Journal of Applied Biology & Biotechnology Vol. 5 (04), pp. 057-065, July-August, 2017
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2017.50409

Encapsulation of Probiotic *Bacillus coagulans* for Enhanced Shelf Life

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ARTICLE INFO

Article history:
Received on: 09/02/2017
Accepted on: 10/05/2017
Available online: 14/08/2017

Key words:
Probiotics, stability studies, *Bacillus coagulans*, Encapsulation, Spray drying, Freeze drying.

ABSTRACT

Probiotic strains must be able to withstand industrial manufacturing conditions and retain maximum viability and functionality during storage. Probiotic cells also have to survive the harsh *mileu* in the stomach and small intestine. Encapsulating probiotic cells with suitable agents helps them resist such adverse conditions and minimize the loss in viability. Four encapsulation materials were evaluated in the current study: Fructooligosaccharide (FOS), Maltodextrin (MD), Skimmed milk (SM) and Xanthan gum (XG). Cells were encapsulated using two commonly used techniques, spray drying (SD) and freeze drying (FD). Accelerated stability study on formulations was carried out as per ICH guidelines. Parameters like viability, water activity, residual moisture content, *etc*. were assessed to determine the shelf life. Viability losses in encapsulation by FD in the range of 4-27.5% were lower than by SD technique (the range of 19-40%). Approximately 60-80% of *B. coagulans* cells survived both SD and FD processing conditions, which is higher than 40-50% viability reported for Lactobacillus strains. Protective abilities of different encapsulation materials were in the order of: SM>FOS>MD>XG. Scanning electron microscopy of the encapsulated probiotics demonstrated that in the course of 90 days, surface of encapsulated particles developed some roughness, indicating their likely less stability.

1. INTRODUCTION

Probiotics have been defined as live microorganisms which when consumed in adequate amounts confer health benefit to the host [1]. In the recent past, there is explosive growth of probiotic-based health products, mostly in the form of fermented dairy products or dietary supplements. Several health benefits have been claimed of probiotic consumption [2, 3]. Before probiotics can be delivered to consumers, microbial cultures must be able to withstand the industrial downstream processing. In addition, they must retain viability and functionality during storage and transportation as dehydrated, frozen or freeze-dried biomass suitable for application. Loss of viability during storage is a major limiting factor for effectiveness of many probiotic products [4, 5]. Therefore, viability of probiotic bacteria in a product at the point of consumption (desirable levels, approximately 10^8-10^9CFUg^{-1}) is an important consideration for their efficacy [6-8]. Bacterial survival during storage, is adversely affected by factors such as temperature, moisture content, Water activity (a_w), nature and quality of carrier media, exposure to light and oxygen, etc. [9]. Once consumed by the humans, probiotics also need to be protected against degradation in the stomach especially at low pH and aggressive intestinal fluids *e.g.* bile and pancreatic juice [10]. Coating the probiotic cells with a suitable encapsulation material for providing protection against adverse physicochemical changes, is currently receiving considerable attention [11-13]. Encapsulation assists in achieving higher cell densities, improved substrate utilization, acts as a barrier against release of entrapped cells and also minimizes the contamination [14]. The encapsulation material may be damaged by mechanical rupture or dissolution of the substance and subsequent release of entrapped cells [15]. An array of encapsulating materials has been used to immobilize probiotics. Examples include a variety of polymers, fats, waxes, pectin, κ-carrageenan, alginate, gellan gum, locust bean gum, galactooligosaccharide (GOS), maltodextrin (MD), skimmed milk (SM), xanthan gum (XG) and fructooligosaccharide (FOS) *etc.* [16-18]. Biopharmaceutical products during storage tend to deteriorate as they age, but they are considered to be stable as long as their characteristics remain within the certain specified limits as laid down in the regulatory guidelines and manufacturer's specifications [7]. The number of days that the product remains stable at the specified storage conditions, as recommended by the manufacturer, is referred to as the shelf life [6].

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Parameters like water activity (a_w), residual moisture content are used as indirect methods of assessing the stability of a formulation whereas viability in terms of colony forming units (CFU), can be used as direct method [18] .The quality of encapsulated material can be also judged by physical methods like electron microscopy to study the smoothness of the walls of the particles beads. The smoother outer surfaces of the beads at the end of stability study are taken as an indication of good stability [19, 20].

Probiotic functionality takes into account the ability of cells to resist gastrointestinal conditions and adhere to intestinal mucosa [4]. Thus, it is important that the encapsulation technique does not reduce viability and / or functionality. Both, spray drying (SD) and freeze drying (FD) can be used for manufacturing of probiotic powders on a large-scale [21, 22]. There are not many reports on encapsulation of the probiotic *B. coagulans* and its shelf life and therefore the present study was undertaken to encapsulate the probiotic *B. coagulans*. The encapsulated probiotic cells were dried using two different methods, spray drying (SD) and freeze drying (FD) and its impact on the shelf life was evaluated.

2. MATERIAL AND METHODS

The microbiological media and media ingredients were purchased from Himedia. The encapsulation materials were purchased locally.

