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# Preparation of probiotic ice cream exploration with acid modified *psyllium* husk and various non-nutritive sweeteners with microencapsulated beads

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**Abstract**

The present investigation was carried out to development of probiotic ice cream using acid modified *psyllium* husk and various non-nutritive sweeteners. Addition of probiotic cultures (*Lactobacillus casei* and *Lactobacillus plantarum*) and acid modified *psyllium* husk with probiotic culture in formulation of probiotic ice cream. Further' the acid modified *psyllium* husk powder with concentration of 0.65% HCL in the ethanol solvent for solvent ratio of 1:6 (w/v) as significantly decreases the hydration capacity, oil absorption capacity & water up-taking rate of *psyllium* husk then it was microencapsulation using probiotics cultures strains by extrusion of the cell polymer solution into calcium chloride solution to form beads. The main aim of this study was the use of microencapsulated probiotics and modified *psyllium* husk in preparation of probiotic ice cream. The prepared probiotic ice cream evaluated for physicochemical, microbial, textural and sensory properties in case of colour, flavour, appearance, texture, and overall acceptability using 9-point hedonic scale.

**Keywords:** *Lactobacillus casei*, *Lactobacillus Plantarum*, *Psyllium* husk, ice cream and microencapsulation

## Introduction

*Psyllium* is a commonly used name for several kinds of plants in the *Plantago genus*. It corresponds to a gel-forming mucilage that is made up of an arabinoxylan with many branching. The secondary chains of *psyllium* are made up of arabinose and xylose, while the backbone is made up of xylose units. *Psyllium* has been identified as a polysaccharide that naturally occurs with medicinal activity that can be used to treat a number of conditions, including diabetes, excessive cholesterol levels, ulcerative colitis, diarrhea, and constipation and bowel disorders such as irritable bowel syndrome. Additionally, *psyllium*, a soluble fiber, has been employed as a prebiotic in certain reports and can encourage bacterial development in the digestive tract. "Non-digestible substances found in food that advantageously affect the host by selectively encouraging the growth and/or activity of a single or small number of bacteria in the colon, and thus promote host health" is how Gibson and Roberfroid represent prebiotics (Farzaneh *et al.*, 2012) [8]. One of the most effective dietary therapies against many health conditions is dietary fiber. No one contradicts the beneficial benefits of fibers, especially viscous dietary fibers, on human health, including their ability to safeguard against and treat chronic illnesses. Additionally, *psyllium* dietary fibers have been widely utilized as ingredients in food and pharmaceutical supplements to help individuals control their weight, regulate their blood sugar levels in diabetic patients, and lower their serum cholesterol levels in hyperlipidemia. (Baljit, 2007) [10]. A potentially sensitive substance can be encapsulated using a mechanical or physicochemical procedure that creates a barrier to protect it from the environment. In in addition to providing resistance against serious gastrointestinal disorders, the microencapsulation matrix improves the stability and survivability of live probiotic cultures under varied temperature and moisture conditions during processing and storage. Consuming products supplemented with live cells of lactic acid bacteria (LAB), specifically their probiotic strains, has been suggested to improve the health of individuals because of the LAB's well-established beneficial effects on the immune system and gastrointestinal tract, as well as because they are able to lower blood cholesterol and appear to possess anticancer properties (Yoon *et al.*, 2006) [7].

