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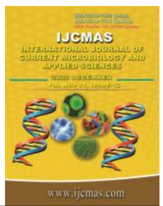

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Exploration of Spray Drying for Microencapsulation of Probiotic with Whey Protein Isolate to Improve Survivability During Gastrointestinal Transit

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A B S T R A C T

In the present era, functional foods are very popular mainly that contain probiotic because of their human health benefits. They play a therapeutic role by modulating immunity, improving lactose tolerance, lowering cholesterol, and preventing some cancers. In the human gastrointestinal tract, survival and stability are being discussed by scientists and researchers. Microencapsulation techniques are getting significant attention to overcome these limitations. It is a process to entrap a substance in a suitable material in order to generate particles with diameters of a few micrometers. It is a useful technique of entrapment of the probiotic cells to protect them from harsh environmental conditions in the gastrointestinal tract. The present study was undertaken to explore the spray drying technique for microencapsulation of probiotic *Lactobacillus fermentum* MTCC 8711 culture to evaluate WPI (whey protein isolate) as a coating material. The probiotic culture *Lactobacillus fermentum* MTCC 8711 was activated and revived in MRS broth, harvested, and the cell biomass was adjusted to >109 CFU/ml in sterile saline solution and then it was added to WPI mixture for feed solution preparation and microencapsulation was carried out by spray drying with suitable parameters. Spray drying process and WPI gave minimum powder particle size of 347.9 d.nm (10% WPI) and better survivability of 90.01% (15% WPI). Microencapsulated powders containing *L. fermentum* MTCC 8711 survived in simulated gastric conditions at pH 3.0 with the survivability of 79.41% (10% WPI) and in simulated bile salts at 1.0% with the survivability of 74.46% (15% WPI) in in vitro condition. The microencapsulated powders were stored at refrigerator (4 °C) and room temperature (37 °C) up to 30 days. The survivability was shown higher at refrigerator temperature than room temperature and they have remained 2.4×107cfu/g which was recommended for probiotic formulations.

Introduction

Lifestyle and dietary patterns are more important features for the general fitness of healthy people. Probiotic is a term that means "for life" and it was described by the Food and Agriculture Organization (FAO) of the United Nations and World Health Organization (FAO/WHO, 2002) which is defined as "live microorganisms which, when administered in sufficient amounts to confer a health benefit on

the host". They are reported to take part in a therapeutic function by modulating immunity, improving lactose tolerance, lowering cholesterol, prevention of some cancers as well (Kailasapathy and Chin, 2000). To generate these beneficial effects for health, probiotic have to be able to stay alive and multiply in the host. With this regard, it should be active, metabolically stable in the product or item, stay alive through the stomach and also reach the digestive tract in bulky amount (Sanz, 2007).

Certain species of *lactobacilli* and *bifidobacteria* are majorly used as probiotics (Sanders, 1998). The probiotic bacteria recommended in food should be in the range of 10^7- 10^9colony forming unit per gram (cfu/g) (Kailasapathy and Sureeta, 2004). In the improvement of safe and effective encapsulated product, it should be necessary to maintain the sufficient amount of viable cells, the shelf life of the food items or products and also during the gastrointestinal tract (GIT) transit after utilization (Solanki *et al.,* 2013).

Different parameters must be examined during the addition of probiotics in food such as, type of bacterial culture used, addition level required to have a physiological impact, survival to the process parameters, different protectants, stability during storage in addition to impact on the sensory properties (Iravani *et al.,* 2015).

In many functional foods, *Lactobacillus fermentum* MTCC 8711 is utilized as a probiotic strain. Because it has capability to avoid the adhesion of Methicillin-resistant *Staphylococcus aureus* (MRSA) to human colon adenocarcinoma cells (Jayashree *et al.*, 2018). Different strains of *L. fermentum* have shown highest cell surface hydrophobicity, high survivability up to four hours (Panicker *et al.,* 2018) and efficient riboflavin producing bacterium showed 2.29 mg/l of riboflavin within media (chemically defined)after 24 h (Thakur *et al.*, 2016).

The viability of probiotic organisms is the most important parameter as the sensitivity of probiotic bacteria affected severely in harsh conditions in GIT environment. Moreover, several factors have an effect on survivability of probiotic cultures in foods and supplements such as, dissolved oxygen, temperature, concentration of acids (lactic and acetic acid), bile salt, hydrogen peroxide (H_2O_2),buffers and digestive enzymes (Iyer and Kailasapathy, 2005; Sarkar, 2010; Sultana *et al.*, 2000). Thus, there is a need to develop technological applications for protecting the probiotic cells from such kind of intrinsic and extrinsic factors during processing and storage of foods as well as to improve survivability inhuman GI tract as control release mechanism.

Microencapsulation appears to be a promising technique because this technique relies on particular applications and parameters such as, particle size, control release mechanisms, physicochemical properties of coating materials and the core as well as process cost (Mahdavi *et al.*, 2014). This technique could potentially stimulate the survival rate of probiotic bacteria within food systems and also increasing the scope of applications (Desai and Park, 2005).

The main purpose of microencapsulation is to protect probiotics from bile salts, low pH and other constituents that meet during GIT (Muthukumarasamy *et al.*, 2006). Moreover, several benefits of microencapsulation of probiotic bacteria comprise of; they are protected from freezing or chilling (Shah and Ravula, 2000), protection from intrinsic factors (i.e. pH, organic acids, water activity), bacteriophages, protection from oxygen (O2) (Sunohara *et al.*, 1995), storage condition (Adhikari *et al.*, 2000) and also acidic condition in GIT (Lee and Heo, 2000) in addition to converting probiotic cells into a powder form that easy to use, as it enhance their uniform delivery throughout the end product (Mortazavian *et al.*, 2007).

There are number of techniques for microencapsulation, but spray drying is one of the advances, most common and also low-cost method to form microencapsulated food materials through which a liquid item is atomized in a hot gas current

to promptly obtain powder form. The energy utilization during spray drying is 6 to 10 times lesser than freeze drying and also producing a good quality end product. In comparison to freeze-drying, the expense of spray-drying process is 30-50 times lower (Desobry *et al*., 1997). Particles size of 2 to 3 microns was acquired at higher atomization gas flow rates, representing initial bead size of 4 to 7 microns atmost whereas lower flow rates gave significantly bigger particles with wider size distribution (Kemp *et al.,* 2013).

