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Development and evaluation of millet based spray dried probiotic health mix

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Abstract
Spray dried millet mix was prepared with incorporation of encapsulated probiotic *viz. Lactobacillus plantarum* NCDC LP 20. Pearl millet and foxtail millet were used for making malted millet mix beverage. Sodium alginate, skim milk powder, whey protein concentrate, cocoa powder and maltodextrin were used in different combinations as wall material for encapsulating the probiotic bacterium. Sodium alginate and cocoa powder at 1:1 ratio yielded the maximum encapsulation efficiency of 90%. The encapsulated probiotic is incorporated into the millet mix beverage for spray drying. The optimized combination of millet mix was arrived based on the sensory scores from 15 semi trained panel members on 9-point hedonic scale and the combination containing sodium alginate and cocoa powder at 1:1 ratio top scored among the four treatments. Response surface methodology was used to optimize the conditions for spray drying process with inlet temperature at 171 ºC, feed flow rate 237ml/hr and proportion of sodium alginate and cocoa powder at 1:0.8.

Keywords: Probiotic, wall material, encapsulation, millet, spray drying, health mix

Introduction
Millets and millet-based foods are gaining importance in our day-to-day life because of their nutritional and functional properties. Millets form a major constituent of the ready to eat products market these days. The United Nations designated the year 2023 as the “International Year of Millets” to increase awareness among consumers and to promote use of millets in our daily dietary component. Millets were once termed as coarse grains but today they are called as “nutri-cereals”, considering their processing and nutritional capabilities. When compared to cereals, millets are nutritionally sound with sulphur containing amino acids like methionine and cysteine. Low glycaemic index potentially helps diabetic patients and gluten free properties prevents incidence of celiac diseases. (Devi *et al.*, 2014) [6]. Pearl millet has a good score of amino acids and also contains omega fatty 3 fatty acids (Promod *et al.*, 2023) [12]. Dietary fatty acids such as alpha linolenic acid, Eicosapentaenoic acid and docosahexaenoic acid are also present in pearl millet (Chandrasekara and Shahidi, 2010) [4]. Foxtail millet is comparatively rich in nutrients with dietary fibre, resistant starch, vitamins and minerals. The time is ripe to take advantage of the underutilized millets to reduce global hunger index and improve food security.

Consumers are the driving force for choice of foods with health benefits that includes functional foods with probiotics (Paim *et al.*, 2016) [7]. Probiotic organisms are the microbes with health benefits when supplied in adequate quantity to the human gut. Probiotics have diverse advantages in regulating the health of the consumers like immunity boosters, reduction of toxins and maintaining right conditions in human gut. (Guo qi *et al.*, 2022) [16]. Enriching of foods and new food formulations with probiotics improved the diversity of food choices to the consumers. (Khater *et al.*, 2010) [13]. *Lactobacillus plantarum* is generally related to the gastrointestinal tract and probiotic strains of the bacterium is known to provide positive health benefits in the gut of human body like prevention of diarrhoea, gut stimulation of human immune system and balancing the microbes in the gut.

Germinated millet mix is a good source of thiamine, lysine and other nutrients. Malted millet mix made of foxtail and pearl millets have increased the digestibility by reducing anti nutrients present in millets. Malted millet mix acts as a good source of nutrients for the growth of probiotics (Budhwar *et al.*, 2019) [18]. Encapsulation is a technique of carrying safely the probiotic into the human gut. Microencapsulation is economically feasible and also provides a higher productivity of probiotics.

A wide variety of methods and materials were used in microencapsulation of probiotics. Development of food grade polymers as wall materials in probiotic encapsulation is a better alternative to protect the probiotics against the harmful effects of spray and freeze drying.

Materials and Methods

Revival and maintenance of probiotic bacterium

Freeze dried culture of *Lactobacillus planatarum* NCDC 20 was sourced from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal and revived as per standard protocol. Stock cultures were maintained by sub culturing once a week. The cultures were frozen and stored at -4 °C by inoculation in 80% glycerol.

Wall materials

Sodium alginate (food grade) was purchased from Venus Essence Pvt. Ltd, Chennai. Whey protein concentrate of Naturaltein brand was obtained from local market of Chennai city and stored in air tight container. Cocoa powder (Hershey's cocoa company) was purchased from local market of Chennai city.

Sodium alginate and skim milk powder

Sodium alginate and skim milk powder (Gul, 2017) [21] were added at a ratio of 1:1 (12.5 g : 12.5 g) and thoroughly mixed. Freshly harvested *Lactobacillus plantarum* (25 ml) was added to the mixture of sodium alginate and skim milk powder.

