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Preparation of probiotic basundi added with modified psyllium husk by microencapsulation

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Abstract

The present investigation was carried out to study the microencapsulation of modified psyllium husk with Probiotic culture in formulation of Basundi. The acid modification studies indicated that psyllium husk with 0.65% HCl treatment in ethanol for ratio of 1:6 (w/v) shown to improve functional properties of psyllium husk. The proximate analysis of modified Psyllium husk was done with respects to moisture, fat, protein, carbohydrates, ash, dietary fiber were investigated. Further, the modified Psyllium husk powder was microencapsulated using *Lactobacillus* strains by extrusion of the cell- Polymer Solution into calcium chloride solution to form beads. The main aim of this study was use of microencapsulated probiotics and modified psyllium husk in preparation of Basundi. The prepared Basundi was then evaluated for sensory properties in case of colour, flavor, mouthfeel, texture and overall acceptability using 9 point hedonic scale. The results indicated that Basundi inoculated with 1 percent each equal proportion of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* with addition of probiotic beads with modified psyllium husk was highly acceptable. Microbial studies revealed that viable probiotic count in probiotic Basundi dependent on level of probiotics beads addition. Finally, Good quality dairy probiotic Basundi can be prepared by encapsulation beads with addition modified psyllium husk

Keywords: Psyllium husk, modification, probiotics beads, sensory evaluation, microbial characteristics

Introduction

Isabgol (Psyllium), the common name in India for *P. ovata*, comes from the Persian words "isap" and "ghol" that mean horse ear, which is descriptive of the shape of the seed. India is largest exporter of isabgol in the world; it has been introduced and cultivated in North Gujarat and adjoining parts of Western Rajasthan and Madhya Pradesh. However, the crop is spreading in non-traditional parts of the country such as Punjab, Haryana, Uttar Pradesh and Karnataka (Jat *et al.* 2015) [13]. The husk is about 10-25% of seed on dry weight basis. The husk obtained after milling is white hydrophilic material forming clear and colorless mucilaginous gel after water absorption. Psyllium seed husk is composed of mucilaginous polysaccharides, with a highly branched acidic arabinoxyylan containing a xylan backbone (Washington *et al.* 1998 and Blumenthal *et al.* 2000) [19, 6].

Dietary fiber is one of the valuable dietary interventions against a number of health disorders. There is no doubt that fibers, in particular viscous dietary fibers, have positive effects on human health, both in the prevention and in treatment of chronic diseases. Further, Dietary fibers from Psyllium have been used extensively both as pharmacological supplements, food ingredients; in processed food to aid weight control, to regulation of glucose control for diabetic patients and reducing serum lipid levels in hyperlipidemias (Baljit, 2007) [5].

Prebiotics 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". Next to inulin and fructo-oligosaccharides, the two most intensively studied prebiotics are arabinoxyylan-oligosaccharides (AXOS) and are being considered as a potential new class of prebiotic components.

Probiotics can be used as single or mixed cultures. The following properties and functions have been attributed to probiotics: they adhere to host epithelial tissue; they are acid resistant and bile tolerant; they are safe, non-pathogenic and non-carcinogenic; they cause improvement of the intestinal microflora; they have a cholesterol lowering, immune stimulating and allergy lowering effect; synthesize and enhance the bioavailability of nutrients (Ouweland *et al.* 2002; Saito, 2004; Grajek *et al.* 2005) [16, 18, 11]. Additionally, probiotics produce a variety of beneficial compounds such as antimicrobials, lactic acid, hydrogen peroxide, and a variety of bacteriocins (Holzapfel *et al.* 2001; Gorbach, 2002) [12, 10].

Probiotics should have the ability to interact with the host microflora and competitive with microbial pathogens, bacterial, viral, and fungal (Gorbach, 2002) ^[10]. Thus, when prebiotic and probiotic works coherently, a synergistic relationship is developed between them and together they are termed as “symbiotic”.

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 byproduct but some are heterofermentative 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.

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.* 2016) ^[9].

The original volume of milk is reduced to about 40 to 50 per cent. In Maharashtra, *Basundi* is mostly served-on ceremonial occasions such as 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 (Aneja *et al.* 2002) ^[4].

