International Food Research Journal 30(4): 1066 - 1073 (August 2023)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of different concentrations of thermoprotectant on microencapsulation of *Lactobacillus rhamnosus* GG by spray-drying, and its effect on physicochemical properties and viability

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Article history

Received:
15 May 2022
Received in revised form:
16 July 2022
Accepted:
19 September 2022

Keywords

probiotics, spray-drying, maltodextrin, microstructure, simulated gastrointestinal digestion

Abstract

Lactobacillus rhamnosus GG (LGG) is an acceptable probiotic strain that can live and grow at a gastrointestinal acidic pH and on a bile-rich medium. The influence of spray-drying microencapsulation of LGG on the physicochemical parameters and survivability was investigated in the present work. LGG was spray-dried with three different maltodextrin concentrations (6, 12, and 18% w/v). The inlet and outlet air temperatures of the spray-dryer were kept at 170 ± 5°C and 75 ± 5°C, respectively. The physicochemical parameters (moisture content (wet basis), water activity, and colour), viability (colony forming unit/g), and simulated gastrointestinal digestion were all investigated. Only 18% MD was selected on the basis of moisture content and log CFU/g. The total soluble solids (TSS) of 16.28 ± 0.93 °Brix were obtained using 18% MD. The end product had a moisture content of 5.40 ± 0.20%, and a water activity of 0.32 ± 0.02 a_w, which were acceptable. The L*, a*, and b* of the final product were 95.14 ± 0.19, -2.33 ± 0.02, and 7.17 ± 0.13, respectively. The spray-dried powder had final probiotic viability of around 10^8 CFU/g. Based on the study, maltodextrin at 18% (w/v) concentration can be considered as an ideal formulation with acceptable powder characteristics for shielding probiotic microorganisms from harmful conditions of spray-drying.

DOI
https://doi.org/10.47836/ifrj.30.4.22

Introduction

The human gastrointestinal system has a diverse microbiota. Gut microbiota are obligate anaerobes that help in the maintenance of human well-being by aiding the digestive system and nutrient assimilation (Rinninella *et al*., 2019). Changes in dietary consumption could cause disruption in the gut microbial system (Arepally *et al*., 2020). *Lactobacillus* spp. belong to the lactic acid bacteria (LAB) group, and are one of the most studied bacteria in the human gut microbiota (Bintsis, 2018). These bacteria are found in abundance in milk, meat, vegetables, cereals, and vertebrates' gastrointestinal systems. LAB are also widely used as starter cultures in the food and beverage industry.

The functional food market has grown immensely in recent years due to increased consumers' awareness. Functional foods contain ingredients that provide health benefits beyond the food's basic nutritional components, and may include vitamins, minerals, and probiotics. Probiotics are live microorganisms associated with various health benefits. Some major health attributes of probiotics are anti-carcinogenic properties, enhancement of calcium absorption, treatment of blood cholesterol, diarrhoea, *Helicobacter pylori* infection, inflammatory bowel disease, irritable bowel

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syndrome, respiratory tract infections, urinary tract infections, inhibition of foodborne pathogens, and associated with vitamin syntheses such as folic acid, nicotinic acid, and vitamin B (Kumar *et al*., 2022). Probiotics must be alive during the manufacturing and processing of food, and they must be viable once they reach the gastrointestinal system (Terpou *et al*., 2019). The viability depends on several factors, including the cultures, strains, drying conditions, fermentation media, growth parameters, and mechanisms of gastrointestinal administration. Probiotic bacterial cells undergo various challenges during production, processing, and gastric transit, such as heat and cold shocks, hydrostatic pressure, and acid exposure during gastric transit (Mishra and Athmaselvi, 2016). Therefore, strain selection plays a crucial role in the production of probiotic-based functional foods. To protect bacteria from all these harsh conditions, microencapsulation is used to improve the viability of probiotics.

