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Studies on Encapsulation Efficiency of Hydrolyzed Psyllium Husk for Microencapsulation of Probiotics

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A B S T R A C T

In the present investigation, sincere efforts have been made to prepare encapsulated beads of sodium alginate and acid hydrolyzed psyllium husk containing probiotic culture of *Lactobacillus acidophilus* and *Lactobacillus plantarum.* The results showed that bead formulation of 1.5 % (w/v) sodium alginate with 0.5 % (w/v) acid hydrolyzed psyllium husk significantly produced spherical beads with narrow size diameter of 1.75 mm and encapsulation efficiency of 99.5 % were achieved. Furthermore, addition of acid hydrolyzed psyllium husk into sodium alginate enhanced the integrity of prepared beads. The results indicated that incorporation of 0.5 % (w/v) acid hydrolyzed psyllium husk in to sodium alginate beads improved viability of the bacteria in acidic conditions as well as bile conditions. The stimulating effect of acid hydrolyzed psyllium husk on the probiotic bacteria was also observed in simulated colonic pH solution.

Introduction

Encapsulation is a mechanical or physicochemical process that traps a potentially sensitive material and provide a protective barrier between it and the external conditions. The various encapsulation technique includes extrusion, spray drying, spray cooling, lyophilization, emulsion etc. Extrusion is the oldest and most common technique to produce capsule with hydrocolloids. From microbiological point of view, microencapsulation can be defined as the process of entrapment, enclosure of cells

of microorganisms by means of coating them with proper hydrocolloid in order to isolate the cells from the surrounding environment, in a way that results in appropriate cell release in the intestinal medium (Jayalalitha, 2013).

Alginate, a commonly used material to encapsulate probiotics, is a naturally occurring biocompatible and biodegradable linear anionic polysaccharide. Preparation of alginate bead, with well retained bacteria in their matrix, can be easily achieved by simple techniques like extrusion or emulsion methods. In spite of the wide application of alginate microcapsules in this area, some problems related to protection efficiency of them have been reported including susceptibility to disintegration in the presence of excess monovalent ions, Ca^{2+} chelating agents, and harsh chemical environments (Mokarram *et al.,* 2009)

Notably, India ranks first in Isabgol production (98%) and is the sole supplier of seeds and husk in the international market. Among medicinal plants, Isabgol is the first ranked foreign exchange earner for the country. India is the largest producer and the main supplier of seed and husk to the world market. USA is the chief importer of Isabgol seeds and husk. It contains a significant amount of proteins and husk yields colloidal mucilage which are valued for medicinal application and is used in Ayurveda, unani and allopathic systems of medicines. It is the main constituent of a number of laxative preparations containing sodium bicarbonate and various flavors used in modern medicine. The psyllium is high in soluble fiber content with detoxing effect over digestive system makes it a very apt nutraceutical (Guo *et al.,* 2008).

Probiotics are live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host (FAO/WHO, 2002). These benefits include maintenance of normal intestinal microflora, defense against entero-pathogen infections, controlling serum cholesterol levels, improving lactose utilization in persons who are lactose maldigesters by production of β-galactosidase, and possessing anticarcinogenic and antimutagenic activities. Most of probiotics are bacteria; among them lactic acid bacteria (LAB), typically associated with the human gastrointestinal tract, are the most widely used probiotic microorganisms. In order to exhibit their potential benefits, probiotics need to pass the harsh conditions of gastric tract and colonize and grow on the epithelium of colon in appropriate population (Kailaspathy *et al.,* 2002). It is suggested that probiotics should be formulated in products in a minimum count of 10^6-10^7 cfu/g or ml of viable probiotic bacteria (FAO/WHO, 2002).

To improve viability and stability of probiotics and efficient delivery of the cells to their active sites, various techniques have been utilized so far. In this regard, encapsulation of probiotics in wide variety of polymers is the most frequently applied method that is cited in numerous studies. Having in mind the pharmacological benefits of psyllium in digestive system as well as its potential to stimulate probiotic growth in the colon, this study aimed to incorporate acid hydrolyzed psyllium in alginate beads containing probiotic culture of *L. acidophilus* and *L. plantarum.*

Materials and Methods

Procurement of Raw Materials and Chemicals

Psyllium husk (98% pure and 40 mesh size) was purchased from local market. MRS broth, MRS agar, pepsin, pancreatin, sodium

alginate, oxgall, sodium hydrogen phosphate, peptone, calcium chloride, sodium hydroxide and hydrochloric acid were collected from Dept. of Food Microbiology and Safety.

