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Exploration of Encapsulated Modified Psyllium Husk for Preparation of Probiotic *Basundi*

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ABSTRACT

Background: 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. 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. It contains a significant number 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. The psyllium is high in soluble fibre content with detoxing effect over digestive system makes it a very apt nutraceutical. *Basundi* is one of the heats desiccated indigenous products popular in Western part of India, mostly Maharashtra and Gujarat. It can be classified in the condensed milk group along with *rabri, khoa, mithai* and *kheer* and can be considered similar to sweetened condensed whole milk.

Methods: The present investigation indicates utilization of encapsulated lactic acid bacteria culture (*Lactobacillus acidophilus, Lactobacillus bulgaricus*) and modified psyllium husk for the preparation of probiotic *basundi.* The prepared probiotic *basundi* was analyzed for sensorial, physicochemical and microbial quality parameters. Probiotic *basundi* was prepared from 1000 ml of milk, 90 gm sugar, 02-03 pieces of cardamom, saffron 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 per cent hydrochloric acid modified psyllium husk. The probiotic *basundi* was then stored at refrigerated conditions at 4°C for 08 hrs.

Result: The organoleptic evaluation of probiotic *basundi* was carried out. As per the score of hedonic scale, basundi with encapsulated 10 per cent probiotic culture (10^9 cfu/gm) with 0.65 per cent hydrochloric acid modified psyllium husk had shown maximum consumer acceptability (8.3) among all samples.

Key words: Encapsulation, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus,* Lactic acid bacteria, Probiotic *basundi*, Psyllium husk.

INTRODUCTION

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 per cent of the total value of output from agriculture derives from this sector contributing about 8 per cent to Gross Domestic Product and placing Indian milk sector in first place (Samal and Pattanaik, 2014).

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. 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.

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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 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.

Basundi is one of the heats desiccated indigenous products popular in Western part of India, mostly Maharashtra and Gujarat. It can be classified in the condensed milk group along with *rabri, khoa, mithai* and *kheer* and can be considered similar to sweetened condensed whole milk (Raghavan and Kumar, 1960). *Basundi* has special importance in various festivals and celebration. *Basundi* is an important indigenous desiccated whole milk product prepared by partial dehydration of the milk with sugar. The dehydration of the milk is done in a karahi on direct fire. The original volume of milk is reduced to about 40 to 50 per cent. *Basundi* is mostly served on ceremonial occasions at feasts and festivals. The market value of product depends upon a relative thick creamy consistency, white to light brown colour, sweetish caramel aroma and soft textured flakes uniformly distributed throughout the product mass (Gaikwad *et al.,* 2015).

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, CO_2 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

The study was carried out at Department of Food Microbiology and Safety, College of Food Technology, VNMKV, Parbhani, Maharashtra during academic year 2019-2020.

Procurement of raw materials

Psyllium husk, buffalo milk, fresh cream sugar, cardamom and saffron 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 4000 rpm/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 (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. 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 0.3 M 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 per cent 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 per cent 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 *basundi* preparation

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

Preparation of *basundi*

The *basundi* was prepared as per standard described by (Gite *et al.,* 2017). Fresh, clean buffalo milk was boiled in iron pan, with constant stirring cum scraping action. When concentration was about one half of original volume of milk, clean, good quality sugar was added @ 5 per cent of original volume of milk, gentle heating was continued for five minutes till final concentration of about 2:1, basundi was then allowed to boil for 10 minutes. Then pan was removed from fire and allowed to cool .The product was then cooled and stored at refrigeration temperature till use.

Flow sheet 3. Preparation of *basundi*

Receiving milk (buffalo milk)
↓
Filtration
↓
Standardization of milk (6 per cent fat)
↓
Heating at simmering temperature (80-90°C)
↓
Vigorously stirring-cum-scraping
↓
Addition of sugar (6 per cent of milk)
↓
Gentle heating for 5 minutes
↓
Cooling to room temperature
↓
Storage at refrigeration temperature (4°C)
↓
Basundi

Preparation of probiotic *basundi* with encapsulation

Preparation of probiotic *basundi* with encapsulated strains, inoculum at 10 per cent of the final *basundi* was encapsulated modified psyllium husk with probiotic beads and the beads were aseptically added to 100 gm *basundi.* The probiotic *basundi* was then stored at refrigerated conditions to (4°C for 5 hrs). The standard recipe with different viable counts of culture is mentioned below in Table 3.

Flow Sheet 4. Probiotic *basundi* with Encapsulation

Basundi
↓
Washing of the probiotic beads
↓
Addition of encapsulated modified psyllium husk with probiotic beads
↓
Incubation at 37°C for 5 hrs
↓
Storage (4°C / 8 hrs)
↓
Probiotic *basundi*

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 per cent 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 and 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 48 hrs for specified acid concentration and PSH and acid solvent ratio. The acid treated psyllium husk product was recovered by vacuum filtration.

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 *basundi.*

Ingredients	Quantity (g/ml)
Milk	1000
Sugar	90
Cardamom and Saffron	02-03 pieces

It can be observed from Table 4 that psyllium husk was having white or pale buff colour. It is evident from the data that the psyllium husk was having translucent and thin boat shaped free flowing flakes broken into smaller fragments in appearance with bland mucilaginous taste and odourless in flavour. It is clear from the Table 4 that psyllium husk was having total ash content 2.60 ± 0.03 w/w and insoluble ash content 0.28 ± 0.004 w/w with swell volume of 40.50 ml/gm. The results revealed that loss in water, light and heavy extraneous matter were 7.21 ± 0.02 w/w, 4.95 ± 0.03 w/w and 1 ± 0.03 w/w respectively. Similar results were reported by Syed *et al.,* (2018). It is revealed from Table 5 that iron and copper content of native psyllium husk was found to be 7.99 ± 0.01 mg/100 g and 0.672 ± 0.04 mg/100 g respectively while manganese and zinc content was found to be 0.600 ± 0.001 mg/100 g and 0.322 ± 0.002 mg/100 g. Iron content was found highest among the minerals assessed. The results are in good agreement with the results reported by Syed *et al.,* (2018).