For probiotic the manufacturing process, a variety of polymeric components, such as alginate in them starch, xanthan gum, fat, Gelatin, and derivatives of glycerides, can be employed as a microencapsulation the matrix. Because it is environmentally friendly, biocompatible, and cost-effective as well as easy to work with, alginate has been identified as the most widely utilized polymerization for probiotic microencapsulation in food items among these coating materials. A potentially sensitive substance can be encapsulated using a mechanical or physicochemical procedure that creates a barrier to protect it from the environment. A probiotic is a type of microbe with positive health benefits. The microbe has to have a number of traits, such tolerance to the breakdown of digestive enzymes and resistance to the stomach and intestinal fluids. The most popular and extensively utilized probiotic microorganisms are lactic acid bacteria. The lactic acid bacteria are catalase-negative, gram-positive, cocci or rods that are typically non-motile and non-sporulating. The only by product of their fermentative metabolism of carbohydrate substrates is lactic acid. The majority of *Lactobacillus* species are homofermentative, meaning they primarily create lactic acid as a metabolic by product; however, certain species are heterofermentative, meaning they also produce $CO_2$, ethanol, and lactate, in that order. (Gaikwad *et al*., 2016) [9]. Beyond only providing energy and nutrients, functional foods also referred to as meals that positively impact particular organs or systems inside the human body are recognized for a number of other uses (Granato *et al.*, 2010) [11]. The current market's explosive growth of functional foods, particularly probiotic dairy products, has been driven by an increase in the number of customers who are worried about their health. Dairy products have a close relationship with probiotics when it comes to functional nutrition. In recognition of the many advantages, they provide to human health, including bacteriocin manufacturing, acid and bile tolerance, epithelial cell compliance, gut survivability and colonization, and endurance to physiochemical conditions of food processing and storage, probiotic strains have gained significant attention in the development of food products (Prado *et al.*, 2008) [12]. Their antibacterial, antimutagenic, anticarcinogenic, and antihypertensive qualities are responsible for these advantages. Frequent consumption has been demonstrated to improve gut resistance to infections, stabilize host gut microbiota, and lessen food allergy symptoms (Liong *et al.*, 2009; Granato *et al.*, 2010) [13, 11]. Probiotics dairy products, fermented drinks, and ice cream are examples of dairy items that are thought to be the primary means of consuming probiotics once they have been supplemented According to (Shah 2007) [14]. Dairy foods should include at least $10^6$ cfu/ml of probiotic cells to offset any future population loss that occurs during transit through the human gut. Probiotic-infused ice cream has been shown to have encouraging health effects (Çaglar *et al*., 2008) [15]. And could act as probiotics' barriers to protection and vehicles during gastrointestinal transit (Cruz *et al.*, 2009) [16]. Due to the rise in illnesses that are not transmissible including obesity and cardiovascular disease, creative formulas for low-fat ice cream that include probiotic cultures, prebiotics, and synbiotics have been developed (Akalin and Erisir, 2008; Chaikham and Rattanasena, 2017) [17, 18].

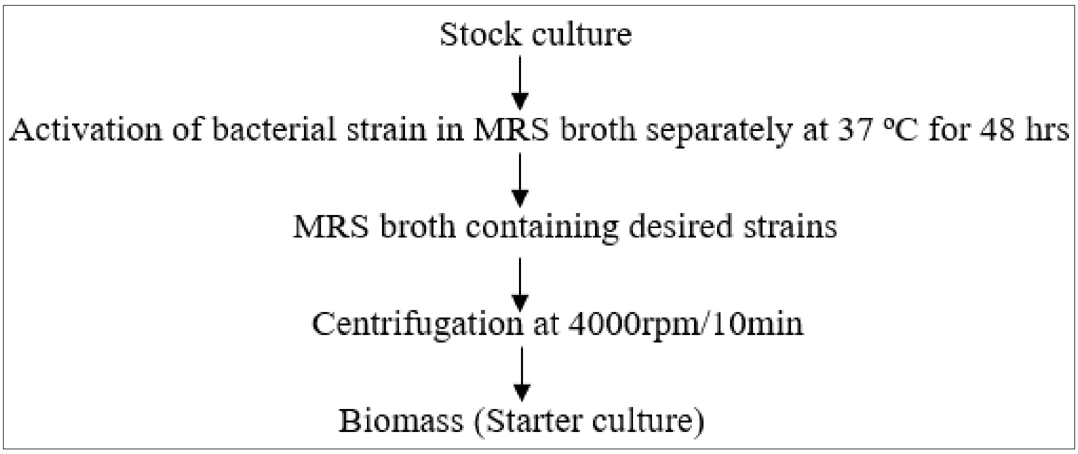
## Materials and Methods

**Materials:** *Psyllium* husk, milk, cream, SMP, non-nutritive sweeteners, color, flavour were procured from the local market of parbhani.

