EFFECT OF COATING MATERIALS ON THE SURVIVAL OF *LACTOBACILLUS PLANTARUM* MTCC2621 IN LITCHI JUICE AND CHARACTERISATION OF ITS MORPHOLOGICAL, PHYSICOCHEMICAL AND TOXICOLOGICAL PROPERTIES

5.1. Introduction

Beneficial microbial cells must be provided with suitable conditions for growth and metabolism and at the same time protected from harsh environmental conditions that they are exposed to. In addition, in case of microbial cells that produce substances of commercial importance, achievement of high cell densities for higher product yields, retention of activity for longer period of time and easy recovery of the cells from the products are often desired.

Probiotic microorganisms must survive past the digestive system before they can grow easily in the gut. They must be resistant to the extreme conditions of the gastrointestinal system such as low pH, gastric enzymes, and bile salts. Also, they must be consumed in a food that allows them to survive passage through the stomach and exposure to bile [1]. Different techniques have been developed for decreasing the fatal effects of the gastrointestinal system on probiotic microorganisms. One of the most promising of these techniques is microencapsulation.

Microencapsulation is a process by which bioactive materials are coated with other protective materials or their mixtures [2]. Microencapsulation protects the core material from environmental stresses such as oxygen, high acidity, and gastric conditions and allows the core material to pass through the stomach with little damaging effects [2]. Protection of the microencapsulated core material, when passing through the stomach, could be increased using water insoluble wall materials or treatments after microencapsulation to decrease solubility. In recent years, many studies have been conducted on microencapsulation for the preservation of probiotic microorganisms during storage and food processing [3]. Proteins, polysaccharides, sugars and their combinations or some liquid food matrices can be used to encapsulate probiotics [4,5].

Spray drying is a common microencapsulation process in the food industry. In this technique, heat and mass transfer occur simultaneously from air to atomised droplets and vice

versa[6]. Furthermore, other advantages of spray drying are that it is a single unit process for particle formation and drying, a low-cost operation, using readily available machinery and can be easily scaled up [7,8,9].

The energy consumption of spray drying is 6 to10 times lower compared to freeze drying and it produces a good quality product. The process involves the dispersion of the core material, forming of an emulsion or dispersion, followed by homogenization of the liquid, and then the atomization of the mixture into the drying chamber [10]. This leads to evaporation of the solvent. It is important to underline that in this technique, the product feed, gas flow and temperature should be controlled. However, it can cause a decrease in microorganism numbers and activity due to high inlet air temperatures and evaporation rates during the process [11].

Prebiotics are simply defined as non digestible foods that are essential for the viability or stimulation of growth of microflora in the gastro-intestinal system[13]. Fructo/galacto oligosaccharides, inulin and maltooligosaccharides are the most widely studied prebiotics [13]. Prebiotics promote the growth of probiotics in the colon by stimulating proliferation and host immunological response or probiotic activity. The effectiveness of prebiotics depends on the ability to influence the growth conditions of probiotics by selective utilization. Other gut microorganisms may also compete with these prebiotics [14]. Since prebiotics and probiotics have a synergistic interaction, consuming both simultaneously might offer some advantage [15]. However, the combined effects of prebiotics and probiotics are highly strain- and substrate-specific [14,16].

Among the various encapsulation materials, maltodextrin is a white granular hygroscopic powder usually soluble in water and its Dextrose Equivalent (DE) varies between 4 and 20 [17]. Pectin is a complex anionic polysaccharide extracted from citrus fruits and apple pomace, primarily consisting of linear chains of α-(1–4)-linked D-galacturonic acid. Pectin is a promising encapsulation polymer and has been used for the oral delivery of drugs [18], natural chemicals and sensitive food ingredients, such as folic acid [19]. Compared to maltodextrin there is considerably less information on the use of pectin-based spray drying for the delivery of probiotic bacteria and their potential incorporation into acidic food products, such as fruit juices. However, recent works have used pectin as part of blends of polymers to

encapsulate probiotic bacteria, provoking an interest in this particular polymer [20,21]. Pectin and fructooligosaccharide (FOS) have been studied in conjunction with maltodextrin to enhance the protection of *Lactobacillus* and *Bifidobacterium* cells in acidic environments [22,23]. FOS is a low molecular weight short chain carbohydrate, which though studied extensively in food applications [24], has been found to create problems in dried products because of stickiness. Stickiness due to FOS is attributed to its low glass transition temperature [25]. On the other hand, maltodextrin has high glass transition temperature and has a protective effect on encapsulated material. By binding a low glass temperature material into a high glass transition material, the overall mixture ends up with a weight fraction-based glass transition temperature. In other words, higher concentration of maltodextrin in the coating material will yield a non rubbery glassy amorphous state [21]. Pectin also has a high glass transition temperature [26].

Previous research showed that encapsulation of *Lactobacillus plantarum* cells within single and double chitosan coated alginate beads enhanced cell survival considerably in the highly acidic pomegranate juice [23]. However, there is very little information on the use of these coating materials in a spray drying encapsulation system for the delivery of probiotic bacteria.

Litchi (*Litchi chinensis* Sonn.) is an important tropical fruit and excellent source of vitamin C (40-90 mg/100 g) [27]. Litchi also contains 21.6 g/100 g of total sugar [24], 0.37 g/100 g titratable acidity (as citric acid) [28], 0.8 mg/g total phenolic compounds [28] and more than 40 aroma volatile compounds [30]. Fortification of litchi juice with probiotics in powdered form could produce an important product for the different food industries. Kingwatee et al. [31] reported the spray drying of litchi juice with *Lactobacillus casei* using maltodextrin and inulin as coating materials.

The subject of the present work was to use maltodextrin as the core encapsulation material and study the effect of additional coating materials like pectin and fructooligosaccharide (FOS) as encapsulating agents on cell survival and on the physical properties of the spray dried powder derived from litchi juice containing probiotic *L. plantarum* MTCC2621.

