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Studies on Process Standardization of Probiotic *Kulfi* by using Encapsulated Modified Psyllium Husk

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A B S T R A C T

In the present investigation efforts were made for utilization of encapsulated lactic acid bacteria culture *(Lactobacillus acidophilus, Lactobacillus bulgaricus)* and modified psyllium husk for the preparation of probiotic *kulfi*. The prepared probiotic *kulfi* was analyzed for sensorial, physicochemical and microbial quality parameters. Probiotic *kulfi* was prepared from 1000 ml of milk, 200 gm cream (containing 25 percent fat), 130 gm sugar, 0.5 percent flavour and encapsulated LAB culture having (10^7, $10^8$and 10^9 cfu/gm containing equal proportions of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*) with 0.65 percent hydrochloric acid modified psyllium husk. The probiotic *kulfi* was then stored at refrigerated conditions to freezing (-8˚C to -10˚C for 12 hrs) and hardening (-18˚C to -20˚C for overnight). The probiotic *kulfi* was then stored at refrigerated conditions (-18˚C). The organoleptic evaluation of probiotic *kulfi* was carried out. As per the score of hedonic scale, *kulfi* with encapsulated 10 percent probiotic culture (10^9 cfu/gm) with 0.65 percent hydrochloric acid modified psyllium husk had shown maximum consumer acceptability (8.8) among all samples.

Introduction

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 fiber 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 flavor's used in modern medicine. In India Gujarat and Rajasthan states are the major producer states of psyllium. Psyllium husk is obtained from genus Plantago. The psyllium is high in soluble fiber content with detoxing effect over digestive system makes it a very apt nutraceutical.

The dairy industry is one of the largest industries in India. The exports were made to 105 countries in the world. Dairy production is one of the major sustenance factors for the rural economy of India. At the national level, about 17 percent of the total value of output from agriculture derives from this sector contributing about 8 percent to Gross Domestic Product and placing Indian milk sector in first place (Samal and Pattanaik, 2014). In India about 0.7 percent of the total milk produced is converted into frozen desserts like ice-cream and *kulfi*.

Kulfi is a frozen dairy product made by suitable blending and processing of Symmetric Multiprocessing (SMP) and other milk products, together with sugar and flavour, with or without stabilizer or colour a typical compositional range for the components used in *kulfi* mix is milk fat 10-16 percent, milk solid not fat 9-12 percent, sucrose 9-12 percent, corn syrup solids 4-6 percent, stabilizers/ emulsifiers 0-0.5 percent, total solids 36-45 percent, and water 55-64 percent. *Kulfi* also known as Malai *kulfi* /Malai-ka-*burfi* and indigenous frozen dairy product, which closely resembles ice cream in composition. Traditionally *kulfi* is prepared by evaporating sweetened and flavoured milk by slow heating with almost continuous stirring to keep milk from sticking to the bottom of the vessel until its volume is reduced by a half thus concentrating the milk. It has a distinctive taste due to caramelization of lactose and sugar during the lengthy heating process. It comes in various flavors, including cream, rose, mango, cardamom, saffron (kesar) and pistachio, the more traditional flavors, as well as newer variations like apple, orange, strawberry, peanut, and avocado.

Lactic acid bacteria are the most well-known and widely used probiotic bacteria. The lactic acid bacteria are gram-positive, usually non-motile, non-sporulating, catalase negative, cocci or rods. They produce lactic acid as a sole product of fermentative metabolism of carbohydrate substrates.

Most lactobacillus species are homo fermentative, producing mainly lactic acid as metabolic by product but some are hetero fermentative i.e., they produce ethanol, CO2 as well as lactate respectively. Thus, to fulfill the nutritional requirement of probiotic bacteria, prebiotics plays a major role. Probiotics are mainly carbohydrates by nature. According to FAO/ WHO the definition of prebiotics is, "non digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria".

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. Microencapsulation is the process of encasing an active component in a shell and is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influences of specific condition.

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

Procurement of Raw Materials

Psyllium husk, buffalo milk, fresh cream sugar, cardamom and *kulfi* moulds were purchased from local market.

Starter culture

The probiotic organisms viz. *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* 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.

Flow Sheet 1. Preparation of starter culture

Stock culture
↓
Activation of bacterial strains in MRS broth separately at 37°C for 48 hrs
↓
MRS broth containing desired strains
↓
Centrifugation at 4000rpm/10min
↓
Biomass (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. Biocompatibility and flexibility are some of the other specifications of this method (Klien *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 percent (w/v) respectively, 10 ml of inoculum (5 ml each of *L. acidophilus and L. bulgaricus*) was mixed in 2 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 formations of beads (2-5mm) and the resulting beads were then stored in 0.1 per cent peptone (Karthikeyan *et al.,* 2014).

