Indian J Dairy Sci 76(1): 38-43
https://doi.org/10.33785/IJDS.2023.v76i01.005

RESEARCH ARTICLE

Spray-dried Probiotic adjunct with *in vitro* acid and bile salt tolerance

Ameeta Salaria[1,2], Shalini Arora,[1,3*], DK Thompkinson[1] and Latha Sabikhi[1]

Received: 06 October 2022 / Accepted: 29 October 2022 / Published online: 20 February 2023

Abstract: Probiotic *Lactobacillus acidophilus* LA1 was encapsulated using spray drying with concentrated skim milk with total solids (17%), and maltodextrin (25%), and gum acacia (15%) as carriers. The encapsulated probiotics were assessed for viability following drying at an inlet temperature of 170°C ± 3°C and an outlet temperature of 80°C ± 3°C. The viable count enumerated after drying was 10^8 log cfu/g. The prepared probiotic adjunct was further assessed for viability through *in vitro* acid and bile salt exposure assay. The results indicated that the cell count decreased at lower pH (1, 2) and high incubation periods (1 hr and 2 hr). A similar trend was observed with bile. However, they showed better survivability for different incubation periods at low pH and in high bile salt concentrations. The spray-dried probiotic adjunct was kept for storage at three different temperatures i.e., 8 °C, 25 °C, and 37 °C for eight weeks, and assessed weekly for cell viability. An inverse relationship between cell survival and storage temperature was observed during storage.

Keywords: Bile; Encapsulation; Gum acacia; Maltodextrin; *Lactobacillus acidophilus*; pH; Skim milk

[1]Department of Dairy Technology, ICAR-National Dairy Research Institute, Karnal, Haryana-132001
[2]Department of Food Science and Technology, Padma Shree Padma Sachdev Govt P.G.
College for Women, Gandhi Nagar, Jammu 180004, Jammu & Kashmir, India
[3]Department of Dairy Technology, College of Dairy Science and Technology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125004, Haryana, India

Shalini Arora (✉)
Department of Dairy Technology,
College of Dairy Science and Technology,
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125004, Haryana, India
Email: shaliniarora.luvas@gmail.com

Introduction

Probiotics are functional ingredients incorporated into foods to enhance their nutritional value. With the growing acceptance of probiotic products, customers usually desire that the health benefits of probiotic strains be kept in the foods they purchase up until the point of consumption. The use of probiotics as an active food ingredient is not easy because, to be beneficial, they must contain at least 10^{6-7} CFU of live microbes per gram or milliliter of food consumed (Huang et al. 2017). However, the FSSAI recommends 10^8 CFU/g of live organisms in food products containing probiotic ingredients also if they are in a lower number than the recommended levels, it must be supported by proven research studies for health benefits as approved by the food authority (FSSAI 2016). Maintenance of probiotic viability throughout product shelf life until consumed is an important consideration. High processing temperatures, low pH, and bile salt sensitivity during gastrointestinal (GI) transit are the most severe threats to survival. A microencapsulation is a promising approach that has appeared recently as a probiotic protective barrier. Numerous microencapsulants and techniques are available to enhance probiotic viability; alginate encapsulation is the most popular among them. However, it suffers from the disadvantage of being expensive and difficult to scale up. Since product cost is an important criterion to survive in the competitive market, some alternatives must be tried.

Spray drying is a well-known technology in the food industry and may serve as an alternative low-cost encapsulating technique to produce significant quantities of dehydrated cells.

However, microorganisms may face survival difficulties due to high temperatures and dehydration during spray drying. Thus, microbial survival is a critical parameter to control here. On the contrary, this technology can elevate survival rates by selectively choosing processing conditions and carriers (Arslan et al. 2015; Gul 2017). It also maintains good viability and functionality of cultures when subjected to simulated gastrointestinal conditions (De Castro-Cislaghi et al. 2012) and increases survival at room temperature (Ananta et al. 2005).

Various approaches to improve the performance parameters of the spray-drying technique have been tried. One method of approach is to use protectants. For instance, adding growth-promoting ingredients like prebiotics and thermoprotectants like trehalose, non-fat milk solids, and adonitol has increased culture viability throughout drying, storage, and gastro-intestinal transit. Regarding entrapment matrix, skim milk and carbohydrates like trehalose and maltodextrin were among the most commonly used and showed significantly improved cell viability during drying (Fu, & Chen 2011).