Maltodextrin (MD) is a popular wall material because it is readily absorbed and digested, non-toxic, inexpensive, and has high solubility and low viscosity. It is a polysaccharide produced by starch hydrolysis. Due to its high activation energy, it can withstand heat stress and oxidative damage during storage. It has a variety of features including high solid concentration, ability to create matrices, and tasteless. The degree of polymerisation (DP) of MD can range from 4 to 22 (Kalita *et al*., 2018). It is used to preserve the probiotic microorganisms during the thermal stress of the spray-dryer. The low cost of MD is a major advantage in utilising it as a carrier agent. It may be used as a non-dairy-based probiotic powder that may be helpful for lactose-intolerant consumers. Therefore, the major goal of the present work was to encapsulate LGG utilising various MD concentrations through spray-drying, and to investigate the consequences of coating material concentrations on TSS, encapsulation yield (%), survivability, simulated gastric digestion, and physicochemical properties of encapsulated probiotic powder.

Materials and methods

Materials

The freeze-dried probiotic bacteria LGG was procured from the NCDC Centre at National Dairy Research Institute (NDRI), Karnal, India. MRS broth, MRS agar, and MD from maize starch were purchased from Hi-Media Laboratories Mumbai, India. Sodium chloride (NaCl), phosphate-buffered saline (PBS), 50 mL centrifuge tubes, and glassware were pre-sterilised (121°C at 15 lbs for 15 min) before use.

Inoculum preparation

The inoculum was prepared by adding a single colony of LGG into 5 mL of MRS broth, and incubated at 37°C for 24 - 48 h under static conditions. Subculturing was done by adding 50 µL of LGG culture to 5 mL of MRS broth, and incubated at 37°C for 24 - 48 h. After that, 5 mL LGG culture was transferred to 500 mL of MRS broth (1% inoculum), and incubated under the same conditions. Initial CFU/g was checked at this stage by using the standard plate count method. Centrifugation at 7,000 rpm at 4°C for 10 min was used to recover the LGG cells. After the removal of the supernatant, the cells were washed twice in sterile PBS solution before being pelleted and employed in the microencapsulation procedure.

Carrier media preparation for spray-drying

MD was disseminated in water and allowed to moisten for 30 min using a magnetic stirrer. The MD solution was mixed with probiotic biomass, and pre-heat-treated at 40°C for 20 min for pre-adaptation to heat, and cooled to room temperature (Yonekura *et al*., 2014). This pre-adaptation provides a high degree of shielding against heat stress (Desmond *et al*., 2002).

Preparation of microencapsulated LGG powder

In a laboratory-scale spray-dryer (SMST Industries, Kolkata, India), MD solution containing probiotic bacteria was spray-dried at an inlet temperature of 170 ± 5°C, and a corresponding outlet temperature of 75 ± 5°C. The atomisation pressure was maintained at 2 kg/cm^2. Three different formulations of MD were prepared for spray-drying namely (a) 6%, (b) 12%, and (c) 18% (w/v) MD, respectively. For adequate mixing of solution, magnetic stirring was used. The spray-dried microencapsulated probiotic powders were stored in polyethylene pouches at 4°C.

Enumeration and quantification of bacteria in spray-dried powder

The viability of spray-dried probiotic powder was performed using the standard plate count method

as defined by Suryabhan *et al*. (2019). The LGG microcapsule powders (1 g) were rehydrated in 9 mL of sterile saline solution (0.85% w/v). To allow complete dissolution, rehydrated samples were kept on a shaker for 2 min. Then the samples were serially diluted, and MRS agar was used for pour-plating. The MRS agar plates were incubated at 37°C for 24 - 48 h. Total plate counts of the live bacteria were represented as log colony forming units per gram (log CFU/g). The viability of probiotics was measured after spray-drying on the same day.