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 *basundi* without culture addition.

A - *Basundi* + encapsulated modified *psyllium* husk with 10 percent probiotic culture having (10^7) cfu/gm.

B - *Basundi* + encapsulated modified *psyllium* husk with 10 percent probiotic culture having (10^8) cfu/gm.

C - *Basundi* + 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

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 per cent 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°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 per cent acid concentration for PSH. solvent ratio @ 1:6 as 2.22 mg/(g×min) and 1.63 mg/(g×min) respectively. 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 (2008), Liangli Yu (2000) 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 per cent while protein content decreased from 2.91 to 1.20 per cent. Similarly, ash and crude fibre decreased from 2.61 to 2.23 and 3.10 to 2.65 per cent respectively. The decrease in fat, protein, ash and crude fibre content resulted

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

due to the partial degradation of the psyllium gel hardness because of acid modification. Further, carbohydrate content increased from 86.48 to 88.95 per cent 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 7 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 affect 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 per cent considering as dietary fibre.

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 *basundi.*

Samples	Colour	Flavour	Taste	Texture	Overall acceptability
MK	8.0	8.0	8.0	8.0	8.0
A	7.9	7.3	7.2	7.2	7.4
B	**8.8**	**8.3**	**8.5**	**8.4**	**8.3**
C	7.9	7.6	7.9	7.7	7.7
SE±	0.018	0.104	0.122	0.132	0.122
CD at 5%	0.054	0.345	0.406	0.438	0.406

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 5 hrs. and after freezing. The sample B obtained higher score for overall acceptability (8.3) as compared to control and other sample. Gaikwad *et al.,* (2015) reported the sensory scores for flavor, body and texture and color and appearance and sensorial characterization of ujani basundi and basundi are 8.29±0.86, 8.32±0.86, 8.64±0.93 and 8±0.93, 8.1±1.43, 7.9±1.43 respectively for ujani basundi and basundi.

The observations in respect of acidity of probiotic *basundi* as influenced by addition of encapsulated modified *psyllium* husk with probiotic beads. The results in this investigation were comparable with the Gaikwad and Hembade (2012) who reported the moisture content of ujani *basundi* was 54.60 per cent data showed in Table 9 revealed that the probiotic *basundi* sample Moisture (53.90 per cent), Fat (10.85 per cent), Protein (7.5 per cent), Carbohydrates (28.3 per cent), Ash (1.56 per cent) .Acidity (0.42 per cent) and Total solid (50.02 per cent). The results were found to be close in agreement with the study conducted by Sonalika Srivastava *et al.,* (2015). The moisture is noted to be in increasing the addition of probiotics beads from 53.90 in control to 51.03 in sample. Fat content of probiotics beads added *basundi* is reduced compared to that of control sample without the addition of *psyllium.* It was observed that the carbohydrate content of prepared probiotic *basundi* was 28.3 percent in sample. (Yadav, 2015). These results are comparable with the results reported by Patel and Upadhyay (2003).

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). It is evident that viable counts were observed at dilutions rate of 10^7, 10^8 and 10^9 (Table 10)

The results from Table 11 shows that, the *basundi* sample was free from *Coliform* and *E. coli* when the sample

Table 9: Physico-chemical characteristics of probiotic *basundi.*

Samples	Physical parameters		Chemical parameters				
	Total Solid %	Acidity (%)	Moisture (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Ash (%)
Control	46.11	0.38	51.03	12.03	9.02	27.25	1.77
Probioticbasundi	50.02	0.42	53.90	10.85	7.05	28.32	1.56

Table 10: Viable counts (LAB) of probiotic *basundi* during storage.

Time in (Days)	Viable counts		
	(cfu/gm)x10^7	(cfu/gm)x10^8	(cfu/gm)x10^9
0	3.3	2.1	1.5
2	4.1	2.6	1.9
4	4.6	2.9	2.1
6	4.9	3.0	2.1

Table 11: Microbial quality of probiotic *basundi* during storage.

Time in (Days)	Total plate count (cfu/g)x10^7	Yeast and mold (cfu/g)x10^4	Coliform count
0	2.9	ND	ND
2	3.9	2.6	ND
4	4.1	2.0	ND
6	5.2	1.4	ND

was fresh and throughout the storage period of 06 days at refrigerator temperature as result of good hygienic and sanitary conditions, during the preparation.

CONCLUSION

Probiotic *basundi* was prepared from 1000 ml of milk, 90 gm sugar, 02-03 pieces of cardamom, saffron 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 per cent hydrochloric acid modified psyllium husk. The probiotic *basundi* was then stored at refrigerated conditions at 4°C for 08 hrs. The organoleptic evaluation of probiotic *basundi* was carried out. As per the score of hedonic scale, *basundi* with encapsulated 10 per cent probiotic culture (10^9 cfu/gm) with 0.65 percent hydrochloric acid modified psyllium husk had shown maximum consumer acceptability (8.3) among all samples.

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