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Research Article

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Stability of Microencapsulated Lactobacillus Casei in Mango Fruit Juice and its Survival at Simulated Human Gastro-Intestinal Condition

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ABSTRACT

Microencapsulation as a state of the art method has far-reaching impact on probiotic survival. In this study, mango fruit juice together with free and Microencapsulated Lactobacillus Casei were investigated. To this end, Lactobacillus Casei was encapsulated by means of calcium alginate, chitosan coating via extrusion technique, and the viability assessment was performed on a weekly basis using plate count method. It was then incubated in simulated gastric juice (along with pepsin, pH=2) and simulated intestinal juice (along with pancreatin and bile salts, pH = 8) for 6 hours at 37°C .

It was found that the survival rate of probiotic bacteria was significantly promoted by microencapsulation in the simulated human gastro-intestinal condition. Likewise, minimal changes were observed in the probiotic count and pH during storage of the beverages at 4 °C. Along the same lines, the shelf-life of the mango fruit juice was estimated to be 28 days under refrigerator condition. The outcome of the study points to the considerable potential of microencapsulated Lactobacillus Casei as a probiotic in fruit juice due to its stability and survival in human gastro intestinal condition.

Keywords: *Microencapsulation, Mango juice, Lactobacillus Casei, Simulated gastro-intestinal condition*

INTRODUCTION

As almost all probiotic foods available at the markets across the globe are milk- oriented, the study of juices has received scant attention. However, there is a mounting interest in the promotion of non-dairy probiotic products given the consumer taste preferences, increasing number of vegetarians, lactose intolerance and milk protein allergies [1, 2, 3]. Over recent decades, the role played by probiotic bacteria in human health has attracted much attention.

Probiotic bacteria are defined as “live microorganisms when, administered in adequate amounts, confer a health benefit on the host” [4, 5].

The health benefits associated with probiotic bacteria are classified into two categories namely, nutritional benefits (enhancing the bioavailability of calcium, zinc, iron, manganese, copper and phosphorus and the synthesis of vitamins) and the therapeutic benefits (antimicrobial activity, ability to assimilate cholesterol, improved lactose intolerance and anti-carcinogenic activity) [6, 7, 8, 9].

Encapsulation process is an effective technique to protect probiotics against unfavorable conditions to which they are exposed. Food is viewed as an ideal tool which delivers probiotic bacteria to the human gastrointestinal tract (GIT). Several factors including pH, storage temperature and processing conditions are considered to be effective in the viability of probiotics in food products. Low pH is among the key factors restricting the growth and stability of probiotic bacteria. Hydrogen ions damage probiotic cells by disrupting the mass transfer through the cell membrane

and acidic starvation of the cells. Storage temperature plays a critical role in probiotic stability in food products [10, 11,1].

Moreover, the probiotics survival during transition through the GIT is still a major challenge for effective delivery of these organisms [12]. In order to prolong their survival in such adverse conditions, encapsulation of probiotics in hydrocolloid beads is deemed to be an effective practice to enhance the viability of probiotics in food products and during gastrointestinal transit.

Encapsulation is a technology that confines the bacterial cells within a protective matrix in a way that they could be released at a controlled rate under specific conditions. Alginate as an encapsulating material is the most widely used polymer for immobilizing viable cells, since alginate gel is deemed gentle to bacterial cells and enjoys non-toxic property. The alginate polymer consists of D-mannuronic acid and L-glucoronic acid bound preferentially to calcium ions (Ca2+).

The release of the entrapped content is the result of solubilizing alginate gels in a solution including sodium citrate that can sequester Ca2+. However, the emerging alginate beads are very porous, which is regarded as a defect when trying to protect cells from low-pH environments. Polycations namely chitosan or poly (amino acids) shape powerful complexes with alginate having stable behavior in the presence of Ca2+ chelators and reduce the porosity of the gel [4, 5].

Chitosan, a de acetylated chitin, is a naturally occurring substance derived from the shells of crustaceans including crabs and shrimps by chemical or microbiological treatments. It is an effective material for coating as it is non-toxic. Chitosan coating has been reported to afford the best protection for Lactobacillus casei when compared to poly-L lysine/alginate or alginate coating. The viability of encapsulated probiotic cells relies on the physicochemical properties of the capsules.