Gum Arabic, or Gum Acacia (G.A.), is a polysaccharide and glycoprotein polymer. It has good solubility, low viscosity, and surface activity and is widely used as an entrapment agent in spray drying because of its excellent emulsifying properties. Higher survival rates have been reported with gum acacia (Gul 2017; Tao et al. 2019). G.A. also inhibits complete water loss of cell components and helps to stabilize microbial cells throughout drying and storage (Liu et al. 2016).

Due to its nontoxicity, affordability, quick rate of dissolving, low viscosity even at high solid content, and ease of availability, maltodextrin (M.D.) is a commonly used coating material for encapsulation. (Silva et al. 2014).

An attempt was made to study the survival rate of *L acidophilus* LA1 spray dried in a combination matrix of three carrier matrices viz skim milk, maltodextrin, and gum acacia. The present study has been undertaken to assess the efficacy of spray-dried microcapsules in enhancing the survival rate of LA during the storage of formulation at different storage temperatures and simulated GI tract conditions.

Materials and Methods

Probiotic organism and culture environment

Lactobacillus acidophilus LA1 was obtained as a pure freeze-dried culture from the National Dairy Research Institute's NCDC National Collection of Dairy Cultures (Karnal, India). The freeze-dried culture was activated in Chalk Litmus milk for 24 hours at 37 °C before being grown in 100 ml MRS Broth at 37 °C for 24-48 h. Before cell encapsulation, the culture was transferred 2-3 times. The reactivated cultures were centrifuged twice in distilled water at 2500 g for 10 minutes at 4 °C using a refrigerated bench-top centrifuge (Hermle Z 382 K, Maschinenfabrik Berhold Hermle A G, Gosheim, Germany).

Growth and maintenance of culture

The culture was subcultured in MRS broth before use and harvested during the stationary phase to avoid cell injury during the spray drying process. The cells were collected and washed twice with saline after being centrifuged at 8000 rpm for 10 minutes at 4°C. Thereafter the cells were kept under refrigeration until used.

Carriers

Whole cow milk procured from the institute's cattle yard was separated in the Dairy Technology division's, Experimental Dairy Plant, and the resulting skim milk was used in the present study. Maltodextrin was procured from Goodrich carbohydrates, Karnal. The gum acacia was acquired from Central Drug House in New Delhi.

Microencapsulation procedure for spray-dried microcapsules

Microencapsulated adjunct containing concentrated skim milk with total solids (17%), maltodextrin (25%), and gum acacia (15%), as well as *L. acidophilus* LA1, was dried in a spray dryer (SSP Pvt Ltd Faridabad) with incoming air at 170°C ± 3°C and an outlet temperature of 80°C ± 3°C. After cooling to room temperature, the microencapsulated powder was vacuum-packed in laminate sachets.

Encapsulation efficiency

The probiotic bacteria's survival rate during spray drying was measured as the encapsulation efficiency (E.E.), which was computed as follows:

$$EE = 100 \times N / N^0$$

Where E.E. is the encapsulation efficiency (%), N^0 is the number of bacteria before drying (log Cfu/g), and N is the number of bacteria after the drying process (log Cfu/g) (Rajam,& Anandharamakrishnan 2015).

Enumeration of encapsulated cells

Eleven gram of powder was dissolved in 99 ml of maximum recovery diluents. After this, about 1ml of the solution was taken, serially diluted, and appropriate dilutions were spread plated with MRS agar. The viable cell count was determined after 48-72 hours of incubation at 37°C. For every sample, three different dilutions were enumerated and averaged. The results were expressed on dry basis.

Stability under simulated GI conditions

Effect of Low pH

The Lee and Heo (2000) approach were used to investigate the impact of pH on the survival of probiotic microbes. As recommended by Rao et al. (1989), simulated gastric solutions containing 0.2% NaCl at pH 1.0, 1.5, and 2.0 (pH adjusted with 0.1 N HCl) were prepared. A simulated stomach solution with a different pH was combined with one g of the microencapsulated culture, and the mixture was then incubated at 37°C for 1, 2, and

3 hours. As a control, free cell culture without encapsulants was kept through the same processes as powder. After being incubated for 48 hours at 37 °C, colony-forming units were counted and noted.

Effect of high bile salt concentration

Lee and Heo (2000) technique was used to examine impact of bile salts. One g of microcapsules was kept in test tubes with 10 ml each of 1.0%, 1.5%, and 2.0% bile salt solution. After incubating at 37 °C for 1, 2, and 3 hours, the cells were counted from each concentration level and compared to a control.

