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Exploration of Acid Modified *Psyllium* Husk for Microencapsulation of Probiotics Cultures

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Abstract:

Microencapsulation is the technique of entrapping or enclosing microorganism cells by coating them with suitable hydrocolloids to separate the cells from their environment, with the objective of obtaining the proper cell release in the intestinal medium. Extrusion technique is used to microencapsulate probiotic microorganisms in order to increase their viability. in the present study encapsulated probiotic microorganism (Lactobacillus casei and Lactobacillus Plantarum) with the addition of acid modified psyllium husk for preparation of encapsulated beads. Psyllium husk improves the functional characteristics of the encapsulated beads. To prepare the encapsulated beads, incorporate 2 grams of modified psyllium husk with 1 percent sodium alginate, 0.8 percent guar gum, and 10 ml of inoculum (5 ml of each Lactobacillus casei and 5 ml of Lactobacillus plantarum). Probiotic culture and modified psyllium husk powder were effectively combined, and then injected into a solution of 0.3M calcium chloride using a syringe. The resultant beads were then kept in peptone solution at 0.1 percent. For Acid modified psyllium were treated

with different concentration 0.65% of HCL in ethanol for solvent ratio 1:6 (W/V) and compered to the Native psyllium husk sample at 37^{0}C temperature to reduce gel hardness and improve the functional properties viz, Oil absorption capacity, hydration capacity, water up taking rate.

Keywords: Psyllium Husk, Encapsulation, Probiotic, Modification, Extrusion Method, Lactobacillus casie, Lactobacillus Plantarum.

Introduction:

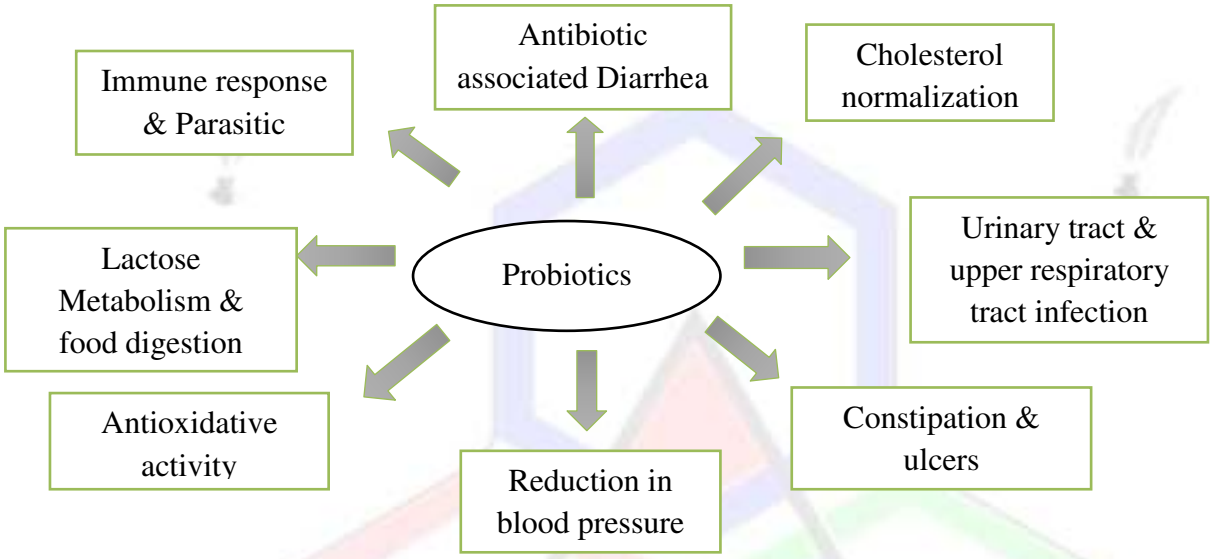
Microencapsulation is a recent technique that enables for the protection of various food ingredients or useful substances. Using a polymeric or nonpolymeric substance to enclose individual food components or functional constituents and allowing for their controlled release under specific circumstances, Additionally, it improves the sensory experience by reducing undesirable odors, aromas, and tastes; moreover, it also increases food safety by preventing the growth of microorganisms (Hasanvand, et al., 2015, Sengupta, et al., 2001). Probiotic bacteria, omega-3 and omega-6 fatty acids, vitamins, phenolic compounds, and carotenoids are just some of the examples of the various bioactive substances that are increasingly frequently used to create goods with a variety of functional qualities that fulfill the growing customer demand. These substances are, however, extremely unstable under particular light, temperature, pH, and oxygen environments. Therefore, microencapsulating those substances is a way that protects them from the severe conditions present during food preparation. Different flavoring agents, lipids, antioxidants, essential oils, pigments, probiotic microorganisms, and vitamins are a few dietary components that are frequently encapsulated (Azeredo 2005). Various coating materials are used depending on their rheological characteristics, their capacity to stabilize and release the active ingredient, as well as their inertness toward the ingredient. For example, lipids like wax, paraffin, beeswax, and diacylglycerols; carbohydrates like starch, maltodextrin, modified starch, cyclodextrin, and cellulose; gums like gum acacia, agar, and carrageenan; and proteins like gluten, casein, and gelatine are some examples of coating materials. Encapsulation is a mechanical or physicochemical procedure that contains a potentially sensitive material and creates a barrier of protection between it and the environment. From a microbiological perspective, microencapsulation is the process of entrapping/enclosing microorganisms' cells by coating them with a particular hydrocolloid(s) in order to separate the cells from their surroundings; this produces an appropriate cell release in the intestinal medium (Krasaekoopt, et al., 2003, Picot and Lacroix, 2003).