Different types of coating materials used for microencapsulation are generally recognized as safe (GRAS) ingredients that can be utilized in food applications (Ei-Salamand Ei-shibiny, 2012). There are various food grade materials which have been used for microencapsulation of probiotic for example, soluble starch, polydextroses, maltodextrins, pectin, cellulose, acacia gum or xanthan gum, alginate, carrageenan andamong proteins i.e. casein, gelatin, skim milk as well as whey protein (Anal and Singh,2007; Rokka and Rantamäki, 2010; Nazzaro *et al.,* 2012).

Milk proteins have excellent functional properties and act as good quality covering material for microencapsulation by spray-drying. Furthermore, they havehigh binding characteristics for the flavor components (Landy *et al*., 1995). Vargas Lopez (2013) suggested that encapsulation of *Lactobacillus delbrueckii ssp. Bulgaricus* and *Streptococcus thermophilus* with whey proteins is favorable to enhance the resistance against bile salts and acidic medium. Gbassi *et al.,* (2009) used whey protein as a wall material to enhance encapsulation efficiency of different *Lactobacillus plantarum* strains (*L. plantarum* 299v, *L. plantarum* 800, *L. plantarum* CIPA159) in calcium alginate beads. Conclusion was that whey proteins are advantageous, cheaper and more effective material for coating alginate beads stacked with bacteria. So, the present study is an attempt to explore the spray drying for microencapsulation of probiotics with WPI to improve survivability during gastrointestinal transit in *in vitro* condition.

Materials and Methods

Revival of probiotic culture

Freeze dried culture of *L. fermentum* MTCC 8711 was inoculated in MRS broth and incubated at 37 °C for 24 h. The fresh culture was prepared by adding 1% inoculums to MRS broth and grow again under the similar conditions for 24 h. In late exponential phase the culture was separated by centrifugation at 10,000 rpm for 15 min.

The cell pellet was washed twice with 0.90 % (w/v) sodium chloride (NaCl). *L. fermentum* MTCC8711 culture stock were kept in 10% glycerol and lyophilized vial at -20 °C for further study.

Feed solution preparation for spray drying

For preparation of feed solution for spray drying, initially overnight culture of *L. fermentum* MTCC 8711 was cultured into MRS broth and incubated at 37°C for 24h to 48 h.

After centrifugation of probiotic cells at 10000 rpm for 15 min at 4 °C, then the cells were washed with sterile 0.90% (w/v) saline solution and it was used further for spray drying with WPI coating material.

Preparation of whey protein isolate (WPI) solution

For this study, WPI was used as a coating material which was purchased from Sinew Nutrition, USA. Three types of feed solutions were prepared with different concentration such as, 10% WPI, 15% WPI and 20% WPI.

WPI powder was mixed as per different concentration in sterile distilled water in a flask and the solution was stirred continuously by using a magnetic stirrer to dissolve it properly. Then the fresh probiotic culture of *L. fermentum* MTCC 8711 (3% w/v) was added. These feed solutions were directly used for spray drying application for microencapsulation (Rajam *et al.,* 2012).

Microencapsulation of probiotic *L. fermentum* MTCC 8711 by spray drying

Microencapsulation of *L. fermentum* MTCC 8711 was carried out using lab scale spray dryer SPD-P-111 (Technosearch instruments, Thane, India). The spray drying system consisted of drying chamber, blower, air heater, scrubber, feed pump, cone, collection bottle and two cyclones (Primary and secondary). The inlet air, heated to 105 °C by an electrical heater, flow rate was maintained at 1.5 mL/min and drying chamber with an outlet temperature of 80 °C. Feed solution was delivered by a peristaltic pump into a fluid stainless steel atomizer.

The microencapsulated powder was collected at the bottom of a cyclone, packed in polythene terephthalate bags by using modified atmospheric packaging machine (MAP 430-GS, mfg. by Elixir technologies), sealed and wrapped with aluminum foil and stored at 4°C & 37 °C. Spray drying of *L. fermentum* MTCC 8711 was carried out with different feed solutions (10% WPI, 15% WPI & 20% WPI) as per parameters indicated in Table 1.

Morphological study of microencapsulated powder

Morphology analysis under Scanning Electron Microscope (SEM)

All the microencapsulated powder samples (10% WPI, 15% WPI and 20% WPI) were carried out for size and surface morphology analysis with the help of scanning electron microscope. Small amount (~2 mg) of powder samples were used for analysis. In this process the sample was coated with gold with the help of vacuum sputtering machine EMITECH SC 7620 sputter coater at 500 kv for 4 min and pressure current was 10mA. Individual powder sample was put in sample holder (aluminum stub). Microscopic analysis of individual powder samples was carried out by using ZEISS EVO-18 SEM having acceleration voltage of 15 kW. An individual powder sample was fixed on aluminum stub with double-sided adhesive tape. SEM image data of powder was collected over a selected area of the powder samples and 2D image was visualized that display properties comprise shape, size and texture of powder samples (Rosenberg and Young, 1993).

Determination of particle size of microencapsulated powder

Particle size and distribution of the microencapsulated powders were analyzed by using a particle size analyzer (Zetasizer Nano-series ZS90, Malvern Panalytical Ltd., UK). Powder samples having different concentrations (10% WPI, 15% WPI and 20% WPI) were analyzed by dissolving in distilled water. Distilled water used as a dispersing medium and taken as a reference having refractive index-1.33, viscosity-0.7920 and dielectric constant-76.75. Disposable four- side plain cuvettes were used under an operating temperature of 30 °C. The average particle size was carried out in triplicate for each solution.

Purity of *L. fermentum* MTCC 8711 in microencapsulated powder

The purity and probiotic property of microencapsulated powders were confirmed using Gram reaction, catalase test and motility test.

Survival of microencapsulated *L. fermentum* MTCC 8711 cells in Gastrointestinal Transit (GIT) condition

Survival in simulated gastric juice (SGJ) condition

As per method given by Krasaekoopt *et al.,* (2004) was used with some modifications. Simulated gastric juice condition was prepared (*in vitro*) by taking 10 ml MRS broth with different pH (3.0, 5.0 and 7.0) adjusting by using 0.5Nhydrochloric acid and sterilized it at 121 °C for 15 min.