Flow sheet 2. Microencapsulation of Strains

Preparation of polymer solution
↓
Addition of probiotic culture and modified *psyllium* husk powder
↓
Extrusion of the cell- polymer solution into calcium chloride solution
↓
Capsule formation by cross linking
↓
Recovery of capsule and storage in 0.1 percent peptone solution at 4°C

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 as per the results of the research study conducted by the Syed *et al.,* (2018) on the standardization of acid concentration and solvent ratio for modification of psyllium husk. Hence, acid modification with concentration of 0.65 percent HCl in the ethanol solvent for solvent ratio of 1.6 (w/v).

Solvent ratio was carried out to improve functional properties of psyllium husk as required for exploration in the value addition of processed food products. The solvent used for *psyllium* husks treatment was vacuum filtered, rinsed with 95 percent ethanol and 100 percent for 2 times each, then dried and stored. Control group was treated with 100 percent ethanol and followed the steps of preparation as in Table 1.

Standardization of probiotic *kulfi* preparation

The processing methodology standardized by using organoleptic evaluation. The recipe used for preparation of probiotic *kulfi* is mentioned below in Table 2.

Preparation of probiotic *kulfi*

Buffalo milk blends was taken for the preparation of kulfi. The required quantity of milks having 6 percent fat was taken in a pan and heated at 50°C of milk, then added cream by 20 percent and sugar by 13 percent. The stabilizer and flavor were added and well mixed. The flavor at the rate 0.05 percent. The prepared kulfi mix were cooled and poured in filled in moulds. The filled moulds were kept for freezing having about -8°C to -10°C at 8 hrs temperature for freezing. It was periodically shaken. After freezing, the moulds were kept in the deep freeze at -18° C to -20°C temperature for overnight for hardening. (Nalkar 2012).

Flow Sheet 3. Preparation of *kulfi*

Milk
↓
Standardization (6 percent fat)
↓
Heating to 50°C
↓
Addition of cream (20 percent by weight)
↓
Addition of sugar @ 13 percent
↓
Addition of flavour @ 0.5 ml/lit
↓
Mixing
↓
Condensing 50 percent of original volume
↓
Cooling to room temperature
↓
Mixing and transferring into moulds
↓
Freezing (-8°C to -10°C) at 8 hrs
↓
Kulfi

Preparation of probiotic *kulfi* with encapsulation

For preparation of probiotic *kulfi* with encapsulated strains, inoculum at 10 percent of the final *kulfi* was encapsulated modified *psyllium* husk with probiotic beads and the beads were aseptically added to 100 gm *kulfi*. The probiotic *kulfi* was then stored at refrigerated conditions to hardening (-18°C to -20°C for 12 hrs to 24 hrs).

Flow Sheet 4. Probiotic *kulfi* with Encapsulation

Kulfi
↓
Washing of the probiotic beads
↓
Addition of encapsulated modified psyllium husk with probiotic beads
↓
Incubation at 37°C for 5 hrs
↓
Freezing (-8°C to -20°C/ 8 hrs)
↓
Hardening (-18° C to -20°C / overnight)
↓
Probiotic kulfi

Results and Discussion

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 as per the results of the research study conducted by the Syed *et al.*(2018) on the standardization of acid concentration and solvent ratio for modification of *psyllium* husk (*Plantago ovata F*.) i.e. The solvent used for *psyllium* husks treatment was ethanol with 34 – 37 percent hydrochloric acid (HCl) at the concentration level of 0.65% (w/v). Hence, further studies were conducted to investigate the effects of selected acid solvent ratios at reaction temperature of 37.5°C on physical/chemical/functional properties of the acid treated *psyllium* husk samples. At reaction temperature of 37.5°C *psyllium* husk –solvent ratios (PSH. Solvent @ 1:6 (w/v)) was tested. After the desired time completion of 48hrs for specified acid concentration and PSH and acid solvent ratio. The acid treated psyllium husk product was recovered by vacuum filtration.