Microbial culture

Lactobacillus acidophilus (NCIM-2903) and *Lactobacillus plantarum* (NCIM-2083) respectively were procured from National Chemical Laboratory (NCL), Pune.

Probiotic culture inoculum

The probiotic organisms viz. *Lactobacillus acidophilus* and *Lactobacillus plantarum* were individually grown in MRS broth at 37°C for 48 hrs. The cultivated MRS broth was then centrifuged at 4000 rpm for 10 min to harvest the cells. The harvested cells were washed twice with sterile water. The biomass was taken as starter culture.

Encapsulation of probiotics

The microencapsulation of probiotic bacteria was performed using the extrusion technique. Extrusion method is the oldest and most common procedure of producing hydrocolloid capsules (King, 1995). It is a simple and cheap method with gentle operations which makes cell injuries minimal and causes relatively high viability of probiotic cells (Klein *et al.,* 1983; Tanaka *et al.,* 1984). Hydrocolloid solution was prepared by using a different combination of sodium alginate with psyllium and then mixed with 10 ml of inoculum (5 ml each of *L. acidophilus and L. plantarum*).

Then they were extruded through a syringe in the form of droplets into 0.3 M calcium chloride solution. Interaction between the two solutions led to formations of beads and the resulting beads were then stored in 0.1 percent peptone solution at 4°C (Karthikeyan *et al.,* 2014).

Partial hydrolysis of psyllium husk

Acid hydrolysis of psyllium husk was carried out as per the method described by Xiaoyin Pei (2008) and Syed *et al.,* (2018) with certain changes in concentration of HCL in ethanol solvent. The solvent used for psyllium husks treatment was ethanol with 34 % to 37 % hydrochloric acid (HCl) at the varying concentration levels of 0.50 %, 0.55 % and 0.60 % (w/v). The study was conducted to investigate the effect of psyllium-solvent ratio and reaction temperature on physical/chemical properties of the acid hydrolyzed psyllium husk samples.

At reaction temperature of 37.5°C three different psyllium-solvent ratios (1:3, 1:5 and 1:7) were tested. After the addition of the solvent, samples were incubated for 48 hours at 37.5°C temperature. Afterward, samples were vacuum filtered, rinsed with 95 % ethanol for 2 times each, then dried and stored. Control group was treated with 100 % ethanol.

Based on the results of acid hydrolysis on functional properties of psyllium husk, acid concentration of 0.60 % HCl in the ethanol solvent for solvent ratio of 1:7 as (Psyllium husk : Solvent) had been selected for encapsulation.

Size analysis of beads

The particle size of beads was assessed using optical microscopy. Data were collected from 50 beads in each sample and mean particle size was reported.

Encapsulation Efficiency (EE) of beads

To determine the encapsulation efficiency, firstly prepared beads were mechanically disintegrated in phosphate buffer (pH = 6.8), then the number of entrapped cells after adequate dilution were measured by pour plate

method. Counts were expressed as number of colony forming units (cfu) and calculated as

$$EE = \frac{Log_{10} N}{Log_{10} N_0} \times 100$$

N = Number of viable entrapped cells released from the beads

N_0 = Number of free cells added to the biopolymer mixture immediately before the production procedure.

Viability of Encapsulated Probiotic Culture at Low pH

Low pH conditions were produced using 9 g/l sodium chloride and 3.0 g/l of pepsin and pH was adjusted to 1.8 with hydrochloric acid. 100 mg beads with entrapped bacteria or 0.1ml of cell suspension were mixed in 20 ml of acid solution and incubated for 120 min at 37°C with constant agitation at 50 rpm (Chavarri *et al.,* 2010).

After incubation, beads were disintegrated in phosphate buffer (pH = 6.8), then 1.0 ml aliquot of the mixture removed and assayed using pour plate method. The survival (%) of the bacteria was calculated as follows.

% Survival = - (log Cfu/g beads after 2 hours exposure to acidic condition/ log Cfu/g beads initial count)× 100.