Material and Method

Material

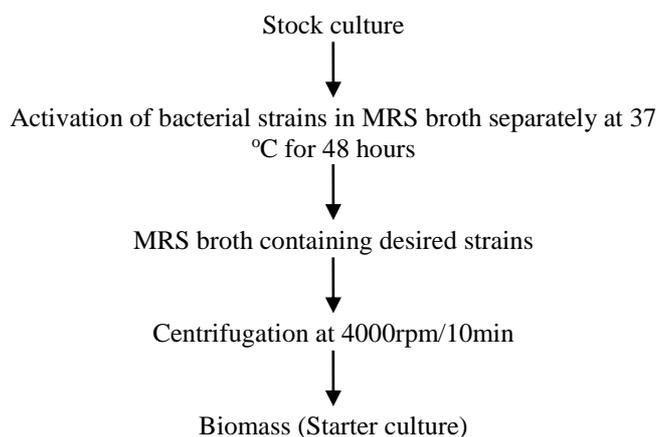
Psyllium husk, Milk and Cardamom were procured from the local market of Parbhani. The cardamom were ground in a laboratory grinder for mixing with Basundi.

Method

Preparation of starter culture

The probiotic organisms viz. *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* were individually grown in MRS broth at 37 °C for 48h. The cultivated MRS broth was then centrifuged at 4,000rpm for 10 min to harvest the cells. The harvested cells were washed twice with sterile water. The biomass was taken as starter culture.

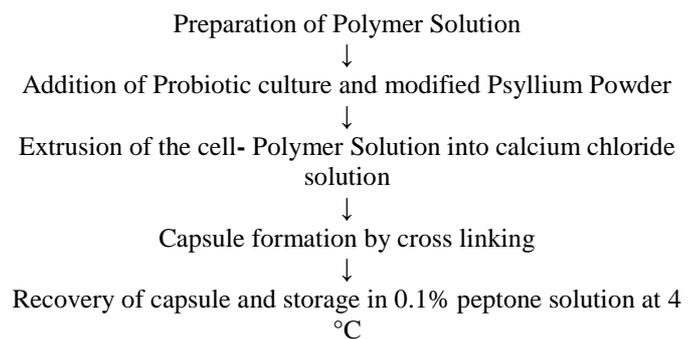
Flow Sheet 1: Preparation of starter culture



Microencapsulation of probiotics

The microencapsulation of probiotic bacteria was performed using the extrusion technique. In this method Hydrocolloid solution was prepared by using a combination of sodium alginate and guar gum at 1 and 0.8 per cent (w/v) respectively for preparation of 100ml Probiotic *Basundi*, 10ml of inoculum (5ml each of *L. acidophilus* and *L. bulgaricus*) was mixed in 2gm of modified *psyllium* powder. Probiotic culture and modified *psyllium* powder were 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 0.1per cent peptone (Karthikeyan *et al.* 2014) ^[14]

Flow sheet: 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) ^[20] with certain changes in concentration of HCL in ethanol solvent. The solvent used for psyllium husks acid treatment was ethanol with 34%-37% hydrochloric acid (HCl) at the varying concentration levels of 0.65% (w/v). The study was conducted to investigate the effect of acid concentration and psyllium-solvent ratio on physico-chemical/functional properties of the acid modified psyllium samples. At reaction temperature of 37.5 °C to psyllium -solvent ratios (PSH: Solvent @ 1:6 (w/v), g/mL) were tested. Thus, 48 g of psyllium husk was divided into 1 groups having 16g PSH each, for treatments with concentrations levels of 0.65% (w/v) of Hydrochloric acid in Ethanol solvent (Table 1). Four samples in each group were designated for three different psyllium-solvent ratios as mentioned earlier. 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 and 100% 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: Acid treatment levels for psyllium husk

