OPTIMIZATION OF SPRAY DRYING CONDITIONS FOR ENHANCED SURVIVABILITY AND RECOVERY OF *LACTOBACILLUS PLANTARUM* MTCC2621 IN FRUIT JUICES

4.1. Introduction

Versatility in consumption with added health factors in addition to nutrition and flavor are characteristics of a functional food. The exorbitantly high-priced health care and medicines and the desire for better quality of life [1] encourages novel functional foods consumption with multiple health benefits apart from basic nutrition. The link between diet and health is growing stronger day by day. Healing an illness through particular food consumption to restore natural defense with fewer side effects than medicine is always appealing to all age groups [2]. Hence research and development on functional food components like phytochemicals, probiotics, and omega fatty acids that act beyond the normal nutrition is presently an important focus of the food industry [3].

Several methods for improving the viability of the probiotics like inclusion of prebiotics, optimizing production operation, selection of a cocktail of probiotic organisms, modification (physically or genetically) of the probiotics prior to microencapsulation are followed to ensure the bioactivity of the probiotic products.

Many protective agents have been used for preserving the function of microorganisms in the dry state. Sucrose and trehalose have been utilised the most [4], although other sugars (e.g. maltose, fructose) and sugar alcohols (e.g. sorbitol, inositol) also offer protective effects [5,6]. Studies on liposomes, as a model for bacterial cell membranes, showed that a mixture of hydroxyethyl starch and glucose stabilized liposomes during drying [7]. The protective effect of sugars on membrane integrity and proteins in dehydrated bacterial cells has been ascribed to the ability of sugars to bind to polar residues of proteins and form an amorphous glass in the dry state; these are important for preserving the function of biological components during and after dehydration [8,4]. In the glassy state, the translational and relaxational motions are severely restricted, and diffusion controlled chemical or enzymatic reactions are arrested [9]. Dry preparations of probiotics are usually supplied as freeze-dried powders. Spray drying of probiotic cultures may be used as an alternative drying method [10]. Higher temperatures used during spray drying may be detrimental to bacteria. However this is not the case for certain lactic acid

bacteria. For example, similar survival rates were obtained on freeze-drying and spray-drying of concentrated cultures of *Lactobacillus bulgaricus* [10]. Cellular damage to probiotics may be reduced and viability preserved through control of drying parameters; specifically, by lowering the outlet temperature of spray dryers [11,12] and the incorporation of appropriate carriers into the drying medium [13]. The addition of sugars to the growth medium also influences the survival of dried probiotic preparations [6].

In this context, microencapsulation of probiotic bacteria is currently drawing more and more attention for being a method to improve the stability of probiotic organisms in functional food products [14,15]. Moreover, according to Ding and Shah [16], microencapsulation may improve the survival of these microorganisms, during both processing and storage, and also during passage through the human gastrointestinal tract. Spray drying is regarded as a microencapsulation method and it has been investigated as a means of stabilizing probiotic bacteria in a number of food matrices, most often composed of proteins, polysaccharides, sugars, and combinations thereof [17]. The survival rate of the culture during spray drying and subsequent storage depends upon a number of factors, which may include the species and strain of the culture, the drying conditions and also the use of encapsulating agents [18].

Another approach to increase the viability of probiotics is the use of prebiotics [19], which are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of bacteria in the colon [20]. These prebiotics may potentially be exploited as carrier media for spray drying and may be useful for enhancing probiotic survival during processing [21]. However, the use of different encapsulating agents for production of microcapsules can result in different physical properties, depending on the structure and the characteristics of each agent [22], and it can also modify functional properties of microcapsules [23]. Additionally, thermoplasticity and hygroscopicity of fruit juice might pose problems during the spray drying causing them to adhere to the chamber wall due to their stickiness, clogging and caking [24]. Adding suitable wall materials such as maltodextrin can reduce the caking and stickiness to the walls and increase the free flowing nature of the spray dried powder.

Therefore, the objective of this study was to optimize the spray drying conditions (inlet temperature, feed ratio, and feed rate) for spray drying of *Lactobacillus plantarum* in

different fruit juices on the basis of survivability and recovery of the product by response surface methodology

4.2. Materials and Methods

4.2.1. *Lactobacillus* strains

The *Lactobacillus* isolates *Lactobacillus plantarum* MTCC2621 was collected from Microbial Type Culture Collection and Gene Bank (MTCC) (IMTECH, CSIR, Chandigarh, India). All the chemicals used were of analytical grade and supplied by Merck, India, Himedia Laboratories and Sigma chemicals, India.

4.2.2. Fruit samples

Three different fruits viz. litchi (*Litchi chinensis* Sonn.), pineapple (*Ananas comosus* L. Merr) and Khasi mandarin orange (*Citrus reticulate* Blanco) were procured from the local fruit market, Tezpur, Assam during the season. The fruits were chosen based on their easy availability and suitability for juice extraction.