Pearl millet flour 55 g, foxtail millet 20 g, skim milk powder 5 g and cocoa powder 10 g were mixed together to prepare malted millet mix. Fifty gram from this malted millet mix is dissolved in 100 ml of skim milk to prepare the malted millet extract. Twenty five percent of this malted millet extract is added to 50% of skim milk containing probiotic and 25% of wall material to prepare the encapsulated probiotic malted millet mix beverage.

Sodium alginate and whey protein concentrate

Sodium alginate and whey protein concentrate (Perez *et al.*, 2020) [22] were added at 1:1 ratio (12.5 g : 12.5 g) and mixed thoroughly. Freshly harvested *Lactobacillus plantarum* (25 ml) was added to the mixture of sodium alginate and whey protein concentrate. Twenty five percent of the malted millet extract (as described above) is added to 50% of skim milk containing probiotic and 25% of this wall material to prepare the encapsulated probiotic malted millet mix beverage.

Sodium alginate and cocoa powder

Sodium alginate and cocoa powder (Erdem *et al.*, 2014) [9] were added at 1:1 ratio (12.5 g : 12.5 g) and mixed thoroughly. Freshly harvested *Lactobacillus plantarum* (25 ml) was added to the mixture of sodium alginate and cocoa powder. Twenty five percent of the malted millet extract is added to 50% of skim milk containing probiotic and 25% of this wall material to prepare the encapsulated probiotic malted millet mix beverage.

Sodium alginate and maltodextrin

Sodium alginate and maltodextrin (Tontul *et al.*, 2017) [24] were mixed at 1:1 ratio (12.5 g : 12.5 g). Freshly harvested *Lactobacillus plantarum* (25 ml) was added to the mixture of sodium alginate and maltodextrin. Twenty five percent of the malted millet extract is added to 50% of skim milk containing probiotic and 25% of wall this material to prepare the encapsulated probiotic malted millet mix beverage.

Table 1: Encapsulation of *Lactobacillus plantarum* with different combination of wall materials

S. No.	Wall Materials	Ratio 1:1 (25 g)	Skim Milk (50 ml)	Malted Millet extract (25 ml)
1	Sodium Alginate + Skim Milk Powder	(12.5 g : 12.5g) 25%	50%	25%
2	Sodium Alginate + Whey Protein Concentrate	(12.5 g : 12.5g) 25%	50%	25%
3	Sodium Alginate + Cocoa Powder	(12.5 g : 12.5g) 25%	50%	25%
4	Sodium Alginate + Maltodextrin	(12.5 g : 12.5g) 25%	50%	25%

Process optimization for spray drying of encapsulated probiotic malted millet mix beverage

The spray drying was carried out as per the methodology of Gong *et al.* (2020) [20]. 75 g of encapsulated probiotic malted millet mix beverage was taken and dissolved in 285 ml of skim milk so as to get a total solid of 15%.

Encapsulation Efficiency

The encapsulation efficiency of the different wall materials was calculated as per the following formula (Semyonov *et al.*, 2010) [23].

Encapsulation Efficiency = $N/N_0 \times 100$

Where N_0 is the number of viable cells (CFU/g) before drying, N is the number of viable cells (CFU/g) in the powders after drying.

Enumeration of viable cells before and after drying

The total viable count (*L. plantarum*) of the malted millet mix was assessed before spray drying and after spray drying with Lactobacillus MRS Agar by pour plate technique (Arriola *et al.*, 2016) [2].

Results and Discussion

The moisture content and water activity of spray dried encapsulated malted millet mix, probiotic viability before and after spray drying and encapsulation efficiency of different wall materials *viz.* sodium alginate, skim milk powder, whey protein concentrate, cocoa powder and maltodextrin in different combinations is presented in Table 2. The Treatment 3 which consisted of sodium alginate and cocoa powder showed the lowest moisture content (4.2%) and water activity (0.36). However, it showed the highest total viable count before and after spray drying *viz.* 10.39±0.04 and 9.17±0.11 log10 cfu/ml respectively and the percentage of encapsulation efficiency being 90.18. Hence Treatment 3 is chosen as the optimum one for further studies.