2.1 Bacterial cultures and maintenance

2.1.1 Probiotic bacterium

B. coagulans was a gift from a private Indian pharmaceutical company located at Hyderabad, India and it was maintained on GYEB medium [3]. The cells were grown in 50ml GYEB medium in 250ml Erlenmeyer flasks at 37°C on a rotary shaker at 150 rpm.

2.1.2 Pathogenic strains

Type strains, *E.coli* NCIM 1025 and *S. typhi* NCIM 1250 were cultured at 37°C on nutrient agar and brain heart infusion agar, respectively.

2.2 Utilization of encapsulation material by probiotic strain and pathogens

To GYEB medium without glucose equivalent amount of individual encapsulation material was added and 50µl of actively growing cultures (OD_{540} =0.1) were inoculated. After 24h incubation at 37°C, 150 rpm growth was checked by turbidity as well as by dilution plating.

2.3 Production of biomass of probiotic cells

Fed batch fermentation was carried out in a 6.6l bench top lab fermentor (Sartorius), to obtain the biomass required for encapsulation as described earlier [23]. Briefly, the process was carried out at 37°C, with agitation range of 200-500rpm and aeration range of 0.75-1 vvm to maintain the dissolved oxygen (DO) between 30-70%. Biomass was obtained by centrifuging the fermented broth at 7500g for 10 min at 4°C.The pellet was washed twice with phosphate buffered saline (pH 7.3) to remove spent medium and cellular wastes. A maximum yield of up to about $30gl^{-1}$ DCW (Dry cell weight) as biomass was achieved. The cell pellet was dried in an oven at 70°C for about 30h to obtain the cellular material to be encapsulated.

2.4 Encapsulation conditions

2.4.1 Spray drying

Cellular material preparation (A): The dried biomass equivalent to 0.6-1.2x10^9 $CFUg^{-1}$ was transferred to a Falcon tube with 15ml DW and left to hydrate for 30min at RT. Encapsulation material preparation (B): Weighed amount of encapsulation material was added to 50ml of DW and homogenized for 15-20 min at approximately 1000 rpm and left to hydrate for 30 min at RT under magnetic stirring. Only stable and homogenous encapsulation material preparations were used for encapsulation. Suspensions A and B were mixed thoroughly and the total volume was made up to 100ml with DW and this suspension was utilized for further processing. The suspension was analyzed for physical properties- refractive index (RI) and viscosity before proceeding with encapsulation. Efficiency of spray drying (SD) process is known to be influenced by the viscosity of the solution and solid content. Hence, RI and viscosity were determined using Abbe's refractometer and Brookfield 620 spindle viscometer as per the procedure recommended by the instrument manufacturers. The probiotic suspension was spray dried using JSIL bench top lab spray dryer (Bombay). Spray drying was performed at 3 different inlet temperatures (110, 120 and 135°C) with fixed flow rate of 1.2ml min^{-1} and compressor air pressure at 0.5MPa. The spray dryer had no provision to control outlet temperature, which changed proportionally with air inlet temperature and feed flow rate conditions. The spray dried encapsulated probiotic formulations collected from the cyclone separator vessel were transferred to labeled sterile 10ml glass vials. The vials were incubated under 3 different sets of conditions, as stated in ICH guidelines, for accelerated stability studies: 4°C, 25°C (65% RH) and 40°C (75% RH).

2.4.2 Cyclone recovery

Cyclone recovery is an important attribute reflecting the efficiency of spray drying process. In a spray dryer the dried powder is separated from fine particles and collected in the cyclone container. The dry probiotic formulations were collected from the cyclone separator vessel on a clean butter paper and then quickly transferred into zip-lock pouches. Weight of the formulation recovered was recorded. Approximately 2g of formulation was transferred to sterile glass vials, under aseptic conditions using a laminar air flow. Cyclone recovery (%) was calculated using the expression [24].

$$\%\text{cyclone recovery} = \frac{\text{Total solid} - \text{recovered solids}}{\text{Total solids}} \times 100$$

2.4.3 Freeze drying

The cellular material and encapsulation material suspensions were prepared and mixed as described above for samples prepared for SD. The samples were stored at 4°C. RI and viscosity of the samples were measured before subjecting them to freeze drying. The freeze drying process consisted of two cycles - primary freezing and Secondary freezing. Primary freeze drying was carried out at -40°C under vacuum where 92-93% of the moisture is usually removed [25]. Secondary freezing was continued for next 6h to obtain the fully dried lyophilized probiotic powder.

2.5 Viability assessment

Encapsulation process efficiency was assessed in terms of CFU remaining after the process of SD or FD. The $CFUg^{-1}$ before and after the drying process was estimated by using the standard technique of serial 10 -fold dilutions followed by plating on GYEA medium. The reduction in viability was calculated by the formula [26].

% Reduction in viability = (A-B) / A x100

where A and B are viable counts before and after the encapsulation process respectively.

2.6 Stability studies

As per the Q5-C ICH guidelines [27], samples were stored for 90 days, at 5˚C (Refrigerator), and in a desiccator at 25°C having saturated solution of sodium nitrite to maintain 65% RH [28] and at 40°C in an environmental chamber with 75% RH. The samples were withdrawn for analysis on day 0, 7, 15,30,60 and 90.