5.2. Materials and Methods

5.2.1. Probiotic strain and growth condition

Lyophilised *Lactobacillus* culture, *Lactobacillus plantarum* MTCC2621 was obtained from Microbial Type Culture Collection and Gene (MTCC) (Chandigarh, India). From this culture, stock solution was prepared by adding sterile glycerol (50% v/v). The glycerol stock culture was stored at frozen condition (-40 ^{o}C) in sterile screw cap tubes for future use. The probiotic organisms were grown individually by inoculating into 10 mL sterile de Man Rogosa and Sharp (MRS) broth (Himedia Laboratories Pvt. Ltd, Mumbai, India) and incubated at 37 ^{o}C for 2 days under aerobic condition. The cells were harvested by centrifuging (Sigma, Germany) at 1500 x g for 15 min at 4^{o}C. Before inoculation into fruit juices, the harvested cells were washed twice with sterile saline water (0.85% w/v NaCl) to remove any residual MRS. The cell pellets were diluted to get a bacterial concentration of 10^{11} CFU/mL by saline water.

All the chemicals used were of analytical grade and supplied by Merck-Millipore, India and Himedia Laboratories, India and Sigma-Aldrich.

5.2.2. Preparation of fruit juice

Sweet variety of ripened litchi (*Litchi chinensis* Sonn.) that was purchased from the local fruit market, Tezpur, Assam during the season was peeled, pitted and cleaned. The juice was extracted using a household juicer (Philips, Bangalore, India). The juice was strained in a muslin cloth and pasteurized at 90 ^{o}C for 1min with consistent stirring. Subsequently, the juice was cooled down to 25 ^{o}C. The pasteurized juice had the pH of 3.65±0.26 and TSS of 14.6 ±0.3 ^{o}Brix. The fruit juice selected for this study was chosen from the four studied fruits reported in Chapter 2 based on their suitability as probiotic carrier.

5.2.3. Spray drying condition

Spray drying was performed using a spray drier (Lab Plant, UK) equipped with nozzle size of 0.1 mm. The drying conditions of the experiment were maintained as per the optimized conditions mentioned in the Chapter IV. One of the dependent variables in Chapter IV was the solid to maltodextrin ratio in the litchi juice used for spray drying. The optimised drying conditions therefore formed the basis for the present study wherein the maltodextrin

level was partly replaced with coating materials like pectin and fructooligosaccharide (FOS) as encapsulating agents and study their effect on cell survival. Pasteurised litchi juice was separately mixed with either 15% (w/v) maltodextrin (≤20 DE, Himedia Laboratories) (coded as M) or in different combinations of 10% (w/v) maltodextrin plus 5% (w/v) pectin (coded as MP) or 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS) (coded as MF) or 5% (w/v) maltodextrin plus 5% (w/v) pectin plus 5% (w/v) fructooligosaccharide (FOS) (coded as MPF). The total soluble sugar (TSS) of the litchi juice was adjusted to 11 °Brix (total solid concentration 0.1 g/L) by appropriate dilution with sterile distilled water because the pure extract was too viscous to be spray dried. Homogenization of this juice and coating material was done to obtain a bacterial concentration of 10^{11} CFU/mL using a magnetic stirrer (LaboTech) just before spray drying. The obtained powder were kept in airtight containers and stored at refrigerated condition (4±1°C) for further studies.

5.2.4. Survival of microencapsulated bacteria

Survival of the microencapsulated bacteria during storage was determined according to the modified method of Kimoto et al. [32] and Crittenden et al. [33]. One gram of microencapsulated probiotic bacteria was suspended in 0.1 M sodium phosphate buffer and before using peptone water solution (1.5 g peptone in 1 L water) for serial dilutions. Plating was carried out using pour-plate method using MRS agar for *L. plantarum*. Plates were incubated at 37 °C for 48 h before colony counting. The survival of microencapsulated bacteria after spray drying was expressed as CFU/g. All survival experiments were repeated 3 times and all analyses were carried out in duplicate (n=6) for statistical analysis [34]. The survival of microencapsulated bacteria during 10 weeks storage was determined as colony forming units (CFU) on 0 day, 3 day, and thereafter on 1, 4, 6, 8 and 10 weeks of storage.

5.2.5. Yield

The yield of the spray drying process was calculated by taking into consideration the total solid content of the feed with maltodextrin and weight of the final dry powder. Yield (%) was calculated using equation **Eq. 5.1.**

$$\text{Yield (\%)} = \frac{\text{Weight of the solids in dried powder}}{\text{Solid Content of the feed material}} \text{x}100 \qquad \textbf{Eq. 5.1}$$

5.2.6. Physicochemical properties of the encapsulated powder

5.2.6.1. Microstructure study of the spray dried powders

To examine the surface morphology of the probiotic litchi juice powder, samples were gold coated with a thin layer of 10 nm. The SEM images were then obtained using a scanning electron microscope (Model JSM-6390LV, JEOL, Japan) at an accelerating voltage of 20 kV.

5.2.6.2. Thermal analysis

The glass-transition temperature (T_g) of the powders were analysed in differential scanning calorimeter (Shimadzu, DSC-60, Japan). In an aluminium pan, 5±1 mg of powder was placed and sealed. The T_g of the powder was scanned at a rate of 10 ^oC /min over a temperature range of 25-150 ^oC using empty pan as a reference. The glass transition value was considered as the midpoint of the glass transition range.