Viability of Encapsulated Probiotics at High Bile Salt Concentration

Prepared beads after 2-hour acid exposure were washed with distilled water, removed, and incubated in 50 ml of high bile condition, containing 6.8 g of monobasic potassium phosphate, and 10 g/l of pancreatin with pH adjusted to 6.8 using sodium hydroxide and 0.5% (w/v) oxgall for 2 hours at 37°C with constant agitation at 50 rpm. (Ding and Shah, 2009). Samples were then taken, and bacterial growth was assayed using pour plate method

Release of Encapsulated Cells in Simulated Colonic pH Solution

The release of the prepared beads was examined at simulated colonic pH solution as described by Mandal *et al.,* 2006. The beads were mixed with 50 ml of simulated colonic pH solution containing 0.1M monobasic potassium phosphate with pH adjusted to 7.4 with sodium hydroxide and incubated for 20 h at 37°C with constant agitation at 50 rpm (Mandal *et al.,* 2006). Samples were taken at different time intervals, and bacterial growth was assayed using pour plate method.

Results and Discussion

As per preliminary study carried out, it was found that uniform and spherical bead preparation by sodium alginate concentrations less than 1 % (w/v) was quite difficult because of decreased viscosity and less ion sites for cross-linkage. Also, sodium alginate concentrations more than 1.5 % (w/v) were too viscose to be extruded from the syringe.

Hence, the sodium alginate concentration of 1.5 % (w/v) was selected. Moreover, 3% (w/v) calcium chloride produced the best result and chosen as optimum hardening solution.

Addition of acid hydrolyzed psyllium husk into sodium alginate gel results in an increase in the viscosity and adherence of resultant gel.

The incorporation of acid hydrolyzed psyllium husk in the concentrations more than 0.5% (w/v) to sodium alginate yields too adherent mixtures to easily fabricate the beads. Consequently, the compositions in Table 1 were selected as the formulations to be further analysed.

Diameter, count and encapsulation efficiency in acid condition of prepared formulations

Table 2 shows results for diameters and encapsulation efficiencies of formulated beads. As it can be seen, beads ranging from 1.60 to 1.70 mm. The mean diameters of beads containing acid hydrolyzed psyllium husk were significantly higher than control that can be attributed to the viscosity of the resultant gel. According to the studies in this regard, an increase in the viscosity of the starter gel leads to the preparation of bigger beads by the extrusion method. Furthermore, narrow range of size distribution was observed for all prepared beads. The results are in good accordance with Kailaspathy *et al.,* (2002).

The initial cell count of *L. acidophilus* and *L. plantarum* before bead preparation was 9.58 (cfu/ml). High bacterial cell entrapping in the range of 9.65 to 9.77 (cfu/g beads) was achieved in resultant beads (Table 2). The results pertaining to encapsulation efficiency indicated that there was no considerable loss of viability for all prepared beads and more than 98.5 % cells for all beads were successfully entrapped that can be due to the gentle method applied.

Viability of Encapsulated Probiotic Culture in Acidic Conditions

The protective effects of different coats of acid hydrolyzed psyllium husk after 2-hour exposure to acid conditions (pH=1.8) are compared to untreated cells, and results are expressed as % survival in Table 3.

It is clearly observed from Table 3 that % survival of probiotic culture consisting of *L. acidophilus and L. plantarum* got increased with addition of acid hydrolyzed psyllium husk. It is in the range of 80.0 % to 85.5 %. Overall, it is clear that survived bacteria after acid exposure, in all prepared beads were significantly higher than those of untreated cells.

There are numerous studies in this regard to protect probiotics by encapsulation in alginates beads using different techniques. Sohail *et al.,* (2011) reported that encapsulation of probiotic bacteria in cross-linked alginate beads is of major interest for improving the survivability in harsh acid and bile environment. Furthermore, Mokarram *et al.,* (2009) showed the efficiency of multistage alginate coating on survivability of probiotic bacteria in simulated gastric and intestinal juices. Sultana *et al.,* (2000) found that encapsulation of bacteria in alginate beads did not effectively protect the organisms from high acidity.

On the other hand, incorporation of acid hydrolyzed psyllium husk into alginate beads resulted in a rise in the viability of probiotic culture in those beads in acid conditions and this effect is more obvious in higher concentrations of acid hydrolyzed psyllium husk. The increase in viability of the bacteria by addition of acid hydrolyzed psyllium husk is in line and it can be attributed to the total concentration of polymers blend used, as the survival of probiotic culture in the beads with the same total amount of polymers showed no significant differences. Moreover, the rising trend in the viability values by increase in the proportion of acid hydrolyzed psyllium husk can be attributed to the alginate concentration. Addition of acid hydrolyzed psyllium husk increases the total polymer concentration and brings it to the appropriate point to remarkably increase the protection of the cells against acid conditions. The results are in good agreement with Albertini *et al.,* (2010) who reported that the incorporation of XG or CAP within the 3% (w/v) of alginate solution increased the survival of the probiotic bacteria in acid conditions.