### Methods

**Preparation of starter culture**

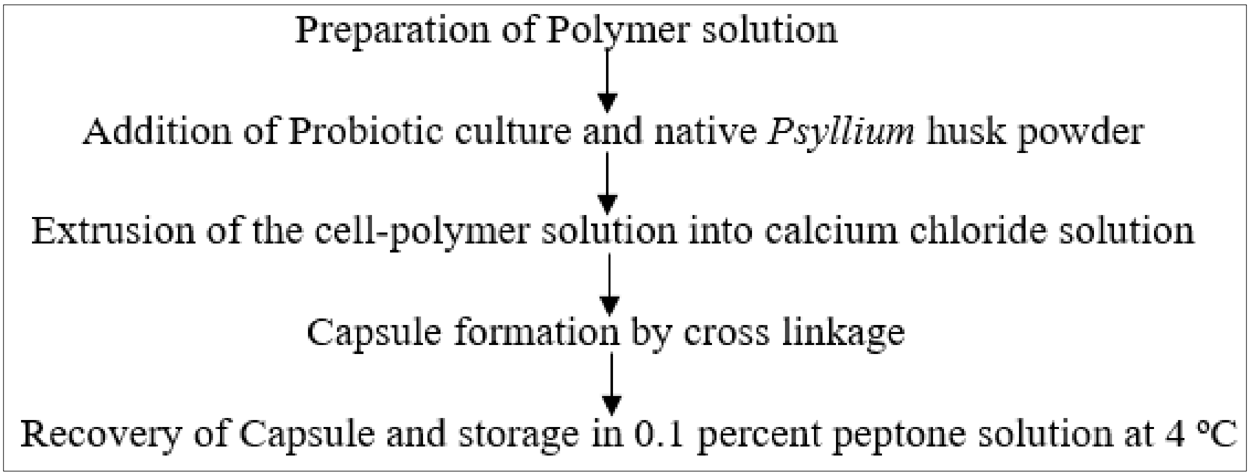
*Lactobacillus casei* and *Lactobacillus plantarum*, two probiotic organisms, were cultured separately in MRS broth for 48 hours at 37 °C. For the purpose of harvesting the cells, the cultured MRS broth was centrifuged for 10 minutes at 4000 rpm. Sterile water was used twice to wash the collected cells. Initiating culture was obtained from the biomass.




**Flow Sheet 1:** Preparation of starter culture

**Encapsulation of probiotics**

Preparing ready to make microencapsulated beads in order to create the hydrocolloid solution, 1 and 0.8 percent (w/v) sodium alginate and guar gum were combined. Ten milliliters of the inoculum - five milliliters of each of *Lactobacillus casei* and *Lactobacillus plantarum* were combined with two grams of acid modified *psyllium* husk powder. Acid modified *psyllium* husk powder and probiotic culture were well combined and then injected into a 0.3M calcium chloride solution using a syringe to create droplets. Beads (ranging in size from 2 to 5 mm) were formed by the interaction of the two solutions, and these beads were then preserved in 0.1% peptone (Karthikeyan *et al.*, 2014) [21].




**Flow Sheet 2:** Microencapsulation of Strains

**Acid modification of *psyllium* husk**

Acid modification of *psyllium* husk was carried out as per the method described by Xiaoyin Pei (2008) [19] with certain changes in concentration of HCl in ethanol solvent as per the results of the research study conducted by the (Syed *et al.*, 2018) [1]. On the establishment of standards of the solvent to acid ratio in order to modify *psyllium* husk. Thus, an acid modification was carried out using an ethanol solvent at a solvent ratio of 1.6 (w/v) and a concentration of 0.65% HCl. In order to enhance the functional qualities of *psyllium* husk and enable further investigation into the value addition of processed food items, a solvent ratio study was conducted. After being vacuum-filtered and twice washed with 95% and 100% ethanol, the solvent used to treat the *psyllium* husks for use was dried and stored. The control group received just 100% ethanol treatment and followed the steps of preparation as in Table 1.

