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Studies on Development of Fibre Rich Probiotic Frozen Yogurt

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ABSTRACT

Background: Yogurt is one of the most popular fermented dairy products worldwide which has great consumer acceptability due to its health benefits other than its basic nutrition. Yogurt is a rich source of calcium in bio-available form and also acts as a probiotic carrier food. Yogurt is reported to be beneficial for the treatment of Inflammatory Bowel Disease (IBD) that includes gastrointestinal disorders. Because of these known health benefits of yogurt, consumer demand for yogurt and yogurt related products has been increased and became the fastest growing dairy category in the global market. Notably, India ranks first in psyllium husk (isabgol) production and is the sole supplier of seeds and husk in the international market. Among medicinal plants, psyllium 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. The psyllium is high in soluble fibre content with detoxing effect over digestive system makes it a very apt nutraceutical.

Methods: The present investigation indicates utilization of acid modified psyllium husk for the preparation of probiotic frozen yogurt. The prepared probiotic frozen yogurt was analyzed for sensorial, physicochemical and microbial quality parameters. Probiotic frozen yogurt was prepared from 1 liter buffalo milk, 10 gm sugar and 0.5 gm of acid modified psyllium husk (0.60% HCl in the ethanol solvent with ratio of 1:7). The probiotic culture (10^7, 10^8, 10^9 cfu/gm) containing equal proportions of *Lactobacillus acidophilus* and *Lactobacillus plantarum* were added in encapsulated form. It was then stored at refrigerated conditions at 4°C for 8 hours.

Result: The organoleptic evaluation of probiotic frozen yogurt was carried out. As per the score of 9-point hedonic scale, probiotic frozen yogurt prepared with 10 per cent encapsulated probiotic culture (10^9 cfu/gm and 5 per cent each of *Lactobacillus acidophilus* and *Lactobacillus plantarum*) and 0.5 gm of acid modified psyllium husk had shown maximum consumer acceptability (8.7) among all samples.

Key words: *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, Modified psyllium husk, Probiotic frozen yogurt, *Streptococcus thermophilus*.

INTRODUCTION

In India, the dairy sector is one of the most important sector. Exports were produced to 105 different nations throughout the world. Dairy farming is an important source of income for India's rural economy. At the national level, this industry accounts for around 17% of overall agricultural production, contributing around 8% to GDP and putting the Indian milk sector in first place (Samal and Pattanaik, 2014).

The word yogurt comes from Turkish work "yogurt" and refers to a tart, thick milk. In 1981, the FDA defined fresh, prepared yogurt in the US and stated that it must include *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yogurt is one of the most popular fermented dairy products worldwide which has great consumer acceptability due to its health benefits other than its basic nutrition. Yogurt is a rich source of calcium in bio-available form and also acts as a probiotic carrier food (Mckinley, 2005, Lourens and Vilijoen, 2001).

Yogurt is reported to be beneficial for the treatment of Inflammatory Bowel Disease (IBD) that includes gastrointestinal disorders such as Crohn's disease, ulcerative colitis and pouchitis (Mckinley, 2005). Because of these known health benefits of yogurt, consumer demand for yogurt and yogurt related products has been increased and became the fastest growing dairy category in the global market. Yogurts are now being manufactured in a numerous styles and varieties with different fat contents, flavors and textures suitable for different meal occasions and plates as a snack, dessert, sweet or savory food.

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Plantago ovata commonly known as 'Psyllium' in English and 'Isabgol' in Hindi belongs to the family of *Plantaginaceae*, is a 10-45 cm short-stemmed annual herb known by different names such as *ashwagolam, aspaghol, aspagol, blond Psyllium*. Isabgol has high fibre content and acts like a sponge serving to clean the bowels and is extensively cultivated in many parts of the globe. It is commercially an important Rabi season crop known for its medicinal properties. Apart from its husk (The seed coat is known as "husk") it is also being used in food industry especially in ice creams, biscuits and candies. The crop is mainly cultivated in the states of Rajasthan, Gujarat, Haryana and Madhya Pradesh.

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 fibre content with detoxing effect over digestive system makes it a very apt nutraceutical.

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 (s) 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).

MATERIALS AND METHODS

The present study was carried out at Department of Food Microbiology and Safety, College of Food Technology, VNMKV, Parbhani, Maharashtra during academic year 2020-2021.

Procurement of raw materials

Psyllium husk, buffalo milk, skim milk, skim milk powder, cream, sugar and cardamom were purchased from local market.