4.2.3. Inoculum preparation

The freeze dried culture of *Lactobacillus* isolates, *Lactobacillus plantarum* MTCC2621 was activated in sterile glycerol (50% v/v). The glycerol stock culture was stored at -20 ^{o}C in sterile screw cap tubes. The cultures were grown at 37 ^{o}C for 24 h in sterile de Man Rogosa and Sharp (MRS) broth (dextrose 20.0 g/L; meat peptone 10.0 g/L; beef extract 10.0 g/L; yeast extract 5.0 g/L; sodium acetate 5.0 g/L; disodium phosphate 2.0 g/L; ammonium citrate 2.0 g/L; tween 80 1.0 g/L; magnesium sulfate 0.1 g/L, manganese sulfate 0.05 g/L) under aerobic conditions. 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 sodium chloride) to remove any residual MRS.

4.2.4. Preparation of fruit juices

Juice from fresh fruits of orange, pineapple and litchi was prepared using electric juicer (Phillips, India). The TSS of the juice was adjusted to 11°Brix (total solid concentration 0.1 g/L) as otherwise the pure extract was too viscous to be spray dried.

4.2.5. Experimental design for optimisation by response surface methodology (RSM)

Process optimizations for spray drying of probiotics in three different juices were performed using Box-Behnken design of RSM with three independent variables and three levels of each variation. The design comprised of 17 experimental runs for each fruit juice having 5 center points, as shown in Tables 4.2-4.4. The independent variables were inlet temperature (X_1, ^oC), ratio of total solids in juice to maltodextrin (X_2), and feed rate (X_3, mL/min) and % recovery and % survival were the dependent variables. Preliminary tests were conducted to establish these variables. In this current study, data obtained by application of RSM was used to build mathematic models to interpret the relationship between the independent and dependent variables. After analyzing the effects of different variables on the probiotics viability and powder recovery, the maximum and minimum values of the each factor were adjusted. Regression analysis was performed based on the experimental data. The data obtained were fitted into a second order polynomial model. The generalised second order polynomial order equation (**Eq.4.1**) used was:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} . \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + e \qquad \textbf{Eq.4.1}$$

where Y was the response variable; βo, βi, βii, βij were the regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively; and xi and xj were independent variables. The results of the experimental design were analyzed and interpreted using Design Expert 9.0. Surface plots were also generated as a function of two variables, while holding the value of a third variable constant (at the central value). The optimization was performed individually by eliminating the insignificant factors in the process equation.

4.2.6. Spray drying process

Mixture of probiotics culture, fruit juice and coating material were homogenized for 1 to 2 min using a magnetic stirrer just before spray drying. Spray drying of the juices with maltodextrin as an additive (wall material) at different ratios i.e. 1:1, 1:1.5, and 1:2 (TSS of the juice: maltodextrin ratio) and mixture of lactobacilli was performed separately using a Lab scale spray dryer (LabPlant UK). The spray dryer was allowed to reach uniform process temperature for 15-20 min prior to the spray drying. The aspiration was maintained at 100% and cyclone air flow rate at $30 m^3/h$. The TSS of the juice was adjusted to 11 °Brix by appropriate dilution with sterile distilled water to prevent clogging of the

spray drying nozzle. A 50 mL volume of fruit juice and maltodextrin at different ratios were homogenized using a magnetic stirrer. A 5 mL volume of probiotic culture (late exponential phase; grown over night; approximately 9.5 log CFU/mL) was centrifuged at 1500 x g for 15 min and dispersed in 1 mL of sterile distilled water and homogenized in fruit juice and maltodextrin mixture by stirring just before spray drying. The juice powder obtained on spray drying was stored in small glass bottles with screw caps and stored at refrigeration temperature (4±2°C) in the dark for further investigations. The RSM trials were done in duplicate.

4.2.7. Recovery (%)

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 the following equation.

$$\text{Recovery (\%)} = \frac{\text{Weight of the solids in dried powder}}{\text{Solid Content of the feed material}} \text{x} 100$$

4.2.8. Survivability (%)

Survival of the microencapsulated bacteria during storage was carried out according to the modified method of Kimoto et al. [45]. One gram of microencapsulated probiotic bacteria was suspended in sodium phosphate buffer 0.1 M before serial dilutions using peptone water solution (1.5 g peptone in 1 L water) were carried out. 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 the CFU/g.

4.2.9. Statistical analysis

All experiments were carried out at least in triplicate and reported as mean ± standard deviation of mean (S.E.M) using SPSS version 11.5. The physicochemical properties were statistically analyzed by Duncan's multiple range test using one-way ANOVA while the colour, phytochemical content and antioxidant properties were subjected to paired-comparison t-test ($p \leq 0.05$).

4.3. Results and Discussion

The optimization of the two dependent variables, % recovery and % survival for all three juices viz. litchi, pineapple and orange (**Tables 4.2 - 4.4**) were performed and insignificant factors in the process equation were eliminated. Viability retention (in terms of log CFU/g) and recovery (%) are discussed in greater detail in later sections. Among the 17 different trials performed for each juice, the best trial with higher shelf life and recovery were chosen for further analysis.

4.3.1. Optimization of spray drying condition for litchi juice with *Lactobacillus plantarum*

The experimental design of 17 runs for optimising independent variables for spray drying of litchi juice with *Lactobacillus plantarum* and the responses of the experiments are shown in **Table 4.1**.