**Table 1:** Acid treatment for psyllium husk

| Concentration of HCL in Ethanol (solvent) | *Psyllium* husk (PSH): Solvent ratio |
|---|---|
| 65% | 1:6 (w/v) |
| 0.00% for control | 1:6(w/v) |

**Preparation of Probiotic ice cream using acid modified *psyllium* husk and non-nutritive sweeteners**

The probiotic ice cream was made in accordance with recommendations provided by P. Prasant (2018) [2]. The ingredient used for preparation of probiotic ice cream is milk, sweeteners (sugar, stevia, fructo-oligosaccharide, jaggery), cream, SMP, flavor, and LAB cultures (*Lactobacillus Casei* and *Lactobacillus plantarum*) were used to make probiotic ice cream. *Psyllium* husk, which is used as an ice cream stabilizer, is a crucial element for improving the consistency, stability, viscosity, and thickness of the dessert. Probiotic ice cream uses acid-modified *psyllium* husk to standardize the recipe with different sweeteners. Standardization of probiotic ice cream using various non-nutritive sweeteners and acid modified *psyllium* husk.

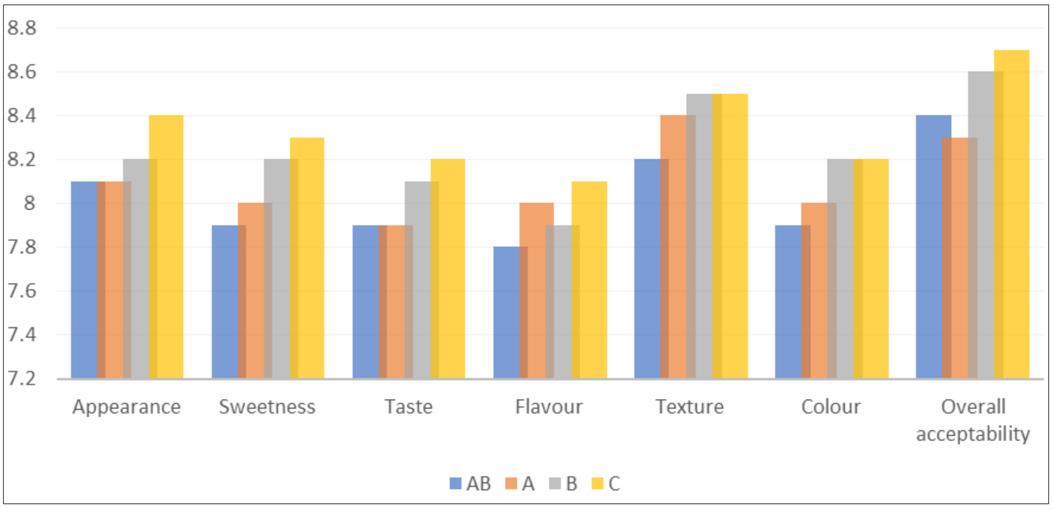
**Sensory analysis:** Probiotic ice cream was organoleptically evaluated using a nine-point hedonic scale, where 1 represents a severe dislike and 9 denotes an extreme like. A panel of semi-trained participants were participating in sensory analysis. Evaluation criteria for probiotic ice cream with different sweeteners included color, texture, flavor, sweetness, appearance, after taste, and acceptability overall.