Statistical analysis

The data obtained in the present study were subjected to a one-way analysis of variance (ANOVA) using SPSS v.16.0 for Windows 246 software (SPSS South Asia (P) Limited, Bangalore, India). The mean values and the standard error were calculated from the data obtained with triplicate trials.

Results and Discussion

Encapsulation efficiency and viability of spray-dried microencapsulated bacteria

Table 1 reveals that following drying, the number of survivors decreased from an initial log count of 9.32 to 8.11 log counts for spray-dried microencapsulated *L. acidophilus* LA1. Further, the overall log count reduction was observed to be 1.21 log counts. Therefore, the encapsulation efficiency calculated in the present study was 87%. The high encapsulation efficiency of matrix materials, more than 80 % for encapsulation of observed organisms in this study, agreed with previous research (Ying et al. 2012). Microcapsules containing gum arabic demonstrated the highest entrapment efficiency of 97.1% (highest viability 7.78 log CFU/g) (Tao et al. 2019). Pereira et al. (2014) reported a higher encapsulation yield of 77 – 82% using maltodextrin and gum arabic. Maltodextrin and gum acacia has long been used as wall materials to encapsulate probiotics and are effective as protectants for culture drying (Gul 2017).

The bar diagram in Fig 1 shows that the percent survival of the microencapsulated probiotic of live cells survived (87.07%) the spray drying process. The overall percent reduction was observed to be 12.93 %. It has been observed that during spray drying there is reduction in the bacterial cells due to heat stress and dehydration effects which may lead to injury at cellular levels. Exposure to higher outlet temperatures also may result in higher viability loss (Ananta et al. 2005). Approaches like use of stationary phase cultures and use of thermoprotectants can be of benefit. In the present study we have employed both approaches that might have resulted in lesser decline in cell from an initial value. According to Lian et al. (2002), the most effective carriers of probiotic *bifidobacteria* for improved survival after spray-drying were skim milk (80% survival) and gum acacia (25 to 60% survival).

Table 1 Log cfu/g of live cells before and after drying

Live cells	Log cfu/g
Before drying	9.32±0.32
After drying	8.11±0.42
Log reduction	1.2±0.31
Encapsulation efficiency	87.07%

Values are Mean±SE (n=3)

Several authors suggest that the effectiveness of dairy carriers in protecting cell viability during drying is related to the presence of lactose, milk proteins (García 2011), and prebiotics (Salaria et al. 2013). As a result of the good viable count obtained after spray drying, the use of gum acacia, maltodextrin, and skim milk as carrier materials is justified in the current experiment.

Stability of *L. Acidophilus* LA1 at simulated pH

The stability of free without encapsulation and microencapsulated *L. acidophilus* LA1 at simulated pH for different time intervals is presented in Table 2.

When free cells were exposed at pH 1.0 for 1 hour, the initial concentration decreased by 4.65 log count, while no colony-forming units could be found after 2-hour incubation at pH 1.0. In contrast, enhanced acid tolerance was observed at pH 2. A 2.77 log and 5.65 log reduction were observed for microencapsulated cells following 1 and 2-hour incubation, respectively.

At pH 2.0 approximately 2.88 log and 4.76 log cycles reduction were observed in free cells after 1 and 2 h of incubation, whereas in the case of microencapsulated cells, the decline was only 0.61 and 1.54 log, respectively. Thus, microencapsulated cells also declined in numbers during incubation at very low pH 1.0. Nonetheless, microcapsules were found to be significantly more acid tolerant than free cells, with > 10^2 cfu/g remaining after 2 hours at pH 1.0.

Milk proteins operate as buffering agents *in vivo*, protecting ingested strains of bacteria throughout upper GI transit, according to Charteris et al. (1998). The current findings agree with Fritzen-Freire et al. (2013), who found that after three hours of incubation at pH 2.0, the number of encapsulated cells decreased by about 2 log. Ilha et al. (2015) also found that encapsulated cells survived well, losing an average of only 1 log and 0.3 log after exposure to pH 2.0 and 3.0, respectively, as opposed to free cells, which lost an average of 4.25 log. It was also reported that in acidic circumstances, *L. casei Shirota* microencapsulated with RSM: GA experienced a modest decline in viability (Gul 2017). An appropriate spray-drying media can shield probiotics from stress