Extrusion, emulsification, spray drying, fluidized beds, and advanced coacervation are some of the common techniques used to create encapsulated probiotics (Olivarez Romero et al., 2018; Rodrigues et al., 2020). The stability of probiotics depends on the selection of the right encapsulating materials since they provide a barrier that can prevent damage or cellular losses. Polymers (polysaccharides and proteins) are among the most common types of materials used for encapsulation because they frequently provide safe biodegradation, absence of toxicity, ease of handling, cheap cost, and biocompatibility. Alginate, xanthan gum, chitosan, carrageenan, gelatine, and dairy proteins are just a few examples of food grade polymers that are frequently used to encase probiotics (Cook, et al., 2012, Rodrigues, et al., 2020). Additionally, the inclusion of prebiotics in encapsulating materials may increase the viability and survival of probiotics in the gastrointestinal system (Burgain, et al., 2011).

Probiotics are live, non-harmful microbes that are beneficial to the host's health when given in sufficient quantities. They also have nutritional benefits. To maintain a healthy balance of helpful germs in the gut flora, it is advised to consume foods containing probiotic microorganisms on a regular basis. Probiotic bacteria like Bifidobacterium and Lactobacillus strains, which are present in

GIT and dietary supplements and are incredibly varied and mind-boggling in their composition and quantity, belong here. A crucial role for the gut microbiota in maintaining human health (Carlos RS, et al., 2010, and Miriam BB, et al., 2012, Mohammad Mehdi SD, et al., 2015).

Health Attribute of Probiotics:



It is crucial that the probiotic strain survives in the location where it is thought to be active. The strain should be able to multiply and colonize at this site specifically for maximal activity. The immune system should also be able to withstand it.It must not be carcinogenic, allergenic, or pathogenic. Probiotics for humans should be "generally recognized as safe," with a shown minimal risk of causing or contributing to the pathogenesis of illness.The probiotic organisms must be able to resist low pH and high concentrations of both conjugated and deconjugated bile acids. They should ideally be of human origin and must be able to live and thrive in the in vivo circumstances at the intended site of administration.The probiotic employed must also be technologically compatible with food processing for successful application in foods. Additionally, the probiotic bacteria-containing meals must retain their conventional counterparts' distinctive sensory qualities (Ravinder Nagpal, et al., 2012).

Table 1: Species used as a probiotic.

List of Probiotic species	Group of Microbes
Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus lactis, Lactobacillus paracasei, Lactobacillus fermentus, L. johnsonii, L. salivarius, L. kefir, L. plantarum, L. rhamnosus, L. delbrueckii.	*Lactic acid producing bacteria*
Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium lactis, Bifidobacterium infantis, Bifidobacterium longum, B. animalissubsp lactis, B. animalissubspanimalis.	*Bifidobacterium species*

Enterococcus faecalis, Enterococcus faecalis, Esterichia coli Nissle, Streptococcus thermophilies, Propinobacterium.	*Non lactic acid producing bacteria*
Sacchromycesboulardii	*Non-pathogenic yeast*
Coccobacillus, Lactobacillus, Leuconostic, Lactococcus lactis subsp. Streptococcus, Pediococcus, Propionibacterium, Bifodobacterium, Bacillus, Bacillus Coagulans, Bacillus subtilis, Enterococcus durans, Saccharomyces cervisiae, Aspergillus niger, A. oryzea, Candida pintolopesii, Bacillus cereus, B. linchenformis, B. clausii, B. pumilus, B. racemilacticus, B. coagulans.	*Non spore forming*

References: (Miriam BB, 2012, Taverniti V, 2013, Mohammad Kazem SY, 2017).