**Table 2:** Sensory analysis of probiotic ice cream containing selected stevia and different viable count $10^7$, $10^8$, $10^9$ cfu/ml

| Sample | AB | A | B | C | SE | CD at 5% |
|---|---|---|---|---|---|---|
| Appearance | 8.1 | 8.1 | 8.2 | 8.4 | 0.079 | 0.245 |
| Sweetness | 7.9 | 8.0 | 8.2 | 8.3 | 0.081 | 0.251 |
| Taste | 7.9 | 7.9 | 8.1 | 8.2 | 0.065 | 0.201 |
| Flavour | 7.8 | 8.0 | 7.9 | 8.1 | 0.066 | 0.205 |
| Texture | 8.2 | 8.4 | 8.5 | 8.5 | 0.073 | 0.228 |
| Colour | 7.9 | 8.0 | 8.2 | 8.2 | 0.070 | 0.219 |
| Overall acceptability | 8.4 | 8.3 | 8.6 | 8.7 | 0.76 | 0.236 |

AB: Ice cream without addition of culture (control).
A: Ice cream + encapsulated acid modified *psyllium* husk with 10% probiotic culture having ($10^7$) cfu/ml.
B: Ice cream + encapsulated acid modified *psyllium* husk with 10% probiotic culture having ($10^8$) cfu/ml.
C: Ice cream + encapsulated acid modified *psyllium* husk with 10% probiotic culture having ($10^9$) cfu/ml.

Sensorial characteristics of probiotic ice cream using acid modified *psyllium* husk and stevia. Probiotic ice cream with different viable cells of *Lactobacillus casei* and *lactobacillus plantarum* 10% of probiotic culture having $10^7$, $10^8$, $10^9$ cfu/ml. as in terms of appearance, sweetness, taste, flavour, texture, colour and overall acceptability are given in Table 2 the overall acceptability of probiotic ice cream 8.7 containing encapsulated acid modified *psyllium* husk with 10% probiotic culture having ($10^9$) cfu/ml.




**Graph 1:** Sensory evaluation of prepared encapsulated probiotic ice cream using stevia

**Proximate composition of *psyllium* husk**

Proximate composition such as fat, protein, moisture, crude fiber, dietary fiber determined by (AOAC 2000) [20] method, carbohydrate by difference method.

**Statistical analysis**

The obtained data was analysed statistically using a Completely Randomized Design (CRD) and significance was tested using analysis of variance at 5% significance level.

**Results and Discussion**

Proximate composition of probiotic ice cream using *psyllium* husk and non-nutritive sweeteners.

**Table 3:** Physicochemical characteristics of probiotic ice cream

| Physical parameter | Probiotic ice cream |
|---|---|
| Overrun (%) | 47 ± 0.64 |
| pH | 6.3 ± 0.1 |
| Acidity (%) | 0.27 ± 0.02 |
| Total solid (%) | 36.4 ± 0.20 |

The physico-chemical properties of probiotic ice cream using acid modified *psyllium* husk containing various non-nutritive sweeteners found the overrun content in ice cream was found 47 ± 0.64, pH content was found 6.3 ± 0.1%, acidity content was found 0.27 ± 0.02%, total solid content was found 36.4 ± 0.20%. The results were in close agreement to the results obtained by (M. Anjum Murtaza 2004) [3].

**Table 4:** Proximate composition of probiotic ice cream

| Chemical parameter | Probiotic ice cream |
|---|---|
| Moisture | 60.04 ± 0.48 |
| Fat | 8 ± 0.30 |
| Protein | 3.51 ± 0.62 |
| Ash | 1.8 ± 0.15 |
| Crude fiber | 1.2 ± 0.15 |
| Carbohydrate | 25.45 ± 0.05 |
| Energy value | 187.8 kcal |

Proximate composition of probiotic ice cream containing acid modified *psyllium* husk was observed and it revealed that moisture content in ice cream was found 60.04 ± 0.48, and fat content was found 8 ± 0.30, protein content was found 3.51 ± 0.62, ash content was found 1.8 ± 0.15, crude fiber content was found 1.2 ± 0.15, carbohydrate was found 25.45 ± 0.05 and energy value content was found 187.8 kcal. Probiotic ice cream using acid modified *psyllium* husk decreases the fat, protein, ash, carbohydrate, crude fiber content in ice cream. Result is similar with (Wael F.E *et al.*, 2022) [4].