The encapsulated formulations were analyzed for the following parameters to gauge the stability.

2.6.1 *Residual moisture content*

Residual moisture content was calculated using the following formula [29]

$$\%\text{moisture} = \frac{\text{Wf} - \text{Wi}}{\text{Wi}} \times 100$$

Where W_i and W_f were the initial and final weights of the probiotic formulations.

2.6.2 Water Activity (a_w)

Water activity (a_w) was measured using a water activity meter (Labswift-a_w Novasina, Switzerland) [30].

2.6.3 Viability assessment

The $CFUg^{-1}$after the drying process was estimated up to 90 days by using the standard technique of serial 10 -fold dilutions followed by plating on GYEA medium.

2.6.4 Scanning electron microscopy (SEM)

Encapsulated probiotic powders (day 0 and 90) were subjected to SEM for cell surface characterization [12]. The samples were examined under the scanning electron microscope model JEOL JSM-6380LA as per the recommended procedure of the manufacturer.

3. RESULTS AND DISCUSSION

3.1 Utilization of encapsulation material for growth

The probiotic *B. coagulans* and pathogenic strains (*E. coli* NCIM 1025 and *S. typhi* NCIM 1250) showed growth with all encapsulation material as the only C sources. Turbidity for *B. coagulans* was almost double as compared to pathogens. As can be seen from **Table I**, FOS and MD as the sole C source showed good growth for *B. coagulans*-($1.4x10^9$ and $1.2x10^9$ $CFUg^{-1}$, respectively). The overall utilization pattern for various encapsulation materials by the probiotic culture and the 2 pathogens was in the order of: FOS>MD>SM>XG. Corresponding cell counts for the media containing XG were lowest, possibly because of the structural complexity of this prebiotic that restricted its utilization [31].The results obtained may be extrapolated as the probiotic culture under evaluation could be able to propagate faster and colonize the GIT better than the harmful pathogenic bacteria.

Table I: EM utilization profile of probiotic and pathogenic strains.

EM	*B. coagulans*		*E. coli* NCIM 1025		*S. typhi* NCIM 1250	
	OD (A_{540nm})	$CFUg^{-1}$ ($x 10^9$)	OD (A_{540nm})	$CFUg^{-1}$ ($x 10^9$)	OD (A_{540nm})	$CFUg^{-1}$ ($x 10^9$)
FOS	0.8 ± 0.3	1.4±0.3	0.4 ±0.11	0.72 ±0.1	0.2 ±0.05	0.36 ±0.1
MD	0.7 ±0.2	1.2±0.5	0.3 ±0.10	0.61 ±0.5	0.1 ± 0.0	0.18 ±0.3
SM	0.6 ±0.1	1.1±0.8	0.3 ±0.12	0.63 ±0.2	0.2 ±0.05	0.37 ±0.2
XG	0.5 ±0.1	0.8±0.2	0.2 ±0.05	0.58 ±0.3	0.2 ±0.05	0.4±0.3

Key :- EM: Encapsulation material

3.2 Assessment of viability

Biomass was estimated as CFU before and after the encapsulation. Higher reduction in viability is a reflection of less protecting ability of the encapsulation material and is also an indication of harshness of the drying process employed. **Table II** shows the optimized process parameters and viability data for SD and FD processes.

As may be noted from the table, the reduction in viability of SD and FD samples was in the order: SM (19% v/s 4%) <FOS (27% v/s 7.2%) < MD (32% v/s 12.3%) < XG (40% v/s 27.5%). The viability loss in FD process (4-27.5%) was much lower than loss incurred in the SD process (19-40%). Thus, FD was milder method of drying for *B. coagulans*. Since, minimum loss of viability occurred for SM coated cells, it was considered to be the best encapsulation material for *B. coagulans*, while XG was the least effective one. The ability of encapsulation material to protect the cellular material from the harsh conditions of drying is crucial for the product viability and stability. Samples containing 20-30% solids had relatively low viscosity (80-100cp) resulting in better SD efficiency (19-40% loss in viability) at the optimum temperature as shown in the **Table II.**

Table II: Process parameters for SD and FD and viability of probiotic samples.

Samples	SM	FOS	MD	XG
Process parameters for SD				
Inlet Temperature	110˚C	135˚C	135˚C	120˚C
Flow rate		1.2ml min^{-1}		
Total solid content (g $100ml^{-1}$)	30	25	30	20
EM: CM	4:1	3:1	7:3	19:1
Viscosity	80cp	100cp	100cp	90cp
Viability				
$CFUg^{-1}$ before spray drying (A)	0.62×10^9	0.98×10^9	0.73×10^9	1.2×10^9
CFU g^{-1} after spray drying (B)	0.5×10^9	0.72×10^9	0.5×10^9	0.72×10^9
% reduction in viability	19	27	32	40
Process parameters for FD				
Total solid content	60	50	60	40
EM: CM	4:1	3:1	7:3	19:1
Viability				
CFU g^{-1} before freeze drying (A)	0.62×10^9	0.98×10^9	0.73×10^9	1.2×10^9
CFU g^{-1} after freeze drying (B)	0.6×10^9	0.91×10^9	0.64×10^9	0.87×10^9
% reduction in viability	4	7.2	12.3	27.5

Key: - EM: Encapsulation material
CM: Cellular material

Though FOS and MD encapsulated samples were subjected to higher temperature of 135°C, the viability loss was lower than XG encapsulated biomass at 120°C. SM was spray dried at 110°C since it had a risk of charring at temperature above 120°C. Irrespective of the drying method, protective abilities of different encapsulation materials were in the order: SM> FOS>MD>XG. Survival was maximum for SM (80%) and minimum for XG (60%) coated cells.

