

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.705.051>

Microencapsulation of Probiotic Culture Beads by Using Modified Psyllium Husk

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ABSTRACT

Keywords

Psyllium husk,
Modification,
Microencapsulation,
Probiotic beads

Article Info

Accepted:
07 April 2018
Available Online:
10 May 2018

Psyllium as a soluble fiber has a potential to stimulate bacterial growth in digestive system, stimulate probiotic growth in the colon, this study aimed to incorporate the modified psyllium husk (MPSH) in alginate beads containing probiotic bacteria viz., *L. acidophilus* and *L. bulgaricus*. Dietary fiber of modified psyllium husk was 79.67% and functional properties also found to be improved which was utilized for further encapsulation studies. The different formulations containing ALG-MPSH were prepared using extrusion technique and characterized in terms of size, morphology and surface properties, encapsulation efficiency (EE), viabilities in acid (pH 1.8, 2 hours) and bile (0.5% w/v, 2 hours) conditions, and release in simulated colon pH conditions. Three different formulations were prepared by using sodium alginate and Modified Psyllium husk with addition of 2% alginate and various levels of modified Psyllium husk 0.1, 0.2 and 0.3% respectively were investigated. The results revealed that the prepared spherical beads had diameter 1.65 ± 0.05 mm with survival $83.2 \pm 0.7\%$ and encapsulation efficiency $99.7 \pm 0.7\%$ was highest achieved for 0.2% Modified Psyllium.

Introduction

Psyllium, the common name used for several members of the plant genus *Plantago*, is gel-forming mucilage composed of a highly branched arabinoxylan. The backbone consists of xylose units, while arabinose and xylose form the side chains. Psyllium has been reported as a medicinally active natural polysaccharide for the treatment of constipation, diarrhea, irritable bowel syndrome, inflammatory bowel disease, ulcerative colitis, colon cancer, diabetes, and hypercholesterolemia. Moreover, psyllium as

a soluble fiber has a potential to stimulate bacterial growth in digestive system, and, in some reports, it has been used as prebiotic. Prebiotics is defined by Gibson and Roberfroid as “non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Farzaneh *et al.*, 2012).

Dietary fibers from Psyllium have been used extensively both as pharmacological supplements, food ingredients; in processed

food to aid weight control, to regulation of glucose control for diabetic patients and reducing serum lipid levels in hyperlipidaemias (Baljit, 2007). Fermentation and water absorption of dietary fibre components result in several beneficial health effects. Dietary fibre intake has been associated with alleviation of constipation, regulation of lipid and glucose/insulin metabolism, and carcinogenesis.

Based on the previous studies, acid modification of psyllium husk presents a competitive potential of being applied in food industry due to its lower cost than enzymatic methods. In the present study an attempt has been made to improve the physico-chemical / functional properties of psyllium for incorporation in foods by acid treatment of the raw psyllium husk, so that it will serve as a source of dietary fiber without disturbing the nutritional and sensorial characteristics of the PSH incorporated processed food products.

Probiotic is a microorganism that provides beneficial health effects. The microorganism must exhibit several characteristics such as resistance against the gastric and intestinal juices and tolerance toward the digestive enzymes degradation.

Such challenges can be addressed by microencapsulating the probiotic bacteria within a protective matrix material.

The microencapsulation matrix functions not only as a protection against harsh gastrointestinal conditions but also increases the stability and viability of live probiotic culture at various heat/moisture conditions during processing and storage.

The consumption of products supplemented with live cells of lactic acid bacteria (LAB), in particular with their probiotic strains, is believed to benefit consumers' health due to

their well-documented positive impact on the function of gastro-intestinal tract and immune system, reduction of blood cholesterol, and apparent anticancer activity (Yoon *et al.*, 2006) Many polymeric materials can be used as a microencapsulation matrix for probiotic formulation, including alginate, starch, xanthan gum, fat, gelatin, and glycerides derivatives. Among these coating materials, alginate has been recognized as the most commonly used polymer for probiotic microencapsulation in food products, because it is non-toxic, biocompatible, low cost, and easy to apply.