Table 2: Comparison of moisture content, water activity, probiotic viability and encapsulation efficiency of different wall materials

Treatments	Combination of Wall Material (1:1)	Moisture Content of spray dried millet mix (%)	Water Activity of spray dried millet mix	Probiotic Viability (Log_{10}cfu/ml)		Encapsulation Efficiency (%)
				Before Spray Drying	After Spray Drying	
T_1	SA + SMP	4.8	0.39	9.12±0.02	7.16±0.14	78.51
T_2	SA + WPC	4.5	0.42	10.12±0.06	7.01±0.13	69.27
T_3	SA+ CP	4.2	0.36	10.39±0.04	9.37±0.11	90.18
T_4	SA+ MD	4.6	0.59	9.34±0.13	8.23±0.14	88.12

Mean ± S.E values are average of six trials
T_1 - SA + SMP: Sodium Alginate + Skim Milk Powder
T_2 - SA + WPC: Sodium Alginate + Whey Protein Concentrate
T_3 - SA+CP: Sodium Alginate + Cocoa Powder
T_4 - SA+MD: Sodium Alginate + Maltodextrin

The lowest moisture content was observed in sodium alginate and cocoa powder and the highest moisture content in sodium alginate and skim milk powder. During the spray drying process, the high temperature of spray drying increased the rate of drying that resulted in low moisture contents. The results of the present study concurred with the findings of Anandharamakrishnan *et al.* (2008) [6]. Survival of the probiotic bacterium was found to be the highest in T_3 with 10.39±0.04 log_{10}cfu/ml and 9.37±0.11with sodium alginate and cocoa powder as wall materials before and after drying respectively. The operating spray drying conditions such as optimum inlet temperature and feed rate are crucial for the viability of thermo-sensitive probiotic strains such as *Lactobacillus plantarum* (Anitha *et al.*, 2022) [1].

Treatment 3 showed the highest encapsulation efficiency of 90.18% with the combination of sodium alginate and cocoa powder. Sodium alginate and skim milk powder, which is a mixture of protein and carbohydrates, has good thermal conductivity and thermal diffusivity. Kou prepared *Lactobacillus paracasei* HD1.7 microcapsules using skim milk powder as protective agent by spray drying and found that the addition of skim milk powder can effectively improve the survival rate and maintain the viability of probiotics. In sodium alginate and whey protein concentrate, the whey proteins were well known for their ability to release sulfur containing amino acids when they are subjected to heat treatments and can act as strong oxygen scavengers, thereby lowering the redox potential (Dave and Shah, 1998; Antunes *et al.*, 2005) [27, 28] and consequently inhibiting the oxidation of the membrane lipid bilayer, which can otherwise be lethal (Garre *et al.*, 2010) [29] and resulted in maintaining the encapsulation efficiency. Cocoa powder is an important component in production of chocolates. It contains a complex structure of proteins, polysaccharides and lipids (Oracz *et al.*, 2020; Sorrenti *et al.*, 2020) [30, 31]. It formed a very good encapsulating agent when added with alginate and fructose oligosaccharide (Hossain *et al.*, 2021) [11].

Sensory evaluation of malted millet mix beverage prepared with different wall materials

Four different combinations of wall materials were used for encapsulating the probiotic bacterium in the malted millet extract and the same was investigated for sensory analysis. The appearance, consistency, flavour, taste and overall acceptability of the reconstituted beverage were analysed on the 9 point hedonic scale by 15 semi-trained panel members and the results are presented in Table 3.

Table 3. Sensory evaluation of reconstituted spray dried probiotic malted millet mix

Treatments	Sensory Characteristics (Mean ± SE)@				
	Appearance	Consistency	Flavour	Taste	Overall acceptability
T_1	6.50±0.42^b	5.66±0.49^b	5.5±0.50^b	6.33±0.33^b	5.83±0.28^b
T_2	5.16±0.40^c	5.50±0.22^{bc}	5.5±0.34^b	5.16±0.30^c	5.79±0.16^b
T_3	8.83±0.16^a	8.17±0.16^a	8.0±0.25^a	8.67±0.21^a	8.17±0.12^a
T_4	6.33±0.21^b	5.50±0.34^{bc}	6.00±0.44^b	6.33±0.42^b	5.67±0.17^b
Control	5.33±0.21^c	4.66±0.21^c	5.0±0.25^b	5.67±0.21^{bc}	5.33±0.21^b
F-Value	23.28**	18.19**	9.82**	18.12**	32.41**

