

Development and Survival Assessment of Microencapsulated *Lactobacillus rhamnosus* GG in Watermelon Juice -Sodium Alginate Beads at Different Storage Conditions

Aruthra Devi^{1*} and Rita Narayanan²

¹PG Scholar, College of Food and Dairy Technology,

²Professor, Department of Food Processing Technology, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 052, Tamil Nadu, India

*Corresponding Author E-mail: aruthrafpe@gmail.com

Received: 5.09.2020 | Revised: 12.10.2020 | Accepted: 20.10.2020

ABSTRACT

The present study adopted microencapsulation of bacteria to enhance its viability. Watermelon juice was found to be a suitable media for the growth of probiotic *L.rhamnosus* GG showing prebiotic effect. No significant difference in the viability of *L.rhamnosus* GG in MRS medium. The average viable count (\log_{10} cfu/ml) of *L.rhamnosus* GG in watermelon and MRS medium were 10.53 ± 0.136 and 10.55 ± 0.132 respectively. The survivability assessment of *L.rhamnosus* GG in microencapsulated beads at different storage conditions was studied. The probiotic level of 10^8 cfu/g of *L.rhamnosus* GG was maintained at both temperatures till 21 days of storage. The viability of *L.rhamnosus* GG was 8.404 ± 0.019 and 8.027 ± 0.008 \log_{10} cfu/ml in microencapsulated beads at refrigeration and ambient temperature respectively on 21st day of storage after which there was a decline in the probiotic count in samples stored at ambient and refrigeration temperature.

Keywords: *Lactobacillus rhamnosus* GG, Microencapsulation, Sodium alginate beads, Probiotics.

INTRODUCTION

The name probiotic comes from the Greek word “pro bios” which means “for life”. The history of probiotics began with the history of man; Cheese and fermented milk were well known to the Greeks and Romans, who recommended their consumption especially for children and convalescents. The concept of

probiotics was introduced by Elie Metchnikoff in the early 20th century (Metchnikoff, 1907). According to Food and Agriculture Organization (FAO) of the United States and World Health Organization (WHO), probiotics are ‘live microorganisms which when administered in adequate amounts confer health benefits to the host’.

Cite this article: Devi, A., & Narayanan, R. (2020). Development and Survival Assessment of Microencapsulated *Lactobacillus rhamnosus* GG in Watermelon Juice -Sodium Alginate Beads at Different Storage Conditions, *Ind. J. Pure App. Biosci.* 8(5), 281-287. doi: <http://dx.doi.org/10.18782/2582-2845.8303>

Alternatively, probiotics have been defined as live microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance (Fuller, 1989).

At present, several well-characterized strains of Lactic acid bacteria are available as potential probiotic organisms which are useful in improvising human health. The largest group of Lactic acid bacteria belongs to genus *Lactobacillus* that comprises of more than 50 different species. *Lactobacillus* species are found in the gut of humans and other animals, while their numbers may vary with species, age of host or their location within the gut. However, species of *Lactobacillus* like *L.acidophilus*, *L.crispatus*, *L.plantarum*, *L.gasseri* are involved in traditional and industrial food fermentations (de Vries et al., 2006).

The inclusion of probiotics in food matrices is a challenging area of research. Over the last few years, the probiotics industry experienced a remarkable market share increase. The development of functional food is associated to a large extent, with products containing probiotics and their contribution in supplementation to the bacterial flora in the intestines. Hence in the current study, the development of synbiotic watermelon beads.

Reid (1999) elaborated the characteristics of *Lactobacillus rhamnosus* GG as a probiotic due to its ability to adhere to the cells, colonize the intestine, exclude or reduce the adherence of the pathogenic strains, persist and multiply, produce compounds that are antagonistic to pathogen growth, resist vaginal microbicides and form a normal, balanced flora.

Land et al. (2005) in their review stated that *Lactobacillus rhamnosus* strain GG was originally isolated from the human intestinal flora and is the most widely used probiotic agent for adults and children. He highlighted its ability to prevent diarrhea and atopy among children.

Lactobacillus rhamnosus GG are large, white, creamy colonies. The colonies were reported to be gram positive, uniform rods in chains (Saxelin et al., 1993).