Table 4.1. Experimental design of the spray drying conditions for litchi juice with *Lactobacillus plantarum* MTCC2621and their responses

Run	Inlet Temp (°C) (X_1)	Solid:Maltodextrin Ratio (X_2)	Feed Rate (mL/min) (X_3)	% Recovery	% Survival
1	100	1:1	50	29.85	81.70
2	130	1:1	50	45.72	56.96
3	100	1:2	50	34.46	76.61
4	130	1:2	50	46.11	51.97
5	100	1:1.5	40	24.85	81.70
6	130	1:1.5	40	36.89	51.97
7	100	1:1.5	60	31.16	77.75
8	130	1:1.5	60	53.39	65.91
9	115	1:1	40	34.17	66.11
10	115	1:2	40	34.95	62.37
11	115	1:1	60	34.17	76.61
12	115	1:2	60	35.72	76.50
13	115	1:1.5	50	31.94	68.19
14	115	1:1.5	50	30.19	69.23
15	115	1:1.5	50	31.55	67.56
16	115	1:1.5	50	27.18	65.59
17	115	1:1.5	50	26.79	65.59

ANOVA (**Table 4.2**) for the model of % recovery as fitted showed significance ($p < 0.05$) and the lack of fit was non- significant ($p > 0.05$). The response surface regression model on % recovery yielded excellent fits with coefficient of determination ($R^2 = 0.89$) for litchi juice with *Lactobacillus plantarum* MTCC2621. The inlet temperature (°C) (X_1) showed a positive ($p<0.001$) effect on % recovery of the spray dried powder.

Table 4.2. Fit statistics for % recovery and % survival surface plot of litchi juice with *L. plantarum* MTCC2621

Variables	Recovery	Survival
X_1	0.0005***	< 0.0001***
X_2	0.4947	0.1072
X_3	0.0535*	0.0025**
X_1^2	0.0145**	0.5853
X_2^2	0.0645*	0.8107
X_3^2	0.4571	0.0647*
$X_1 X_2$	0.5757	0.9860
$X_1 X_3$	0.1995	0.0122**
$X_2 X_3$	0.9171	0.5174
R^2	**0.89**	**0.94**
Adj R^2	0.77	0.92
Adeq Precision	7.74	15.49
Lack of Fit	3.85	5.02
Model (F-value)	6.92	20.77

X_1 = inlet temperature (^oC); X_2 = solid: maltodextrin ratio and X_3= feed rate (mL/min)
*Significant at $p<0.1$, **Significant at $p<0.05$, ***Significant at $p<0.001$

In order to optimize processing conditions for spray drying of litchi juice with probiotic and maltodextrin by numerical optimization, which finds a point that maximizes the desirability function, importance of 3 was given to recovery and 4 to cell survival (**Table 4.3**).

Table 4.3. Optimized parameters in the response optimizer

Response	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Recovery	Maximum	24.85	53.39	1	1	3
Survival	Maximum	51.97	81.69	1	1	4

The optimal combination for inlet temperature, juice solid to maltodextrin ratio and feed rate was 130°C, 1.5 and 60 mL/min (**Table 4.4**). At the optimised conditions, the predicted responses gave 53.39% recovery and 67.95% survival.

Table 4.4. Optimized solution obtained using the response optimizer

Optimal solution			**Predicted responses**	
X_1	**X_2**	**X_3**	**Recovery (%)**	**Survival (%)**
130	1.5	60	53.39	67.95

X_1 = inlet temperature (^oC); X_2 = solid: maltodextrin ratio and X_3= feed rate (mL/min)

a) Effect on % recovery

The response plot for % recovery with respect to total solids: maltodextrin ratio and inlet temperature is presented in **Fig 4.1**. From the response plot, it was observed that rise in inlet temperature (X_1) up to 130^{o}C resulted in an increase in recovery of spray dried powder. The recovery varied between 24.85% and 53.39% depending on changes in inlet temperature and feed rate. The wall of the spray drier was layered completely with the litchi juice solids by the end of spray drying due to the stickiness of the solutions which led to major loss in product recovery. The surface plot indicates the overall optimum condition with respect to maximum % recovery (53.39 %) at input conditions of 130°C inlet temperature, a total solids to maltodextrin juice ratio of 1.5 and an inlet feed rate of 60 mL/min (**Fig. 4.1a**). The interaction effect between the three variables showed a positive effect on the response. The regression equation obtained for % recovery is given below (**Eq. 4.2**)

$$Recovery = 29.53 + 7.72\,X_1 + 2.95\,X_3 + 5.66\,X_1^2 + 3.84\,X_2^2 \qquad \textbf{Eq. 4.2}$$

Where, X_1, $X_2$and X_3 are coded variables of inlet temperature (°C), total solids to maltodextrin ratio and inlet feed rate (mL/min), respectively

From the response plot presented in **Fig 4.1a**, both the independent variables (inlet temperature and feed rate) positively affected the recovery of the spray dried powder. As the inlet tempratute increased from 100°C to 130°C, the recovery of powder increased by 53%. Similar increased was also seen when the feed rate increased upto 60 mL/min.