@ Average Scores of 15 semi trained panel members
**Highly Significant
Means bearing different superscripts across column differ significantly
T_1 - SA + SMP: Sodium Alginate + Skim Milk Powder
T_2 - SA + WPC: Sodium Alginate + Whey Protein Concentrate
T_3 - SA+CP: Sodium Alginate + Cocoa Powder
T_4 - SA+MD: Sodium Alginate + Maltodextrin
Control -Unencapsulated reconstituted malted millet powder

It can be inferred from the Table 3 that the Treatment 3 containing sodium alginate and cocoa powder scored the highest sensory scores when compared to Treatment 1, 2, 4 and control. The high inlet temperature intensified the light colours of skim milk powder, maltodextrin and whey protein concentrate and thereby reduced the appearance scores of T_1, T_2 and T_4 and control. Cocoa powder, because of its darker colour and sticky nature, retained the appearance even after spray drying at high inlet temperature. Consistency denotes the degree of solubility of the powder and its ability to mix well when reconstituted in water. Skim milk powder retained some lumps and dairy components like whey protein concentrate has less consistency and hence scored less (Maleki *et al.*, 2020) [32]. Cocoa powder and sodium alginate with fine texture received high consistency score. Similar type of results were obtained in study of sensory properties involving spray dried kefir powders (Cruz *et al.* 2013) [33].

Optimization of Spray Drying by using response surface methodology (Box-Behnken Design): In order to reduce the number of actual experiments and efficiently use the available time and materials, Box-Behnken Design (BBD) is adopted and the same is presented in Table 4.

Table 4: Optimization of variables by using response surface methodology

S. No	Runs	Inlet Temperature (degree C)	Wall material ratio	Feed flow rate (ml/h)	Survival%	Recovery%	Solubility%
1	1	180	1	200	72.46	28.08	70.73
2	2	160	1.5	100	71.72	38.46	64.21
3	3	170	1.5	200	84.7	46.23	84.18
4	4	160	1	200	69.9	27.41	63
5	5	160	1.5	300	73.21	37.74	78.14
6	6	180	1.5	300	73.46	39.44	71.46
7	7	170	2	300	81.4	47.1	74.54
8	8	170	2	100	74.14	36.73	64.04
9	9	170	1	300	72.16	32.46	60.71
10	10	180	1.5	100	70.93	45.3	70.14
11	11	170	1	100	70.41	25.47	62.84
12	12	180	2	200	78.82	44.69	73.23
13	13	160	2	200	77.52	40.74	71.61

As a result, it is possible to probe into potential interactions between the independent parameters investigated and their effects on the dependent parameters, because the analyses performed on the results are straightforward to realise and experimental errors are minimized.

Process optimization for spray drying of probiotic incorporated malted millet mix beverage was performed using the Box-Behnken design of response surface methodology (RSM). The design comprised 13 experimental runs for millet mix having 5 centre points. Independent variables were inlet temperature, ratio of sodium alginate to cocoa powder, feed rate and dependent variables were recovery percentage, survival percentage and solubility percentage. The optimum inlet temperature is 171ºC, wall material ratio 1:0.8, feed flow rate 237 ml/hr, survival 85%, recovery 42.65%, solubility 82.851% and desirability 0.906 as presented in Table 5.

Table 5. Optimized conditions for spray drying process

S. No.	Inlet Temperature (degree C)	Wall Material Ratio	Feed Flow Rate (ml/h)	Survival%	Recovery%	Solubility%	Desirability
1	170.914	1:0.8	236.721	85.335	42.605	82.851	0.906

The interactive effect of inlet temperature and wall material on recovery percentage, survival percentage and solubility percentage are presented in Fig. 1, 2 and 3