Microbial culture

Lactobacillus bulgaricus (NCIM-2056), *Streptococcus thermophilus* (NCIM-2412), *Lactobacillus acidophilus* (NCIM-2903) and *Lactobacillus plantarum* (NCIM-2083) respectively were procured from National Chemical Laboratory (NCL), Pune.

Probiotic culture

The probiotic organisms *viz. Lactobacillus bulgaricus* (NCIM-2056), *Streptococcus thermophilus* (NCIM-2412), *Lactobacillus acidophilus* (NCIM-2903) and *Lactobacillus plantarum* (NCIM-2083) 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 combination of sodium alginate and guar gum at 1 and 0.8 per cent (w/v) respectively, 10 ml of inoculum (5 ml each of *L. acidophilus* and *L. plantarum*) was mixed in 0.5 gm of modified psyllium husk powder. Probiotic culture and modified psyllium husk powder normal mixed properly and passed through a syringe in the form of droplets into 0.3M calcium chloride solution. Interaction between the two solutions led to forknown as "husk") it is also being used in food industry mations of beads (2-5 mm) and the resulting beads were then stored in 0.1 per cent peptone solution (Karthikeyan *et al.,* 2014).

Acid modification of psyllium husk

Acid modification 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 modified psyllium 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 and followed the steps of preparation mentioned above Table 1.

Table 1: Acid treatment levels for psyllium husk.

Concentration of HCl in ethanol solvent	Psyllium husk (pH): solvent ratio
0.50%	1:3, 1:5 and 1:7 (w/v)
0.55%	1:3, 1:5 and 1:7 (w/v)
0.60%	1:3, 1:5 and 1:7 (w/v)
Control	1:3, 1:5 and 1:7 (w/v)

Preparation of probiotic frozen yogurt by using modified psyllium husk

Yogurt was prepared by adding native psyllium husk powder as well as its hydrolysates to claim the health benefits. The flow chart of the method followed is as:

Buffalo Milk
↓
Cream separation
↓
Skim milk (Standardized SNF 8.5%)
↓
Added with 8% sugar in powder form
↓
Mixed well
↓
Heated at 95°C for 10 min
↓
Cooled to 42°C
↓
Starter added @ 2% (109 cfu/ml)
[1:1 ratio of *L. bulgaricus* (NCIM-2056)
and *S. thermophilus* (NCIM-2412)]
↓
Incubated at 42°C for 6 hours
↓
Addition of encapsulated psyllium husk powder
hydrolysate (0.5, 0.75 and 1.0%)
↓
Addition of probiotic starter culture 10% (109 cfu/ml)
[1:1 ratio of *L. acidophilus* (NCIM-2903)
and *L. plantarum* (NCIM-2083)]
↓
Blended with flavour and color
↓
Packaged and stored at 4°C

Flow Sheet 1: Preparation of probiotic frozen yogurt.

Proximate composition

Proximate composition such as moisture, fat, crude protein, ash and crude fibre were determined as per A.O.A.C. (1995) and carbohydrate by difference method.

Dietary fibre

Determination of dietary fibre was done using the method described by Prosky *et al.* (1992)

Functional properties

Oil absorption and hydration capacities were determined by the method of Rosario and Flores (1981). Water uptake rate was determined by the method described by Yu and Perret (2003).

RESULTS AND DISCUSSION

Proximate analysis and mineral composition of native psyllium husk

The results shown in Table 2 revealed that the moisture content of native psyllium husk was 7.18 per cent on whole weight basis, fat content was 1.85 per cent and protein content was 2.94 per cent. It is clear from the Table 2 that ash and total carbohydrate content of native psyllium husk were found to be 2.61 per cent and 85.42 per cent respectively. Calculated energy value was found 372 Kcal/ 100 g. The results were in close agreement with the finding of Guo *et al.* (2008). It is revealed from Table 3 that Iron and Copper content of native psyllium husk was found to be 8.00 mg/100 g and 0.675 mg/100 g respectively while manganese and zinc content was found to be 0.600 mg/100 g and 0.325 mg/100 g. Iron content was found highest among the minerals assessed.

Functional properties of native psyllium husk

The data presented in Table 4 shows the various functional properties of native psyllium husk. The hydration capacity of native psyllium husk was found to be 3.1 ml/g while the oil absorption capacity was found 1.1 ml/g. However, it is clearly revealed from Table 4 that water up-taking rate of native psyllium husk was found to be 2.22 mg/g × min Kamaljit *et al.* (2011) reported similar findings for native psyllium husk functional properties as an ingredient for high fibre bread.