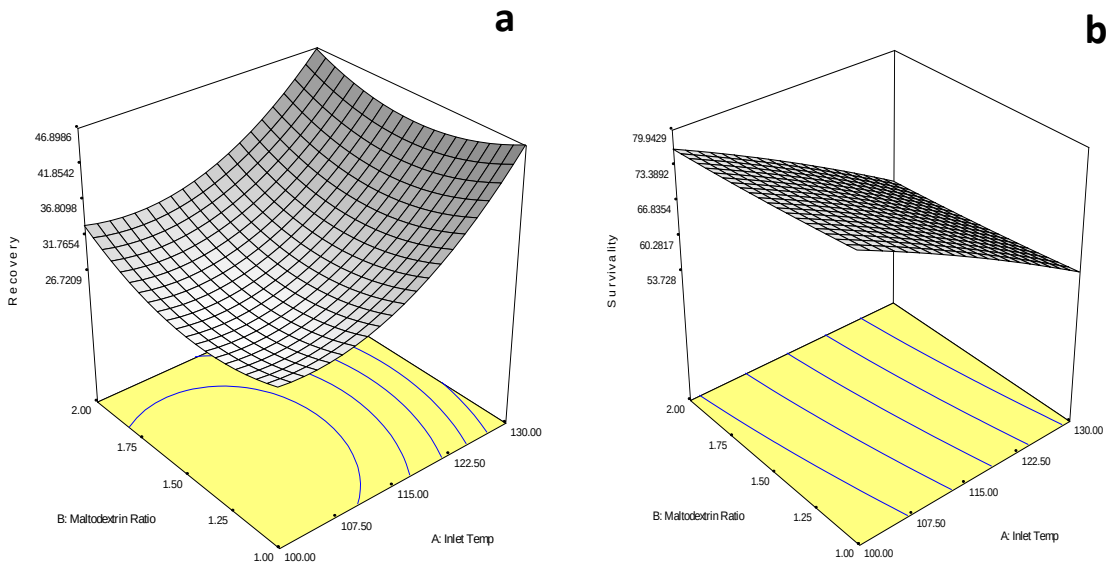

Fig 4.1. Effect of response variables on (a) recovery and (b) survival in spray dried *Lactobacillus plantarum* MTCC2621 with litchi juice

The optimized equation (**Eq 4.2**) follows a two factor linear form. From the analysis, solid to maltodextrin ratio did not have a significant role in product recovery. Inlet temperature and feed rate were the significant factors in the optimized predictive model. The R^2 value of the master model was 0.89 whereas the predictive model had an R^2 value of 0.77 (**Table 4.3**). The ANOVA table and effect estimates for % recovery of the master and predicted model are presented in APPENDIX.

b) Effect on % survival

Prediction of microbial survival is essential to optimize any potential industrial process. High viability is required in the final probiotic product. The surface response plot for % survival with respect to varying temperature (°C) and solids to maltodextrin ratio is presented in **Fig. 4.1b.** The surface plot indicates maximum % survival (81.69%) at the input conditions of 100°C inlet temperature, a total solids in juice: maltodextrin ratio of 1:1 and an inlet feed rate of 50 mL/min (**Fig. 4.1b**).

As expected from the response plot presented in **Fig 4.1b**, the processing temperature had a major effect on the probiotic's survival. The cell survival dropped down to almost 36% (9.5 to 5 log CFU/mL) when the inlet temperature was raised to 130°C. The solids to maltodextrin ratio did not have a very significant role comparatively. It was

also noted that the survivablity % varied with the inlet feed rate. From **Table 4.1** it is seen that under same inlet temperature conditions both high and low feed rate decreased survival of the bacterial cells. This indicates that the survival is mostly dependent on inlet temperature (**Fig. 4.1b**).

The % survival optimized model equation (**Eq 4.3**) follows a two factor linear form where temperature plays the most important role. The R^2 value of the master model was 0.94 whereas the optimized predictive model had an R^2 value of 0.92 (**Table 4.2**). The ANOVA table and effect estimates for % survival of master and predicted model are presented in APPENDIX.

$$Survival = 67.23 - 11.34\,X_1 + 4.33\,X_3 + 2.84\,X_3^2 + 4.47\,X_1X_3 \qquad \textbf{Eq. 4.3}$$

Where, X_1, $X_2$and X_3 are coded variables of inlet temperature (°C), solids to maltodextrin ratio and inlet feed rate (mL/min), respectively.

4.3.2. Optimization of spray drying condition for pineapple juice with *Lactobacillus plantarum*

The optimized results of the spray drying for pineapple juice with *Lactobacillus plantarum* are shown in the **Table 4.5**.

Table 4.5. Optimization of the spray drying condition for pineapple juice with *Lactobacillus plantarum* MTCC2621

Run	Inlet Temp (°C) (X_1)	Solid:Maltodextrin Ratio (X_2)	Feed Rate (mL/min) (X_3)	% Recovery	% Survival
1	100	1:1	50	25.4	84.33
2	130	1:1	50	36.8	58.8
3	100	1:2	50	30.75	82.62
4	130	1:2	50	36.3	68.03
5	100	1:1.5	40	32.1	84.33
6	130	1:1.5	40	38.8	56.87
7	100	1:1.5	60	25.6	80.26
8	130	1:1.5	60	47.1	53.65
9	115	1:1	40	32.5	68.24
10	115	1:2	40	35.5	69.10
11	115	1:1	60	31.1	70.39
12	115	1:2	60	41.5	64.38
13	115	1:1.5	50	35.2	71.14
14	115	1:1.5	50	35.2	67.70
15	115	1:1.5	50	36.3	69.74
16	115	1:1.5	50	32.9	67.70
17	115	1:1.5	50	27.6	71.46