3.4 Cyclone recovery

Generally, the combination of high temperature and low feed resulted in low particle stickiness and hence higher cyclone recoveries. **Table III** shows cyclone recovery of different samples after SD. FOS and MD samples which were spray dried at 135°C showed higher cyclone recoveries of 48% and 56% respectively. While, SM and XG encapsulated probiotics which were spray dried at 110 and 120°C resulted in lesser cyclone recovery of 46 and 40% recovery.

Table III: Cyclone recovery after spray dried encapsulated probiotic *B. coagulans*.

EM	Solid content (%)	Total solid content (EM+CM)(g)	Recovered solids (encapsulated powder) (g)	Cyclone recovery (%)
SM	30	60	28	46
FOS	25	50	24	48
MD	30	60	34	56
XG	20	40	16	40

Key: - EM: Encapsulation material
CM: Cellular material

The amount of the dried product recovered via cyclone separation is influenced by many parameters, such as drying air flow, local velocities, the spatial geometry of the separator and the adhesiveness and cohesiveness of the particles while interacting with the drying chamber [32]. At higher temperatures the evaporation of water (i.e. drying) is faster and tendency of samples to stick to the drying chamber is very less [12]. Hence, recovery in the cyclone separator was higher for MD and FOS, as against the recoveries for SM and XG.

3.5 Stability studies on the optimized final product

3.5.1 Residual moisture content and Water activity (a_w):

Figure I depicts the trend of residual moisture content and a_w for the spray dried products. Residual moisture content is represented by the line graph. Residual moisture content of samples ranged between 3.8 to 5.5% during the storage tenure. With increase in storage temperature, the residual moisture content also increased but it remained below 5.5%. Thus, the residual moisture content in all the spray dried samples was within the recommended limit of under 5.5% as per the ICH guidelines.

The a_w values are represented as bar graph. For FOS-SD samples stored at 5°C, a_w of was almost the same throughout storage period. With rise in temperature, an increase in a_w was indicative of the negative effect of temperature on water activity and thus product stability. In our study, a_w remained below 0.6, within the recommended limits [27] and therefore the product can be considered as stable. Residual moisture content and a_w values for MD encapsulated spray dried samples were higher than those of FOS encapsulated spray dried samples. Both the parameters did not show any proportional increase with rise in storage temperature and RH conditions. Stability of samples prepared with SM and spray dried was in the order: 5°C >25°C (65% RH) >40°C (75% RH). Corresponding a_w values were all within 0.6. For 40°C with 75% RH, residual moisture content was somewhat higher (5.2%) but still within the permissible limit. This could be because of the higher water binding property of SM compared to other encapsulation material [33]**.** Water activity of the samples prepared with XG and spray dried was stable during 90 days storage but residual moisture content exceeded the recommended limit of 5.5% indicating some instability of the formulation. Also XG was not an effective barrier to protect the cells from elevated storage conditions. Similar findings have also been reported in the literature [34].

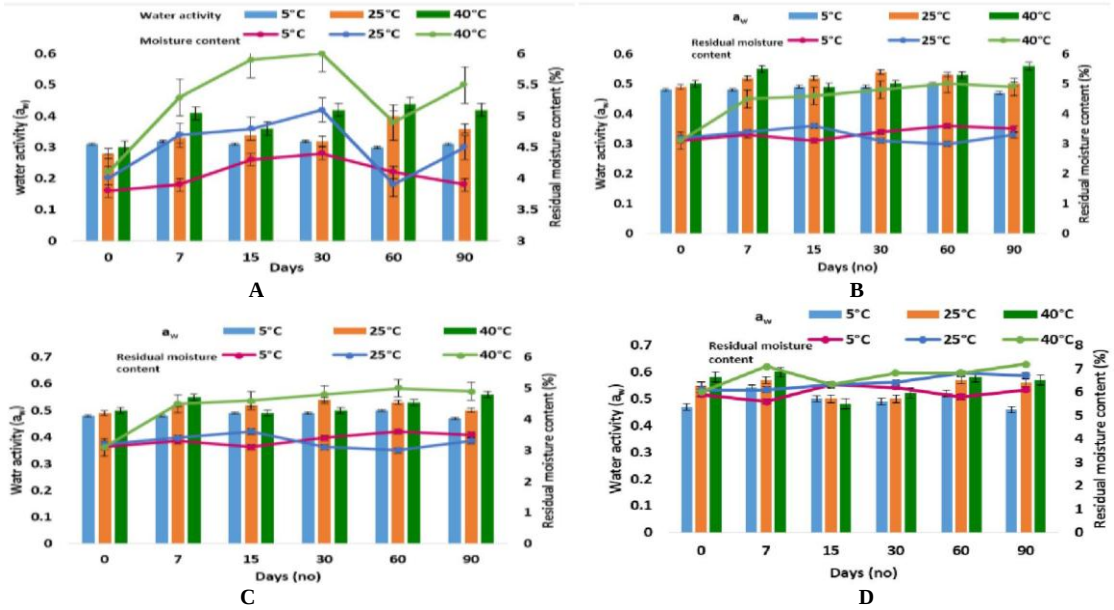


Fig. I: Water activity (aw) and residual moisture content of spray dried probiotic preparations of B. Coagulans using a) FOS, b) MD, c) SM and d) XG.