Table 2: Proximate composition of psyllium husk.

Parameters (%)	Native psyllium husk (NPH)
Moisture	7.18
Fat	1.85
Protein (N×6.25)	2.94
Ash	2.61
Carbohydrate	85.42
Dietary fibre	76.66
Energy value	372 Kcal/100 g

Table 3: Mineral composition of psyllium husk.

Parameters (mg/100 g)	Native psyllium husk (NPH)
Iron (Fe)	8.00
Copper (Cu)	0.675
Manganese (Mn)	0.600
Zinc (Zn)	0.325

Table 4: Functional properties of native psyllium husk.

Parameters	Native psyllium husk (NPH)
Hydration capacity (ml/g)	3.1
Oil absorption capacity (ml/g)	1.1
Water up-taking rate [mg/(g×min)]	2.22

Effect of acid modification on functional properties of psyllium husk

It is revealed from the Table 5 that the hydration capacity of psyllium husk was decreased with the increased level of acid concentration used for treatment from 2.88 to 1.62 ml/g. Significant decrease in hydration capacity were observed in case of psyllium husk sample treated with 0.60% acid concentration having lowest 1.62 ml/g for 1:7 ratio. It can be observed from the Table 5 that the oil absorption capacity of 0.60% acid treated psyllium husk for the psyllium husk: solvent ratio as 1:7 was found to be lowest as 0.50 ml/g. The data from the Table 5 indicates that the OAC of treated psyllium husk decreased with the increased level of acid concentration from 0.91 ml/g to 0.50 ml/g. The data from the Table 5 indicates that water up-taking rate is lowest for 0.60% acid treated psyllium husk for the psyllium husk: solvent ratio as 1:7 sample as 1.68 mg/(g×min).

Based on the results of acid modifications on functional properties of psyllium husk, acid modification concentration of 0.60% HCl in the ethanol solvent for solvent ratio of 1:7 as PH:Solvent ratio, had been selected considering previous reported studies for further studies on its effect on proximate composition and utilization in the probiotic frozen yogurt.

Effect of acid modification on proximate composition of selected acid modified psyllium husk

The results related to the effect of acid modification of the selected concentration of HCl in ethanol *i.e.*, 0.60% (0.60 ml of 34%-37% concentrated hydrochloric acid (HCl) in 100 ml pure ethanol) based on the assessment of functional properties of psyllium husk such as oil absorption, hydration capacity, water uptake rate and swelling capacity on proximate composition of psyllium husk are shown in Table 6. It can be observed from Table 6 that moisture content increased from 7.18 to 7.35 per cent upon acid modification. Fat content decreased after acid modification from 1.85 to 0.62 per cent while protein content decreased from 2.94 to 1.21 per cent. Similarly, ash and protein decreased from 2.61 to 2.24 and 2.94 to 1.21 per cent respectively. The decrease in fat, protein and ash content resulted due to the partial degradation of the psyllium gel hardness because of acid modification. Further, carbohydrate content increased from 85.42 to 88.55 per cent and energy value decreased from 372 to 367 Kcal/100 g.

Effect of acid modification on hardness and adhesiveness of selected acid modified psyllium husk

Gelling properties were analyzed using TA-*XT2 PLUS* Texture Analyzer (Stable Micro System, Surrey, UK) having 60 kg load cell was used for Texture profile analysis (TPA). Measurements were performed with a pretest speed of 2.0 mm/sec, a test speed of 5.0 mm/sec, a post test speed of 5.0 mm/sec and a distance of 6 mm. All measurements were made in triplicate. Two and one-half grams (2.5 g) of psyllium (40 mesh) was added to 50 ml. of distilled water kept for overnight and this was used to compare the gelling and water-absorbing properties of modified psyllium. The results were expressed as in gram force for hardness and adhesiveness. All results are shown in Table 7. Hardness and adhesiveness are the maximum force (g) measured on for peaks of the "texture profile" graph provided according to analytical testing with a texture analyzer. These properties correspond to the first positive peak and the first negative peak. Comparative data of hardness and adhesiveness from Table 7 indicates that there was a substantial decrease in hardness and adhesiveness of the acid modified psyllium husk.