Encapsulation yield (%)

Spray-dried probiotic powder encapsulation yield (%) was calculated as a percentage of the total microencapsulated powder and the weight of the carrier agents (Mahdi *et al*., 2020) using Eq. 1:

$$\text{Encapsulation yield (\%)} = \frac{\text{weight (g) of the powder collected}}{\text{weight (g) of the carrier agents}} \times 100 \quad \text{(Eq. 1)}$$

Moisture content

The hot-air oven method was employed to determine the moisture content of the probiotic powders. The weight loss after drying the sample in the oven at 105°C for 3 h was used using Eq. 2:

$$\text{Moisture content (\%)} = \frac{\text{Sample weight (g)} - \text{Dry sample weight (g)}}{\text{Sample weight (g)}} \times 100 \quad \text{(Eq. 2)}$$

Water activity

The water activity of the sample was measured using a water activity analyser (Aqua Lab 4TE, METER Group, USA) at 25°C (Chandra *et al*., 2020).

Colour of spray-dried powder

The colour of 1 g of LGG powder placed in plastic cuvettes was determined by a handheld colorimeter (Model CR-400, Konika Minolta, Japan). The L* (lightness), a* (green to red), and b* (blue to yellow) values were determined according to Kumar *et al*. (2021).

Survival of free and encapsulated LGG cells in simulated gastrointestinal conditions

The simulated gastric juices (SGJ) and simulated intestinal juices (SIJ) were prepared according to Minekus *et al*. (2014). The SGJ was prepared by dissolving sodium chloride (2.05 g/L), potassium chloride (0.37 g/L), calcium chloride (0.11 g/L), potassium dihydrogen phosphate (0.60 g/L), pepsin (0.0133 g/L), and lysozyme (0.01 g/L), and the pH was adjusted to 3 using 1 M HCl. The SIJ was made by mixing pancreatin (0.1 g/L) and bile salt (3 g/L) in an 8.5 g/L sodium chloride solution with sodium phosphate dibasic heptahydrate, and the pH was adjusted to 8 using sterile 100 mM sodium hydroxide. A 0.22 µm membrane filter was used to sterilise the SGJ and SIJ solutions. Next, 1 g sample of probiotic powder was inoculated in 9 mL of sterile SGJ solution, and incubated in an orbital shaker at 120 rpm for 120 min at 37°C. After incubation, CFU/g were counted by standard pour plate method using MRS agar. Furthermore, the survival rate of microencapsulated cells in SIJ conditions was investigated, and the remaining pellet was placed in 9 mL of SIJ, and incubated in an orbital shaker at 120 rpm for 180 min at 37°C. The viable bacteria were measured after the incubation period (Moayyedi *et al*., 2018).

Scanning electron microscopy (SEM)

The probiotic powder samples were deposited directly over adhesive support, and gold-coated under vacuum conditions. The SEM (EVO® 50, Carl Zeiss, USA) was employed to obtain the microstructure at two different magnifications (1000× and 2500×) using a 20 kV accelerating voltage (Kumar *et al*., 2021).

Statistical analysis

The statistical analyses were conducted using Statistical Package for the Social Sciences (IBM SPSS statistics 20). Analysis of variance (ANOVA) and the Duncan's test was used to investigate the significant differences between the samples. All of the tests were done in triplicate, and the mean ± standard deviation was calculated.

Results and discussion

Total soluble solids (°Brix)

Significant ($p \leq 0.05$) increase in TSS of powder samples from 4.94 ± 0.03 to16.28 ± 0.93 was observed with an increase in the addition level of MD (6 - 18%). TSS indicates the amounts of soluble solids in a liquid, and expressed in °Brix. The more concentrated the solution, the higher the TSS in the solution (Hadiwijaya *et al*., 2020). The level of concentration of a solution was 18 > 12 > 6. The

highest TSS was obtained in 18% MD concentration, as shown in Table 1. The solid content of the feed sample plays a crucial role in determining the high viability of different strains of LAB (Yonekura *et al*., 2014). Würth *et al*. (2016) recommended that the highest total solid content for a practicable spray-drying process is 35%. An increase in total solid content is associated with an increase in viscosity. In this experiment, total solid content for a spray-drying process was well below the recommended limit. Total solid content also affects the particle size of spray-dried powders (Muhammad *et al*., 2017).