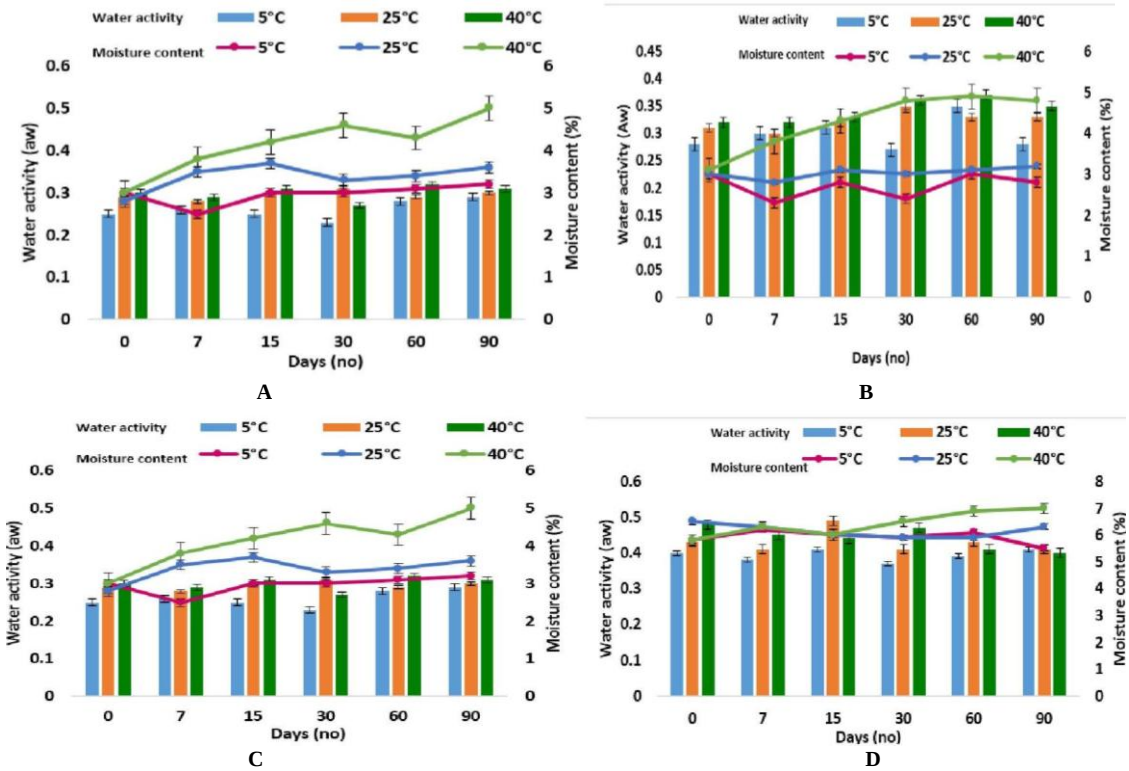


Fig. II: Water activity (aw) and residual moisture content of freeze dried cells of encapsulated probiotic B. Coagulans using a) FOS, b) MD, c) SM and d) XG.

For samples showing lower a_w, the RI at the time of encapsulation was between 1.33-1.371, very close to RI of pure water [21]. Therefore, the samples were rather free flowing due to low viscosity and spray drying efficiency was expected to be better [35].Reduced a_w also minimizes other undesirable chemical changes occurring during storage [36].

Figure II displays residual moisture content and a_w (bar graph) values for freeze dried probiotic samples encapsulated using the 4 encapsulation materials. The a_w values on day 0 and 90, for FOS-FD samples stored at 5°C, were almost unchanged indicating their inertness. Residual moisture content trend was also stable for 5°C samples. At temperatures 25°C (65% RH) and 40°C (75% RH) the samples displayed non-linear increments in a_w as well as residual moisture values, possibly indicating some product degradation. Maximum residual moisture and a_w readings for MD-FD samples were 4.8% and 0.38 respectively. The MD –FD

samples under all the conditions were found to be stable throughout the storage period. Similar results were obtained for SM-FD samples where the stability of formulations was in the order: 5°C >25°C (65% RH) >40°C (75% RH). a_w values of XG-FD samples were within 0.6 but residual moisture content had crossed 7% (higher than recommended value of 5.5%). This could be detrimental to the product viability and thus quality.