Materials Methods:

Psyllium husk procured from local market of parbhani. The experiment was conducted in Department of Food Microbiology and Safety, College of Food Technology, VNMKV, Parbhani, Maharashtra.

Psyllium Husk:

Plantago ovata, also known as "Psyllium" in english and "Isabgol" in Hindi, is a 10- to 45-cm-tall annual plant that is also known by other names including "blond psyllium," "ashwagolam," "aspaghol," and "aspagol." Isabgol is widely grown around the world and has a high fiber content. It functions like a sponge to help clear the intestines. Due to its medicinal qualities, it is a significant crop during the Rabi season commercially. In addition to its husk (the seed coat is referred to as "husk"), it is utilized in the culinary sector, particularly in ice creams, cookies, and sweets. The states of Rajasthan, Gujarat, Haryana, and Madhya Pradesh are where the crop is mostly grown. Plantago ovata is the source of psyllium. The seeds themselves have also been given, however the husks are more frequently employed. The polysaccharides pentoses, hexoses, and uronic acids make up psyllium. Husk preparations typically include 67–71% soluble fiber and around 85% total fiber by weight, while seed preparations typically contain about 47% soluble fiber by weight (Ahmadi, et al., 2012).

Psyllium or Ispaghula is the common name for a number of Plantago species whose seeds are used to make mucilage for commercial purposes. P. ovata seed is often referred to as white or blonde psyllium, Indian plantago, or Isabgol in trade circles. Isabgol, also known as Ispaghol in Pakistan, is the popular name for P. ovata in India. It is derived from the Sanskrit words asp and ghol, which mean "horse flower," which describes the seed's form. Psyllium is made from the husks of blonde psyllium seeds and is a natural source of high soluble fiber. Isabgul's primary product is psyllium husk. The husk is the seed's outermost skin, and it is removed mechanically. Around 25 to 26% of the husk from the seed is recovered in total. Under normal and customary storage circumstances, psyllium husk has a shelf life of only six months. On a dry weight basis, the husk makes up around 10–25% of the seed. P. ovata is a crop that matures in 119 to 130 days and grows well in cold, dry conditions. P. ovata is grown mostly in north gujarat in india as a "rabi" crop from

october to march. Average temperatures during this monsoon-following season vary from 15 to 30 °C (59 to 86 °F), and moisture levels are low.

Acid Modification of Psyllium Husk:

Psyllium husk was acidified according with the procedure given out by (Xiaoyin Pei, 2008), with certain modifications to the HCL content in the ethanol solvent. A mixture of ethanol and 34%–37% hydrochloric acid (HCl) at different concentrations of 0.65% (w/v) was the solvent used to treat the psyllium husks with acid. The objective of the study was to determine how the physico-chemical and functional characteristics of the acid modified psyllium samples were affected by the acid concentration and psyllium to solvent ratio. Three distinct psyllium-solvent ratios (PSH: Solvent @ 1:6 (w/v), g/ml) were investigated at a reaction temperature of 37.5^0C. In order to test different concentrations of 0.65% (w/v) of hydrochloric acid in ethanol solvent, 48 g of psyllium husk were split into 4 groups, each containing 16 g of PSH. As previously noted, psyllium to solvent ratios were performed on four samples from each group. Samples were incubated for 48 hours at 37.5^0C after the solvent was added. Samples were vacuum filtered after that.

A) Plantago Ovata Plant

B) Acid modified Psyllium Husk Powder

Microencapsulation Techniques;

Encapsulation of food ingredients into coating materials can be achieved by several methods. As fallows:

1) Spray drying
2) Spray Cooling
3) Extrusion
4) Fluidized bed coating
5) Coacervation
6) Emulsification
7) Cyclodextrin inclusion
8) Lyophilization
9) Cocrystallization
10) Centrifugal Suspension Separation.

The physical and chemical characteristics of the core and coating materials as well as the intended utilization of food components determine the choice of the microencapsulation technique. Table lists the microencapsulation procedures used to encapsulate food components.