Encapsulation is a mechanical or physicochemical process that traps a potentially sensitive material and provides a protective barrier between it and the external conditions.

From a microbiological point of view, microencapsulation can be defined as the process of entrapment/enclosure of microorganisms cells by means of coating them with proper hydrocolloid(s) in order to segregate the cells from the surrounding environment; in a way that results in appropriate cell release in the intestinal medium (Krasaekoopt *et al.*, 2003; Picot and Lacroix, 2003a).

Materials and Methods

Material

Raw materials

The raw materials such as Psyllium husk were procured from the local market of Parbhani.

Strains of LAB: Two LAB strains were used

Lactobacillus acidophilus
Lactobacillus bulgaricus

Methods

Preparation of stock culture

The pure cultures i.e. *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* were cultured on MRS media slants. This was incubated at 37°C for 48 hours and stored at 4°C for further use.

Preparation of starter culture

The probiotic organisms viz. *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* were individually grown in MRS broth at 37°C for 48hrs. The cultivated MRS broth was then centrifuged at 4,000 RPM for 10 min to harvest the cells. The harvested cells were washed twice with 1per cent peptone water.

Acid modification of psyllium husk

Acid modification of psyllium husk was carried out as per the method described by Xiaoyin Pei (2008) with certain changes in concentration of HCL in ethanol solvent. The solvent used for psyllium husks acid treatment was ethanol with 34% - 37% hydrochloric acid (HCl) at the varying concentration levels of 0.65% (w/v). The study was conducted to investigate the effect of acid concentration and psyllium to solvent ratio on physico-chemical/functional properties of the acid modified psyllium samples. At reaction temperature of 37.5°C three different psyllium –solvent ratios (PSH: Solvent @ 1:6 (w/v), g/mL) were tested. Thus, 48 g of psyllium husk was divided into 4 groups having 16g PSH each, for treatments with different concentrations levels of 0.65% (w/v) of Hydrochloric acid in Ethanol solvent. Four samples in each group were designated for psyllium to solvent ratios as mentioned earlier. 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 and 100% for 2 times each, then dried and stored. Control group was treated with 100% ethanol and followed the steps of preparation mentioned above.

Microencapsulation of strains

The microencapsulation of probiotic bacteria was performed using the extrusion technique. In this method Hydrocolloid solution was prepared by using a combination of sodium alginate at 1 and 0.8 per cent (w/v) respectively. 10ml of inoculum (5ml each of *L. acidophilus* and *L. bulgaricus*) was mixed in 2gm Modified psyllium husk. Probiotic culture and modified *Psyllium* husk powder were mixed properly and passed 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 (2-5mm) and the resulting beads were then stored in 0.1per cent peptone (Karthikeyan *et al.*, 2014).

Size and morphological analysis

The particle size of beads was assessed using optical microscopy (Dinolite, Taiwan) by Scion image analyzer software. Data were collected from 60 beads in each sample, and mean particle size was reported. The topographical properties of prepared beads were investigated by scanning electron microscopy (SEM) (Philips XL30, Holland) at an accelerating voltage of 20 KV. Prior to examination, samples were prepared on aluminum stubs and coated with gold under argon atmosphere by means of a sputter coater.

Encapsulation Efficiency (EE)

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, and counts were expressed as number of colony forming units (CFU), and calculated as

$$EE = \left(\frac{\text{Log}10N}{\text{Log}10N0} \right) \times 100$$

Where N is the number of viable entrapped cells released from the beads and $N0$ is the number of free cells added to the biopolymer mixture immediately before the production procedure.