3.5.2 *Viability*

Loss of viability in non-encapsulated cells was faster and continued till the 90th day of storage as was anticipated **(Figure III)**. Rate of loss of viability was lesser at 5°C, higher at 25°C (65% RH) and highest at 40°C (75% RH). The CFU count of samples stored at 40°C (75% RH) was reduced to less than half of the initial value (0.38 x 10^9 $CFUg^{-1}$), by 90 days. These results show that the rise in temperature coupled with humid environment initiated the bacterial metabolism and hence process of degradation and loss in viability was accelerated [37,38].

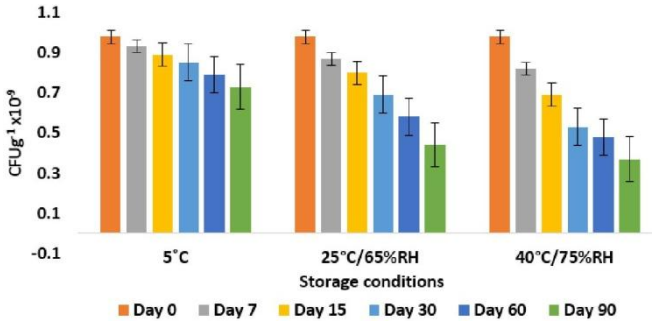
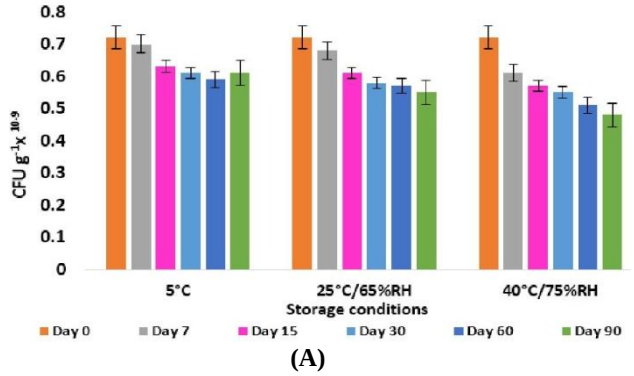
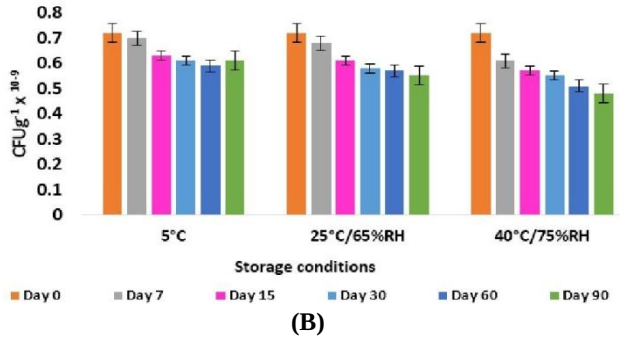


Fig. III: Viability profile of non-encapsulated probiotic *B. coagulans.*

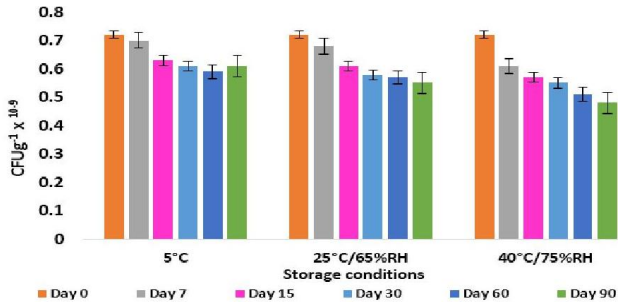


(A)

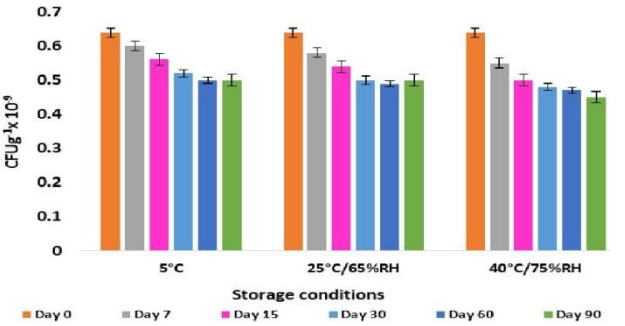


(B)

Fig. IV: Viability profile of a) FOS-SD and b) FOS-FD encapsulated cells of probiotic *B. Coagulans.*



(A)



(B)

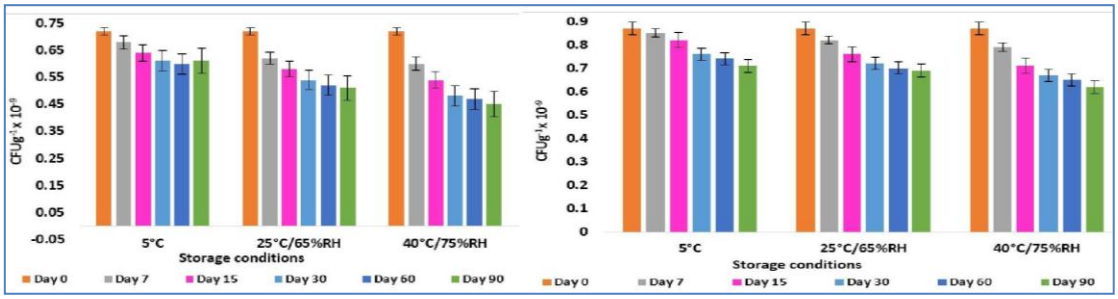
Fig. V: Viability profile of a) MD-SD and b) MD-FD samples of probiotic *B. coagulans.*