It is revealed from the Table 6 that the hydration capacity of *psyllium* husk was decreased with the acid concentration treatment from 3.0 to 1.6 ml/g. Substantial decrease in hydration capacity was observed in case of PSH sample treated with 0.65 percent acid concentration having lowest 1.6 ml/g while control sample having 2.8 ml/g. It can be observed from the Table 6 that the oil absorption capacity of 0.65 percent acid treated psyllium husk for the PSH. solvent ratio @ 1:6 was found to be lowest as 0.5ml/g, indicating that the OAC of treated psyllium husk decreased with the acid treatment from 1.0 ml/g (native PSH) to 0.5 ml/g. According to Oladele and Aina (2007), the major chemical component affecting OAC is protein, which is composed of both hydrophilic and hydrophobic parts. Higher OAC might be due to the partial denaturation of proteins with exposition of high hydrophobic proteins which show superior binding to hydrocarbon chains of lipids. The effects of psyllium husk - solvent ratio (1:6) and acid concentration on the water up-taking rate of psyllium samples was also investigated

at a reaction temperature of 37.5^{0}C. The data from the Table 6 indicates that the water up-taking rate is lowest for 0.65 percent acid treated psyllium husk for the PSH. solvent ratio @ 1:6 sample as 1.63 mg/(g×min). Moreover, substantial water up-take rate reduction was observed between the raw psyllium husk sample and PSH treated with 0.65 percent acid concentration for PSH. solvent ratio @ 1:6 as 2.22 mg/(g×min) and 1.63 mg/(g×min) respectively.

Table.1 Acid treatment levels for psyllium husk

Concentration of HCl in Ethanol	Psyllium Husk (PSH). Solvent Ratio
0.65 %	1.6 (w/v)
0 % for Control	1.6 (w/v)

Table.2 Standard recipe for preparation of probiotic *kulfi*

Ingredients	Quantity (g/ml)
Milk	1000
Sugar	130
Cream containing 25 % fat	200
Flavor	0.05 %

Table.3 Standardization of recipe with different viable counts of cultures

Samples	Inoculum %	Viable count (cfu/g)
MK	0	0
A	10	10^7
B	10	10^8
C	10	10^9

MK - Milk *kulfi* without culture addition
A - *Kulfi* + encapsulated modified *psyllium* husk with 10 percent probiotic culture having (10^7) cfu/gm
B - *Kulfi* + encapsulated modified *psyllium* husk with 10 percent probiotic culture having (10^8) cfu/gm
C - *Kulfi* + encapsulated modified *psyllium* husk with 10 percent probiotic culture having (10^9) cfu/gm

Table.4 Quality characteristics of *psyllium* husk

Parameters	Results
Colour	White or pale buff
Appearance	Translucent
Taste	Bland Mucilaginous
Flavour	Odourless
Total ash (w/w)	2.60 ± 0.03
Acid insoluble ash(w/w)	0.28 ± 0.004
Swell volume(ml/gm)	40.50
Loss on drying(w/w)	7.21 ± 0.02
Light extraneous matter (w/w)	4.95 ± 0.03
Heavy extraneous matter (w/w)	1 ± 0.03

Table.5 Mineral composition of native psyllium husk

Parameters	Results (mg/100 g)
Iron (Fe)	**7.99 ± 0.01**
Copper (Cu)	**0.672 ± 0.04**
Manganese (Mn)	**0.600 ± 0.001**
Zinc (Zn)	**0.322 ± 0.002**

Table.6 Effect of acid modification on functional properties of *psyllium* husk

Concentration of HCl in Ethanol	Psyllium Husk : Solvent Ratio	Hydration capacity (ml/g)	Oil absorption capacity (ml/g)	Water up-taking rate (mg/(g×min))
Control	1:6	2.8	0.8	**1.88**
0.65%	1:6	1.6	0.5	**1.63**
Native Psyllium Husk	**--**	**3.0**	**1.0**	**2.22**

Table.7 Effect of acid modification on proximate composition of *psyllium* husk

Parameters (%)	Native psyllium husk	Modified psyllium husk
Moisture	7.15	**7.32**
Fat	1.82	**0.63**
Protein (N x 6.25)	2.91	**1.20**
Ash	2.61	**2.23**
Carbohydrate	86.48	**88.95**
Crude fibre	3.10	**2.65**
a) Dietary fibre	75.59 ± 0.26	**77.65 ± 0.82**
b) Arabinoxylan	46.20 ± 0.21	**47.80 ± 0.48**
Energy value (Kcal / 100g)	**371 Kcal/100g**	**366 Kcal/100g**

