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Technology standardizing for probiotic frozen yogurt and deserts using psyllium husk modified by acid

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Abstract
The current study aimed to standardize the partial hydrolysis process of psyllium husk using hydrochloric acid. Psyllium husk was subjected to 0.50%, 0.55%, and 0.60% hydrochloric acid in ethanol at a reaction temperature of 37.5 °C, with three different psyllium husk: solvent ratios (1:3, 1:5, and 1:7 g/ml). Based on the functional properties of psyllium husk after partial hydrolysis, an acid concentration of 0.60% HCl in the psyllium husk: solvent ratio of 1:7 was chosen for incorporation into probiotic frozen yogurt.
Probiotic frozen yogurt were produced utilizing varying amounts of acid modified psyllium husk, specifically 0.5, 0.75, and 1 gm. Encapsulated probiotic culture, with concentrations ranging from 107 to 109 cfu/gm and consisting of equal parts *Lactobacillus acidophilus* and *Lactobacillus lactis*, was incorporated into the mixture. Subsequently, the product was stored under refrigerated conditions at 4 °C for a duration of 8 hours. According to the sensory evaluation conducted using a 9-point hedonic scale, the probiotic frozen yogurt and frozen dessert formulated with 10 percent encapsulated probiotic culture and 0.5 gm of acid modified psyllium husk exhibited the highest level of consumer acceptance compared to the other samples.

Keywords: Psyllium husk, Isabgol, frozen yogurt, LAB, probiotic food

Introduction
Soluble fibers consist of non-cellulosic polysaccharides such as pectin, gums, and mucilage, whereas insoluble fibers primarily comprise cell wall components like cellulose, hemicellulose, and lignin (Yoon *et al.*, 2005) [17]. Chau and Huang (2003) [5] emphasize the significance of food fibres, which has resulted in a substantial and promising market for fibre-rich products and ingredients. In recent years, there has been a push to discover new sources of dietary fibre that can be utilized in the food industry.
Psyllium is cultivated in India for its medicinal properties (Karimzadeh and Omidbaigi, 2004) [8]. Isabgol husk, derived from the Plantago ovata plant, consists mainly of polysaccharide chains with ß-xylan systems. Its primary use is as a bulk-forming agent in constipation, but it also has applications in colorectal cancer, ulcerative colitis, haemorrhoids, diabetes, hypercholesterolemia, and other medical conditions (Sharma and Bhattacharya, 2009) [13]. Psyllium is categorized as a mucilaginous fiber due to its strong capacity to create a gel when mixed with water. Research conducted on both animals and humans has shown that a gel-forming component, comprising approximately 55-60 percent of the husk, is accountable for the laxative and cholesterol-lowering effects of psyllium (Marlett and Fischer, 2002) [10].

Materials and Methods
Raw materials
A local market in the Cuddalore district provided 98% pure indigenous psyllium husk. A 60-mesh sieve was used to screen and grind the husk. Until it was needed, the powder was stored in an airtight plastic container. To make frozen yoghurt, buffalo milk, cream, sugar, skim milk powder, and corn kernel oil were bought at a nearby market in the Chidambaram area.

Reagents and Chemicals
The Department of Microbiology at Annamalai University, Chidambaram, Cuddalore district

provided the necessary chemicals for the processing of raw materials, manufacture, and analysis of formulated goods. Yoghurt starter culture. It comprised of *Lactobacillus delbrueckii* and *Streptococcus thermophilus* respectively. The culture was used to formulate yogurt.

To isolate the probiotic bacteria from food sources

We purchased pickled lemon and buttermilk from the Chidambaram local market. *Lactobacillus acidophilus* and *Lactobacillus lactis*, which would be employed as probiotic cultures in the current study, were isolated using them.

Culture media

The Department of Food Microbiology created a number of culture media, including MRS agar, nutritional agar, nutrient broth, Methyl Red and Vogues-Proskauer (MR-VP) agar, Triple Sugar Iron (TSI) agar, etc.

Quality features of psyllium husk

The quality features of psyllium husk were evaluated based on the technical criteria set by the Bureau of Indian Standards (IS 13662: 2021).

Acid-modified psyllium husk

The acid modification of psyllium husk was carried out following the method described by Syed *et al.*, (2018) [14] with adjustments made to the hydrochloric acid content in the ethanol solvent. Psyllium husks were treated with ethanol (solvent) containing 34-37% hydrochloric acid (HCl) at concentrations of 0.50%, 0.55%, and 0.60% (w/v). The main objective of this research was to investigate how the psyllium-solvent ratio and reaction temperature impacted the physical and biochemical characteristics of the acid-modified psyllium samples. The psyllium husk and solvent ratio of (1:3, 1:5, 1:7) in grams per ml were evaluated at a reaction temperature of 37.5 °C.

A total of 48 grams of psyllium husk was divided into four groups, each consisting of 16 grams of psyllium husk, to undergo treatments with varying concentrations of hydrochloric acid in ethanol solvent (0.50%, 0.55%, and 0.60% w/v). Each group had four samples with different psyllium-solvent ratios. Following the addition of the solvent, the samples were left to incubate for 48 hours at 37.5 °C. Subsequently, the samples were vacuum filtered washed twice with 95% ethanol and once with 100% ethanol, dried, and stored. The control group was treated with 100% ethanol and underwent the same experimental procedures.