Viability of encapsulated and free *L. acidophilus* and *L. bulgaricus* at low pH condition

Low pH conditions were produced using 9 g/L sodium chloride and 3.0 g/L of pepsin and pH adjusted to 1.8 with hydrochloric acid. 100 mg beads with entrapped bacteria or 0.1mL of cell suspension were mixed in 20mL of acid solution and incubated for 120 min at 37°C with constant agitation at 50 rpm. After incubation, beads were disintegrated in phosphate buffer (pH = 6.8), then 1.0mL aliquot of the mixture removed and assayed using pour plate method. The survival (%) of the bacteria was calculated as follows:

% Survival = $\left(\frac{\log \text{CFU/g beads after 2 hours exposure to acidic condition}}{\log \text{CFU/g beads initial count}} \right) \times 100$.

Viability of encapsulated and free *L. acidophilus* and *L. bulgaricus* at high bile salt concentration

Prepared beads after 2-hour acid exposure were washed with distilled water, removed, and incubated in 50mL of high bile condition, containing 6.8 g of monobasic potassium phosphate, and 10 g/L of pan creatine with pH adjusted to 6.8±0.1 using sodium hydroxide and 0.5% w/v ox gall for 2 hours at 37°C with

constant agitation at 50 rpm. Samples were then taken, and bacterial growth was assayed using pour plate method.

Release of encapsulated cells and free *L. acidophilus* and *L. bulgaricus* in simulated colonic pH solution

The release of the prepared beads was examined at simulated colonic pH solution as described by Mandal *et al.*, the beads were mixed with 50mL of simulated colonic pH solution containing 0.1M monobasic potassium phosphate with pH adjusted to 7.4±0.1 with sodium hydroxide and incubated for 20 h at 37°C with constant agitation at 50 rpm. Samples were taken at different time intervals, and bacterial growth was assayed using pour plate method as described in Section 2.2.5.

Statistical analysis

Statistical testing was carried out using SPSS19. All of the experiments were performed in triplicates. Data are presented as mean ± SD. The One-Way ANOVA test was performed to assess the difference between different beads and control groups and $P < 0.05$ considered as a statistically significant difference.

Results and Discussion

Effect of acid modification on functional properties of psyllium

The data pertaining to the effect of acid treatments on functional properties such as hydration capacity, oil absorption capacity and water up-taking rate of native psyllium husk were studied and obtained results are presented in Table 1.

It is revealed from the Table 1 that the hydration capacity of psyllium husk was decreased with the increased level of acid

concentration used for treatment from 3.0 ± 0.03 ml/g to 1.6 ± 0.05 ml/g. Significant decrease in hydration capacity were observed in case of PSH sample treated with 0.65% acid concentration having lowest 1.6 ± 0.05 ml/g for 1:6 - PSH : Solvent ratio followed by 1:4 PSH : Solvent ratio with 1.8 ± 0.07 ml/g.

Similarly, it was found that there was a substantial decrease in the hydration capacity of PSH sample treated 0.55% acid concentration for 1:6, 1:4 and 1:2 - PSH : Solvent ratios with 2.3 ± 0.02 , 2.4 ± 0.01 and 2.5 ± 0.04 ml/g respectively in comparison with the raw PSH having 3.0 ± 0.03 ml/g and Control having 2.8 ± 0.02 , 2.8 ± 0.06 and 2.9 ± 0.01 ml/g respectively followed by 0.50% acid concentration with 2.6 ± 0.05 , 2.6 ± 0.08 and 2.7 ± 0.02 ml/g respectively. For the water absorbing capacity and swelling volume results respectively, for acid treated PSH at

different acid concentrations, PSH: Solvent ratios and reaction temperatures.

Proximate composition of selected acid modified psyllium husk

It can be observed from above Table 2 that moisture content increased from 7.19 to 7.36 per cent upon acid modification. Fat content decreased after acid modification from 1.84 to 0.65 per cent while protein content decreased from 2.95 to 1.22 per cent. Similarly, ash and crude fiber decreased from 2.95 to 1.22 and 3.13 to 2.69 per cent respectively. The decrease in fat, protein, ash and crude fiber content resulted due to the partial degradation of the psyllium gel hardness because of acid modification. Further, carbohydrate content increased from 85.38 to 88.50 per cent and energy value decreased from 370 to 365 Kcal/100g.