0.5g of microencapsulated powders were dissolved in 9.5 mL of sterile simulated gastric juice and incubated at 37 °C for 12 h with constant agitation at

50 rpm in incubator with shaker (Make: REMI Elecktrotechnik). Then after, 0.1mL culture was spread on MRS agar plate for observation of gastric juice tolerance capacity, if bacterial cells are grown in plate or tubes which considered as gastric juice tolerance.

Survival in simulated intestinal fluids (SIF) condition

To evaluate the intestinal fluids tolerance capacity of *L. fermentum* MTCC 8711, method suggested by Krasaekoopt *et al.,* (2004) was used with some modification. In this process, simulated intestinal fluid condition (*in vitro*) was prepared by dissolving bile salt (Hi Media Laboratories, Mumbai, India) in 10 mL MRS broth with different concentrations (0.5%, 1.0% and 1.5%)by adjusting the pH8.0 with 0.1 N NaOH and sterilized at 121 °C for 15 min.

0.5g of microencapsulated powders were dissolved in 9.5 mL of sterile simulated intestinal fluids and incubated at 37 °C for 12 h with constant agitation at 50 rpm in a incubator with shaker (Make: REMI Elecktrotechnik). Then after, 0.1mL culture was spread on MRS agar plate for observation of intestinal fluids tolerance capacity, if bacterial cells are grown in plate or tubes which considered as intestinal fluids tolerance.

Water activity and moisture content of microencapsulated powder

After spray drying, the water activity of microencapsulated powder samples i.e., 10% WPI, 15% WPI and 20% WPI was measured by using a water activity meter at 27°C (novasina, Lab swift a_w, Switzerland).

The average moisture content of microencapsulated powder samples were determined by oven drying at 102 °C for 2h. Moisture content was analyzed by determination of the difference in weight before and after drying, expressed as a percentage of the initial powder weight (IDF, 1993). In this method, known mass of sample (0.5 g) was taken in aluminum dish and dried in a hot air oven (Make: Macro scientific works Pvt. Ltd.) at 102 ± 2 °C until it reaches constant weight.

Moisture content (%) = (weight of samples before drying–weight of samples after drying) × 100

Survivability of microencapsulated probiotic cells at different storage time

For the enumeration of viable counts, take 1.0 gm of microencapsulated powder samples and added to 9 mL of saline sterile solution, the serial dilutions were prepared before spreading on to the MRS agar plate. This sample solution was stirred on the magnetic stirrer to dissolve the powder and release of entrapped probiotic bacteria. The plates were incubated for 24 - 48 h at 37 °C and the microencapsulated probiotic cells were enumerated with the help of digital colony counter.

Microencapsulated powders were packed in polyethylene terephthalate bags with the help of modified atmospheric packaging machine and kept at 4 °C & 37 °C for the time interval of 0day, 10days, 20days and 30days. The survivability of *L. fermentum* MTCC 8711 was determined for their stability at given specific time intervals.

Statistical Analysis

The collected data were subjected to statistical analysis. Data were analyzed by analysis of variance (ANOVA) and critical difference test at 5% level of significance (P<0.05) to compare the different treatments means with the help of SPSS software version 26.0 (SPSS, Inc., Chicago, IL, USA).

Results and Discussion

Microencapsulation of *L. fermentum* MTCC 8711

Microencapsulation of *L. fermentum* MTCC 8711 was carried out using lab scale spray dryer SPD-P-111 (Technosearch instruments, Thane, India). In this exploration, the prepared feed solutions were

directly utilized for microencapsulation process and the process with different parameters of spray drying like inlet temperature, feed flow rate and outlet temperature. Then microencapsulated powders were examined and analyzed for further analysis such as surface morphology study by scanning electron microscope, powder particle size by particle size analyzer, survivability in gastrointestinal tract and during storage, water activity and moisture content determination.

Surface morphology study of microencapsulated powder with Scanning Electron Microscope

The surface morphology of different microencapsulated powder samples was observed by scanning electron microscopy (SEM) and it has been applied for the micro structure evaluation of different coating materials (i.e. 10% WPI, 15% WPI and 20% WPI). The size of microencapsulated powder particle was obtained in the range of 3.80µm to 17.50µm in case of WPI coating material. The minimum size of microencapsulated powder particle was 3.856µm with 10% WPI (Figure 1A) and maximum size of microencapsulated powder particle was 17.39µm with 20% WPI (Figure 1C). SEM images which shown in Figure 1 highlighted that probiotic cells were not observable in microencapsulated powder and it indicates that the coating material used in present study entirely encapsulated the probiotic bacterial cells.

Surface morphology of microencapsulated powders with WPI by SEM was showed spherical shape with concavities in the outer surface (Figure 1). There was no any crack or fractures observed on the external surface of coating materials and from that result of SEM images indicate good mechanical force with higher protection from oxidation reactions and avoiding their undesired release. Rajam *et al.,* (2012) studied whey protein–alginate as wall material for microencapsulation of *Lactobacillus plantarum* and they found highly porous and spongy like structure of microencapsulated powder, our results are in opposition to them.

Sheu and Rosenberg (1998) reported that surface of microcapsules was entirely smooth without any cracks shows the smooth surface texture of whey protein and it also prevents loss of coating material. In this study, they observed as concentration of coating material increased, it generates concavities in microencapsulated powder. Our results are similar to them.

Particle size study of microencapsulated powder

Particle size and distribution are very important physical characteristics which has direct impact for its successful utilization in food fortification. The particle size was analyzed through particle size analyzer (Zetasizer Nano-series ZS90, Malvern Panalytical Ltd., UK). In this study, size of microencapsulated powders particle with WPI was obtained in the range of340 d.nm to 750 d.nm. The minimum size of microencapsulated powder particle was 347.9 d.nm with 10% WPI (Table 2 & Figure 2A) and maximum size of microencapsulated powder particle was 746.1 d.nm with 20% WPI (Table 2 & Figure 2C).

Table 2 summarizes the size of particles of different microencapsulated powder samples using WPI. In general, microencapsulated powder with large particle size gives additional protection to probiotic bacteria as compared to microencapsulated powder particles with small size, because it contains low concentration of coating material.