5.2.6.3. Moisture content, bulk density and tapped density of the powder

Moisture content of the spray dried powder was determined by the AOAC method [35]. Bulk density (g/mL) of the spray dried powder was calculated by weighing 5g of sample powder into a 10 mL graduated measuring cylinder and measuring the volume occupied by the powder. Tapped density was determined by tapping the cylinder manually for 50 times and then the volume occupied by the sample was measured as for bulk density [36]. From the bulk and tapped density values, the Hausner ratio (HR) and Carr index (CI) were calculated to determine the cohesiveness and flowability property of the spray dried powder [37.38].

$$\text{Hausner ratio, HR} = \frac{TD}{BD}$$

$$\text{Carr index, CI} = \frac{TD-BD}{BD} \text{x}100$$

Where, TD is tapped density and BD is bulk density

5.2.6.4. Solubility

The solubility (%) was determined (**Eq. 5.2**) following the method described by Chau et al. [39]. Briefly, the spray dried powder were mixed with distilled water (1:10 w/v) and stirred for 1 h at room temperature. The mixture was then centrifuged at 3000 x g for 5 min. After that, the supernatant was collected, dried and weighed.

$$\text{Solubility (\%)} = \frac{\text{Weight g of supernatant after drying (g)}}{\text{Weigh of the sample (g)}} \; x \; 100 \qquad \textbf{Eq. 5.2}$$

5.2.6.5. pH and titratable acidity (TA) of the powdered samples

The pH of the sample was measured using a pH meter (Eutech, Merck). Briefly, 1 g of sample was dissolved in 5 mL deionised water and pH was measured at 27°C. Titratable acidity (TA) was determined by titration method [35]. To 1 g of sample dissolved in deionised water, 2-3 drops of phenolphthalein indicator was added and titrated against 0.1N sodium hydroxide. Titratable acidity was expressed as citric acid equivalent (**Eq. 5.3**).

$$\text{Titratable acidity (\%)} = \frac{\text{Titre value} \times 0.1\text{N NaOH} \times 64 \times 100}{\text{Sample volume taken} \times \text{weight sample} \times 1000} \qquad \textbf{Eq. 5.3}$$

5.2.6.6. Hygroscopicity

Hygroscopicity of the spray dried powder was determined following the method described by Cai and Corke [40] with some modifications. Briefly, 2 g of spray dried powder sample was placed in pre-weighed glass vial and placed in an airtight desiccator containing saturated sodium chloride solution (75.09% RH) at 30 ^{o}C and kept for 7 days. After the incubation period, sample vial was weighed and hygroscopicity was expressed as grams of adsorbed moisture per 100 g sample.

5.2.6.7. Water activity (a_w)

The a_w of spray dried powder sample was measured by a Water Activity Meter (Model-4TE, AQUA® Labs) throughout the storage period at every 10 days interval.

5.2.6.8. Colour of the spray dried powder

Colour values (*L, a, b)* were measured for feed and reconstituted fruit juice powders by using a Hunter colour spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA). The "L" value indicates degree of lightness. "L" value in the range between 0 - 50 indicates dark and 51-100 indicates light. Similarly, "a" measures red (+ve values) and green colours (-ve values); "b" measures the yellow (+ve value) and blue (-ve values) colours. The overall colour change (ΔE) of the samples was calculated using the equation (**Eq. 5.4**) reported by [41].

$$\Delta E = \sqrt{\{(\Delta L^2) + (\Delta a^2) + (\Delta b^2)\}} \qquad \textbf{Eq. 5.4}$$

5.2.6.9. Polydispersity index and particle size distribution of the spray dried powder

Polydispersity index (Đ) and particle size distribution of the spray dried probiotic powder was determined using a particle size analyzer (NanoPlus, Particulate Systems, Atltanta, GA). The particle size was analysed based on the principle that when laser beams are irradiated to particles under the Brownian motion, scattered light from the particles shows fluctuation corresponding to individual particles. The fluctuation is observed according to the pinhole type photon detection method, from which particle size and particle size distributions are calculated. A small quantity of powder was suspended in water and analysed at 25 ^{o}C. The particle size (µM) was depicted with respect to its intensity (%) while particle size distribution was represented by span and calculated using the equation given below.

$$\text{Span} = \frac{D_{90} - D_{10}}{D_{50}}$$

where, D_{10}, D_{50} and D_{90} are the diameters of sample at the 10th, 50th and 90th percentiles.

5.2.7. Cytotoxicity assay

For cytotoxicity assay, human embryonic kidney (HEK293) cells were cultured in Dulbecco Modified Eagle's Medium (DMEM) with 10% fetal bovine serum and 1% penicillin-streptomycin solution. The cells were sub-cultured and maintained in 5% CO_2 at 37 ^{o}C in a CO_2 incubator (Eppendorf, Germany). Cultures were periodically monitored for confluency under inverted microscope and 10,000 cells were seeded in each well of a 96 well plate with 200µL media incubated for 24 h. Probiotic fruit juice sample (0.05g/mL) that was diluted to attain concentrations 50 mg/mL, 25mg/mL, 5mg/mL, and 0.5mg/mL were added to the HEK293 cells. After 24 h of incubation, 20 µL of MTT reagent (1mg/mL in phosphate buffer saline) was added to each well and incubated for 3 h at 37 ^{o}C until a purple precipitate was observed. Media was removed without disturbing cells and 200µL of DMSO solution was added in the well for stabilizing the formazone crystals and the absorbance was read at 570 nm. Percent cytotoxicity of the juice was calculated from the standard curve and plotted using varying concentration of silver nitrate as control.

5.2.8 Statistical analysis

All experiments and analyses were done in triplicate. The results are expressed as mean ± standard deviation. The analysis of variance followed by a Duncan multiple range test ($p < 0.05$) was used for the mean comparison, using SPSS v21.0 software package (SPSS Inc.,Chicago-Illinois-USA).

5.3. Results and Discussion

5.3.1. Survival of microencapsulated bacteria

Spray drying is one of the viable and low cost techniques to entrap probiotic microorganisms. But the main drawback of this process is that it causes extreme cell damage and loss of viability [42]. Microbial survivability is influenced by the operating conditions such as drying temperature, carrier materials used and other storage conditions [43]. Survival during spray drying does not necessarily correlate or ensure survival during storage. Indeed, a series of optimal combinations of conditions are necessary to ensure survival during drying as well as during storage [44]. Due to the stress involved during spray drying, the cells tend to lose viability over the period of storage and hence it is essential to check that the required standard counts (> 10^6-10^8CFU/g) are maintained throughout the shelf life of the product [45]. Carrier materials like maltodextrin and gum arabic are extensively used to spray dry fruit juices due to their high solubility and low viscosity which are essential aspects for achieving good product quality [6].