**Table 5:** Effect of encapsulated acid modified *psyllium* husk with probiotic beads on melting time

| Parameters (%) | Control |
|---|---|
| Melting time (minutes to collect 20 ml of melt) | 47 min |

Melting time of probiotic ice cream sample decreases after acid modification of *psyllium* husk containing different probiotics (*Lactobacillus casei* and *Lactobacillus plantarum*). The melting time of probiotic ice cream was observed 47 min results are closely related with (Ismail E. A. 2013) [5].

**Table 6:** Mineral composition of probiotic ice cream

| Minerals (mg/100 g) | $S_1$ (mg/100 g) |
|---|---|
| Calcium | 223±1.52 |
| Phosphorus | 105±1.20 |
| Potassium | 336±1.52 |
| Iron | 4.95±0.01 |

Mineral composition in probiotic ice cream using acid modified *psyllium* husk was found calcium was found 223±1.52, phosphorus was found 105±1.20, potassium was found 336±1.52, iron 4.95±0.01.

**Table 7:** Viable counts (LAB) of probiotic ice cream during storage

| Time in (weeks) | Viable counts | | |
|---|---|---|---|
| | (cfu/ml) × $10^7$ | (cfu/ml) × $10^8$ | (cfu/ml) × $10^9$ |
| 1 | 3.4 | 2.5 | 1.8 |
| 2 | 4.2 | 2.7 | 2.0 |
| 3 | 4.7 | 3.0 | 2.4 |
| 4 | 4.9 | 3.2 | 2.6 |

**Table 8:** Microbial quality of probiotic ice cream during storage

| Time in (weeks) | Total plate count (cfu/ml) ×$10^7$ | Yeast/Mold (cfu/ml) ×$10^7$ | Coliform (cfu/ml) ×$10^7$ |
|---|---|---|---|
| 1 | 1.9 ×$10^7$ | ND | ND |
| 2 | 2.5 ×$10^7$ | ND | ND |
| 3 | 2.9 ×$10^7$ | 1.2 ×$10^4$ | ND |
| 4 | 3.3 ×$10^7$ | 1.5 ×$10^4$ | ND |

The microbial analysis of probiotic ice cream was found in Table 7 and 8. Probiotic ice cream containing total viable count, total plate count, yeast and mold and coliform count as found during period as per adopted by (Cappuccino and Sherman 1996) [6]. Conclusion the probiotic ice cream can be prepared by incorporation with non-nutritive sweeteners and acid modified *psyllium* husk and probiotic beads using equal amount of 1 percent stater cultures of LAB i.e., *Lactobacillus casei* and *Lactobacillus plantarum*. Viable probiotic count in probiotic ice cream sample is dependent on level of probiotics beads addition, good quality dairy probiotic ice cream can be prepared by encapsulation.

## Conclusion

The present investigation was carried out the incorporation into ice cream and non-nutritive sweeteners and acid modified *psyllium* husk it enhances the health benefits. Probiotic cell as live microorganism when administered in suitable amounts confer a health benefit to the host. Probiotic ice cream was prepared from using ingredients milk, non-nutritive sweeteners, cream, SMP, colour and flavour and encapsulated LAB cultures having ($10^7$, $10^8$, $10^9$ cfu/ml) contains equal proportion of *Lactobacillus casei* and *Lactobacillus plantarum* with 0.65% hydrochloric acid and modified *psyllium* husk. The probiotic ice cream was then stored (-28 °C) overall acceptability of ice cream (8.7) among all samples, the supplemented of ice cream with encapsulated live probiotic cell can improve the organoleptic characteristics of products.

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