Table 4.6. Fit statistics for % recovery and % survival surface plot for litchi juice with *L. plantarum* MTCC2621

Variables	Recovery	Survival
X_1	0.0018***	< 0.0001***
X_2	0.0888*	0.7834
X_3	0.5109	0.2734
X_1^2	0.9090	0.1481
X_2^2	0.5740	0.3074
X_3^2	0.1401	0.0672*
$X_1 X_2$	0.4003	0.1045
$X_1 X_3$	0.0579*	0.8889
$X_2 X_3$	0.2947	0.2799
R^2	**0.84**	**0.95**
Adj R^2	0.65	0.89
Adeq Precision	9.23	14.08
Lack of Fit	0.71	4.82
Model (F-value)	4.28	15.93

X_1 = inlet temperature (^oC); X_2 = solid: maltodextrin ratio and X_3= feed rate (mL/min)
*Significant at p<0.1, **Significant at p<0.05, ***Significant at p<0.001

Table 4.7. Optimized parameters in the response optimizer

Response	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Recovery	Maximum	25.4	47.1	1	1	3
Survival	Maximum	53.6	84.3	1	1	4

The optimized conditions derived from the response surface were an inlet temperature of 100°C, juice solid to maltodextrin ratio of 1: 2 and feed rate of 40 mL/min (**Table 4.8**). At the optimised conditions, the predicted responses were recovery of 34.11% and 82.82% survival.

Table 4.8. Optimized solution obtained using the response optimizer

Optimal solution			Predicted responses	
X_1	X_2	X_3	Recovery (%)	Survival (%)
100	2	40	34.11	82.82

X_1 = inlet temperature (^oC); X_2 = solid: maltodextrin ratio and X_3= feed rate (mL/min)

a) Effect on % recovery

The response surface regression model on % recovery yielded excellent fits with coefficient of determination (R^2 = 0.84) for pineapple juice with *Lactobacillus plantarum* MTCC2621. The inlet temperature (°C) (X_1) showed a positive ($p<0.001$) effect on % recovery of product. The response plot for % recovery with respect to total solids: maltodextrin ratio and inlet temperature is presented in **Fig. 4.2a**. From the response plot, it was observed that rise in inlet temperature resulted in an increase in recovery of spray dried powder. The recovery varied between 25.4% and 47.1% depending on the change in inlet temperature from 100 to 130°C. The grid on the top indicates the overall optimum condition with respect to maximum % recovery (47.1 %) at input conditions of 130°C inlet temperature, a maltodextrin to total juice solids in juice ratio of 1:5 and an inlet feed rate of 60 mL/min (**Table 4.6**). The interaction effect between the three variables showed a positive effect on the response. The regression equation obtained for % recovery is given below (**Eq. 4.4**)

$$Recovery = 33.44 + 5.64\ X_1 + 3.7\ X_1\ X_3 \qquad \textbf{Eq. 4.4}$$

Where, X_1, $X_2$and X_3 are coded variables of inlet temperature (°C), maltodextrin ratio and feed rate (mL/min), respectively

From the response plot in **Fig. 4.2a**, inlet temperature and maltodextrin ratio had significant effects on % recovery of produced powder from pineapple juice. Both variables positively affected the recovery percentage. When the inlet temperature was raised from 100 to 130°C, the recovery increased by 46%. Similarly when the feed rate increased upto 60 mL/min the recovery decreaed by 20%. This may be due to the stickiness of the solutions which led to major losses in product recovery.

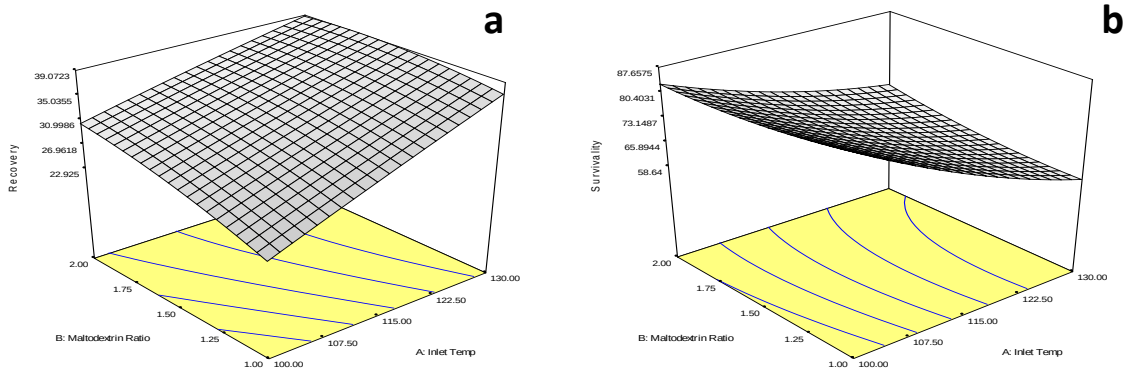

Fig 4.2. Effect of response variables on (a) recovery (%) and (b) survival (%) in spray dried *Lactobacillus plantarum* MTCC2621 with pineapple juice

The optimized equation (**Eq 4.2**) follows a two factor linear form. From the analysis feed rate did not have a significant role in product recovery. Inlet temperature and maltodextrin ratio were the significant factors in the optimized predictive model. The R^2 value of the master model was 0.84 whereas the predictive model had an R^2 value of 0.65 (**Table 4.6**). The ANOVA table and effect estimates for % recovery of the master and predicted model are presented in APPENDIX

b) Effect on % survival

The surface response plot for % survival of bacterial cells in pineapple juice powder with respect to varying temperature (°C) and maltodextrin ratio is presented in **Fig. 4.2b.** The response plot indicates the optimized conditions with respect to maximum % survival

(84.3%) at the input conditions of 100°C inlet temperature, a maltodextrin: total solids in juice ratio of 1:1.5 and an inlet feed rate of 40 mL/min (**Table 4.6**).