Table.1 Different formulations of beads

Formulation	Sodium Alginate (% w/v)	Hydrolyzed Psyllium Husk (% w/v)
T (Control)	1.5	0.0
T1	1.5	0.3
T2	1.5	0.4
T3	1.5	0.5

Table.2 Diameter, count and encapsulation efficiency in acid condition of prepared formulations

Formulation	Diameter (mm)	Count (cfu/g)	Encapsulation Efficiency (%)
T (Control)	1.61	9.60	98.3
T1	1.60	9.65	98.5
T2	1.65	9.70	99.0
T3	1.70	9.75	99.5

Table.3 Viability of Encapsulated Probiotic Culture in Acidic Conditions

Formulation	% Survival
T (Control)	75.5
T1	80.0
T2	82.0
T3	85.5

Table.4 Viability of Encapsulated Probiotic Culture at High Bile Salt Concentration

Formulation	Cell Number log (cfu/g)
T (Control)	4.5
T1	2.0
T2	2.2
T3	2.5

Moreover, encapsulation of probiotic in alginate-starch blend also showed improved level of protection against acidic condition (Muthukumarasamy *et al.*, 2010).

Viability of Encapsulated Probiotic Culture at High Bile Salt Concentration

The effect of 2 hours exposure to 0.5 % (w/v) oxgall on the survival of probiotic culture (passed through acidic conditions) in prepared beads and untreated cells is demonstrated in Table 4. It is clear that probiotic culture encapsulated in acid hydrolyzed psyllium husk beads showed better survivability (less than 2.5 log reduction) after 2 hours bile exposure compared to those of untreated that is dropped by around 4.5 log cfu/ml after 2 hours bile exposure. Survivability of encapsulated probiotics against harsh environmental conditions especially bile tolerance is highly dependent on the strain type. Sohail *et al.*,

(2011) who reported that encapsulation of *L. acidophilus* in extruded macro beads was effective in maintaining cell viability.

Release of Encapsulated and Free Bacteria in Simulated Colonic pH Solution

In the case of beads prepared using acid hydrolyzed psyllium husk, not only the bacteria completely released from the beads but also different rates of bacterial growth after 3 hours was observed indicating a stimulating effect of acid hydrolyzed psyllium husk on the bacteria. Higher concentrations of acid hydrolyzed psyllium husk produced the greater stimulation effect on bacteria, as 0.5% (w/v) showed more than 4 log rise in bacterial count. The stimulation effect of acid hydrolyzed psyllium husk on probiotic culture can be probably attributed to its structure as a soluble fiber (Elli *et al.,* 2008). Based on prebiotic definition, nondigestible food ingredients such as carbohydrates in the form of soluble fiber can stimulate the growth and/or activity of bacteria. Indeed, in some cases, acid hydrolyzed psyllium husk has been used as prebiotic in different situations.

In the present investigation, sincere efforts have been made to prepare encapsulated beads of sodium alginate and acid hydrolyzed psyllium husk containing probiotic culture of *Lactobacillus acidophilus* and *Lactobacillus plantarum.* Different bead formulations of 1.5 % (w/v) sodium alginate with 0.3 %, 0.4 % and 0.5 % (w/v) acid hydrolyzed psyllium husk were prepared by using extrusion technique. The prepared beads were characterized in terms of size, encapsulation efficiency, viabilities in acid (pH 1.8 for 2 hours) and bile (0.5 % w/v for 2 hours) conditions. The results showed that bead formulation of 1.5 % (w/v) sodium alginate with 0.5 % (w/v) acid hydrolyzed psyllium husk significantly produced spherical beads with narrow size diameter of 1.75 mm and encapsulation efficiency of 99.5 % were achieved. Furthermore, addition of acid hydrolyzed psyllium husk into sodium alginate enhanced the integrity of prepared beads. The results indicated that incorporation of 0.5 % (w/v) acid hydrolyzed psyllium husk in to sodium alginate beads improved viability of the bacteria in acidic conditions as well as bile conditions. The stimulating effect of acid hydrolyzed psyllium husk on the probiotic bacteria was also observed in simulated colonic pH solution.

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