Protection of probiotics has been proposed for various dairy fermentation, with

microencapsulation in hydro colloidal beads for improving their viability in both the food products and in intestinal tract (Champagne et al., 1992).

Viability of probiotic bacteria in a product at the point of consumption is an important consideration for their efficacy, as they have to survive during processing and shelf life of food and supplements, transit through high acidic conditions of the stomach and enzymes and bile salts in the small intestine (Kebary, 1996).

Microencapsulation is the process of encasing an active component in a shell and is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influence of specific conditions (Kailasapathy & Masaondole, 2005).

According to Ding and Shah (2008), microencapsulation provides a favourable environment for sensitive probiotic bacteria as well as a physical barrier from the harsh environment. He also highlighted that food products containing microencapsulated probiotic bacteria were more stable than those containing free probiotic organisms.

Encapsulation is a process whereby cells are retained within a wall material to reduce cell injury. Encapsulation in hydrocolloid bead has been investigated as a means to protect and improve viability of probiotic microorganisms in food products and in the intestinal tract (Rao et al., 1989).

Microencapsulation by extrusion involves projecting an emulsion core and coating material through a nozzle at high pressure. Extrusion of polymer solutions through nozzles to produce capsules is mainly reported on a laboratory scale, where simple devices such as syringes are applied. If the droplet formation occurs in a controlled manner (contrary to spraying) the technique is known as prilling (Heinzen, 2002).

The term synbiotic is used when a product contains both probiotic and prebiotic ingredients (Schrezenmeir & Vrese, 2001). Synbiotic are used not only for the improved survival of beneficial micro organisms added to food or feed but also for the stimulation of

the proliferation of specific native bacterial strains present in the gastrointestinal tract (Gourbeyre et al., 2011).

According to Sivudu et al. (2014), fruits and vegetables are rich in nutrients and can be considered as a carrier to support probiotic and prebiotic delivery.

Watermelon is one of the underutilized fruits and is liked by consumers due to its flavour and attractive colour. The colour in the watermelon is due to the presence of lycopene that has potential to act as a bio-colour and as an anticancerous agent (Huor et al., 1980).

Wang et al. (1996) reported that the antioxidant activity, mineral and phenolics of pomegranate maintained the viability and stability of *L. plantarum* in microencapsulated beads.

Fruit juices represent a promising carrier for probiotic bacteria (Marianne et al., 2015).

MATERIALS AND METHODS

Materials

Lactobacillus rhamnosus GG (ATCC 53103) was obtained from National Dairy Research Institute, Karnal, Haryana. De Man Rogosa and Sharpe (MRS) broth (Himedia GM369) and De Man Rogosa and Sharpe (MRS) Agar (Himedia M641) were used for the propagation and enumeration of the freeze dried culture respectively. Food grade Sodium alginate and Calcium chloride was purchased from Loba Chemie Pvt Ltd. Watermelon was purchased from from local market, Chennai, TamilNadu.

Methods

Propagation and enumeration of freeze dried *Lactobacillus rhamnosus* GG

The freeze dried culture of *L. rhamnosus* GG (ATCC 53103) was inoculated in MRS broth and incubated overnight at 37°C. The propagated culture was then enumerated in MRS agar. Stock cultures were maintained by sub-culturing once in 15 days, whereas the working cultures were freshly prepared in skim milk as and when needed.

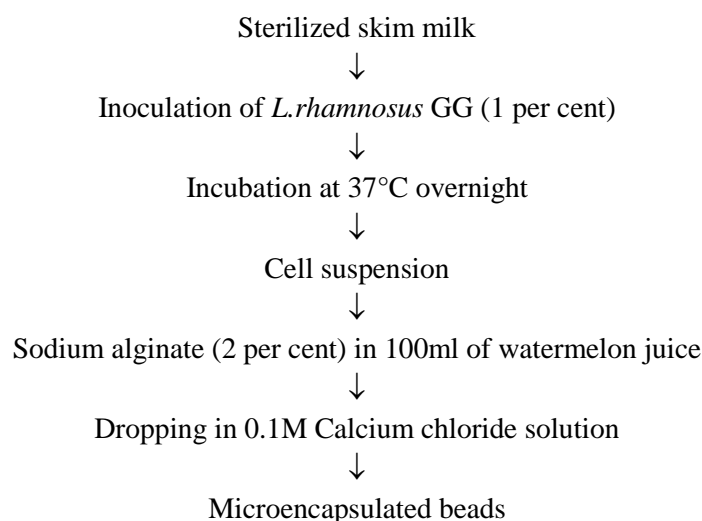
Preparation of microencapsulated *L. rhamnosus* GG beads