Figure IV presents the viability ($CFUg^{-1}$) of probiotics encapsulated with FOS. As seen from the figure, viability decreased during the 90 days span from 0.72x10^9 (day 0) to 0.48x10^9 (day 90) for samples stored at 40°C (75% RH). The drop in cell count was rapid and high in the first 15 days of storage. Further loss in viability was slow, thus storage in air tight glass vials helped in extending the shelf life of the formulations. Cell counts of FOS-FD samples also decreased with increase in storage temperature. The lowest counts recorded for FOS-SD and FOS-FD samples at 40°C (75% RH) were 38 and 70% respectively.

Viability profiles of MD-SD and MD-FD samples are represented in **Figure V.** MD-SD sample showed the decline in cell counts from 0.5x10^9 (day 0) to 0.31x10^9 (day 90) representing 32% reduction. Cell counts for MD-FD samples dropped from 0.64x10^9(day 0) to 0.46x10^9(day 90) *i.e.* 12.3% loss in viability.

Figure VI displays the viability profile recorded for probiotic cells encapsulated using SM. Cell counts at 40°C (75% RH) for SD and FD samples were 54% and 68% respectively. As is evident, FD method was milder than SD and hence viable counts were higher.

Finally, **Figure VII** represents the viability of cells encapsulated with XG, spray dried and freeze dried. As seen in the figure, for samples stored at 40°C (75% RH) for SD samples, the viability decreased during the span of 90 days from 0.72x10^9 to 0.45x 10^9 equivalent to 37.5% loss. The drop in cell count was rapid and high in the first 15 days. Similar was the profile for FD samples stored at 40°C (75% RH) where cell counts declined from 1.2x 10^9 to 0.87 x 10^9 equivalent to a viability loss of 27.5%.



A **B**

Fig. VI: Viability profile of a) SM-SD and b) SM-FD samples of probiotic B. coagulans.

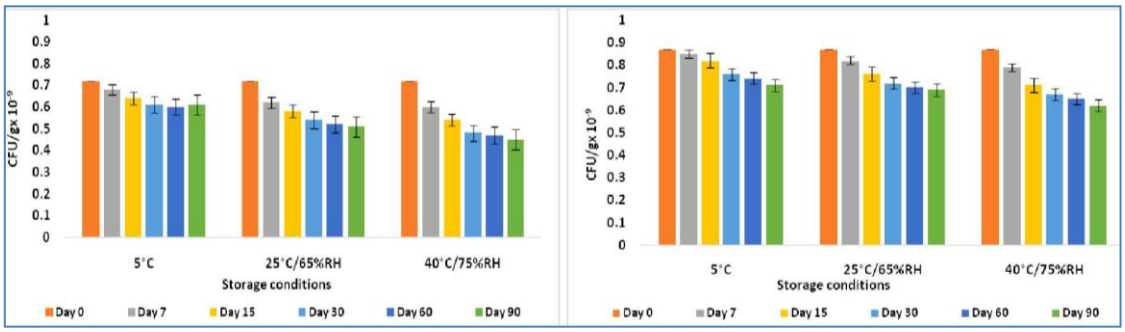
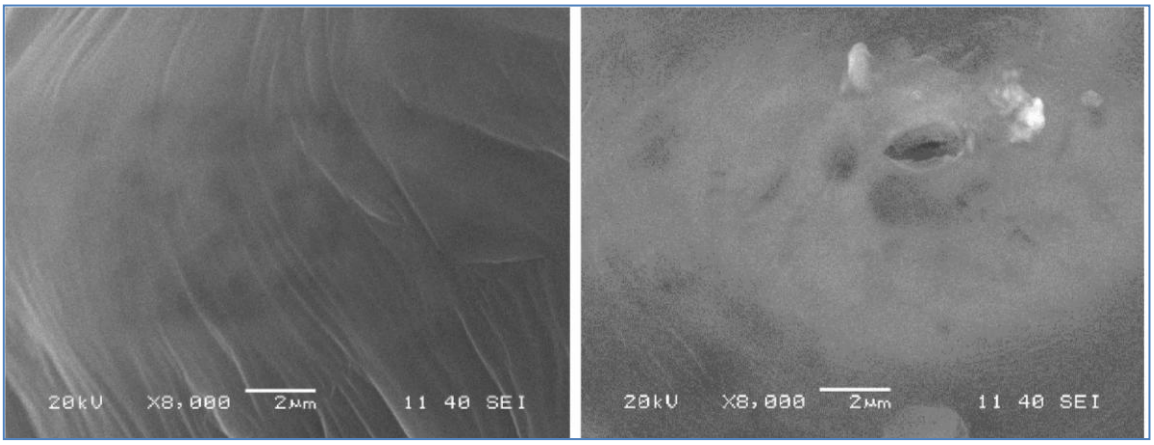


Fig. VII: Scanning electron micrograph of MD encapsulated B. coagulans cells on a) day 0 and b) day 90.