Table.1 Effect of acid modification on functional properties of psyllium

Concentration of HCl in Ethanol (Solvent)	Psyllium Husk (PSH): Solvent Ratio	Functional properties		
		Hydration capacity (ml/g)	Oil absorption capacity (ml/g)	Water up-taking rate [mg/(g×min)]
Control	1:2	2.9 ± 0.01	0.9 ± 0.05	2.1 ± 0.05
	1:4	2.8 ± 0.06	0.9 ± 0.01	2.0 ± 0.03
	1:6	2.8 ± 0.02	0.8 ± 0.07	1.9 ± 0.08
0.50 %	1:2	2.7 ± 0.02	0.8 ± 0.01	1.91 ± 0.03
	1:4	2.6 ± 0.08	0.7 ± 0.06	1.88 ± 0.02
	1:6	2.6 ± 0.05	0.6 ± 0.03	1.85 ± 0.04
0.55%	1:2	2.5 ± 0.04	0.6 ± 0.07	1.82 ± 0.01
	1:4	2.4 ± 0.01	0.6 ± 0.01	1.78 ± 0.03
	1:6	2.3 ± 0.02	0.5 ± 0.08	1.77 ± 0.02
0.65%	1:2	2.1 ± 0.06	0.5 ± 0.05	1.74 ± 0.04
	1:4	1.8 ± 0.07	0.5 ± 0.03	1.71 ± 0.06
	1:6	1.6 ± 0.05	0.5 ± 0.01	1.68 ± 0.07
Raw / Native Psyllium Husk		3.0 ± 0.03	1.0 ± 0.02	2.20 ± 0.03
SE ±		0.3692	0.0243	0.1682
CD @ 5%		1.1077	0.0731	0.5046

* Each value is average of three determinations.

Table.2 Effect of acid modification on proximate composition of psyllium husk

Particulars (g/100g)	Native Psyllium Husk PSH (N)	Modified Psyllium Husk PSH (M)
Moisture	7.19	7.36
Fat	1.84	0.65
Protein	2.95	1.22
Ash	2.62	2.25
Carbohydrate	85.38	88.50
Crude Fiber	3.13	2.69
a) Dietary fiber	77.66 ± 1.28	79.67 ± 0.89
b) Arabinoxylan	47.60 ± 0.65	48.73 ± 0.78
Energy Value	370 Kcal/100g	365 Kcal/100g

* Each value is average of three determinations.

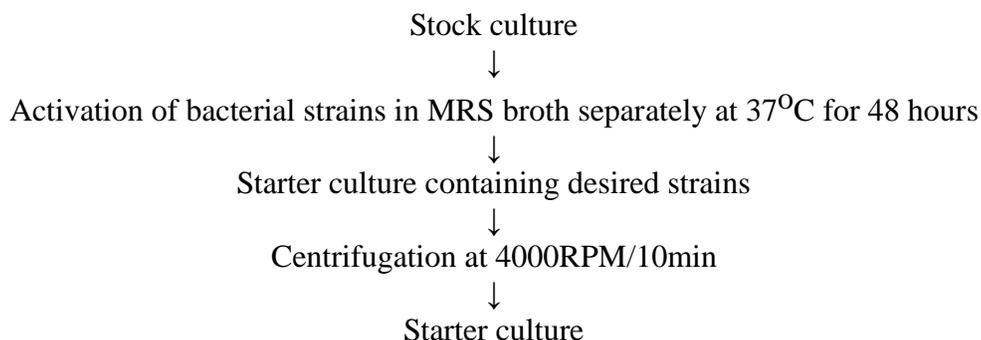
Table.3 Composition of the studied formulation

Formulation	Sodium Alginate (%w/v)	Modified Psyllium Husk (%w/v)
F1	2	0.1
F2	2	0.2
F3	2	0.3