Extrusion Method for Microencapsulation:

A physical encapsulation technique is extrusion. This process involves extruding a mixture of concentrated probiotic bacteria cells or any other food bioactive and a hydrocolloid solution. The mixture is subsequently dripped through a syringe needle or nozzle to produce droplets that fall into a hardening solution, delivering the hydrocolloid solution's rapid transition to gel and resulting in the formation of beads, which are subsequently gathered and dried (Riaz Masud, 2013, Abd El-Salam & El-Shibiny, 2015, Yeung, et al., 2016, Rodrigues, et al., 2020).The creation of solutions that harden, such calcium as calcium chloride, typically involves the use of multivalent cations. Extrusion as an encapsulating technique has a number of benefits, including being a straightforward, affordable, and accessible process that doesn't harm probiotic bacteria cells and results in a high viability of probiotic microorganisms. Flexibility and biocompatibility are further benefits of this technique (Huq, et al., 2013, Riaz & Masud, 2013). However, this process has certain limitations, including the difficulty expanding up since beads form slowly and the need for low to moderate viscosity hydrocolloid solutions (Riaz & Masud, 2013, Rodrigues, et al., 2020). The produced beads particles via extrusion typically have a diameter that falls between 2 and 5 mm in size. The diameter of the nozzle, the distance between the hydrocolloid solution's outflow and the hardening solution, the viscosity and concentration of the hydrocolloid-cell combination are among the variables that affect the size of the produced capsules (Huq, et al., 2013, Riaz & Masud, 2013, Abd El-Salam & El-Shibiny, 2015).

Microencapsulation Of Strains By Using Acid Modified Psyllium Husk:

The extrusion process was used to microencapsulate probiotic microorganisms. In this approach, sodium alginate was combined with 0.8 and 1 percent (w/v) of water to create a hydrocolloid solution. In 2gm of modified psyllium husk, 10ml of inoculum (5ml each of L. acidophilus and L. bulgaricus) were added. Probiotic culture and modified Psyllium husk powder were well combined before being injected into a 0.3 M calcium chloride solution using a syringe. Beads (2–5 mm) were formed as a result of the interaction between the two solutions and were then kept in 0.1 percent peptone. (Karthikeyan, et al., 2014).

Flow sheet 1:Microencapsulation of strains

Preparation of polymer solution
↓
Addition of Probiotic culture and modified Psyllium powder
↓
Extrusion of the cell- Polymer Solution into calcium chloride solution
↓
Capsule formation by cross linking
↓
Recovery of capsule and storage in 0.1% peptone solution at 4°C

Microencapsulated Beads

Results and Discussion:

Table 2: Acid Treatment Levels of psyllium husk

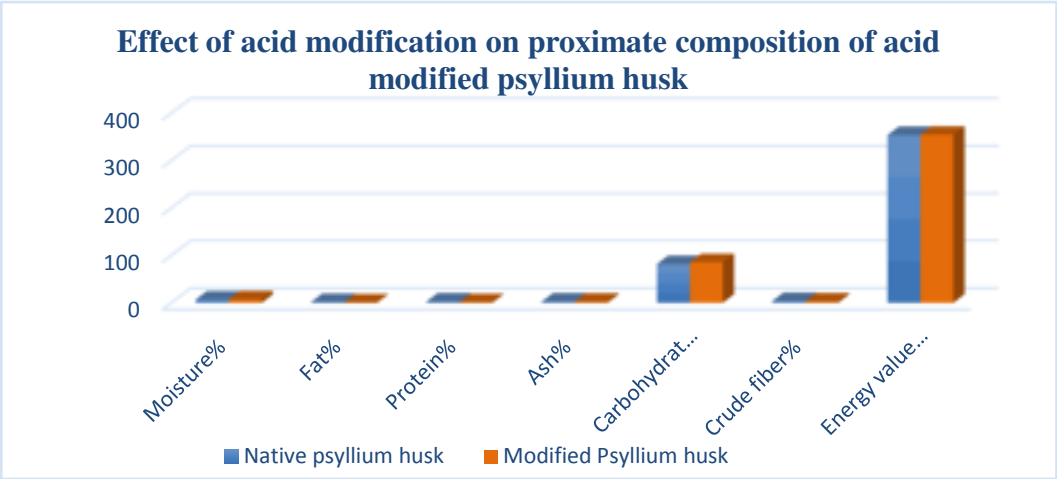
Concentration of HCL in Ethanol	Psyllium Husk (PSH): Solvent Ratio
0.65%	1:4 and 1:6 (W/V)
0% for Control	1:6 (W/V)

Acid modification of psyllium husk was carried out as per the method described by xiaoyinpei 2008 with certain changes in concentration of HCl in ethanol as per the results of the research study. Acid concentration and solvent ratio for the modification of psyllium husk solvent used for psyllium husk treatment was ethanol with 34-37 per cent hydrochloric acid (HCL) at the concentration level of 0.65% (W/V), acid solvent ratio at reaction temperature of 37.5^0C therefore, it is observed functional properties of psyllium husk improved after acid modification of psyllium husk viz, water-up taking rate, oil, absorption level, hydration capacity.