From the response plot presented in **Fig. 4.2**, the temperature of spray drier and maltodextrin ratio had major effect on the probiotics' survival. The survival dropped down to almost 36% when the inlet temperature was raised to 130°C. The feed rate did not show significant role on survivability of the cells. From **Table 4.7,** it is observed that under same inlet temperature conditions, a high solid to maltodextrin ratio showed an increased survival rate. This indicates that the survival is mostly dependent on inlet temperature and coating material (**Fig. 4.2b**).

The % survival optimized model equation (**Eq. 4.5**) follows a two factor linear form where temperature plays the most important role. The R^2 value of the master model was 0.95 whereas the optimized predictive model had an R^2 value of 0.89 (**Table 4.7**). The ANOVA table and effect estimates for % survival of master and predicted model are presented in APPENDIX

$$Survival = 69.55 - 11.77\ X_1 - 3.09 X_3^2 \qquad \textbf{Eq. 4.5}$$

Where, X_1, X_2 and X_3 are coded variables of inlet temperature (°C), maltodextrin ratio and inlet feed rate (mL/min), respectively.

4.3.3. Optimization of spray drying condition for orange juice with *Lactobacillus plantarum*

The optimized results of the spray drying for orange juice with *Lactobacillus plantarum* are shown in the **Table 4.9**.

Table 4.9. Optimization of the spray drying condition for orange juice with *Lactobacillus plantarum* MTCC2621

Run	Inlet Temp (°C) (X_1)	Maltodextrin Ratio (X_2)	Inlet Feed Rate (mL/min) (X_3)	% Recovery	% Survival
1	100	1:1	50	40.3	62.59
2	130	1:1	50	36.8	48.20
3	100	1:2	50	29.1	61.44
4	130	1:2	50	39.4	52.44
5	100	1:1.5	40	34.5	66.30
6	130	1:1.5	40	51.3	64.09
7	100	1:1.5	60	29.1	66.14
8	130	1:1.5	60	35.5	46.48
9	115	1:1	40	35.6	48.22
10	115	1:2	40	35.1	49.60
11	115	1:1	60	28.2	50.40
12	115	1:2	60	37.8	48.16
13	115	1:1.5	50	37.4	51.40
14	115	1:1.5	50	49.6	52.41
15	115	1.5	50	35.1	47.66
16	115	1.5	50	28.6	51.45
17	115	1.5	50	29.1	55.12

Table 4.10. Fit statistics for % recovery and % survival surface plot for orange juice with *L. plantarum* MTCC2621

Variables	Recovery	Survival
X_1	0.0387**	0.0017**
X_2	0.0872*	0.8150
X_3	0.0571*	0.1058
X_1^2	0.0342**	0.0014**
X_2^2	0.1152	0.0597*
X_3^2	0.1219	0.5331
$X_1 X_2$	1.0000	0.4336
$X_1 X_3$	0.0261**	0.0311**
$X_2 X_3$	0.0984*	0.5943
R^2	**0.85**	**0.91**
Adj R^2	0.66	0.78
Adeq Precision	10.07	8.96
Lack of Fit	0.04	2.10
Model (F-value)	4.45	7.44

X_1 = inlet temperature (^oC); X_2 = solid: maltodextrin ratio and X_3= feed rate (mL/min)
*Significant at p<0.1, **Significant at p<0.05, ***Significant at p<0.001

In order to optimize processing conditions for spray drying of orange juice with probiotic and maltodextrin by numerical optimization, which finds a point that maximizes

the desirability function, importance of 3 was given to recovery and 4 to cell survival (**Table 4.11**).

Table 4.11. Optimized parameters in the response optimizer

Response	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Recovery	Maximum	28.2	51.3	1	1	3
Survival	Maximum	46.48	66.3	1	1	4

The optimized condition derived from the response surface was an inlet temperature 100°C, juice solid to maltodextrin ratio 1:1.68 and feed rate 40 mL/min (**Table 4.12**). At the optimised conditions, the predicted responses were recovery of 40.61% and 63.64% survival.

Table 4.12. Optimized solution obtained using the response optimizer

Optimal solution			Predicted responses	
X_1	X_2	X_3	Recovery (%)	Survival (%)
100	1.68	40	40.61	63.64

X_1 = inlet temperature (^oC); X_2 = solid: maltodextrin ratio and X_3= feed rate (mL/min)

a) Effect on % recovery

The response surface regression model on % recovery yielded excellent fits with coefficient of determination (R^2 = 0.85) for orange juice with *Lactobacillus plantarum* MTCC2621. The inlet temperature (°C) (X_1) showed a positive ($p<0.001$) effect on % recovery of product.