Assessing the prebiotic property of watermelon on the viability of *L. rhamnosus* GG

The prebiotic property of watermelon was assessed as per the modified procedure of Saranyambiga et al. (2017).

Preparation of microencapsulated *L. rhamnosus* GG beads

Microencapsulation technique in the present study was adopted from Krasaekoopt et al. (2003). *Lactobacillus rhamnosus* GG was inoculated in skim milk and incubated at 37°C overnight. The overnight culture was suspended in the carrier solution containing 2 per cent sodium alginate in sterilized watermelon juice. This was then filled in a pre sterilized syringe and dropped slowly into 0.1M Calcium chloride solution from a height of 20cm get pink coloured *L. rhamnosus* GG microencapsulated beads. The flow diagram for the preparation of *L. rhamnosus* GG beads is given below.



Assessment of survivability of *Lactobacillus rhamnosus* GG in microencapsulated beads during the storage period at different temperatures

L.rhamnosus GG was enumerated in microencapsulated beads stored at ambient and refrigeration temperatures as per the procedure followed by Savedboworn et al. (2015) for a period of 28 days at 7 days interval.

Statistical analysis

The Statistical analysis was done for the data obtained using VETSTAT as per the standard procedure of Snedecor and Cochran (1980). Results were expressed as Mean ± Standard Error.

RESULTS

Comparative study on viability of *Lactobacillus rhamnosus* GG in MRS broth and Watermelon juice.

Table shows the viability of *L.rhamnosus* GG in MRS broth and watermelon juice

The Mean±SE values for the growth (\log_{10} cfu/ml) of *L.rhamnosus* GG in MRS broth was 10.55 ± 0.132 and in watermelon juice was 10.53 ± 0.136 .

Statistical analysis revealed no significant difference in the counts of *L.rhamnosus* GG in MRS broth and watermelon juice.

Table 1: Comparative study on viability of *Lactobacillus rhamnosus* GG[#] in MRS broth and Watermelon juice

Name of the culture	Different media		t test
	MRS broth	Watermelon juice	
<i>Lactobacillus rhamnosus</i> GG	10.55 ± 0.132	10.53 ± 0.136	NS

[@]Average of six trials

[#] \log_{10} cfu/ml

NS Non significant

Table 1 presents the viability of *L.rhamnosus* GG in pasteurized water melon juice. The log values of $10.53\pm 0.136 \log_{10}$ cfu/ml showed no significant difference with MRS medium and suggest that water melon can be considered as a suitable medium for the growth of *L.rhamnosus* GG. The results concur with the observations of Tuorila and Gardello (2002) that fruit and vegetable juices may be considered as an alternate vehicle for the delivery and incorporation of probiotics into human intestine. The medium can be

considered as an ideal vehicle for probiotics as per the findings of Sivudu et al. (2014) that watermelon juice is rich in lycopene, minerals, vitamins and sugars which encourages the growth of probiotics like *L.fermentum* and *L.casei* for several weeks both at refrigeration and room temperature. The results are in consonance with the observation of Marianne et al. (2015) that fruit juices represent a promising carrier for probiotic bacteria.



Microencapsulated beads



Single microencapsulated bead

Table 2 shows the assessment of survivability of *L.rhamnosus* GG in microencapsulated beads at different storage temperatures. The Mean \pm SE values of viable count (\log_{10} cfu/g) of *L.rhamnosus* GG in microencapsulated beads at 4°C at 0, 7, 14, 21 and 28 days were 8.427 \pm 0.016, 8.416 \pm 0.015, 8.423 \pm 0.017, 8.404 \pm 0.019 and 7.818 \pm 0.052 respectively.