In this study, three potential carrier materials in different combinations (already mentioned) used for spray drying of *L. plantarum* MTCC2621 in litchi juice were investigated for effect on cell viability and the results are shown in **Table 5.1**. Cell viability (CFU/g) was found in all types of coating materials used for spray drying up to 6 weeks in refrigerated condition. After 6 weeks, viability of cells declined sharply in MPF, MP and M, in that order.

Table 5.1. Viability of spray dried *L. plantarum* in different coating materials during storage (4±1^{o}C)

Time	CFU/g			
	M	MP	MF	MPF
0 day	$6.4 \pm 1.3 \times 10^8$	$5.8 \pm 0.9 \times 10^8$	$1.4 \pm 0.3 \times 10^8$	$6.4 \pm 1.3 \times 10^8$
3 day	$2.5 \pm 0.8 \times 10^8$	$3.5 \pm 0.6 \times 10^8$	$8.5 \pm 0.2 \times 10^7$	$2.5 \pm 0.8 \times 10^8$
1 week	$8.2 \pm 0.4 \times 10^7$	$9.5 \pm 0.5 \times 10^7$	$10.6 \pm 0.4 \times 10^6$	$3.5 \pm 0.6 \times 10^7$
4 week	$4.2 \pm 2.2 \times 10^7$	$3.5 \pm 0.6 \times 10^6$	$4.2 \pm 0.8 \times 10^6$	$5.5 \pm 0.6 \times 10^6$
6 week	$4.0 \pm 0.8 \times 10^5$	$9.5 \pm 0.5 \times 10^5$	$1.4 \pm 0.3 \times 10^6$	$4.2 \pm 2.2 \times 10^3$
8 week	<10CFU/mL	<10CFU/mL	$8.5 \pm 0.2 \times 10^5$	$4.0 \pm 0.8 \times 10^2$
10 week	<10CFU/mL	<10CFU/mL	$7.6 \pm 0.4 \times 10^5$	<10CFU/mL

Mean and standard deviation for n = 3. M: 15% (w/v) maltodextrin; MP: 10% (w/v) maltodextrin plus 5% (w/v) pectin; MF: 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS); MPF: 5% (w/v) maltodextrin plus 5% (w/v) pectin 5% (w/v) plus fructooligosaccharide (FOS)

Spray drying with MF (10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide enabled highest survival of cells (up to 5 log CFU/g) after 10 weeks of cold storage compared to all other combinations of coating materials. This is due to firm and uniform coating of the bacterial cells which protected the cells from the high outlet temperature of drying process, which is also supported by the scanning electron micrograph in **Fig. 5.2**. Storage at lower temperature ensured longer shelf life and higher cell count retention at the end of 30 days which is in agreement with previous research [46]. FOS appeared to have greater compatibility with bacterial cells than the other coating materials. Lipid oxidation of the cell walls during storage and subsequent permanent damage are considered as the main causes for low shelf life of spray dried cultures [47].

The coating material studied decreased in their ability to provide cell protection in the following order: MF>MPF>MP>M. The study also showed that prebiotics like fructooligosaccharide (FOS) and pectin have positive effect on cell viability during storage of spray dried cells.

5.3.2. Physicochemical parameters of the spray dried fruit juice powders

The physicochemical properties of the spray dried probiotic juice powders of different combination of coating materials are illustrated in **Table 5.2**. Highest yield (77.50%) was found in fruit juice spray dried with MF. Addition of MP and MPF in juice showed no significant difference in the yield. Water activity (a_w) and presence of oxygen are factors which affect the viability of probiotics during storage [48]. There was no proper correlation between a_w and survival during storage because cells survived even when a_w was between 0.245 and 0.338. It was reported that stable a_w <0.3 (moisture content of less than 5%) is essential for a good survival of the probiotics during storage [4]. The water activity of the four spray dried samples ranged from 0.171 to 0.564. Powders containing pectin exhibited most inferior properties, i.e. high a_w and low solubility due to effect of high hygroscopicity. On the other hand, powder prepared with maltodextrin and FOS showed superior characteristics of low a_w and good solubility. Ideally a_w should be between 0.11 and 0.23 (moisture content of 4-5 %) for most *Lactobacillus* species [49]. Increased a_w encourages a faster death rate of the probiotics during storage as it encourages other microorganisms and fungi to grow and also accelerates undesirable chemical reactions [4, 44].

All the *L. plantarum*-litchi-juice powders showed acidic pH in the range of 3.71-3.98 and titratable acidity of 0.30% - 0.36%. The solubility percentage of the samples ranged

between 62.11% and 68.77%. Solubility was found high in case of fruit powder coated only with maltodextrin and decreased significantly with addition of other coating materials. Higher molecular weight of the coating material influences the solubility of the spray dried powder[36].

Table 5.2. Physicochemical parameters of the spray-dried *L. plantarum* litchi juice powders

Sample	Yield (%)	Water activity (a_w)	pH	Titratable acidity (%)	Solubility (%)	Hygroscopicity (g/100g)
M	75.27^{b}± 0.31	0.564	3.71^{a}±0.10	0.36^{d}±0.02	68.11^{c} ± 0.14	14.99^{b} ± 0.19
MP	68.77^{a}± 0.20	0.412	3.88^{c}±0.12	0.38^{c}±0.09	63.25^{a} ± 0.18	15.18^{a} ± 0.14
MF	77.50^{c}± 0.19	0.288	3.81^{b}±0.11	0.32^{b}±0.04	66.64^{b} ± 0.12	13.12^{b} ± 0.13
MPF	67.25^{a}± 0.11	0.171	3.90^{c}±0.10	0.30^{a}±0.05	62.77^{a} ± 0.15	17.34^{a} ± 0.17

M: 15% (w/v) maltodextrin; MP: 10% (w/v) maltodextrin plus 5% (w/v) pectin; MF: 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS); MPF: 5% (w/v) maltodextrin plus 5% (w/v) pectin 5% (w/v) plus fructooligosaccharide (FOS)

Means ± SD of triplicates values with the same letter within the column are not significantly different at $P \leq 0.05$ by DMRT.