Bacterial population

The log CFU/g of spray-dried powders varied from 4.27 ± 1.05 to 8.83 ± 1.67 (Table 1). There was no significant difference ($p > 0.05$) in the initial counts (10^{11} CFU/mL) of live bacteria before spray-drying. Tripathi and Giri (2014) recommended that at least 10^6 CFU/g probiotic bacteria should be present in food at the time of consumption to achieve the intended health benefits. In 12% and 18% MD solutions, the total viability was above the recommended level of > 6 log CFU/g. The maximum survival rate was 8.83 log CFU/g obtained with 18% MD solution after spray-drying. This might have been due to MD's rigid and homogeneous covering over the bacterial cells, which reduced the stress of spray-drying on the bacteria (Kalita *et al*., 2018).

Encapsulation yield (%)

The yield % for 6, 12, and 18% MD were 8.13 ± 0.81, 19.27 ± 0.69, and 41.32 ± 1.31%, respectively. According to Arslan *et al*. (2015), the inlet temperature had no significant ($p > 0.05$) effect on product yield, while the wall materials had a significant effect ($p < 0.05$). The yield % of close to 50% is considered an efficient drying in the lab-scale spray-dryer (Bhandari *et al*., 1997). The low drying yield in 6% and 12% MD solutions could have been due to the higher moisture content of the final powder. Due to this high moisture content, the spray-dried powder became more rubbery, and stuck to the spray-dryer wall, thus lowering the yield (Pandey and Mishra, 2021). Arslan *et al*. (2015) reported a 40.39% microencapsulation yield for *S. boulardii* with MD as a coating material through spray-drying. Various factors, including the type of substrate used, temperature, and total solid content could influence the product yield.

Moisture content and water activity

The moisture content and water activity of microencapsulated probiotic powder were not affected by the inlet air temperature. These mainly depend on the outlet air temperature (Arslan *et al*., 2015). The spray-drying inlet temperature of 170 ± 5°C was used with a corresponding outlet temperature of 75 ± 5°C, which led to an alteration in the moisture content from 5.40 ± 0.20 to 8.84 ± 0.19% (wet basis, w.b.) as shown in Table 1. There was a decrease in moisture content with an increase in drying temperature, which can be ascribed to an increased rate of heat transfer to the particle at a higher drying temperature, thus generating a large impact on moisture evaporation (Mishra and Athmaselvi, 2016). The moisture content also decreased with an increase in MD concentration which might have been due to the firm coating of microcapsules. Based on several studies, the level of moisture in spray-dried powders should be around 4 - 5 g/100 g for greater storage stability (Chávez and Ledeboer, 2007). Rajam and Anandharamakrishnan (2015) observed higher moisture content (7 - 9 g/100 g) in several experiments. The water activity values of spray-dried powder ranged from 0.32 ± 0.02 to 0.62 ± 0.11 (Table 1). The recommended range of water activity for spray-dried microcapsules is 0.15 to 0.30 to ensure microbiological stability (Corcoran *et al*., 2004).

Table 1. TSS, moisture content, water activity, and bacterial count of microencapsulated probiotic powder at 6, 12, and 18% MD (w/v) solutions.

Parameter	6% MD	12% MD	18% MD
TSS (°Brix)	4.94 ± 0.03^{a}	11.03 ± 0.18^{b}	16.28 ± 0.93^{c}
Moisture content (%)	8.84 ± 0.19^{c}	6.84 ± 0.13^{b}	5.40 ± 0.20^{a}
Water activity	0.62 ± 0.11^{b}	0.38 ± 0.06^{a}	0.32 ± 0.02^{a}
Bacterial count (log CFU/g)	4.27 ± 1.05^{a}	6.54 ± 1.54^{ab}	8.83 ± 1.67^{b}

Means followed by different lowercase superscripts in a column differ significantly ($p \leq 0.05$) as calculated by Duncan's test.