The Mean \pm SE values of viable count (\log_{10} cfu/g) of *L.rhamnosus* GG in microencapsulated beads at 32°C at 0, 7, 14, 21 and 28 days were 8.427 \pm 0.016, 8.898 \pm 0.017, 8.408 \pm 0.011, 8.027 \pm 0.008 and 7.201 \pm 0.262 respectively.

Statistical analysis revealed a highly significant difference ($P \leq 0.01$) in the survivability of *L.rhamnosus* GG between 0, 7, 14, 21 and 28 days of storage at refrigerated

temperature. There was a one log reduction in the viable count of *L.rhamnosus* GG on the 28th day of enumeration.

Statistical analysis revealed a highly significant difference ($P \leq 0.01$) in the survivability of *L.rhamnosus* GG between 7th day and other days of storage at ambient temperature. The maximum viable count was enumerated on the 7th day followed by 0 and 14th day. There was a gradual decline of viability on the 21st day of storage and a one log reduction in the viable count of *L.rhamnosus* GG on the 28th day of enumeration.

The survival rate and the probiotic level of 10⁸ cfu/g of *L.rhamnosus* GG were maintained at both temperatures till 21 days of storage.

Table 2: Assessment of survivability of *Lactobacillus rhamnosus* GG in microencapsulated beads during the storage period at different temperatures

Days	Storage Temperature	
	4 ^o C	32 ^o C
0	8.427 ^b \pm 0.016	8.427 ^c \pm 0.016
7	8.416 ^b \pm 0.015	8.898 ^d \pm 0.071
14	8.413 ^b \pm 0.017	8.408 ^c \pm 0.011
21	8.404 ^b \pm 0.019	8.027 ^b \pm 0.008
28	7.818 ^a \pm 0.052	7.201 ^a \pm 0.262
F value	125.28^{**}	38.65^{**}

Assessment of survivability of *Lactobacillus rhamnosus* GG[#] in microencapsulated beads during the storage period at different temperatures (Mean \pm SE)[@]

[@]Average of six trial

[#] \log_{10} cfu/g

^{**}Statistically highly significant ($P \leq 0.01$)

Means bearing various superscripts in the same column differs highly significantly ($P \leq 0.01$)

The viability of *L.rhamnosus* GG was maintained up to $8\log_{10}$ cfu/g for 21 days at refrigerated temperature and ambient temperature after which there was a decrease in the viable count. This may be due to the reasoning of Charteris et al. (2002) that stress associated with encapsulation and other adverse conditions such as refrigerated storage may cause culturable cells to enter a growth phase that does not produce colonies on media that normally support their growth.

CONCLUSION

Probiotic foods are becoming increasingly popular due to their contribution to good health. *Lactobacillus rhamnosus* species has a long history of being used in probiotic foods and possess a genome that allows it to adapt to a range of environments including the human gastrointestinal and urogenital tracts. In order to exert health promoting probiotic effects, it is important for the bacteria to survive the inhospitable environment of the human gastrointestinal tract. The present study adopted microencapsulation of bacteria to enhance its viability. Watermelon juice was found to be a suitable media for the growth of probiotic *L.rhamnosus* GG showing prebiotic effect. No significant difference in the viability of *L.rhamnosus* GG in MRS medium. The average viable count (\log_{10} cfu/ml) of *L.rhamnosus* GG in watermelon and MRS medium were 10.53 ± 0.136 and 10.55 ± 0.132 respectively. The survivability assessment of *L.rhamnosus* GG in microencapsulated beads at different storage conditions was studied. The probiotic level of 10^8 cfu/g of *L.rhamnosus* GG was maintained at both temperatures till 21 days of storage. The viability of *L.rhamnosus* GG was 8.404 ± 0.019 and 8.027 ± 0.008 \log_{10} cfu/ml in microencapsulated beads at refrigeration and ambient temperature respectively on 21st day of storage after which there was a decline in the probiotic count in samples stored at ambient and refrigeration temperature.

REFERENCES

Champagne, C. P., Morin, N., Couture, R., Gagnon, C., Jelen, P., & Lacroix, C.