Similarly, hygroscopicity of spray dried powders coated with M and MF showed no significant variation in values. Hygroscopicity was found highest in spray dried powder having maltodextrin plus pectin plus fructooligosaccharide. **Table 5.2** presents the physical properties of the *L. plantarum*-litchi-juice powder samples having different coating material.

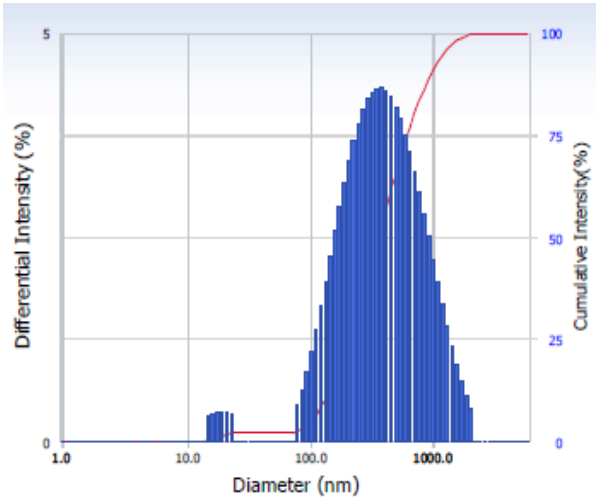
Table 5.3. Bulk density, tapped density, hausner's ratio, carr index and polydispersity index and span value of the spray-dried *L. plantarum*-litchi-juice powders

Sample	Bulk density (g/mL)	Tapped density (g/mL)	Hausner ratio	Carr index (%)	Polydispersity index (Đ)	Span value
M	0.367^{a} ± 0.015	0.423^{a} ± 0.003	1.15	13.23	0.335	14.32^{b} ±0.11
MP	0.377^{b} ± 0.003	0.444^{c} ± 0.005	1.60	37.61	0.272	36.13^{a} ±0.17
MF	0.357^{b} ± 0.009	0.555^{b} ± 0.003	1.51	33.87	0.599	8.47^{a} ±0.07
MPF	0.383^{a} ± 0.011	0.454^{a} ± 0.002	1.36	26.65	0.305	6.15^{c} ±0.19

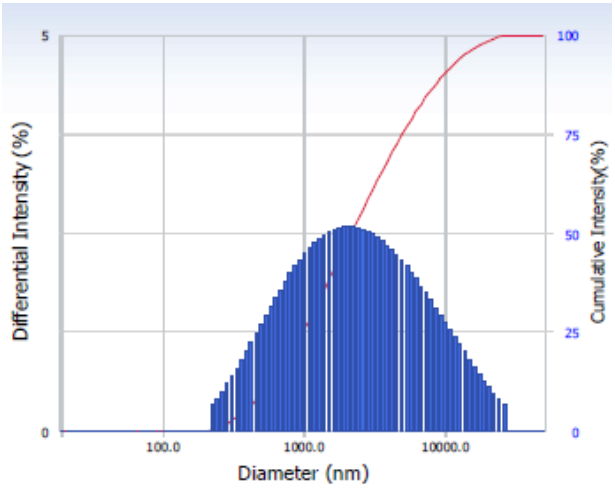
M: 15% (w/v) maltodextrin; MP: 10% (w/v) maltodextrin plus 5% (w/v) pectin; MF: 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS); MPF: 5% (w/v) maltodextrin plus 5% (w/v) pectin 5% (w/v) plus fructooligosaccharide (FOS)

Means ± SD of triplicates values with the same letter within the column are not significantly different at $P \leq 0.05$ by DMRT.

The Hausner ratio ranged between 1.15 and 1.60 and Carr index range was between 13.23 and 37.61%. According to the classification of powder on the basis of Hausner ratio and Carr index [33], powder spray dried with only 15 % (w/v) maltodextrin (M) had low cohesiveness, powders having 10% (w/v) maltodextrin plus 5% (w/v) FOS (MF) and 10% (w/v) maltodextrin plus 5% (w/v) pectin (MP) had high cohesiveness and powder having 5% (w/v) maltodextrin, 5% (w/v) pectin and 5% (w/v) FOS (MPF) had intermediate cohesiveness.



M



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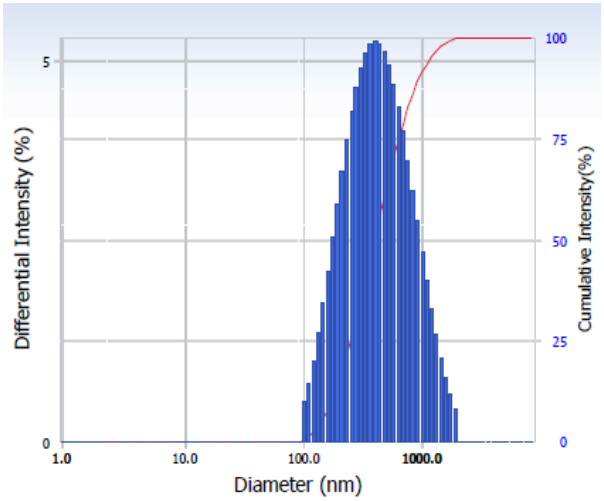
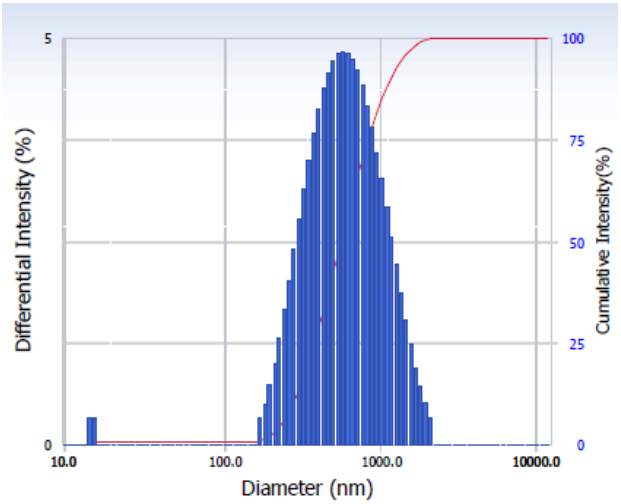


MF



MPF

Fig. 5.1. Particle size distribution of the spray-dried *L. plantarum*-litchi-juice powders
M: 15% (w/v) maltodextrin; MP: 10% (w/v) maltodextrin plus 5% (w/v) pectin; MF: 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS); MPF: 5% (w/v) maltodextrin plus 5% (w/v) pectin 5% (w/v) plus fructooligosaccharide (FOS)

Further, the flowability as per Carr index was very good in powder with M, fair for MPF and bad for MP and MF powders. SEM micrographs in **Fig.5.2** also confirmed this finding.