Purity of *L. fermentum* MTCC 8711 in microencapsulated powder

To check the contamination during process and storage periods in microencapsulated powder, purity study of *L. fermentum* MTCC 8711 was carried out by three tests (Gram staining, catalase test and motility test) to confirm the presence of any other type of contaminants.

After performing Gram staining, the observation of glass slide by using optical microscope (Make: Labomed) under oil immersion lens and it was

observed that all cells are grampositive with rod shape and occurring in pairs. It confirmed that there was no any other contamination in the end products.

In catalase test, the probiotic cells did not produce air bubbles which indicates the negative result as well as probiotic cells were found non-motile during the observation of cavity slide under 40X and after in oil immersion lens. Thus our results found at par to Thummar and Ramani (2016).

Survival of microencapsulated probiotic culture cells in Gastrointestinal Transit (GIT) condition (*in vitro* study)

Survival of probiotic cells in simulated gastric juice (SGJ)

Ability of probiotics to tolerate digestive system is one of the most significant properties for the successful incorporation of probiotic cells into functional food (Ross *et al.*, 2005). For this study, survivability of microencapsulated probiotic cells was carried out in simulated gastric juice at different pH (3.0, 5.0 and 7.0) for 12 h incubation at 37 °C(*in vitro* study). Invariable loss was observed in survivability of free probiotic cells which indicates the requirement of protection or encapsulation. Increasing order of percent age survivability (%) of microencapsulated *L. fermentum* MTCC 8711 with WPI coating materials as follows:

10% WPI (79.41%) > 20% WPI (78.86%) > 15% WPI (70.80%) > Free cells (41.69%)

The survivability of *L. fermentum* MTCC 8711 was decreased after 12 h exposure to acid at pH 3.0 & 5.0 at 37 °C. We found highest survivability of 79.41% with 10%WPI (6.79 log cfu/g) coating material and then 78.86% with 20% WPI (6.68 log cfu/g)coating material as compared to rest of other microencapsulated powder with minimum survivability of 70.80% with 15% WPI (5.99 log cfu/g) coating material, while in case of free cells without encapsulation (control) showed much more reduction in survivability i.e. 41.69% (3.44 log cfu/g) that showed in Table 3 and Figure 3 Rajam *et al*. (2012) reported increasing cell viability of encapsulated *Lactobacillus plantarum* at low pH (2.0)by using whey protein isolate and alginate as wall materials, our results are similar to them for this study.

As per observations, we concluded that microencapsulation of *L. fermentum* MTCC 8711 by spray drying using WPI give better protection to this probiotic cells at low pH and shows better survivability.

Table.1 Different parameters for spray drying system

Sr. No.	Different Parameters	Value
1.	Inlet temperature (°C)	105
2.	Outlet temperature (°C)	80
3.	Temperature of plate (°C)	40
4.	Cooling temperature (°C)	60
5.	Aspiration flow rate (Nm^3/h)	70
6.	Feed pump flow rate (ml/min)	1.5
7.	Stirrer speed (rpm)	20

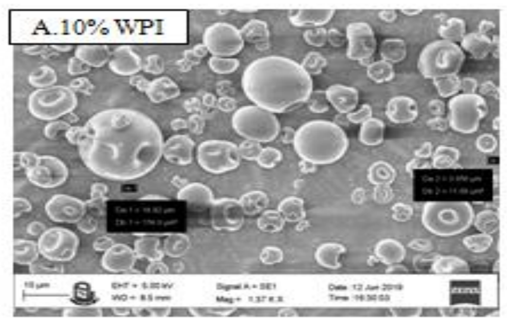
Table.2 Particle size of microencapsulated powders

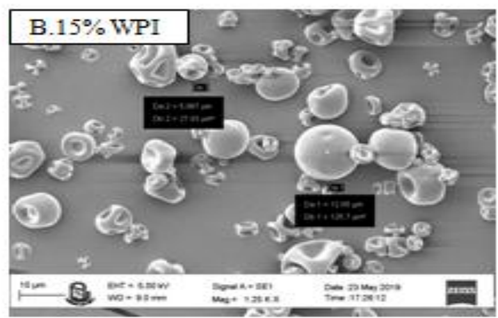
Microencapsulated powder samples	Particle size (d.nm)
A.10% WPI	347.9
B.15% WPI	425.2
C.20% WPI	746.1

Table.3 Survivability (%) (log number cfu/g) of free and microencapsulated *L.fermentum* MTCC 8711 in simulated gastric juice using acid with WPI (*in vitro* study)

Samples	Tolerance condition & Incubation time					
	0 h		After 12 h at Acid (pH-3)		After 12 h at Bile salt (1.0%)	
	Log no.	Survivability (%)	Log no.	Survivability (%)	Log no.	Survivability (%)
Free cells	8.25	100	3.44	41.69	3.5	42.42
10% WPI	8.55	100	6.79	79.41	6.07	70.99
15% WPI	8.46	100	5.99	70.80	6.30	74.46
20% WPI	8.47	100	6.68	78.86	5.94	70.12

Fig.1 SEM photomicrography of microencapsulated powder containing *L. Fermentum*MTCC 8711 with different coating materials (A) 10% WPI, (B) 15% WPI, (C) 20% WPI





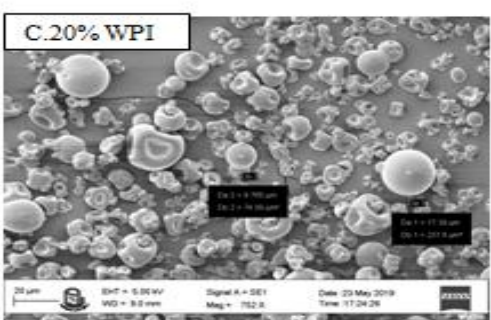


Table.4 Water activity (a_w) and moisture content of microencapsulated powder with *L. Fermentum* MTCC 8711 using different coating materials

Storage time (After spray drying)	Storage temperature	Different concentration of WPI	Different Parameters	
			Water activity (a_w) (at 27 °C)	Moisture content (%)
0 day		10% WPI	0.26	4.60
		15% WPI	0.27	4.82
		20% WPI	0.24	4.91
After 30 days	4 °C	10% WPI	0.27	4.82
		15% WPI	0.28	4.94
		20% WPI	0.26	5.00
	37 °C	10% WPI	0.28	4.86
		15% WPI	0.27	4.90
		20 % WPI	0.27	5.10