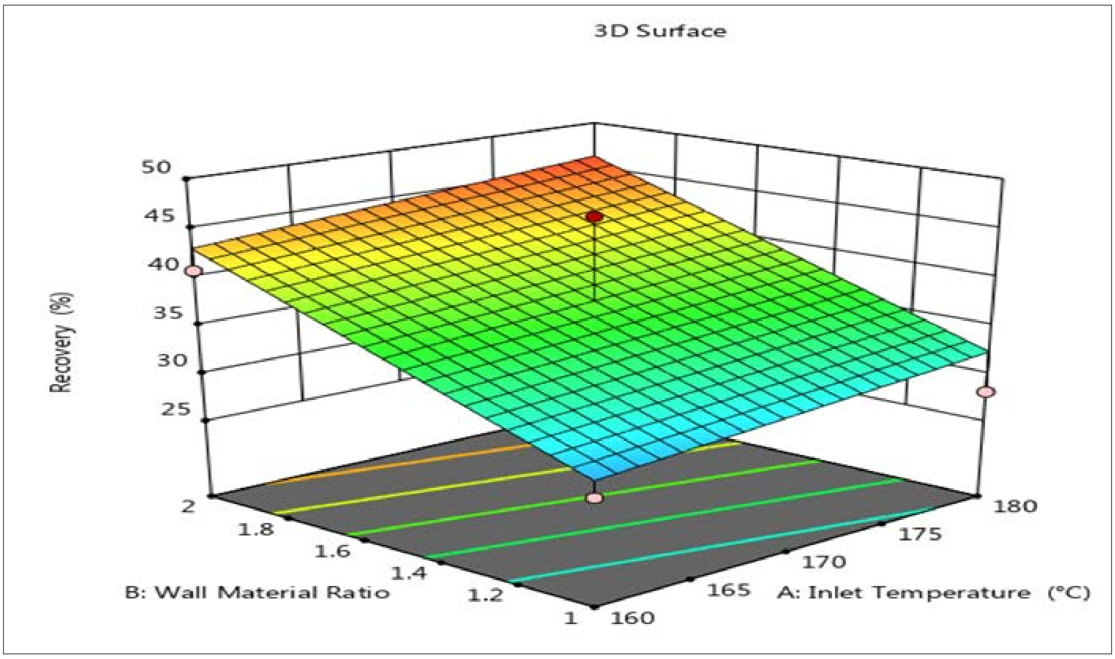

Fig 1: 3D diagram showing x-axis wall material vs y-axis inlet temperature vs z-axis recovery %

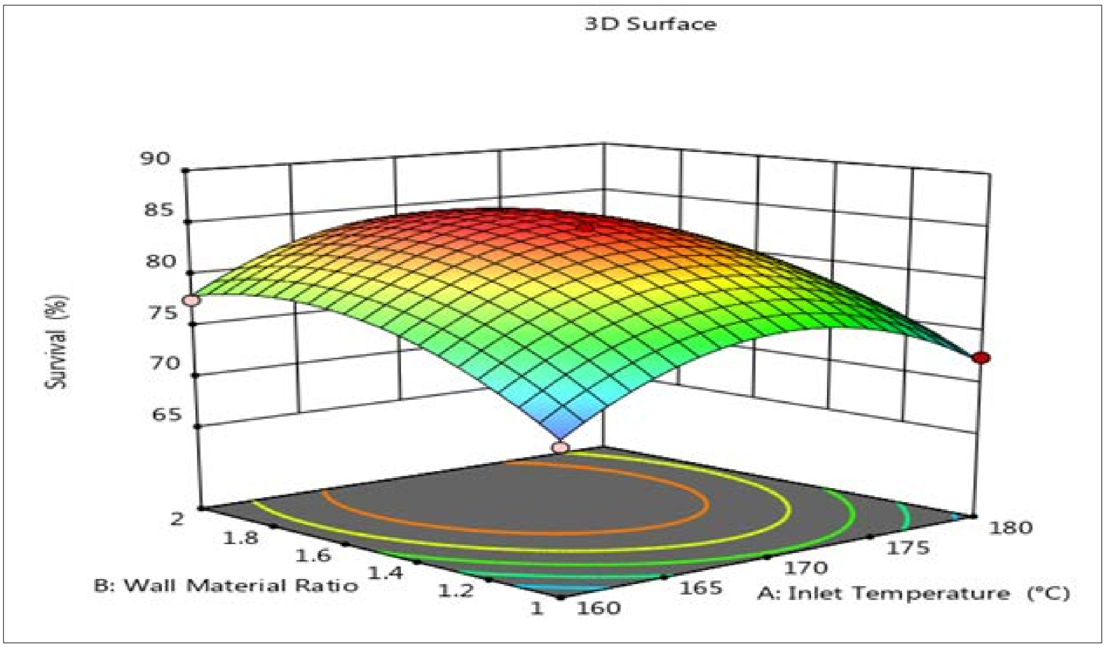

Fig 2: 3D diagram showing x-axis wall material vs y-axis inlet temperature vs z-axis survival%