Particle size of the spray dried powder was reflected in span value for the samples (**Table 5.3**). In case of MF and MPF, span was 8.47 and 6.15, respectively whereas, it was 14.32 and 36.13, respectively for powder with M and MP. Span values indicated the uniformity in particle size for MF and MPF powders. It can be assumed that low viscosity of powder with MF and MPF had led to the formation of smaller particles [36] which were also reflected in their higher polydispersity index (**Fig. 5.1**).

In general, powder with high glass transition temperature (T_g) shows low hygroscopicity and becomes more sticky [50]. **Fig. 5.2** demonstrates that T_g, the midpoint of the glass transition range, of the powders coated with 10% (w/v) maltodextrin and 5% (w/v) pectin was much higher than the powder spray dried with only 15 % (w/v) maltodextrin or 10% (w/v) maltodextrin plus 5% (w/v) FOS because the T_g has a positive relationship to molar mass of the molecule [51].

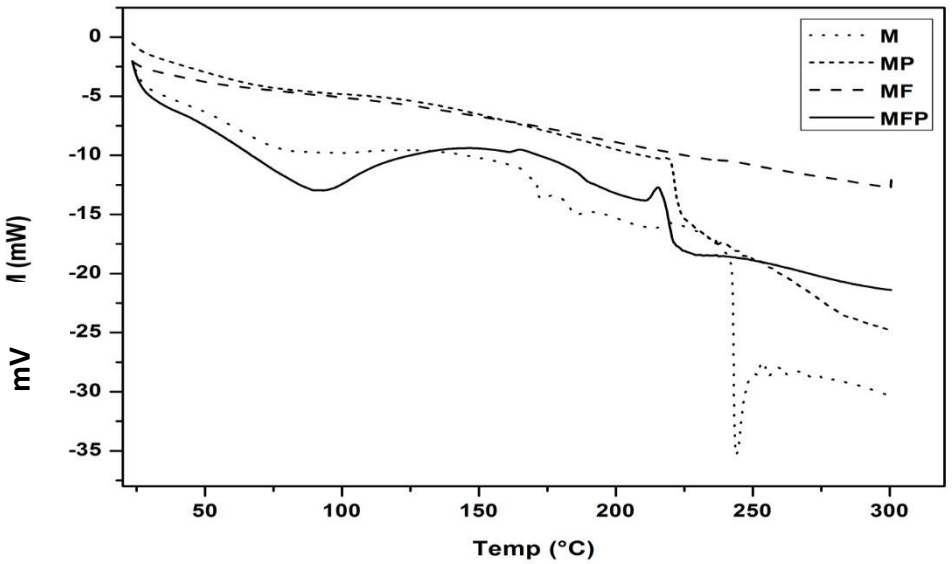


Fig.5.2. DSC thermograph of the spray-dried *L. plantarum*-litchi-juice powders with carrier materials

M: 15% (w/v) maltodextrin; MP: 10% (w/v) maltodextrin plus 5% (w/v) pectin; MF: 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS); MPF: 5% (w/v) maltodextrin plus 5% (w/v) pectin 5% (w/v) plus fructooligosaccharide (FOS)

5.3.3. Surface morphology study of the spray dried juice powder

The protective effect of maltodextrin and FOS was ascertained from the morphology of the particles as seen in the scanning electron micrographs in **Fig 5.3**.