The response plot for % recovery with respect to total solids: maltodextrin ratio and inlet temperature is presented in **Fig. 4.3**. From the response plot, it was observed that rise in inlet temperature from 100°C to 130°C resulted in increased in recovery of spray dried powder. The recovery varied between 28.2% and 51.3% depending on the change in inlet temperature from 100 to 130°C. The response surface plot indicates the overall optimum condition with respect to maximum % recovery (51.3 %) at input conditions of 130°C inlet temperature, a maltodextrin to total solids in juice ratio of 1:5 and feed rate of 50 mL/min

(**Table 4.10**). The interaction effect between the three variables showed a positive effect on the response. The regression equation obtained for % recovery is given below (**Eq. 4.6**)

$$Recovery = 33.16+2.88X_1+ 2.25\ X_2+ 2.25\ X_3 + 4.09X_1^2 +4.5\ X_1\ X_3 - 3.05\ X_2\ X \quad \textbf{Eq. 4.6}$$

Where, X_1, $X_2$and X_3 are coded variables of inlet temperature (°C), maltodextrin ratio and feed rate (mL/min), respectively

From the fit statistics **Table 4.10**, all the vriables viz. inlet temperature and maltodextrin ratio and feed rate had significant effects on % recovery of produced powder. All the variables positively affected the recovery percentage of the spray dried powder. When the inlet temperature raised from 100°C to 130°C without changing the other conditions, the recovery increased by 32%. Whereas increase in the fruit solid to maltodextrin ratio from 1:1 to 1:2, decline of the output was observed. The feed rate has negative impact on yield and an increased upto 60 mL/min the recovery decreased by 44%. This may be due to the stickiness of the solutions which lead to major losses in product recovery.

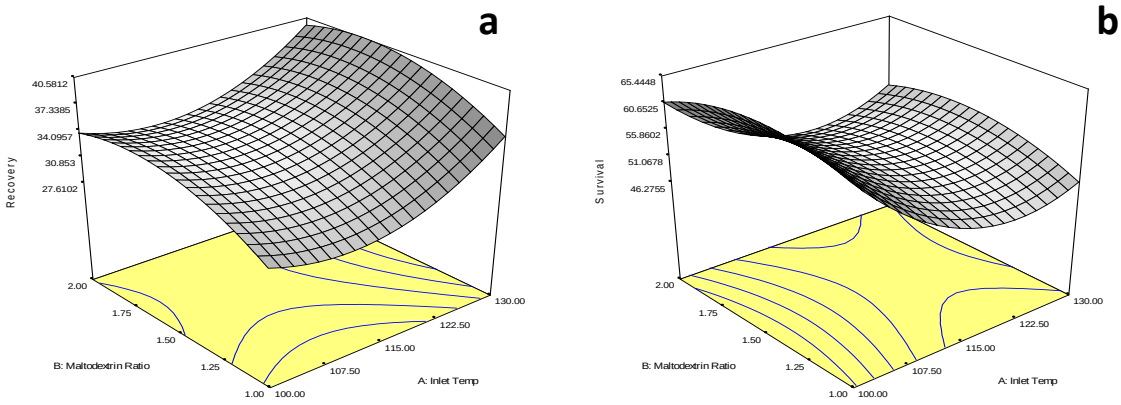

Fig 4.3. Effect of response variables on (a) recovery (%) and (b) survival (%) in spray fried *Lactobacillus plantarum* MTCC2621 with orange juice

The optimized equation (**Eq 4.6**) follows a two factor linear form. The inlet temperature (°C), maltodextrin ratio and feed rate (mL/min) were the significant factors in the optimized predictive model. The R^2 value of the master model was 0.85 whereas the predictive model had an R^2 value of 0.66 (**Table 4.11**). The ANOVA table and effect estimates for % recovery of the master and predicted model are presented in APPENDIX.

b) Effect on % survival

The surface response plot for % survival of bacterial cells in orange juice powder with respect to varying temperature (°C) and maltodextrin ratio is presented in **Fig. 4.3**. The grid on top indicates the optimized conditions with respect to maximum % survival (66.3%) at the input conditions of 100°C inlet temperature and minimum (46.48%) was observed at 130°C (**Table 4.10**).

From the response plot presented in **Fig.4.3**, the temperature of spray drier had major effect on the probiotics' survival as expected. The survival dropped down to almost 46% when the inlet temperature was raised to 130°C. The maltodextrin ratio and feed rate did not have shown significant role on survivablity of the cells. From **Table 4.9,** it is observed that under same maltodextrin ratio higher inlet temperature showed an decreased cell survival. This indicates that the survival of cells is mostly dependent on inlet temperature (**Table 4.11**) in case of orange juice. The % survival optimized model equation (**Eq 4.7**) follows a two factor linear form where temperature plays the most important role. The R^2 value of the master model was 0.91 whereas the optimized predictive model had an R^2 value of 0.78 (**Table 4.11**). The ANOVA table and effect estimates for % survival of master and predicted model are presented in APPENDIX

$$Survival = 51.61-5.66X_1+8.11X_1^2 - 3.55\ X_2^2 - 4.36X_1X_3 \qquad \textbf{Eq. 4.7}$$

Where, X_1, X_2 and X_3 are coded variables of inlet temperature (°C), maltodextrin ratio and inlet feed rate (mL/min), respectively.