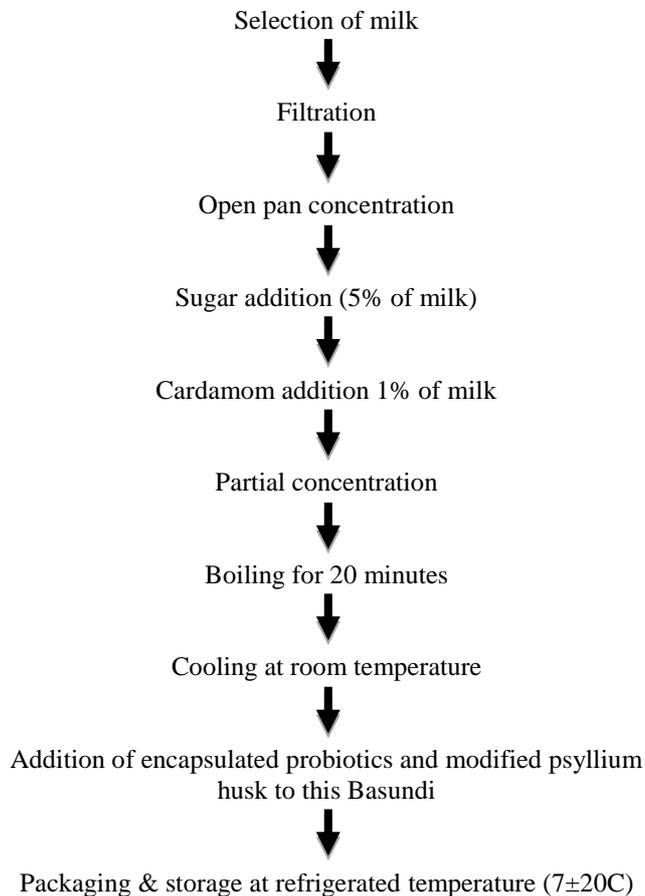
Concentration of HCl in Ethanol (Solvent)	Psyllium Husk (PSH): Solvent Ratio
0.65%	1:2, 1:4 and 1:6 (w/v)
0.00% for Control	1:2, 1:4 and 1:6 (w/v)

Preparation of probiotic Basundi

The Basundi was prepared as per standard described by Patel and Upadhyay (2003) ^[17]. 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% 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 and subsequently after cooling at room temperature in encapsulated probiotics and modified psyllium husk were added to mixed.

Flow chart for preparation of probiotic Basundi



Proximate composition of psyllium husk

Proximate composition such as moisture, fat, protein, ash and crude fiber were determined as per (A.A.A.C. 2000) and carbohydrate by difference method.

Physico-chemical analysis of Buffalo Milk

The pH of buffalo Milk was measured through electronic digital pH meter (Gemachu *et al.* 2015). Titratable acidity will be measured by titrimetric method. The acidity of milk sample was calculated by standard method (A.O.A.C, 2002). The parameter of milk like lactose, protein, fat, lactose, ash and acidity of buffalo milk were according to method (A.O.A.C, 2002)

Proximate analysis of probiotic basundi

All samples were analyzed for moisture, crude protein, crude fat, total ash and total carbohydrate, total solid and crude fiber contents according to their respective standard methods as described in (A.O.A.C, 2003) ^[2]

Sensory Evaluation

The sensory characteristics of probiotic beads added basundi will be evaluated at weekly intervals using 9-point Hedonic scale test by a panel of ten semi trained members. Sensory

parameters of Basundi. Color, body, flavor, Overall Acceptability. (Chougule *et al.* 2014) ^[8]

Microbial analysis of probiotic basundi

Microbial examination is the perfect quality assessment protocol performed in quality analysis of food products. However, in a probiotic product it is a mandatory one. Total Plate count, Yeast and mold count and *coliform* count were analysed.

Results and Discussion

Proximate composition of selected acid modified psyllium husk (0.65%/1.6)

It can be observed from Table 2 that moisture content increased from 7.10 to 7.36 per cent upon acid modification. Fat content decreased after acid modification from 1.80 to 0.65 per cent while protein content decreased from 2.90 to 1.22 per cent. Similarly, ash and crude fiber decreased from 2.55 to 2.25 and 3.13 to 2.69 per cent respectively. The decrease in fat, protein, ash and crude fiber content resulted due to the partial degradation of the psyllium gel hardness because of acid modification. Further, carbohydrate content increased from 85.30 to 88.50 per cent and energy value decreased from 370 to 365 Kcal/100 g obtained by Xiaoyin Pei (2008) ^[20].

Table 2: Effect of acid modification on proximate composition of psyllium husk

Particulars (g/100g)	Native Psyllium Husk PSH (N)	Modified Psyllium Husk PSH (M)
Moisture	7.10	7.36
Fat	1.80	0.65
Protein	2.90	1.22
Ash	2.55	2.25
Carbohydrate	85.30	88.50
Crude Fiber	3.13	2.69
a) Dietary fiber	77.66±1.28	79.67±0.89
b) Arabinoxylan	47.60±0.65	48.73±0.78
Energy Value	370 Kcal/100g	365 Kcal/100g

* Each value is average of three determinations

Physicochemical characteristics of buffalo milk

The physicochemical characteristics of buffalo milk were found to contain pH (6.32), acidity (0.12%), moisture content (82.97%), fat (6.32%), protein (4.28%), lactose (5.12%) and ash (1.15%) Buffalo milk is twice as rich in fat as compared to cow milk and most important fraction responsible for its high energetic and nutritive value. The results were in close agreement to the results obtained by Mohammad *et al.* (2007).