Formulation of probiotic frozen yogurt incorporated with acid modified psyllium husk

The trial formulations of the present investigation are highlighted in Table 8. The formulations of probiotic frozen yogurt were carried out in laboratory, fermented by inoculating 2 per cent starter cultures. For preparation of probiotic frozen yogurt, the quantity of psyllium husk (native and modified) was kept in various proportions *viz.* 0, 0.5, 0.75 and 1.0 per cent respectively.

Effect of psyllium husk on physico-chemical characteristics of probiotic frozen yogurt

The physico-chemical characteristics of psyllium husk added probiotic frozen yogurt is highlighted in the Table 9. It can

Table 5: Effect of acid modification on functional properties of psyllium.

Functional properties	Psyllium husk: Ethanol ratio	Concentration of HCl in ethanol (%)			
		Control	0.50	0.55	0.60
Hydration capacity (ml/g)	1:3	2.88	2.70	2.50	2.12
	1:5	2.83	2.65	2.38	1.82
	1:7	2.79	2.62	2.29	1.62
Oil absorption capacity (ml/g)	1:3	0.91	0.80	0.63	0.52
	1:5	0.89	0.73	0.60	0.51
	1:7	0.84	0.61	0.54	0.50
Water up-takingrate [mg/(g×min)]	1:3	2.11	1.90	1.81	1.73
	1:5	2.01	1.88	1.78	1.71
	1:7	1.92	1.85	1.76	1.68
SE±		0.37	0.02	0.17	0.16
CD @ 5%		1.11	0.07	0.50	0.49

be observed from Table 9 that the probiotic frozen yogurt containing acid modified psyllium husks had higher values for total soluble solids contents than probiotic frozen yogurt containing native psyllium husks for the corresponding addition levels of psyllium husk as 0.5, 0.75 and 1 per cent respectively. The pH was found higher in probiotic frozen yogurt prepared by addition of native psyllium husks as 0.5, 0.75 and 1 per cent followed by the probiotic frozen yogurt prepared by addition of acid modified psyllium husks as 0.5, 0.75 and 1 per cent respectively. Moreover, it can be observed from Table 9 that the probiotic frozen yogurt containing native psyllium husks had higher values for acidity contents than probiotic frozen yogurt containing acid modified psyllium husks for the corresponding addition levels of psyllium husk as 0.5, 0.75 and 1 per cent respectively.

Table 6: Effect of acid modification on proximate composition of psyllium husk.

Particulars (g/100 g)	Modified psyllium husk (MPH)
Moisture	7.35
Fat	0.62
Protein	1.21
Ash	2.24
Carbohydrate	88.55
Dietary fibre	79.66
Energy value	367 Kcal/100 g

Table 7: Effect of acid modification on hardness and adhesiveness of selected acid modified psyllium husk.

Particulars	Hardness (g)	Adhesiveness (-g)
Native psyllium husk	88.0	11.0
Modified psyllium husk	55.0	9.0

It was observed that even though there was increase in moisture with the increase in level of psyllium husk addition in the probiotic frozen yogurt but high rate was found in case of native psyllium husk incorporated probiotic frozen yogurt, while in case of modified psyllium husk, due to decrease in hydration capacity, moisture increase rate was less than native psyllium husk. Significant decreasing trend was found from the data of the fat and protein percentage of probiotic frozen yogurt for the various treatments. As noted earlier in the comparative proximate composition, modified psyllium husk had slightly higher dietary fibre content than native pH, which reflected in the carbohydrate contents of the probiotic frozen yogurt. Due to increased psyllium husk level as a compositional constituent, fibre provides sufficient amount of ash to the recipe resulted in the momentous increase of ash in various treatments.

Sensory evaluation of probiotic frozen yogurt

The sensorial quality characteristics of probiotic frozen yogurt play a vital role in attracting consumers to purchase the product. Sensorial evaluation was done using 9-point Hedonic scale (9-Like Extremely and 1-Dislike Extremely). The probiotic frozen yogurt was evaluated for acceptability in terms of characteristics such as colour, flavour, taste and texture. The sensorial characteristics of probiotic frozen yogurt are summarized in Table 10. Overall acceptability is based on multiple organoleptic quality parameters *i.e.*, colour, flavour, taste, texture *etc.* and shows the accumulative perception and acceptance by the sensory panelists. The maximum score for overall acceptability was recorded in sample YM_1 (8.7) added with 0.50% acid modified psyllium husk.