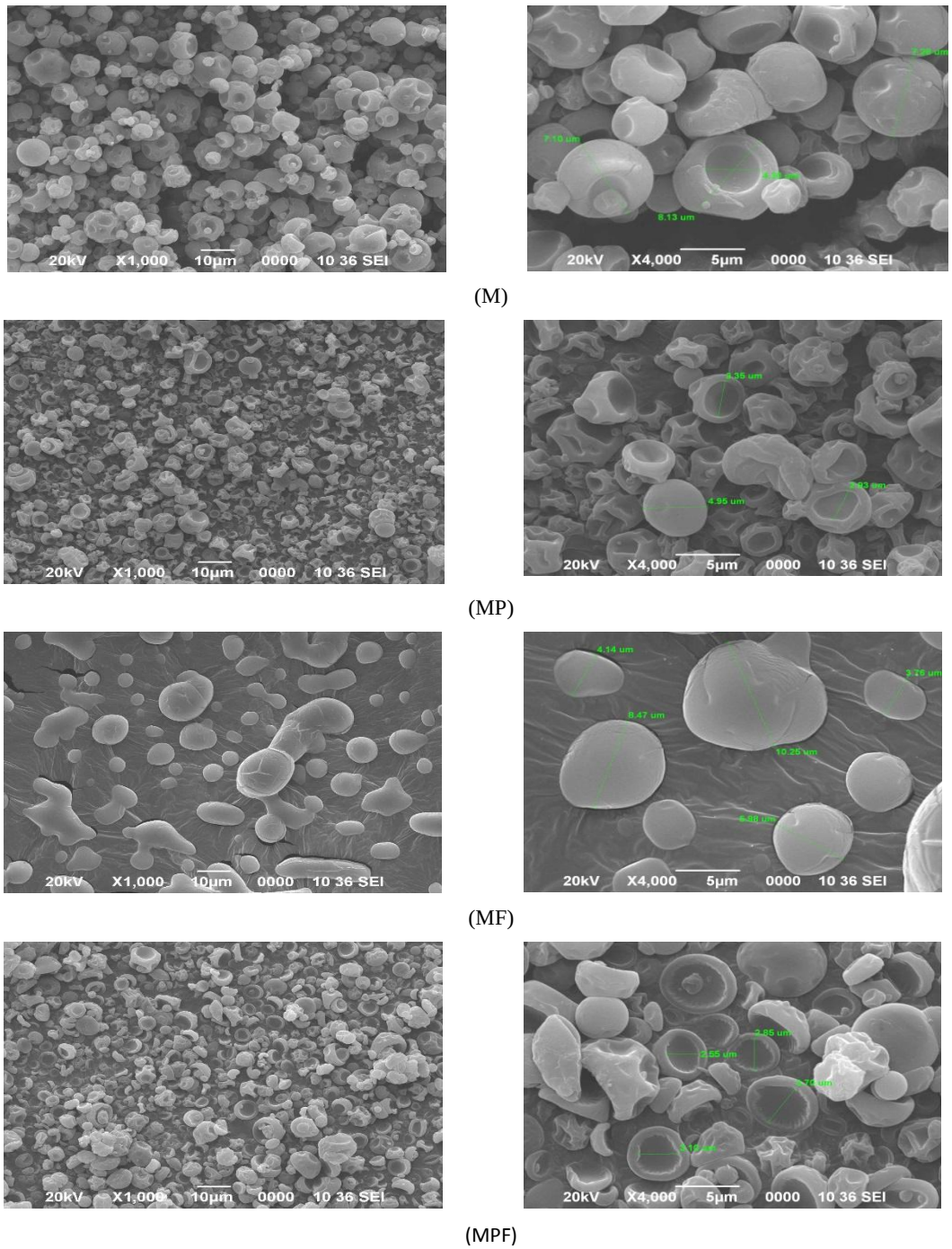


Fig. 5.3. Scanning electron microscopy (SEM) photographs of the spray-dried *L. plantarum*-litchi-juice powders with different carrier materials.

M: 15% (w/v) maltodextrin; MP: 10% (w/v) maltodextrin plus 5% (w/v) pectin; MF: 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS); MPF: 5% (w/v) maltodextrin plus 5% (w/v) pectin 5% (w/v) plus fructooligosaccharide (FOS)

Table 5.4. Colour parameters of the fresh litchi juice and spray-dried *L. plantarum*-litchi juice powders

Sample	*L*		*a*		*b*		Overall colour difference, △E
	FS#	RS#	FS	RS	FS	RS	
M	51.29± 0.16*	44.25±0.12*	17.27±0.05*	-0.51±0.07*	0.75±0.08*	0.69±0.09*	31.12±0.12
MP	49.55±0.13	33.17±0.10	15.69±0.07*	0.64±0.02*	0.55±0.02*	0.84±0.08*	34.14±0.16
MF	44.28±0.11*	37.15±0.14*	25.14±0.02*	-0.42±0.06*	0.51±0.24*	-0.27±0.02*	28.42±0.10
MPF	41.69±0.17*	35.27±0.18*	29.11±0.06*	-0.46±0.05*	0.49±0.01*	-0.19±0.01*	36.61±0.14

M: 15% (w/v) maltodextrin,
MP: 10% (w/v) maltodextrin plus 5% (w/v) pectin
MF: 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS)
MPF: 5% (w/v) maltodextrin plus 5% (w/v) pectin 5% (w/v) plus fructooligosaccharide (FOS)
* denotes statistically significant difference at $p \leq 0.05$ between FS and RS
# FS denotes feed sample; RS denotes reconstituted sample

Litchi juice powder coated with M, MP, and MPF formed spherical particles of which some were smooth surfaced while others appeared to be shrunk with dents on the surface. On comparison, particles with MF were larger in size with smooth surface.

Goula and Adamopoulos [36] stated that void formation resulted from the shrinking process that occurred after the hardening of the outer surface followed by the expansion of the air bubbles trapped inside the droplet. MF coated powder exhibited irregular shapes having smooth surface with no voids, which could be an effect of FOS coated on the outer surface to hinder heat transfer through the inner particle, as a result more protection of probiotic cells and enhanced cell survivability were observed (**Table 5.1**). It was also noted that the particle size of powder coated with maltodextrin plus FOS were much bigger than those coated only with maltodextrin or maltodextrin plus pectin. Similar finding was also reported in spray dried raspberry juice by Anekella and Orsat [10] and Arslan et al. [52].

5.3.4. Colour of the spray dried fruit juice powders

The '*L*', '*a*' and '*b*' values between feed and reconstituted spray dried powder exhibited significant difference ($P \leq 0.05$) except in "*L*" value of the powder with MP (**Table 5.4**). Decrease in '*L*' and '*a*' and '*b*' values was observed in all reconstituted samples indicating that the colour of reconstituted juices were lighter than the feed stock. The overall colour change (ΔE) was more prominent in powder having maltodextrin, pectin and FOS.

5.3.5. Cytotoxic activity of the spray dried powder

MTT assay is a reliable way to examine cell proliferation and cytotoxic effect of drugs or chemicals which is based on the reduction capacity of the dehydrogenase enzymes of the metabolically active cells [53]. The viable cells reduce the yellow tetrazolium MTT to form intercellular needle shaped purple formazon crystals (**Fig. 5.4**).

These crystals were solubilized in an organic solvent and the intensity was measured spectrophotometrically. In this study, HEK-293 cells showed survivability of 83.79%, 80.03%, 94.91% and 79.70% in 5 mg/ mL of water soluble fraction of the M, MP, MF and MPF coated spray dried powder, respectively (**Fig. 5.5**).

Highest survival was found in powder with MF whereas lowest was seen with MPF. From the figure it was confirmed that there is very nominal cytotoxic effect of the

spray dried powder on HEK-293 cells. The cells were adherent and no viable dead cells or cell debris was observed.