Table.8 Sensory evaluation of the prepared encapsulated probiotic *kulfi*

Samples	Colour	Flavour	Taste	Texture	Overall acceptability
MK	7.8	7.6	7.6	7.5	**7.7**
A	8.2	7.8	7.5	7.6	**7.9**
B	8.4	8.4	8.2	8.0	**8.3**
C	**8.5**	**8.8**	**8.8**	**8.5**	**8.8**
SE±	0.0881	0.0816	0.1040	0.0957	**0.0955**
CD at 5%	**0.2640**	**0.2448**	**0.3110**	**0.2871**	**0.02862**

Table.9 Physico-chemical characteristics of probiotic *kulfi*

Samples	Physical parameters		Chemical parameters				
	Total Solid%	Acidity (%)	Moisture (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Ash (%)
Control	33.20	0.18	64.78	8.10	4.50	40.32	**1.34**
Probiotic kulfi	**35.60**	**0.26**	**70.66**	**7.60**	**3.46**	**43.64**	**1.75**

Table.10 Effect of probiotic beads on melting time and hardness of probiotic *kulfi*

Parameters (%)	Control	Probiotic *kulfi*
Melting time (min)	35.40	**39.07**
Hardness (mm/5sec)	**36.95**	**38.50**

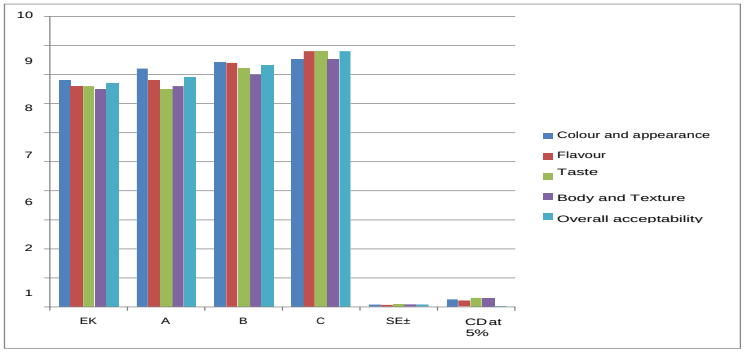
Table.11 Viable counts (LAB) of probiotic *kulfi* during storage

Time in (weeks)	Viable counts		
	$(cfu/gm)x10^7$	$(cfu/gm)x10^8$	$(cfu/gm)x10^9$
1	3.3	2.1	**1.5**
2	4.1	2.6	**1.9**
3	4.6	2.9	**2.1**
4	**4.9**	**3.0**	**2.1**

Table.12 Microbial quality of Probiotic *kulfi* during storage

Time in (weeks)	Total Plate Count $(cfu/g)x10^7$	Yeast and Mold $(cfu/g)x10^4$	Coliform Count
1	2.30	ND	**ND**
2	2.70	1.60	**ND**
3	3.00	1.20	**ND**
4	**3.30**	**1.00**	**ND**

Fig.1 Sensory Evaluation of prepared encapsulated probiotic kulfi using Hedonic Scale



The data for the water up-taking rate showed that acid treatment of PSH at 1:6 @ PSH. solvent ratio highly affects the water up-taking rate, particularly it helps in reduction of water up-taking rate of the psyllium husk. The results for the water up-taking rate are in good agreement with the results found by the Xiaoyin Pei and Liangli Yu (2008) and Zhihong *et al.,* (2009) for water up-taking rate for acid treated PSH. Similar results were also reported by Syed *et al.,* (2018).

It can be observed from Table 7 that moisture content increased from 7.15 to 7.32 percent upon acid modification. Fat content decreased after acid modification from 1.82 to 0.63 percent while protein content decreased from 2.91 to 1.20 percent. Similarly, ash and crude fibre decreased from 2.61 to 2.23 and 3.10 to 2.65 percent respectively. The decrease in fat, protein, ash and crude fibre content resulted due to the partial degradation of the psyllium gel hardness because of acid modification. Further, carbohydrate content increased from 86.48 to 88.95 percent and energy value decreased from 371 to 366 Kcal/100g. The results are in good agreement with results reported by Syed *et al.,* (2018). The results from the Table 14 also indicates that Dietary fibre and Arabinoxylan contents as 75.59 ± 0.26 and 46.20 ± 0.21 percent for native psyllium husk while for acid modified psyllium husk 77.65 ± 0.82 and 47.80 ± 0.48 percent respectively. The results are in good agreement with results reported by Syed *et al.,* (2018). Slight increase in dietary fibre might be due to marginal increase in the total carbohydrate content resulting from the sugar hydrolysis giving by products such as oligosaccharides, and possibly along with acid salts that may form by reaction of psyllium husk components or other reaction by products as reported by the Liangli Yu (2000) in the Patent No. WO1999062342 A9. The acid modified psyllium husk degraded on the external surface structure only due to hydrolysis occurred by excursion, that's why dietary fibre did not affected by the acid treatment. Considering psyllium husk as source of dietary fibre some researchers inferred that arabinoxylan as the active fraction helpful to manage various physiological ailments (Fischer *et al.,* 2004; Saghir *et al.,* 2008; Van-Craeyveld *et al.,* 2008). Guo *et al.,* (2008) explored the chemistry of psyllium husk and noted total carbohydrates up to 84.98 percent considering as dietary fibre.