A **B**

Fig. VII: Viability profile of a) XG-SD and b) XG-FD samples of probiotic B. coagulans.

3.5.3 Evaluation of encapsulated formulations using SEM

It is known that the morphology of the beads prepared with encapsulation of biomass varies based on the coating material used and drying conditions employed [39]. The particulate structure of the encapsulated product can be viewed using SEM [19]. The scanning revealed that both the drying procedures (SD and FD) had resulted in partially collapsed particles with the appearance of deflated ball like spherical structures. Day 0 samples appeared smooth due to the even spreadability of encapsulation material. During the course of storage, the encapsulation material as well as probiotic cells might have come in contact with the moisture and oxygen present in the glass vial and integrity may have got compromised due to hygroscopicity of encapsulation material and/or growth of cells, resulting in formation of some roughness on surface of particles. The loss of integrity of outer surface increases the surface area for interactions with the environment and may negatively affect the stability of the product. A representative electron micrograph for the particle surfaces where probiotic cultures encapsulated with MD were spray dried is shown in **Figure VIII**.

As may be noted from the electron micrograph, the external surface of the day 0 sample was smooth spherical and non-porous, indicating the inertness of the product (**Figure VIIIa**). The smoother the surface, the lesser or no interaction pockets for oxygen, humidity etc. and thus more stable is the formulation. Allan-Wojtas and colleagues have reported that they could effectively see the morphological difference in the empty and bacteria loaded calcium alginate microcapsules [19].

Exposure of microorganisms to stress conditions decreases their viability in any product. In fact, several researchers have demonstrated that numerous probiotic food products including traditional fermented dairy products, have failed to meet

the requirement of minimal level of viable bacteria [40-42]. Viability of probiotic cells in food products and their activity at the target site depends on various factors like conditions during fermentation, downstream processing, storage as well as the conditions prevailing during passage through gastrointestinal tract [29, 43]**.**

The traditional spray dried probiotic products containing Lactobacillus strains have shown 15-40% viability which can be considered as less than desirable [44]. In our study, the *B. coagulans* could withstand the spray drying conditions better showing 60-80% viability **(Table II)**. This can be partly attributed to better spore forming ability of our *B. coagulans* probiotic strain. It is known that spores are able to withstand harsh environmental conditions [45]. The improved survival can also be due to choice of suitable encapsulation material and tightly controlled conditons during spray drying and freeze drying. A study done by Kearney et al. has demonstrated the importance of spray drying conditions for maintaining the viability of probiotic yoghurt containing *Lactobacillus paracasei* NFBC 338 [39].

Our results confirm that the process of spray drying is harsher than the freeze drying. The viability of bacterial population drops drastically at high temperatures and dehydration conditions during the SD process as against the freeze drying process [9,46,47]. Loss of viability is principally caused by cytoplasmic membrane damage although the cell wall, ribosome and DNA are also affected at higher temperatures [9, 46, 48, 49]. The stationary phase cultures are more resistant to heat compared to cells in exponential growth phase [12]. Our best results, viability of 96% and 81% for FD and SD samples respectively, with SM as encapsulating material is largely in agreement with previous reported results by others. It was shown that a rifampicin resistant variant of *Lactobacillus paracasei* NFBC 338, showed survival rate of >80% during spray-drying in reconstituted skimmed milk, at 100-110°C [50], while under similar conditions, a survival rate of >60% for *L. rhamnosus* GG has also been reported [51]. In another study *B. coagulans* cells were spray dried with calcium alginate and the loss of viability was restricted to 30% [47]. These reports also highlight the fact that survival of cells varies depending on the species under investigation. Though freeze drying results in lesser loss in viability and frozen cultures occupy less volume and require less storage space at sub-zero temperatures, the cost of cooling is prohibitively high for freeze drying and hence spray drying may still be preferred as the SD process is 5-10 times cheaper than FD process [52].

4. CONCLUSION

The encapsulated formulations showed a significant drop in viability only during the first 15 days of storage. This confirmed the fact that irrespective of the encapsulation material used, encapsulation was able to slow down the cell death. With spray drying as well as freeze drying the least loss in viability was seen with skimmed milk as encapsulation medium. The process of freeze drying resulted in milder drying conditions than the spray drying. It was concluded that amongst the 4 encapsulation materials evaluated, skimmed milk was the most effective agent. The work described here contributes to the better understanding of the encapsulation process for the probiotic *B. coagulans* as very few reports are available on choice of encapsulating materials for this probiotic. Also data obtained with scanning electron microscopy supports the work by other researchers to develop it as a useful, independent tool for studying the integrity of particle surface of encapsulated microbial cells.

5. ACKNOWLEDGMENT

We wish to acknowledge the help provided by Institute of Chemical Technology, Matunga, Mumbai, India for spray drying and SEM analysis of encapsulated formulations.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Pandey KR, Vakil BV. Encapsulation of Probiotic *Bacillus Coagulans* for Enhanced Shelf Life. J App Biol Biotech. 2017; 5 (04): 057-065.