Table 1: Acid modification treatments for psyllium husk

Concentration of HCl in Ethanol (%)	Psyllium Husk: Solvent Ratio (w/v)
0.50	1:3, 1:5 and 1:7
0.55	1:3, 1:5 and 1:7
0.60	1:3, 1:5 and 1:7
Control	1:3, 1:5 and 1:7

Production of probiotic frozen yogurt involves the utilization of both native and acid modified psyllium husk.

Yogurt samples were created using fresh buffalo milk based on the methods outlined by Bhat *et al.*, (2017) [4]. The fresh raw buffalo milk was divided into four equal portions, with three of them having 0.5, 0.75, and 1.0 % psyllium husk added. The batch without any additional fiber served as the control. The formulation for yogurt preparation can be found in Table 2. Homogenization of all batches took place at 60 °C using a two-stage homogenizer with pressures of 150 kg/cm2 and 50 kg/cm2 in the first and second stages, respectively. Following this, all batches were heated in a water bath at 95 °C for 10 minutes, cooled to around 42 °C, inoculated with 2 % starter cultures, transferred to plastic cups, and then incubated at 42 °C for 6 hours. The samples were refrigerated at 4 °C overnight before analysis.

Table 2: Formulation of frozen yogurt by using native and modified psyllium husk

Contents	Control	Native Psyllium Husk			Modified Psyllium Husk		
		YN_1	YN_2	YN_3	YM_1	YM_2	YM_3
Milk (ml)	90	89.5	89.25	89	89.5	89.25	89
Sugar (gm)	8	8	8	8	8	8	8
Starter Culture (ml)	2	2	2	2	2	2	2
Psyllium Husk (gm)	0	0.5	0.75	1.0	0.5	0.75	1.0

Control = Probiotic frozen yogurt without addition of psyllium husk

YN_1 = Probiotic frozen yogurt added with 0.5 gm native psyllium husk

YN_2 = Probiotic frozen yogurt added with 0.75 gm native psyllium husk

YN_3 = Probiotic frozen yogurt added with 1.0 gm native psyllium husk

YM_1 = Probiotic frozen yogurt added with 0.5 gm modified psyllium husk

YM_2 = Probiotic frozen yogurt added with 0.75 gm modified psyllium husk

YM_3 = Probiotic frozen yogurt added with 1.0 gm modified psyllium husk

Results and Discussion

Evaluation of the utilization of psyllium husk as prebiotic agents.

Prebiotics are indigestible compounds that stimulate the host by promoting the growth or activity of a select few beneficial bacteria. The curd underwent serial dilution with *Lactobacillus acidophilus*, *Lactobacillus lactis*, and varying levels of psyllium husk. Dilution aliquots ranging from 10^7 to 10^9 were plated on MRS growth media and then cultured at 37 °C for 48 hours to assess the potential of psyllium husk as a prebiotic. The microbial count increased gradually with higher concentrations of psyllium husk, as indicated.

Encapsulation of probiotic culture

The encapsulation of probiotic cultures was achieved through the extrusion process, which is a widely utilized method for producing hydrocolloid capsules (King, 1995) [9]. This technique is characterized by its cost-effectiveness and user-friendliness, employing gentle operations that minimize cell mortality and enhance the survival rate of probiotic microorganisms

The feasibility of probiotic culture encapsulation in acidic environments

Sohail *et al.*, (2011) [15] found that encapsulating probiotic bacteria in crosslinked alginate beads is beneficial for enhancing survival in harsh acid and bile conditions. Furthermore, the addition of psyllium husk to alginate beads

improved the viability of probiotic culture in acidic conditions, with a more pronounced effect as the psyllium husk content increased. The increase in polymer content due to the addition of psyllium husk provides greater protection for the cells against acidic conditions. Albertini *et al.*, (2010) [3] also observed that adding XG or CAP to a 3 per cent (w/v) alginate solution enhanced the survivability of probiotic bacteria in acidic environments.

Sensory evaluation of probiotic frozen yogurt

The sensory attributes of probiotic frozen yogurt play a vital role in enticing consumers to purchase the product. Factors such as color, flavor, taste, and texture are utilized by consumers to evaluate the quality of probiotic frozen yogurt. A 9-point Hedonic scale was employed to measure sensory perception. The acceptability of the probiotic frozen yogurt was determined based on its color, flavor, taste, and texture. Table 3 provides a summary of the sensory characteristics of probiotic frozen yogurt. Overall acceptability is influenced by various organoleptic quality factors, including color, flavor, taste, and texture, reflecting the cumulative perception and acceptance of the sensory panelists.

Table 3: Sensory evaluation of probiotic frozen yogurt

Sample	Colour	Flavour	Taste	Texture	Overall Acceptability
Control	8.0	8.2	8.5	8.2	8.1
YN_1	7.9	7.6	7.9	7.7	7.7
YN_2	7.8	7.8	7.7	7.5	7.6
YN_3	7.7	7.3	7.2	7.2	7.4
YAM_1	8.6	8.4	8.8	8.6	8.7
YAM_2	8.5	7.9	8.4	8.3	8.5
YAM_3	8.0	7.5	7.4	7.5	7.6
SE ±	0.04	0.06	0.04	0.09	0.10
CD at 5%	0.12	0.18	0.13	0.27	0.30

Conclusion

The results of this study indicate that both native and modified psyllium husks are suitable for creating fiber-rich value-added food products. Additionally, the physical, chemical, sensory, and functional characteristics of these psyllium husk-enriched food items can be improved by treating them with a 0.60 percent hydrochloric acid (HCl) solution in an ethanol solvent ratio of 1:7.

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