Table 3: Physicochemical characteristics of buffalo milk

Parameters	Value
pH	6.32
Acidity (%)	0.12
Moisture (%)	82.97
Fat (%)	6.32
Protein (%)	4.28
Lactose (%)	5.12
Ash (%)	1.15

*Each value represents the average of three determinations

Chemical analysis of probiotic Basundi

The data pertaining to various chemical properties of probiotic Basundi is depicted in Table 4. Data showed in table 4 revealed that the probiotic Basundi sample Moisture (53.2%), Fat (10.5%), Protein (9.5%), Carbohydrates (16.5%),

Ash (1.9%), Crude fiber (0.2%) and Total solid (38.41%). The results are 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 52.6 in control to 53.2 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 16.5 percent in sample. (Yadav, 2015) [21]

Table 4: Chemical analysis of probiotic Basundi

Sample	Control	Probiotic Basundi
Moisture %	52.6	53.2
Ash %	1.7	1.9
Crude Protein %	9.9	9.5
Crude Fat %	11.6	10.5
Total Solid %	37.63	38.41
Total carbohydrates %	16	16.5
Crude Fiber %	0	0.2%

Sensory Evaluation

The sensory characteristics of probiotic beads added basundi will be evaluated at weekly intervals using 9 point Hedonic scale test by a panel of ten semi trained members. Sensory parameters of Basundi. Color, body, flavor, Overall Acceptability.

The sensorial quality characteristics of Probiotic Basundi play a vital role in attracting consumers to purchase the product. Consumer judges probiotic Basundi quality on the basis of its sensory parameters such as color, flavor, taste, texture etc. Sensorial evaluation was done using 9 point Hedonic scale. The Probiotic Basundi was evaluated for acceptability based on characteristics such as color, flavor, taste and texture. The sensorial characteristics of Probiotic Buttermilk are summarized in table 5.

Where, probiotic Basundi inoculated with 1 percent each equal proportion of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* but variation only in addition of probiotic beads with addition modified psyllium husk. Control= probiotic Basundi prepared from 0 percent addition of probiotics beads and probiotic beads addition of good flavor, color, mouthfeel and over acceptability.

Table 5: Sensory evaluation of probiotic Basundi

Samples	Color	Flavor	Mouth feel	Texture	Overall Acceptability
Control	7.5	7	7	7.5	7.5
Probiotic Basundi	8	8.5	8.5	8.5	9

Microbial analysis of probiotic Basundi

Microbial examination is the perfect quality assessment protocol performed in quality analysis of food products. However, in a probiotic product it is a mandatory one. Total Plate count, Yeast and mold count and coliform count were analyzed.

Table 6: Microbial analysis of probiotic Basundi during storage period day

Storage period (day)	Total Plate Count (cfu/gm) x 10 ⁷	Yeast & Mold Count (cfu/gm) x 10 ⁸	Coliform Count (cfu/gm) x 10 ³
0	2.8	ND	ND
2	3.8	2.3	ND
4	4.2	1.1	ND

The microbial analysis of probiotic Basundi is tabulated in table 6. The accepted sample was subjected to microbial studies for total plate count, yeast and mold count and Coliform growth during the storage period as per method adopted by Cappuccino and Sherman (1996) [7].

In conclusion, the Probiotic Basundi can be prepared by utilizing sugar and cardamom and probiotics beads using equal amount of 1 percent starter culture of LAB *viz.* *L. acidophilus* and *L. bulgaricus*. Viable probiotic count in probiotic Basundi samples are dependent on level of probiotics beads addition. Good quality dairy probiotic Basundi can be prepared by encapsulation beads.

Conclusion

The incorporation of probiotics into Basundi could offer a good alternative to common dairy Products and allow to broaden the health claims. Probiotic cells as live microorganisms, when administered in suitable amounts, confer a health benefit to the host. The consumption of products supplemented with live cells of lactic acid bacteria (LAB), in particular with their probiotic strains, with addition of modified psyllium husk is believed to benefit consumers' health due to their well-documented positive impact on the function of gastro-intestinal tract and immune system. The development of Probiotic Basundi with microencapsulated probiotic strains with addition of modified psyllium husk can be advisable.

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