Reducing the maltodextrin ratio can decrease the recovery however it also affect the survival of the probiotic due to reduced encapsulation efficiency. Inceasing the feed flow rate could be an alternative approach to increase product recovery since it had minimal effect on probiotic survival. But the possible disadvantage with increased feed rate is the increase in residence time within the drying chamber which exposes the probiotics to longer thermal stress. Low dextrose equivalent additives have been demonstrated to increase the product recovery by reducing the stickiness of orange juice and similarly higher Dextrose Equivalent (DE) value increased moisture content of the final product [25]. In the study conducted by Phongpipatpong et al. [26], on longan juice

spray drying with maltodextrin as an additive, maltodextrin had a positive impact on the recovery of spray dried powder by reducing product stickiness.

Increases in inlet temperatures is the major factor affecting the cell survival, more importantly than feed rate as expected from previous studies [27]. In general outlet temperatures greater than 85°-90°C are lethal for probiotics [28,29,30] but sub-lethal temperature pretreatment enabled cells to survive in that range with cell death occurring only after 100°C outlet temperature in our current study. Heat shock proteins produced during sub-lethal stress aid the probiotics during subsequent stress. They usually assist during the refolding of denatured proteins or removal of denatured proteins before they cause death [31]. However, the outlet temperature of a spray dryer is difficult to predict, control or fix for any given set of operating conditions. The effect of temperature on survival of the probiotics is also strain dependent as there are varying results from strain to strain [32].

In reported research, maltodextrin offered good adherence to the probiotics during drying, storage and also gastric transit [33]. Other studies have proven that probiotics encapsulated in starch were able to exert their health benefits and stress tolerance in the gut [34,35]. The survival is attributed to the strong adherence to the carrier, which protects cells from high acidic and bile conditions [36]. Overall, maltodextrin is confirmed to serve as a good encapsulating matrix as well as a moderate prebiotic for high survival of probiotics [37].

The molecular nature of heat damage (above 90°C) is not clearly known but denaturation of critical proteins, DNA and ribosomes are few vital events [38]. The cell membrane heat damage is one of the most susceptible target during spray drying. High temperatures during spray drying cause the cellular pores to leak the intracellular substances from the cell [28,29]. Loss of metabolic activity might also be observed due to the denaturation of proteins[39]. Critical components like ribosomes, DNA/RNA and their related enzymes may be lost which account for the loss of viability. The glycolytic enzymes production which is responsible for higher survival during and after spray drying, are also reduced due to the thermal stress [40]. Another mechanism which causes thermal

cell death is the fact that Mg^{+2} ions ooze out of the cells during thermal stress and these ions are necessary for ribosome stability [41].

From the above optimization experments for three different juices three optimized conditions were derived (**Table 4.13**). Overall parameter optimization is essential for scale up of any lab process to industrial scale. The three independent variables were optimized simultaneously to obtain the condition at which there is maximum % recovery and the highest % survivability.

Table 4.13. Summary of optimized conditions obtained using the response optimizer and experimental values for three different juices

Juice	Optimized conditions			Predicted values		Experimental values	
	Temp (^{0}C)	Solid:Maltodextrin ratio	Feed rate (mL/min)	Recovery (%)	Survival (%)	Recovery (%)	Survival (%)
Litchi	130	1:1	60	53.39	67.95	54.42	68.60
Pineapple	100	1:2	40	34.11	82.82	35.15	81.50
Orange	100	1:1.68	40	40.61	63.64	40.50	64.00

The optimization equations suggests that litchi juice with an inlet 130°C inlet temperature, a maltodextrin to total solids in juice ratio of 1:1 and feed rate of 50 mL/min gave highest survivabily of 67.95 % and a better recovery of 53.39%. The experimental values for the responses were found to be quite comparable and with agreement with that of the predicted value (**Table 4.13**). The optimization equations also suggests that of survival is a function of processing conditions as well as the chemical nature of the suspension. These results are in agreement with some of the previous studies where conditions of spray drying as well as composition of suspension had equal effect on probiotic survival and during storage as well. [42,37]

Litchi juice showed improved cell recovery and cell survivality as compared to pineapple and orange juices on microencapsulation with maltodextrin. This may probably be due to the protective effect of TSS and titratable acidity of litchi juice on the probiotic microorganism. Litchi juice had highest TSS (17.0 ^{o}Brix) and lowest titratable acidity (0.19%) among the studied juices.

4.4. Conclusion

Inlet temperature had a positive effect on % recovery during spray drying. Interactive terms of other independent variables of inlet feed rate and maltodextrin ratio

were also found significant. Juice solid content to maltodextrin ratio and inlet feed rate have a major effect on recovery. The R^2 values of all the three master models were found to be higher than the predicted model. Solid to maltodextrin ratio had a major effect on recovery and survivability of the cells in orange and litchi juice model. Flow rate had positive effect on recovery in litchi juice but had negative effect on survivability of the cells in spray dried powders of pineapple juice. Optimization conditions were found to vary among the fruits studied. Litchi juice showed higher recovery and cell survival compared to the other two juices. The optimization data revealed that 130^{o}C inlet temperature, 1:1 ratio of juice solid to maltodextrin and 60 mL/min flow rate will give highest recovery and desirable survivability of the spray dried powder.

4.5. Bibliography

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