Effect of storage on physico-chemical parameters of probiotic frozen yogurt

The effect of storage for a period of 21 days on the physico-chemical characteristics were assessed in terms of pH and Titrable acidity. The results are summarized in the Table 11.

Table 8: Standardization of the use of psyllium husk.

Samples	Buffalo milk (liter)	Psyllium husk (gm)	Sugar (gm)	Starter culture (v/v)	Probiotic culture (v/v)
Control	1	0	10	2%	10%
N_1	1	0.5	10	2%	10%
N_2	1	0.75	10	2%	10%
N_3	1	1.0	10	2%	10%
M_1	1	0.5	10	2%	10%
M_2	1	0.75	10	2%	10%
M_3	1	1.0	10	2%	10%

Where,
Control= Sample without addition of psyllium husk.
N_1= Addition of 0.5 g native psyllium husk.
N_2= Addition of 0.75 g native psyllium husk.
N_3= Addition of 1.0 g native psyllium husk.
M_1= Addition of 0.5 g modified psyllium husk.
M_2= Addition of 0.75 g modified psyllium husk.
M_3= Addition of 1.0 g modified psyllium husk.

Table 9: Effect of psyllium husk on physico-chemical characteristics of probiotic frozen yogurt.

Samples	Physical parameters				Chemical parameters			
	TSS (°Brix)	pH	Acidity (%)	Moisture (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Ash (%)
Control	19	4.2	1.02	38.80	11.49	6.69	42.31	0.71
YN_1	21	4.3	1.25	40.50	10.80	5.53	42.42	0.75
YN_2	24	4.6	1.20	42.50	8.20	4.98	43.50	0.82
YN_3	28	4.7	1.19	45.20	7.63	3.69	42.63	0.85
YM_1	24	4.2	1.21	39.30	11.00	5.70	43.27	0.73
YM_2	27	4.3	1.17	41.40	8.45	5.10	44.24	0.81
YM_3	30	4.5	1.14	43.10	7.84	3.90	44.33	0.83
SE±	1.55	0.60	0.26	1.25	0.72	0.36	1.53	0.03
CD at 5%	4.64	1.81	0.77	3.74	2.16	1.08	4.59	0.08

Where,

Control = Probiotic frozen yogurt without addition of psyllium husk.

YN_1 = Probiotic frozen yogurt with addition of 0.5 g native psyllium husk.

YN_2 = Probiotic frozen yogurt with addition of 0.75 g native psyllium husk.

YN_3 = Probiotic frozen yogurt with addition of 1.0 g native psyllium husk.

YM_1 = Probiotic frozen yogurt with addition of 0.5 g modified psyllium husk.

YM_2 = Probiotic frozen yogurt with addition of 0.75 g modified psyllium husk.

YM_3 = Probiotic frozen yogurt with addition of 1.0 g modified psyllium husk.

Table 10: Sensory evaluation of probiotic frozen yogurt.

Samples	Colour	Flavour	Taste	Texture	Overall acceptability
Control	8.0	8.4	8.5	8.2	8.1
YN_1	7.9	7.6	7.9	7.7	7.7
YN_2	8.2	8.0	8.3	8.2	8.3
YN_3	7.9	7.3	7.2	7.2	7.4
YM_1	8.6	8.2	8.8	8.6	8.7
YM_2	8.5	7.9	8.4	8.3	8.5
YM_3	8.0	7.5	7.4	7.5	7.6
SE±	0.49	0.40	0.46	0.53	0.42
CD at 5%	1.46	1.19	1.38	1.60	1.27

The data from Table 11 reveals that the pH of accepted probiotic frozen yogurt sample was found to be 4.20, 4.18, 4.15 and 4.12 on the day of preparation, seventh, fourteen and twenty first days after preparation respectively. This may be due to the conversion of lactose into lactic acid and other organic acids by the starter cultures that reduced the pH of yogurt. Data indicated that the storage time significantly affected the acidity level in the probiotic frozen yogurt; titrable acidity increased, while the pH was decreased.

Table 11: Effect of storage on physico-chemical parameters of probiotic frozen yogurt.

Storage time (in days)	pH	Titrable acidity (%)
0	4.20	1.014
7	4.18	1.016
14	4.15	1.018
21	4.12	1.020

Viable counts (LAB) present in probiotic frozen yogurt during storage

The total viable counts of probiotic frozen yogurt were observed at different time interval during storage period on MRS agar media. It is evident from the Table 12 that, the viable counts were observed at dilution rate of 10^7, 10^8 and 10^9. Cell viability on MRS agar plates in 1st week and 4th week of storage are shown in Table 12. A minimum range of 10^6-10^7 plate microorganisms per gram or milliliter should

Table 12: Viable counts (LAB) of probiotic frozen yogurt during storage.