Table.5 Storage stability study of microencapsulated *L. fermentum* MTCC 8711 before, after, at 0 day, 10 days, 20 days and 30 days

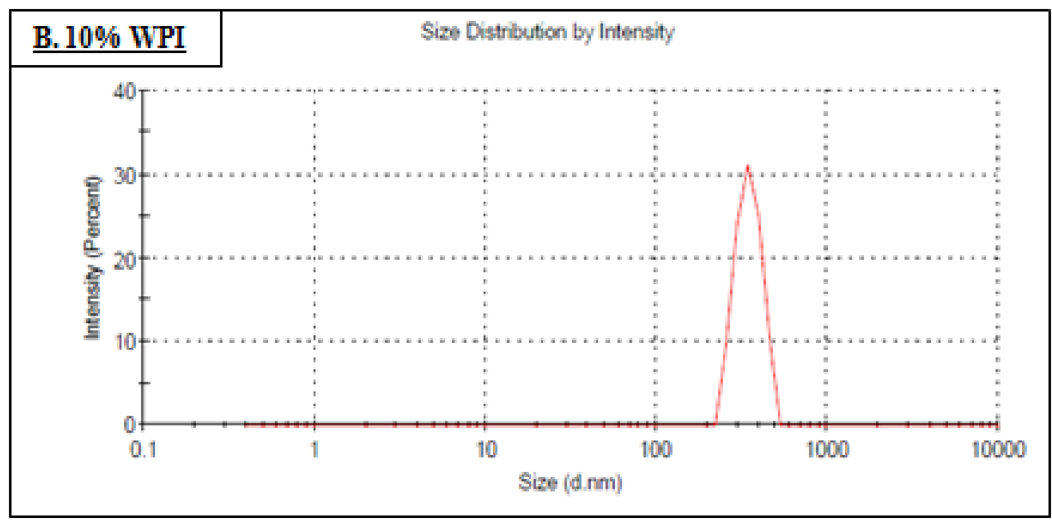
Coating materials	Viable cell Count (cfu/g)							
	Before spray drying	After spray drying (0 day)	After 10 days storage		After 20 days storage		After 30 days storage	
			4 °C	37 °C	4 °C	37 °C	4 °C	37 °C
10 % WPI	3.2×10^9	5.2×10^8	4.3×10^8	3.1×10^8	3.7×10^8	2.6×10^8	2.4×10^7	2.9×10^6
15 % WPI	4.1×10^9	4.4×10^8	4.0×10^8	2.3×10^7	3.2×10^7	3.9×10^6	3.6×10^6	1.4×10^5
20 % WPI	4.3×10^9	3.8×10^8	9.8×10^7	7.4×10^7	1.8×10^7	7.4×10^6	6.2×10^6	5.6×10^5

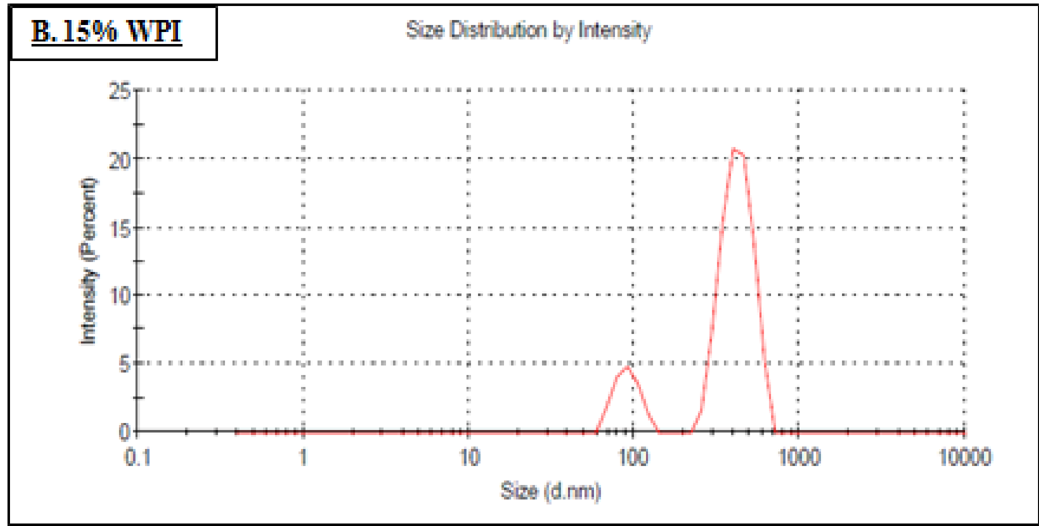
Table.6 Effect of WPI on the size of microencapsulated powder particle

Sample	Concentration	Range* (d.nm)	Average±SE (d.nm)
WPI	10%	344.8 - 350.1	347.6 ± 1.53^a
	15%	425.2 - 430.6	428.03 ± 1.56^b
	20%	741.3 - 752.8	746.73 ± 3.33^c

***Data represent mean±SE of three determinations.**
CD value: 8.12

Fig.2 Particle size and size distribution by intensity of microencapsulated powder using different coating materials (A) 10% WPI, (B) 15% WPI and (C) 20% WPI





