prepared in previous step, was sterilized (121 °C for 15 min) prior to the encapsulation procedure. After being cooled to room temperature, 5 mL of isolated cells with the initial concentration of 10.01 Log CFU/g were mixed with 10 mL of hydrogel solutions and stirred for 30 min. Finally the suspensions were injected dropwise through a 5-ml sterile syringe (AVA LUERLOCK, LS20280 Tehran, Iran) into $CaCl_2$ (0.2 M) crosslinking solution. The hydrogels encapsulated cells were collected washed with distilled water, filtered and sealed in sterilized conical tubes for the following experiments.

The encapsulation efficiency (EE) of *L. plantarum* was calculated as follows: Eq. (2):

$$EE(\%) = (\mathrm{Log}_{10} N / \mathrm{Log}_{10} N_0) \times 100 \quad (2)$$

Where, N and N_o represent the viable CFUs after and before encapsulation.

2.5. Morphology analysis

The cross-sections of bacteria encapsulated hydrogels were analyzed by scanning electron microscopy (SEM) (Philips XL 20, Oregon, and USA). An optical microscope (OlympusBX61) with 4, 10, 40 and 100 × objective lenses was also utilized to capture image of *L. plantarum* entrapped in the hydrogels. Meanwhile, the microscope eyepiece was fitted with a calibrated micrometer scale to measure the average size of the unencapsulated hydrogels from the mean diameter of 50 particles of each batch as the mean size standard deviation (SD).

2.6. . enumeration of L. plantarum cells

The free *L. plantarum* cells were 10 times serially diluted with PBS solution (pH 7.4, 50 mM KH_2PO_4) and 100 μL aliquots were plated on MRS agar. The plates were incubated at 37 °C for 72 h.