Time (weeks)	Viable counts		
	(cfu/gm)×10^7	(cfu/gm)×10^8	(cfu/gm)×10^9
1	3.1	2.0	1.3
2	4.0	2.5	1.7
3	4.5	2.7	2.0
4	4.7	2.8	2.0

Table 13: Microbial quality of probiotic frozen yogurt.

Storage period	Microbial quality		
(days)	Total plate count (cfu/g)×10^8	Yeast and mold (cfu/g)×10^4	Coliform count
0	2.20	ND	ND
7	2.60	1.50	ND
14	2.90	1.20	ND
21	3.10	1.00	ND

Table 14: Textural profile analysis (TPA) of probiotic frozen yogurt.

Samples	TPA				
	Hardness (g)	Cohesiveness	Adhesiveness (g)	Springiness (m)	Gumminess (g)
Control	52.60	0.403	-1.38	1.021	26.58
YM_1	61.30	0.414	-1.43	1.028	25.38
SE±	0.487	0.002	0.022	0.002	0.434
CD at 5%	1.614	0.006	0.073	0.006	1.439

be present in food product in order to meet the requirements of a probiotic food, as recommended by the Japanese Fermented Milk and Lactic Acid Bacteria Drinks Association (Ishibashi and Shimanura, 1993). Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002 and Ganguly *et al.,* 2011).

Microbial quality of probiotic frozen yogurt

The accepted probiotic frozen yogurt sample was subjected to microbial studies for total plate count, yeast and mould count and *Coliform* count during the storage period as per the method adopted by Cappuccino and Sherman (1996). The results recorded are presented in Table 13. It is evident from the Table 13 that the total plate count was the highest in probiotic frozen yogurt after 21 days of storage period. However, the total plate count (TPC) was lowest in the modified psyllium husk added probiotic frozen yogurt sample on the preparation day. The yeast and mold count observed on Potato Dextrose Agar, increased gradually with storage period. The probiotic frozen yogurt sample was free from *Coliform* and *E. coli* when the sample was fresh and throughout the storage period of 21 days at refrigerator temperature of 4°C as result of good hygienic and sanitary conditions, during the preparation of probiotic frozen yogurt.

Texture profile analysis of probiotic frozen yogurt

The textural profile analysis (TPA) was conducted for probiotic frozen yogurt samples for all experiments to obtain textural responses *viz.*, hardness, springiness, adhesiveness, cohesiveness and gumminess. The TPA scores are laid down in Table 14. It is revealed from Table 14 that the increase in hardness, cohesiveness, springiness and gumminess of probiotic frozen yogurt with the addition of modified psyllium husk.

CONCLUSION

In the present investigation, sincere efforts have been made to study the effect of native and acid modified psyllium husk and its exploration in probiotic frozen yogurt. At the reaction temperature of 37.5°C, psyllium husk was treated with 0.50%, 0.55% and 0.60% of (34% to 37%) hydrochloric acid (HCl) in ethanol as a solvent in three different psyllium : solvent ratios (1:3, 1:5 and 1:7 g/mL). Based on the results of acid modifications on functional properties of psyllium husk, acid modification concentration of 0.60% HCl in the ethanol solvent ratio of 1:7 was selected to be incorporated in probiotic frozen yogurt.

Probiotic frozen yogurt was prepared from 1 liter buffalo milk, 10 gm sugar and 0.5 gm of acid modified psyllium husk (0.60% HCl in the ethanol solvent with ratio of 1:7). The probiotic culture (10^7, 10^8, 10^9 cfu/gm) containing 5 percent equal proportions of *Lactobacillus acidophilus* and *Lactobacillus plantarum* were added in encapsulated form. It was then stored at refrigerated conditions at 4°C for 8 hours. The organoleptic evaluation of probiotic frozen yogurt was carried out. As per the score of 9-point hedonic scale, probiotic frozen yogurt prepared with 10 per cent encapsulated probiotic culture (10^9 cfu/gm and 5 per cent each of *Lactobacillus acidophilus* and *Lactobacillus plantarum*) and 0.5 gm of acid modified psyllium husk had shown maximum consumer acceptability (8.7) among all samples.

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