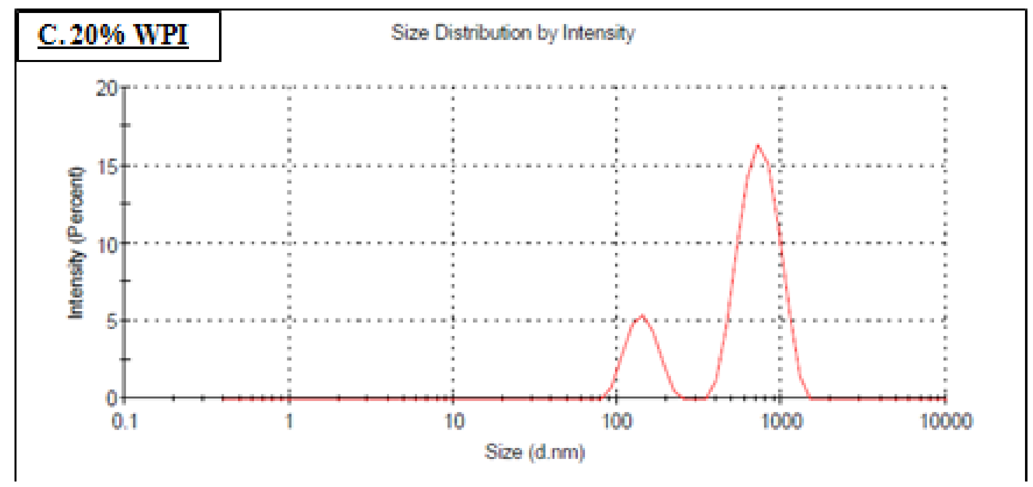


Fig.3 Survivability (log number) of microencapsulated *L. fermentum* MTCC 8711during incubation period at pH-3.0 using WPI as a coating material

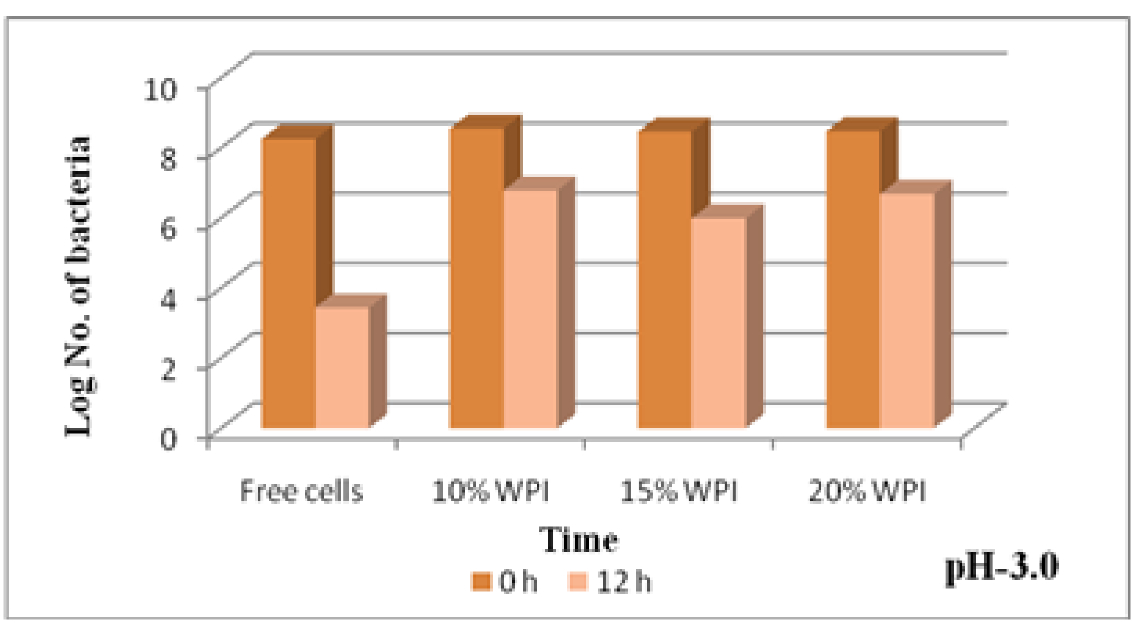


Fig.4 Survivability (log number) of microencapsulated *L. fermentum* MTCC 8711during incubation period with 1.0% bile salt mixture with different concentration of WPI

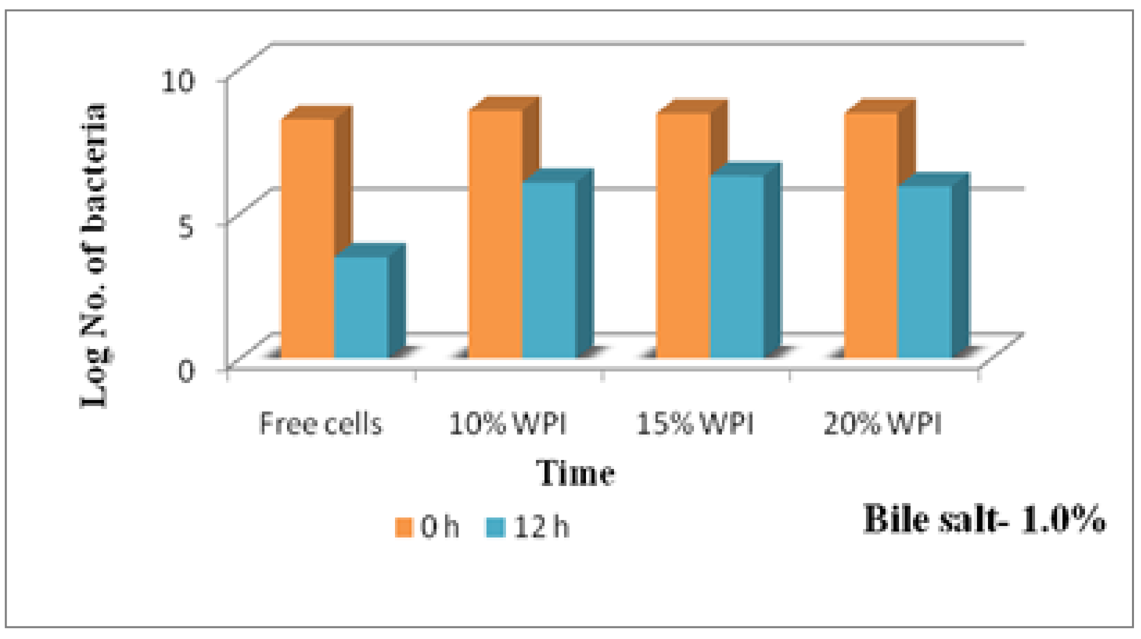
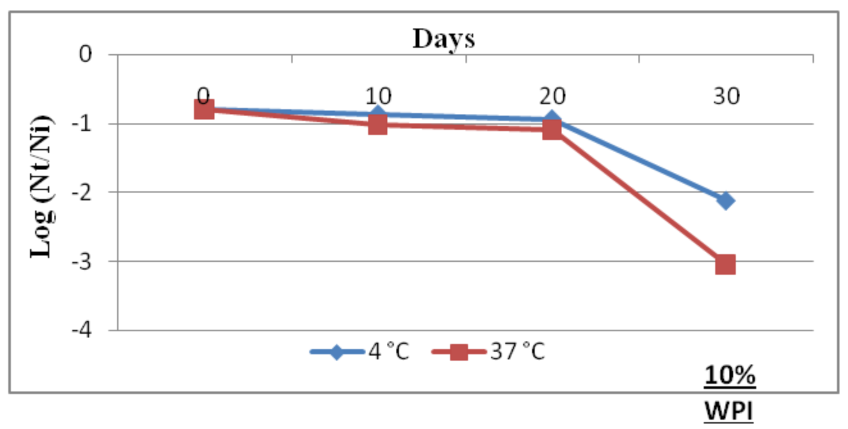
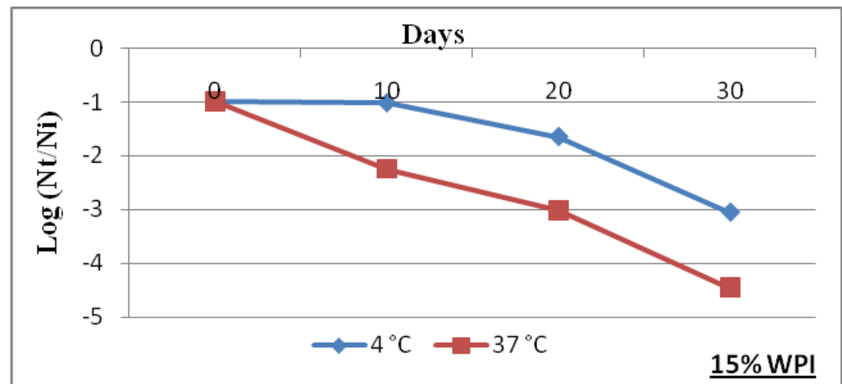