Table 3: Proximate Composition of Psyllium Husk

Constituents (g/100g)	Native Psyllium Husk	Modified Psyllium Husk
Moisture	7.15±0.02	7.34±0.01
Fat	1.84±0.03	0.63±0.01
Protein	2.62±0.02	1.31±0.02
Ash	2.58±0.02	2.24±0.02
Carbohydrate	82.01±0.01	85.84±0.05
Crude Fiber	3.8±0.15	2.64±0.02
Energy Value	355 Kcal/100g	354 Kcal/100g

The results similar to the effect of acid modification of the selected concentration of HCL in ethanol i.e., 0.65% percent (0.65 ml of 34 per cent to 37 per cent concentrated hydrochloric acid (HCL) in 100 ml pure ethanol. proximate composition of psyllium husk results showed that moisture content increased from 7.15±0.02 to 7.34±0.01per cent after acid modification of psyllium husk. Fat content decreased upon acid modification from 1.84±0.03 to 0.63±0.01 per cent while protein content also decreased from 2.62±0.02 to 1.31± 0.02per cent. Similarly, ash and crude fiber also decreased from 2.58±0.02 to 2.24±0.02 and 3.8±0.15 to 2.62±0.02 per cent respectively. The decreases in fat, protein, ash, and crude fiber content resulted due to the partial degradation of the psyllium gel hardness because of acid modification of psyllium husk. Further, carbohydrate content increased from 82.01±0.01 to 85.86±0.05 per cent and energy value decreased 355 to 354 Kcal/100g. the following chart is showing the effect of acid and native psyllium husk.



Proximate composition of psyllium husk

A) **Native psyllium husk**

B) **Acid modified psyllium husk**

Table 4: Effect of acid modification on functional properties of psyllium husk

Concentration of HCL in Ethanol	Psyllium Husk: Solvent Ratio	Hydration Capacity (ml/g)	Water up-taking rate (mg/ (g× min)	Oil absorption capacity (ml/g)
Control	1:06	2.6±0.12	1.86±0.01	0.9±0.15
0.65%	1:06	1.5±0.16	1.69±0.02	0.6±0.15

The result obtained from table 3. Revealed that different functional properties of native psyllium husk and acid treated psyllium husk. the hydration capacity was 2.6±0.12 ml/g and water up taking rate was 1.86±0.01 mg/ (g× min) and oil absorption capacity was 0.9±0.15ml/g. and for acid treated 0.65% Concentration of HCL in ethanol 1.5±0.16 ml/g, 1.69±0.02 mg/ (g× min) and 0.6±0.15 ml/g was obtained after acid treatment improve the functional properties of psyllium husk.

Minerals composition of psyllium husk:

Represented that copper and iron content of native psyllium husk was found 0.675±0.002mg/100g and 7.89±0.0021 mg/100g respectively, while manganese and zinc were found 0.665±0.004 mg/100g and 0.345±0.004mg/100g. iron is higher than other minerals.

Table 5: Minerals composition of psyllium husk:

Parameters	Results (mg/100g)
Copper (Cu)	0.675±0.002
Iron (Fe)	7.89±0.0021
Manganese (Mn)	0.665±0.004
Zinc (Zn)	0.345±0.004

Conclusion:

Psyllium is obtained from the seed of the plantago ovataforsk it is ritch source of dietary fiber. Psyllium consists of mixed viscous polysaccharide in which about 35% soluble and 65% insoluble polysaccharide are present. In present study the effect of acid modification with concentration of 0.65% of 37% hydrochloric acid (HCL) in ethanol as a solvent. The effect of acid treatment for psyllium husk solvent ratio 1:6 which improves functional properties of psyllium husk without affecting the dietary fiber. Modified psyllium husk was better functional properties than the native psyllium husk without affecting the nutritional properties.

Future Scope:

Psyllium husk rich in dietary fiber also called as a prebiotic.it is also used as alternative of stabilizer. It helpful for the weight control, constipation, blood pressure. Psyllium is typically used as a to treat constipation and mild diarrhea symptoms. It can also be utilized as a food thickening.

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