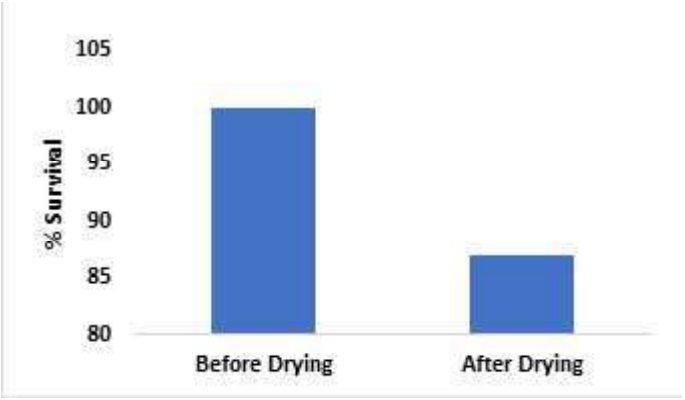

Fig. 1 Percent (%) survival in *L. acidophilus* LA1 following spray drying

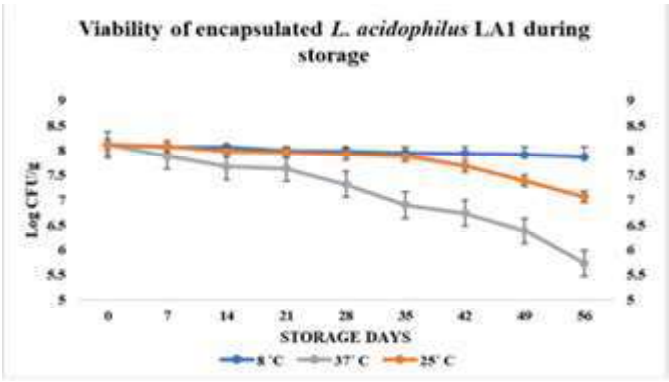

Fig.2 Effect of temperature on the viability of encapsulated *L. acidophilus* LA1 during storage

Table 2 Effect of simulated gastric pH on the viable count of *L. acidophilus* LA1 (Log cfu/g)

pH	Time(h)	Initial count	Free cells	Initial count	Protected cells	t stat
1.0	1	9.32	4.59±0.09	8.11	5.34±0.16	4.14*
	2	9.32	0.00±0.00	8.11	2.46±0.23	10.57**
2.0	1	9.32	6.36±0.18	8.11	7.50±0.24	3.86**
	2	9.32	4.48±0.20	8.11	6.57±0.25	6.49**

Values are Mean±SE (n=3), ** highly significant, * significant

Table 3 Effect of bile salt concentration on the viable count of microcapsules containing free and encapsulated *L. acidophilus* LA1 (Log cfu/g)

Bile %	Time(h)	Free cells	Protected cells	t stat
1%	1	7.32±0.17	7.91±0.06	3.27*
	2	6.80±0.08	7.74±0.06	9.63**
	3	6.46±0.26	7.57±0.11	3.93**
1.5%	1	7.14±0.09	7.73±0.12	3.86**
	2	6.52±0.19	7.41±0.24	11.92**
	3	6.05±0.12	6.78±0.07	5.32**
2%	1	6.87±0.03	7.29±0.23	1.81^{NS}
	2	5.48±0.08	6.79±0.08	11.92**
	3	4.57±0.11	6.24±0.23	6.61**

Values are Mean±SE (n=3), ** highly significant, * significant, NS=Not Significant

during digestion, according to *in vitro* research (Arslan et al. 2015).

Stability of *L. acidophilus* LA1 to simulated bile concentrations

L. acidophilus LA1 containing spray-dried adjunct was exposed to varying concentrations of bile salts (1%, 1.5%, and 2%). It is clear from the results (Table 3) that a similar trend was followed as with low pH. The cell numbers steadily declined with increasing bile levels and incubation time. The reduction rate was more for free cells. Statistically, the difference in the reduction of the free cells (F.C.) and encapsulated cells (E.C.) of *L. acidophilus* LA1 was significant at all bile levels ($p<0.01$) (Table 3). These findings agree with several earlier reports. Milk proteins and gum acacia may be responsible for the protective effects against bile (Gul 2017).