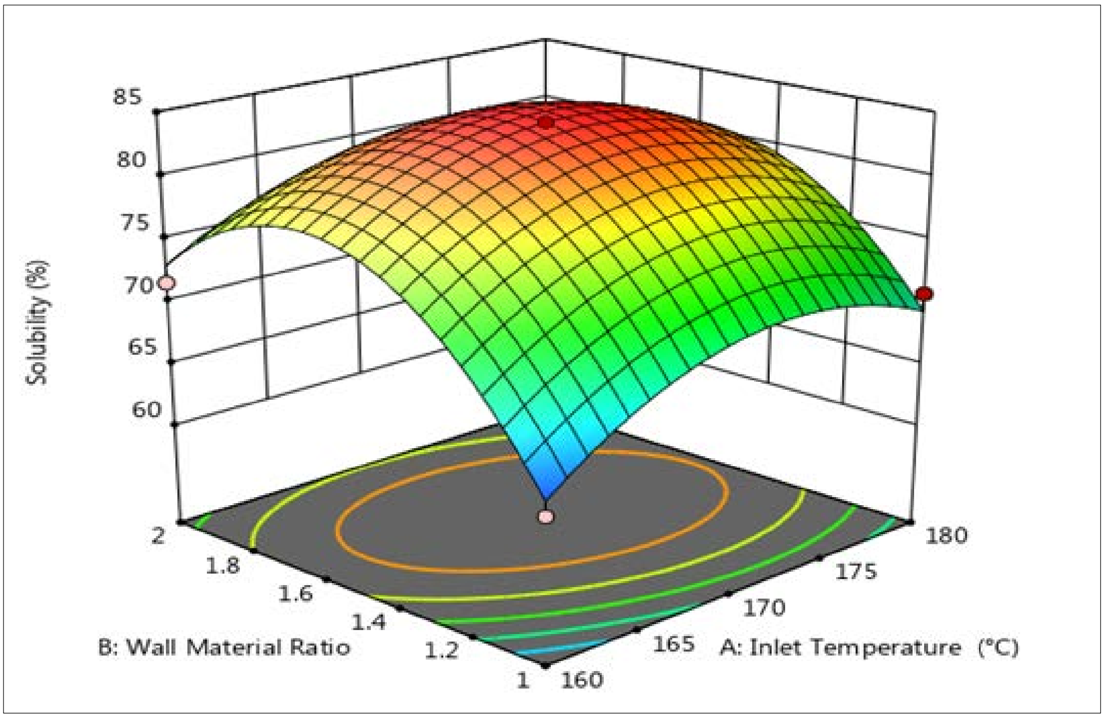

Fig 3: 3D diagram showing x-axis wall material vs y-axis inlet temperature vs z-axis solubility%

Survival Percentage

The percentage of survival of the probiotic is dependent on variables *viz.* inlet temperature, feed flow rate and ratio of sodium alginate to cocoa powder as mentioned in Table 4. The surface response plot for survival percentage of the probiotic is 85.34% since the inlet temperature is set at 171ºC, feed flow rate 237ml/hr and ratio of sodium alginate and cocoa powder is 1:0.8.

Recovery Percentage

The recovery percentage is dependent on variables *viz.* inlet temperature, feed flow rate and wall material ratio mentioned above. Based on response plot, the result of recovery percentage is 42.605%. The recovery percentage ranged between 27.41% to 42.60% depending on changes in inlet temperature and feed flow rates. The surface plot indicated the overall optimum condition with respect to maximum percentage recovery (42.60%) at input conditions of 171°C inlet temperature, sodium alginate to cocoa powder ratio of 1:0.8 and an inlet feed rate of 237 mL/min.

Solubility

The ability of a powder to completely dissolve in water is termed solubility (Gaiani *et al.*, 2011) [3]. Solubility of the spray dried malted millet powder from the surface plot ranged between 60.71 -84.18%. The sodium alginate and cocoa powder ratio influenced the solubility of the powder.

Conclusion

The results of the present study optimized the process for the microencapsulation of probiotic *Lactobacillus plantarum* by spray drying with sodium alginate and cocoa powder as wall material. Response surface methodology was used to optimize the process conditions for spray drying to obtain superior results. The conditions optimized by response surface methodology include the inlet temperature at 171 ºC, feed flow rate 237ml/hr and ratio of wall material *viz.* sodium alginate and cocoa powder at 1:0.8. To conclude, sodium alginate and cocoa powder and are better encapsulating agents with 90.18% of encapsulation efficiency. The addition of cocoa powder not only increased the encapsulation efficiency but also appearance, flavour and other sensorial properties of the product. The spray dried powder has good solubility (82.51%), moisture content 4.2% and water activity 0.36 which are important parameters to reconstitute. *Lactobacillus plantarum* has good viability under hot and harsh conditions of spray drying and good survival percentage (85.33%).

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