Colour analysis

For colour analysis, only 18% MD solution was selected because the selection of the optimised sample was done on the basis of moisture content and final log CFU/g. Only 18% MD solution gave a satisfactory value of moisture content and log CFU/g. The probiotic powder had L*, a*, and b* values of 95.14 ± 0.19, -2.33 ± 0.02, and 7.17 ± 0.13, respectively. The obtained sample was light, slightly green, and yellow. The colour of the dried product was influenced by increased inlet temperature due to non-enzymatic browning reactions (Mishra and Athmaselvi, 2016). In general, higher L* value indicates lighter colour, while lower L* value indicates darker colour. According to Aryana and McGrew (2007), higher L* values of microcapsules are desired. Microcapsules with higher a* and b* values represent more red and yellow hues, respectively (De Castro-Cislaghi *et al*., 2012).

Survivability in simulated gastrointestinal conditions

As shown in Table 2, free cells decreased to 3.23 and 3.21 log CFU/g after 2 h of exposure time with 18% and 12% MD solutions, respectively, while the encapsulated cell's viability was 5.31 and 4.19 log CFU/g at the end of the analysis at pH 3 of SGF solution. Free cells had a viability of 1.91 log CFU/g under simulated intestinal fluid (SIF) conditions (pH 8), while encapsulated cells had a viability of 3.72 and 2.13 log CFU/g at the end of 3 h of exposure time with 18% and 12% MD solutions, respectively. It was observed that the SIF solution had a minor effect on the viability of cells.

Table 2. Viability of *Lactobacillus rhamnosus* GG (log CFU/g), with and without encapsulation at **(a)** 18% and **(b)** 12% MD (w/v) solutions during exposure to simulated gastric juice (SGJ, pH 3.0) and simulated intestinal juice (SIJ, pH 8.0).

(a)			**(b)**		
Time (h)	**Encapsulated cell (log CFU/g)**	**Free cell (log CFU/g)**	**Time (h)**	**Encapsulated cell (log CFU/g)**	**Free cell (log CFU/g)**
SGF condition (18% MD), pH 3.0			**SGF condition (12% MD), pH 3.0**		
0	8.83	11	0	6.54	11
2	5.31	3.23	2	4.19	3.21
SIF condition, pH 8			**SIF condition, pH 8**		
0	5.31	3.23	0	4.19	3.21
3	3.72	1.91	3	2.13	1.91