Viability of encapsulated *L. acidophilus* LA1 during storage

The results of the survival of microencapsulated *L. acidophilus* LA1 kept at different storage temperatures is presented in Fig. 2. It is evident from the figure that the numbers declined steadily as the temperature and storage period increased. The rate of decrease was more significant ($p<0.01$) at higher temperatures. The results may also be observed that there was no significant reduction at 8°C and 25 °C temperatures for the entire storage period. However, at 37°C, there was a gradual and significant ($p>0.05$) decline from an initial 8.11 log count value to 5.73 and was more rapid. At the end of the storage period, the 7.86 log

survival at 8 °C, 7.07 at 25°C, and 5.73 at 37 °C was reported. Thus, it can be inferred from the results that temperature has a marked effect on probiotic survival. The results indicated that eight weeks of storage of microencapsulated *L. acidophilus* LA1 at 8°C and 25°C corresponds to the advised therapeutic minimum dose of 10^{6-7} Cfu/g.

Statistical analysis also showed a highly significant ($p>0.01$) effect of temperatures on the survival and decline dynamics of probiotic *L. acidophilus* LA1. There has been a significant effect of temperature and interval interaction. The log count declined significantly at 37°C from an initial value and followed a non-linear trend, whereas, at refrigeration storage, it followed a linear pattern with no significant loss in viability until the end of the storage period. At ambient temperature during the initial part, there has been a linear trend for up to 5 weeks; afterward, a sharp decline can be observed. The statistical comparison showed significant differences ($p>0.05$) in survival kinetics during storage interval and temperature.

Low storage temperatures boost microbiological survival rates while reducing the stability in terms of cell survival of spray-dried samples (Kearney et al. 2009; Dimitrellou et al. 2008; Behboudi-Jobbehdar et al. 2013). Significant losses in viability were observed during storage at 37°C. Other researchers reported similar results (Ranadheera et al. 2015; Simpson et al. 2005). At higher storage temperatures, such as 37°C, a more significant loss of cell viability has been observed (Kearney et al. 2009; Strasser et al. 2009).

Our results agree with other studies that evaluated *L. acidophilus* viability using different encapsulating agents during refrigerated and higher storage temperatures.

Conclusions

In this work, a probiotic microorganism (*L. acidophilus* LA1) was spray-dried to protect the probiotic and check its efficacy against *in vitro* acid and bile salt conditions. Skim milk, maltodextrin, and gum acacia gave satisfactory effects as protectants. The viability of the probiotic strain was enhanced significantly by adding protectants during spray drying and storage. On the other hand, storage temperature was a crucial factor impacting strain viability. As was predicted, higher storage temperature led to a higher inactivation rate.

Acknowledgements

The authors acknowledge the National Dairy Research Institute, Karnal (India), for the smooth conduct of research work.

References

Ananta E, Volkert M, Knorr D (2005) Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* G.G. Int Dairy J 15:399–409

Arslan S, Erbas M, Tontul I, Topuz A (2015) Microencapsulation of probiotic *Saccharomyces cerevisiae var. boulardii* with different wall materials by spray drying. LWT-Food Sci Technol 63: 685-690

Behboudi-Jobbehdar S, Soukoulis C, Yonekura L, Fisk I (2013) Optimization of Spray-Drying Process Conditions for the Production of Maximally Viable Microencapsulated *L acidophilus* NCIMB 701748. Drying Technol 31: 1274-1283 https://doi.org/10.1080/07373937.2013.788509

Charteris W P, Kelly P M, Morelli L, Collins, J K (1998) Development and application of an *in vitro* methodology to determine the transit tolerance of potentially probioitc *Lactobacillus* and *Bifidobacterium* species in the upper human gastro-intestinal tract. J of Appl Microbiol 84: 759-768

De Castro-Cislaghi FP, Silva CDRE, Fritzen-Freire CB, Lorenz JG, Sant'Anna ES (2012) *Bifidobacterium* Bb-12 microencapsulated by spray drying with whey: Survival under simulated gastro-intestinal conditions, tolerance to NaCl, and viability during storage. J Food Eng 113:186-193 https://doi.org/https://doi.org/10.1016/jfoodeng.2012.06.006

Dimitrellou D, Tsaousi K, Kourkoutas Y, Panas P, Kanellaki M, Koutinas A (2008) Fermentation efficiency of thermally dried immobilized kefir on casein as starter culture. Process Biochem 43:1323–1329

Food Safety and Standards (2016) Health supplements, nutraceuticals, food for special dietary use, food for special medical purpose. Functional Food and Novel Food Regulations, 1-153.