Fig.5 Survivability of microencapsulated *L. fermentum* MTCC 8711 at different storage time at 4 °C and 37 °C with 10% WPI, 15% WPI and 20% WPI





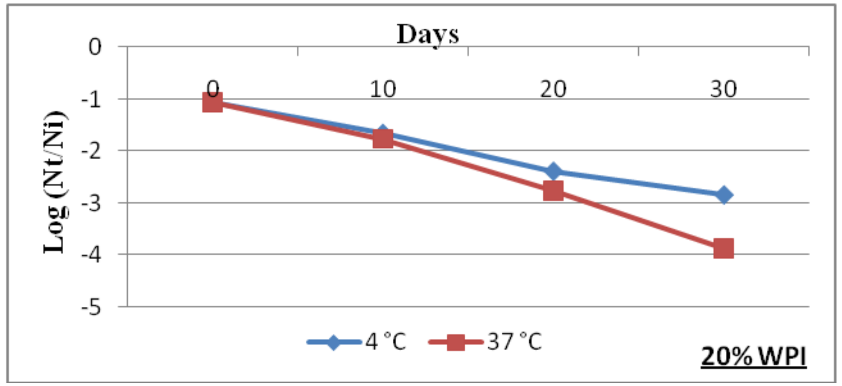
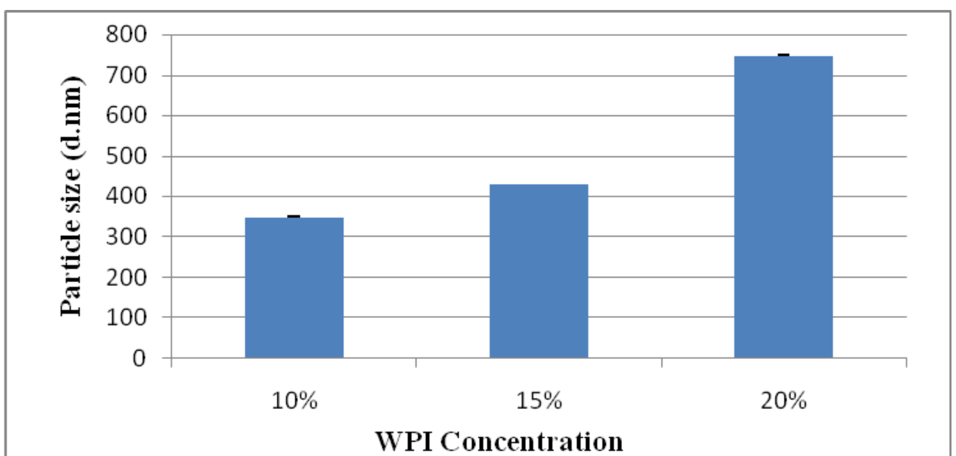


Fig.6 Effect of WPI with different concentration on the size of microencapsulated powder particle



Survival of probiotic cells in simulated intestinal fluids (SIF) condition

For this study, survivability of microencapsulated *L. fermentum* MTCC 8711 was carried out in simulated intestinal fluids condition (*in vitro*) with different bile salt concentration (0.5%, 1.0% and 1.5%) at 37 °C for 12 h. We observed better growth in simulated intestinal fluids condition with bile salt mixture concentration up to 1.0%.Increasing order of percentage survivability (%) of microencapsulated *L. fermentum* MTCC 8711 with WPI coating material with 1.0% bile salt concentration as follows:

15% WPI (74.46%) > 10% WPI (70.99%) > 20% WPI (70.12%) > Free cells (42.42%)

By using WPI coating material, we found maximum survivability of 74.46% for microencapsulated *L. fermentum* MTCC 8711 with 15% WPI (6.30 log cfu/g) coating material and minimum survivability of 70.12% with 20% WPI (5.94 log cfu/g) coating material, while free cells (control) without encapsulation showed much more reduction of survivability i.e. 42.42% (3.5 log cfu/g) when exposed to bile condition (Table 3). Figure 4 represents the viable count of microencapsulated *L. fermentum* MTCC8711 at 0 h and after 12 h exposure to bile salt at 1.0%. Rajam *et al.,* (2012) studied the survivability of microencapsulated *Lactobacillus plantarum* in simulated gastrointestinal condition by using whey protein and alginate by spray drying in which they found better tolerance capacity in bile condition up to 1%, our results for microencapsulated *L.fermentum* MTCC 8711 at par to them.