(1992). The potential of immobilized cell technology to produce freeze-dried, phage-protected cultures of *Lactococcus lactis*. *Food Research International*, 25(6), 419-427.

Charteris, W. P., Kelly, P. M., Morelli, L., & Collins, J. K. (2002). Edible table (bio) spread containing potentially probiotic *Lactobacillus* and *Bifidobacterium* species. *International Journal of Dairy Technology*, 55(1), 44-56.

De Vries, M. C., Vaughan, E. E., Kleerebezem, M., & de Vos, W. M. (2006). *Lactobacillus plantarum*—survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal*, 16(9), 1018-1028.

Ding, W. K., & Shah, N. P. (2008). Survival of free and microencapsulated probiotic bacteria in orange and apple juices. *International Food Research Journal*, 15, 219–232.

Fuller, R. (1989). Probiotics in man and animals. *Journal of applied bacteriology*, 66(5), 365-378.

Heinzen, C. (2002). Microencapsulation by prilling and coextrusion. *Nutraceuticals and Probiotics (Abstract) Workshop* (No. 53).

Huor, S. S., Ahmed, E. M., Rao, P. V., & Cornell, J. A. (1980). Formulation and sensory evaluation of a fruit punch containing watermelon juice. *Journal of food science*, 45(4), 809-813.

Kailasapathy, K., & Masaondole, L. (2005). Survival of free and microencapsulated *Lactobacillus acidophilus* and *Bifidobacterium lactis* and their effect on texture of feta cheese. *Australian Journal of Dairy Technology*, 60, 252-258.

Kebary, K. M. K. (1996). Viability of *Bifidobacterium bifidum* and its effect on quality of frozen Zabady. *Food Research International*. 29(5), 431-437.

- Krasaekoopt, W., Bhandari, B., & Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*, 13(1), 3-13.
- Land, M. H., Rouster-Stevens, K., Woods, C. R., Cannon, M. L., Cnota, J., & Shetty, A. K. (2005). *Lactobacillus sepsis* associated with probiotic therapy. *Pediatrics*, 115(1), 178-181.
- Perricone, M., Bevilacqua, A., Altieri, C., Sinigaglia, M., & Corbo, M. R. (2015). Challenges for the Production of Probiotic Fruit Juices. *Beverages*, 1, 95-103.
- Metchnikoff, E. (1907). Lactic acid as inhibiting intestinal putrefaction in the prolongation of life: Optimistic studies. W. Heinemann, London: 161-183.
- Rao, A. V., Shiwnaraiin, N., & Maharaj, J. (1989). Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juices. *Canadian Institute of Food Science and Technology Journal*, 22, 345-349.
- Reid, G. (1999). The scientific basis for probiotic strains of *Lactobacillus*. *Applied and Environmental Microbiology*, 65(9), 3763-3766.
- Saranyambiga, D., Narayanan, R., & Vadivoo, V. S. (2017). Development Of Jamun Synbiotic Smoothie. *International journal of science,environment and technology*,6(4).
- Savedboworn, W., & Wanchaitanawong, P. (2015). Viability and probiotic properties of *Lactobacillus plantarum* TISTR 2075 in spray-dried fermented cereal extracts. *Maejo International Journal of Science and Technology*, 9(3), 382.
- Saxelin, M., Ahokas, M., & Salminen, S. (1993). Dose response on the faecal colonisation of *Lactobacillus* strain GG administered in two different formulations. *Microbial Ecology in Health and Disease*, 6(3), 119-122.
- Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics—approaching a definition. *The American journal of clinical nutrition*, 73(2), 361s-364s.
- Sivudu, S. N., Umamahesh, K., & Reddy, O. V. S. (2014). A Comparative study on Probiotication of mixed Watermelon and Tomato juice by using Probiotic strains of *Lactobacilli*. *International Journal of Current Microbiology and Applied Sciences*, 3(11), 977-84.
- Tuorila, H., & Cardello, A. V. (2002). Consumer responses to an off-flavor in juice in the presence of specific health claims. *Food Quality and Preference*, 13(7-8), 561-569.
- Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural Food Chemistry*, 44, 701-705.