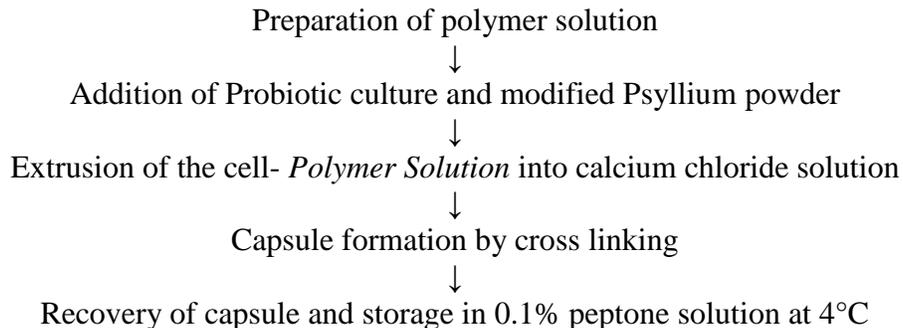
Table.4 Size, encapsulation efficiency, and % survival in acid condition of prepared formulations

Formulation	Diameter(mm)(n=60)	Count (CFU/g) after preparation	Encapsulation efficiency (%)	% Survival
F1	1.66 ±0.04	9.79 ±0.07	99.8± 0.9	82.3± 0.2
F2	1.65± 0.05	9.79 ±0.06	99.7± 0.7	83.2± 0.7
F3	1.64 ±0.03	9.76 ±0.05	99.4 ±0.6	86.6± 0.3

Flowchart for preparation of starter culture



Microencapsulation of strains



Characterization of prepared beads

Size, morphology, encapsulation efficiency, and surface characteristic: In the preliminary experiments, different concentrations of Sodium Alginate (0.75 to 3% w/v) and CaCl₂ as hardening solution (1 to 6% w/v) were examined. According to the results of this step, it was found that uniform and spherical bead preparation by ALG 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 2% (w/v) were too viscous to be extruded from the syringe. Hence, the Sodium Alginate concentrations between 1 to 2% w/v were selected. Moreover, according to our tests, 4% w/v CaCl₂ produced the best result and chosen as optimum hardening solution. In the second step of preliminary tests, the concentrations of Modified Psyllium Husk to be incorporated in Sodium Alginate beads were optimized. Addition of Modified Psyllium Husk into Sodium Alginate gel results in an increase in the viscosity and adherence of resultant gel.

Indeed, incorporation of Modified Psyllium Husk in the concentrations more than 0.1, 0.2 and 0.3 w/v to Sodium Alginate in the concentrations of 2% w/v, respectively, yields too adherent mixtures.

Table 4 shows results for diameters and encapsulation efficiencies of different ALG and Sodium Alginate- Modified Psyllium Husk beads. As it can be seen, beads ranging from 1.65 to 1.67mm for ALG and from 1.66 to 1.80mm for Sodium Alginate- Modified Psyllium Husk formulations were achieved. The mean diameters of beads containing PSL were significantly higher than those without Modified Psyllium Husk ($P < 0.02$) 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 and no significant differences in size ($P > 0.02$) were observed between beads contained or not *L. acidophilus* loads.

The consumption of products supplemented with live cells of lactic acid bacteria (LAB), in particular with their probiotic strains, can benefit consumers health due to their positive impact on the function of gastro-intestinal tract and immune system. This study concluded that incorporation of modified psyllium husk (MPSH) in alginate beads containing probiotic bacteria viz., *L. acidophilus* and *L. Bulgaricus*. As modification psyllium husk shown high dietary fiber 79.67% and functional properties also found to be improved which can be suitable for encapsulation of probiotic culture.

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How to cite this article:

Hashmi, S.A.S., H.W. Deshpande, A.S. Farooqui, H.M. Syed and Sontakke, M.D. 2018. Microencapsulation of Probiotic Culture Beads by Using Modified Psyllium Husk. *Int.J.Curr.Microbiol.App.Sci*. 7(05): 392-400. doi: <https://doi.org/10.20546/ijemas.2018.705.051>