Fritzen-Freire CB, Prudêncio ES, Pinto SS, Muñoz IB, Amboni RDMC (2013) Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to simulated gastro-intestinal conditions and heat treatments. LWT - Food Sci Technol 50: 39-44 https://doi.org/https://doi.org/10.1016/j.lwt.2012.07.037

Fu N, Chen XD (2011) Towards a maximal cell survival in convective thermal drying processes. Food Res Int 44:1127–1149

García AH (2011) Anhydrobiosis in bacteria: From physiology to applications. J Biosci 36 :939–950 https://doi.org/10.1007/s12038-011-9107-0

Gul O (2017) Microencapsulation of *Lactobacillus casei* Shirota by spray drying using different combinations of wall materials and application for probiotic dairy dessert. J Food Process Preserve 41: e13198.

Huang S, Vignolles ML, Chen XD, Le Loir Y, Jan G, Schuck P, Jeantet R (2017) Spray drying of probiotics and other food-grade bacteria: A review. Trends Food Sci Technol doi: 10.1016/j.tifs.2017.02.007.

Ilha EC, da Silva T, Lorenz JG, de Oliveira Rocha G, Sant Anna ES (2015) *Lactobacillus paracasei* isolated from grape sourdough acid bile salt and heat tolerance after spray drying with skim milk and cheese whey. Europ Food Res and Technol 240: 977–984

Kearney N, Meng X C, Stanton C, Kelly J Fitzgerald GF, Ross RP (2009) Development of a spray dried probiotic yoghurt containing *Lactobacillus paracasei* NFBC 338. Int Dairy J 19: 684–689

Lee KY, Heo T (2000) Survival *Bifidobacterium longum* immobilized in calcium alginate beads in simulated gastric juices and bile salts solution. Appl Environ Microbiol 66:869–873

Lian WC, Hsiao HC, Chou CC (2002) Survival of *Bifidobacteria* after spray drying. Int J Food Microbiol 74: 79–86

Liu H, Gong J, Chabot D, Miller SS, Cui SW, Ma J, Zhong F, Wang Q (2016) Incorporation of polysaccharides into sodium caseinate-low melting point fat microparticles improves probiotic bacterial survival during simulated gastro-intestinal digestion and storage. Food Hydrocolloids 54: 328-337

Pereira ALF, Almeida FDL, Lima MA, Costa JMC, Rodrigues S (2014) Spray-drying of probiotic cashew apple juice. Food Bioprocess Technol. 7: 2492-2499. https://doi.org/10.1007/s11947-013-1236-z

Rajam R, Anandharamakrishnan C (2015) Spray freeze drying method for microencapsulation of *Lactobacillus plantarum*. J Food Eng 166 :95–103

Ranadheera CS, Evans CA, Adams MC, Baines SK (2015) Microencapsulation of *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Propionibacterium jensenii* 702 by spray drying in goat's milk. Small Ruminant Res 123: 155-159

Rao AV, Shiwnarain N, Maharaj I (1989) Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juices. Can Inst Food Sci Technol J 22: 345-349

Salaria A, Tompkinson DK, Satish MHK, Sabikhi L (2013) Prebiotics in the microencapsulation matrix enhance the survival of *Lactobacillus acidophilus* LA1 Int J Fermented Foods 2: 33-45

Silva VM, Vieira GS, Hubinger MD (2014) Influence of different combinations of wall materials and homogenisation pressure on the microencapsulation of green coffee oil by spray drying. Food Res Int 61:132-143

Simpson PJ, Stanton C, Fitzgerald GF, Ross R P (2005) Intrinsic tolerance of *Bifidobacterium* species to heat and oxygen and survival following spray drying and storage. J Appl Microbiol 99 :493-501

Strasser S, Neureiter M, Geppl M, Braun R, Danner H (2009) Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria. J Appl Microbiol 107:167-177

Tao, Dinga Z, Houa D, Prakash S, Zhaoa Y, Fana Z, Zhangc D, Wanga Z, Liua M, Hana J (2019) Influence of polysaccharide as co-encapsulant on powder characteristics, survival and viability of microencapsulated *Lactobacillus paracasei* Lpc-37 by spray drying. J Food Eng 252:10-17

Ying D, Sun J, Sanguansri L, Weerakkody R, Augustin MA (2012) Enhanced survival of spray-dried microencapsulated *Lactobacillus rhamnosus* G.G. in the presence of glucose. J Food Eng 109:597-602 https://doi.org/https://doi.org/10.1016/j.jfoodeng.2011.10.017