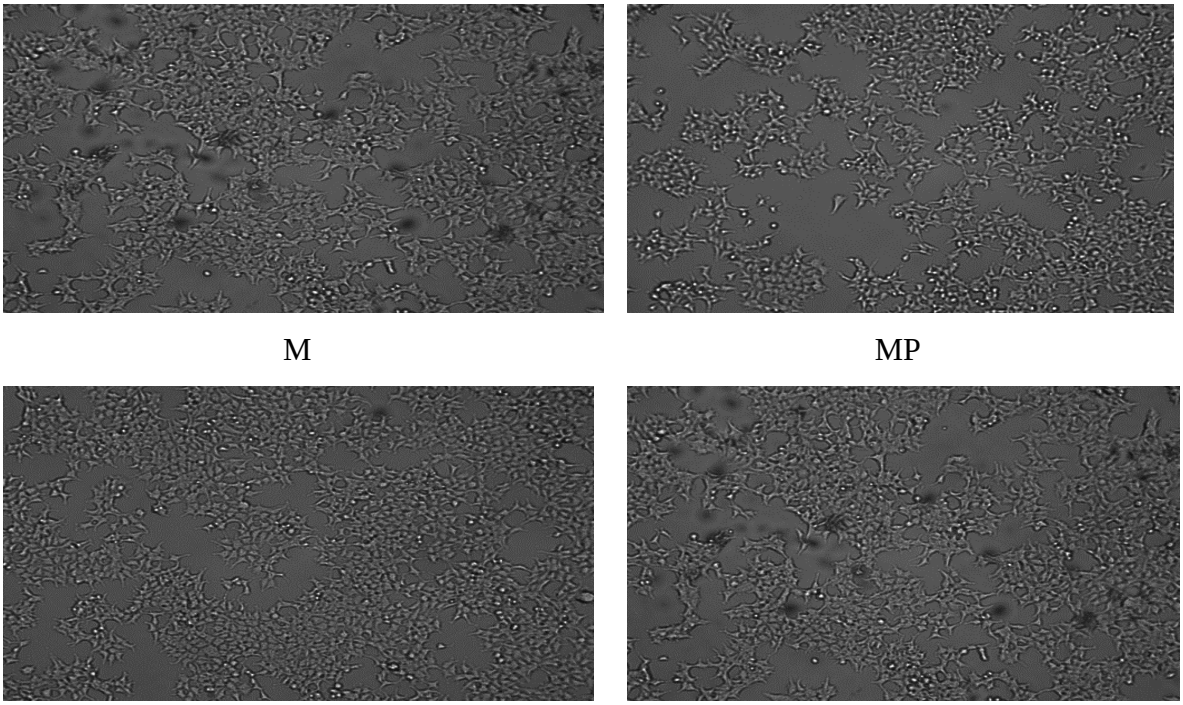


Fig. 5.4. MTT proliferation assay on HEK 293 cell lines of the of the spray-dried *L. plantarum*-litchi-juice powders:
M: 15% (w/v) maltodextrin; MP: 10% (w/v) maltodextrin plus 5% (w/v) pectin; MF: 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS); MPF: 5% (w/v) maltodextrin plus 5% (w/v) pectin 5% (w/v) plus fructooligosaccharide (FOS)

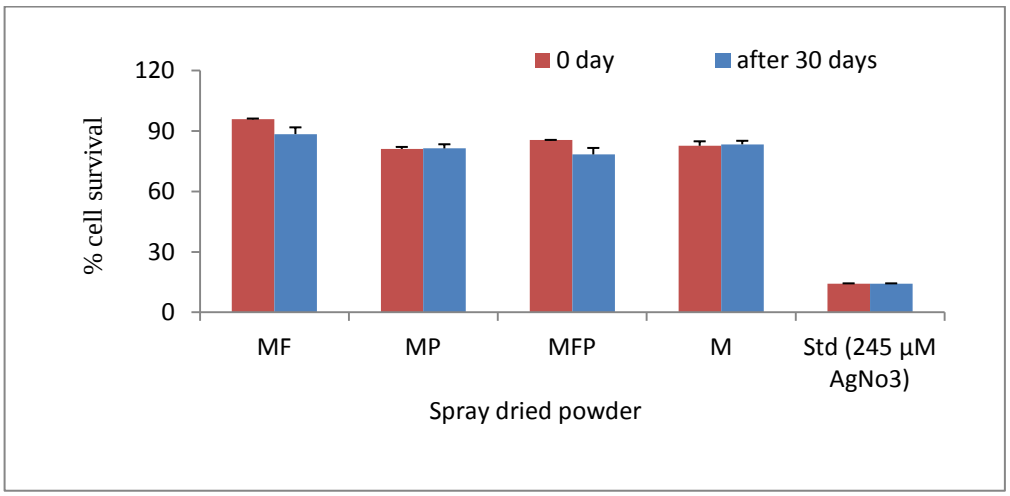


Fig. 5.5. MTT- cytotoxicity assay for cell survival in different spray dried powders during storage
M: 15% (w/v) maltodextrin; MP: 10% (w/v) maltodextrin plus 5% (w/v) pectin; MF: 10% (w/v) maltodextrin plus 5% (w/v) fructooligosaccharide (FOS); MPF: 5% (w/v) maltodextrin plus 5% (w/v) pectin 5% (w/v) plus fructooligosaccharide (FOS)

5.4. Conclusion

Spray drying of probiotic *L. plantarum* in litchi juice with different prebiotics had both positive and adverse effects on the powder quality. The physicochemical properties such as yield, colour, solubility, water activity and hygroscopicity of the final product varied depending on carrier material used. Maltodextrin 10% (w/v) plus 5% (w/v) FOS gave highest yield and viability of the encapsulated bacteria than the other combinations. No significant change in colour was observed with varied carrier materials. Maltodextrin plus FOS coated spray dried powder had superior flowability and solubility. Spray drying of probiotic *L. plantarum* in litchi juice with the coating material did not show any cytotoxicity to the human cells. Spray drying is a convenient method to enhance the viability of the probiotics in fruit juice powder. It has also the convenience of reconstitution just prior to consumption. Maltodextrin plus fructooligosaccharide retained relatively higher number of viable cells for ten weeks and can therefore be concluded to have the most protective effect on probiotics.

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