As per observations shown, the survivability of microencapsulated *L. fermentum* MTCC 8711 in simulated intestinal fluids condition, WPI especially with 15% concentration gave better results to survive.

Water activity and Moisture content of microencapsulated powder

In the current study, we found the water activity values of all microencapsulated powder samples in the range 0.19 to 0.31 at 0 day to 30 days storage time which shown in Table 4. Reyes *et al*. (2018) stated that if water activity is less than 0.1, cell membrane lipids could be oxidized that lead to decrease viability of cells, our results are opposition to them. On the other hand, Manojlovi´c *et al.,* (2010) reported that water activity values around 0.2 have determined as an ideal value for the survival of microorganisms during storage period and for probiotics around 0.25 aw should be suggested for long-lasting storage and our results are at par to them. Fazilah *et al.,* (2019) studied the effect of spray drying for microencapsulation of *Lactococcus*

lactis Gh1 with gum Arabic and *Synsepalum dulcificum* and they found a_w below 0.3, our results are affirmation to them for this study.

The viability of microorganisms was influenced by moisture content in dried products. In this exploration, we found the result of moisture content for different microencapsulated powder in the range of 4.50% to 5.10%. The result of sample 20% WPI showed highest moisture content as compared to other samples after spray drying (0 day) which is depicted in Table 4. We observed that the moisture content of microencapsulated powder were increase from 0 day to 30 day. In this study, our results are opposition to Rajam *et al.,* (2012) in which they obtained the moisture content value of 2.90% to 3.60% by using WPI as a wall material.

Survival of microencapsulated probiotic cells at different storage time

To check the survivability of microencapsulated *L. fermentum* MTCC 8711 using two different coating materials with different concentration i.e. 10% WPI, 15% WPI and20% WPI. The viable count of microencapsulated *L. fermentum* MTCC 8711 was found more in all coating materials and remained more than 10^8 cfu/g. In this study, we have obtained the viable cell count of 5.2×10^8 cfu/g incase of WPI (10% WPI) coating material after spray drying (0 day).

Similarly, viable counts for all other coating materials were mentioned in Table 5. Then the microencapsulated powders were packed in pre sterilized polyethyleneterephthalate bags using modified atmospheric packaging (MAP) machine and stored under refrigerator condition (4 °C) and room temperature (37 °C). Survivability of microencapsulated cells was checked at different time interval of 0 day, 10 days, 20 days and 30 days storage shown in Table 5 and Figure 5.

The viable count of microencapsulated cells under refrigerated (4 °C) condition remained almost stable up to 20 days and then decreased in both coating materials. Similarly, viability of probiotic cells was found stable up to 10 days at room temperature (37°C) and then gradually decreased at the end of 30 days (Figure 5).

Initially, we found viable counts of 5.2×10^8cfu/g with 10% WPI after spray drying (0 day) and then they were remained higher viable count i.e. 2.4×10^7cfu/gat 4 °C and 2.9×10^8cfu/g at 37 °C as compared to 15% WPI & 20% WPI at the end of 30 days.

The survivability of *L. fermentum* MTCC 8711 was found better at 4°C storage conditions which indicate that microencapsulated powder can be stored at refrigerated condition up to 20 days and storage up to10 days at room temperature. The results obtained are slightly similar to result of Thummar and Ramani (2016) who studied the viability of *L. fermentum* MTCC 8711using soymilk maltodextrin and non-fat skim milk as an encapsulating material for microencapsulation by spray drying.

Statistical analysis

Statistical analysis was conducted using analysis of variance (ANOVA) through SPSS 26.0 software (SPSS, Inc., Chicago, IL, USA) with statistical significance difference value determined at $p \leq 0.05$. One-way analysis of variance followed by least significant difference test was used to determine significant difference in microencapsulated powder particle size. In the present exploration, various concentration of milk proteins (i.e. WPI) and microencapsulation method (i.e. spray drying) are taken as independent variables while particle size is taken as dependent variables for analysis.

Effect of concentration of whey protein isolate (WPI) on the size of microencapsulated powder particle by ANOVA

It was found that particle size using WPI coating material ranges from 344.8d.nm to 350.1 d.nm with average value of 347.6 d.nmwith10% WPI coating

material, in case of 15% WPI coating material, particle size ranges from 425.2 d.nm to 430.6 d.nm with average value of 428.03 d.nm and in case of 20% WPI coating material it ranges from 741.3 d.nm to 752.8 d.nm with average value of 746.73d.nm that is presented in Table 6.

It was observed that WPI concentration and microencapsulation process has significant effect on the microencapsulated powder particle size. As the strength of the WPI concentration increase the particle size of microencapsulated powder was significantly ($p<0.05$) increase. However, the significant difference of particle size could be observed in WPI concentration (Figure 6). Thus, it could be concluded that particle size were directly proportional to concentration of WPI.

The purpose of this investigation was to improve the survivability of probiotic during gastrointestinal transit with different concentration of coating material i.e. WPI and to explore the spray drying process which gives the minimum particle size and the maximum survivability. Spray drying technique was used for microencapsulation because of it decrease the transportation and storage costs, lower process cost, avoiding chances of biological & chemical degradations. The method of microencapsulation and concentration of coating material have a significant impact on the probiotic survivability and size of powder particle obtained. It gave minimum powder particle size of 347.9 d.nm (10% WPI) and maximum survivability of 90.01% (15% WPI).

The microencapsulated powders containing *L. fermentum* MTCC 8711 were stored at refrigerator (4 °C) and room temperature (37 °C) upto 30 days. The survivability of *L. fermentum* MTCC 8711 was found better at 4 °C storage conditions which indicate that microencapsulated powder can be stored at refrigerated condition up to 20 days and storage up to 10 days at 37 °C. However they were remained in the level of recommendation probiotic cell population of 1.4×10^5 to 2.6×10^6 cfu/g at the end of 30 days.

From the present exploration it is recommended to use spray drying process for microencapsulation of probiotic bacteria. Milk protein especially WPI can be used to get micro particles which can be stored even at room temperature (37 °C) conditions with higher survivability up to 10 days. Further research can be tackled to use these microencapsulated powder to expand various functional food products for effective intake of probiotics.

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