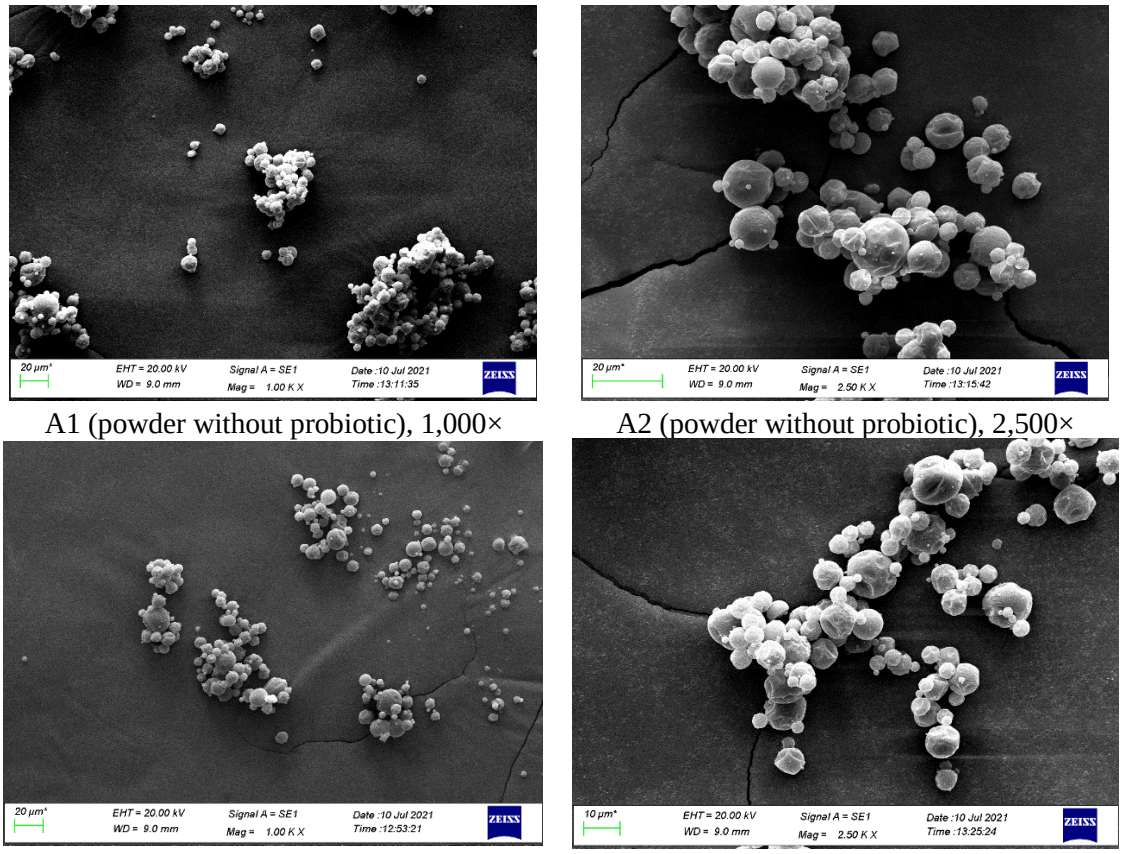
Note: Residual cells were transferred to SIF conditions.

Scanning electron microscopy (SEM)

As shown in Figure 1, the morphology of the particles showed the protective effect of MD concentration. For the SEM study, only 18% MD concentration was selected due to its high viability during simulated gastrointestinal conditions. The microcapsules were all spherical and regular in shape, and some had dents on the surface, which might represent rapid evaporation of the liquid drops from the surface, and variations in the droplets' surface tension (Saénz *et al*., 2009; Slavutsky *et al*., 2017). The spherical and uniform shape is essential for increased core retention and reduced water and oxygen permeability (Hijo *et al*., 2015; Kalita *et al*., 2018; Moayyedi *et al*., 2018). There was no bacterial appearance outside the capsules, thus indicating that all bacterial cells were properly microencapsulated in wall components (Gul, 2017; Pandey and Mishra, 2021). The smooth and void-free microcapsules improve the protective properties of coated microcapsules. The probiotic protection is demonstrated by the absence of a void. Kalita *et al*. (2018) reported similar morphology of spray-dried probiotic (*Lactobacillus plantarum*) litchi juice with 15% MD (w/v) solution.

Conclusion

In the present work, MD solutions (6, 12, and 18% w/v) were used to spray-dry probiotic LGG using a lab-scale spray-dryer. High viability of probiotics was obtained at 18% MD concentration.



A1 (powder without probiotic), 1,000× A2 (powder without probiotic), 2,500×

B1 (probiotic powder), 1,000× B2 (probiotic powder), 2,500×

Figure 1. Scanning electron microscopic (SEM) images of the spray-dried powder at 18% MD concentration (1,000× and 2,500× magnification).

MD as a thermoprotectant improved the survivability of LGG after spray-drying and during simulated gastrointestinal digestion. The physicochemical properties of the final product were also affected by the concentration of the carrier material. The SEM images showed spherical and regular shape of microcapsules, and rapid evaporation of the liquid drops might have caused some dents on the surface. Based on the proposed work, probiotic powders could be produced in a more sustainable, productive, and low-cost way by utilising spray-drying.

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