It is evident from the Table 8 that among various sensory characteristics color, flavor and taste were significantly affected by the various levels encapsulated modified psyllium husk with probiotic culture i.e., *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* ranging from 10^7 to 10^9 cfu/gm and its incubation time period 5hr and after freezing (-8°C to -20°C/8hrs).

The observations in respect of acidity of probiotic *kulfi* as influenced by addition of encapsulated modified *psyllium* husk with probiotic beads. The acidity content of accepted probiotic *kulfi* sample were found to be 0.26 percent the acidity of probiotic *kulfi* remained higher than control. Similarly, the moisture content was 70.66 percent in probiotic *kulfi* and control samples 64.78 respectively. The fat content was lowest in probiotic kulfi and highest in control sample. It was 7.60 percent in probiotic *kulfi* samples and 8.10 percent in control samples. The market samples had lower fat content. The protein content was lowest in samples of probiotic *kulfi*. The protein content of 3.46 percent was, respectively, in control samples 4.50 percent. The results of present study on protein content of market samples as well as in control samples were lower than that of Yarriswamy *et al.,* (1985) but are in fair agreement of PFA (1955) for control samples. The carbohydrate content was lowest in

control samples 40.32 percent and highest in samples of probiotic *kulfi* 43.64 percent (Kumar *et al.,* 2012).

The encapsulated modified *psyllium* husk with probiotic beads on had significant effect on the melting time of the probiotic *kulfi* samples. The melting time for control was found as 35.40 min and probiotic *kulfi* was found as 39.07 min respectively. This showed that with an increase in the level of probiotic *kulfi*, there was significant decrease in melting time of control *kulfi*. This showed improvement in body and texture of *kulfi* due to beads addition. The control *kulfi* sample recorded the hardness as 36.95 mm/5sec.whereas probiotic *kulfi* samples showed hardness as 38.50 mm/5 sec at probiotic beads, it can be observed from the table that with an increase in the level of probiotic beads, there was an increase in the penetration depth values. The results revealed that there was an increase in the penetration depth with an increase in the level of probiotic beads, causing more firmness of the product and presence of large number of viable cells in the treated samples. Shivaprakash (2002) reported that, extent of inoculums has significant effect on hardness of ice cream. Similar observation was made by Salem *et al.,* (2005) and Taha *et al.,* (2007).

A minimum range of 10^7–10^8 plate microorganisms per gram or milliliter should be present in food product in order to meet the requirements of a probiotic food, as by the Japanese Fermented Milk and Lactic Acid Bacteria Drinks Association (Ishibashi and Shimanura 1993).

The results from table shows that, the *kulfi* sample was free from *Coliform* and *E. coli* when the sample was fresh and throughout the storage period of 4 weeks at refrigerator temperature (-18ºC) as result of good hygienic and sanitary conditions, during the preparation.

In conclusion probiotic *kulfi* was prepared from 1000 ml of milk, 200 gm cream (containing 25 percent fat), 130 gm sugar, 0.5 percent flavour and encapsulated LAB culture having (10^7, $10^8$and 10^9 cfu/gm containing equal proportions of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*) with 0.65 percent hydrochloric acid modified psyllium husk. The probiotic *kulfi* was then stored at refrigerated conditions to freezing (-8˚C to -10˚C for 12 hrs) and hardening (-18˚C to -20˚C for overnight). The probiotic *kulfi* was then stored at refrigerated conditions (-18˚C). The organoleptic evaluation of probiotic *kulfi* was carried out. As per the score of hedonic scale, *kulfi* with encapsulated 10 percent probiotic culture (10^9 cfu/gm) with 0.65 percent hydrochloric acid modified psyllium husk had shown maximum consumer acceptability (8.8) among all samples.

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