The type as well as the concentration of coating material, particle size, initial cell number and bacterial strains are among the key factors to take into account. Encapsulation of probiotics using suitable concentrations of carriers may improve the survival of probiotics under unfavorable conditions during storage and gastrointestinal transition [13, 14]. In this regard, this study sets out to investigate the impact of microencapsulation, on the stability and viability of Lactobacillus casei added in the mango fruit juice and its resistance to simulated gastric and enteric conditions.

2.MATERIALS AND METHODS

2.1. Microencapsulation

The extrusion technique of encapsulation was adopted from [15]. Sodium alginate (Sigma-Aldrich, Steinheim, Germany) solution with various concentrations (20, 30 and 40 gL-1) was prepared in distilled water and sterilized at 121°C for 15 min. Low-molecular-weight chitosan (=75% de acetylation, Sigma-Aldrich) solution of various concentrations (2, 4 and 10 gL-1) was prepared in 90 ml of distilled water acidified with 0.4 ml of glacial acetic acid. The pH was then adjusted to 5.7–6 by adding1 Mol L-1 NaOH. Chitosan solution was filtered using Whatman #4 filter paper and the volume of which was adjusted to 100 ml before being autoclaved at 121°C for 15 min. The best result obtained at higher dosage in this case. For encapsulation, 5 ml of bacterial culture [Probiotic strain was from Chr. Hansen (5.1×109colony-forming units (CFU) mL-1)] was suspended in 10 ml of sodium alginate solution. The suspensions were thrusted in drops by means of a 0.11 mm needle into sterile 0.1 mol L-1 CaCl2 as a hardening solution. Following a 30 min gelification in CaCl2, the beads were cleaned using distilled water and immersed in100 ml of chitosan solution under gentle shaking at 100 rpm for 40 min on an orbital shaker. The beads were cleaned with distilled water and then utilized on the same day.

2.2. Production of mango fruit juice

A set of 400 ml portions of the mango beverage were poured into sterile PET bottles. The ingredients in the fruit juice drink were, mango purees, natural flavors, ascorbic acid and beta-carotene. The products had been heat treated under the pasteurization condition. Inoculating the juice beverages with 4 ml of fresh cell suspension of Lactobacillus Casei and microencapsulated Lactobacillus, we stored it at 4 °C under aerobic conditions. Then, constant care was taken to maintain the temperature in question during the sampling.

2.3. pH measurement and Lactobacillus casei count

The self-life of beverages was defined as refrigerator storage (4 °C) during which pH remained around 4.0 and the viable counts of cell were above 10 9cfu/ml. viable counts and pH analyses were performed in 0, 7, 14, 21 and 28 days. The pH of the mango juices was measured by means of a digital pH meter (Hanna Instruments, Singapore). Viable cell counts were obtained by pouring plating the adequate Lithium chloride Sodium propionoate (LP) MRS

agar. The plates are incubated at 37 °C for 72 h under aerobic condition.

2.4. Survival of probiotics under simulated gastro-intestinal conditions

The evaluation of probiotic's survival in mango juice submitted to gastric and enteric simulated conditions was performed according to the method described by [16] with modifications. At 28 days of sampling, 10 ml of each triplicate dilution of mango juice with 0.5% NaCl solution was transferred to a sterile flask, and pH was adjusted to 1.4–1.9 with 1N HCl. Pepsin (from porcine stomach mucosa, Sigma-Aldrich, St. Louis, MO, USA) and lipase (Amano lipase F-AP15 from Rhyzopusoryzae, Aldrich Chemical Company, Milwaukee, WI, USA) solutions were added to samples to reach a concentration of 3 g/l and of 0.9 mg/l, respectively. Flasks were incubated at 37°C, with the agitation of approximately 150 RPM (shaker incubator Lab Tech), during 2 h (gastric phase). In the next step, the pH of the samples was increased to 4.3–5.2 using an alkaline solution (150 ml of 1N NaOH, 14 g of $PO_4H_2Na.2H20$ and distilled water up to one 1 l). Bile (bovine bile, Sigma-Aldrich) and pancreatin (pancreatin from porcine pancreas, Sigma-Aldrich) were added to reach a concentration of 10 g/l and of 1 g/l, respectively. Samples were incubated again at 37 °C for 2 h under agitation (enteric phase 1). In the last step, the pH was increased to 6.7–7.5 using the same alkaline solution, bile and pancreatin concentrations were adjusted (10 g/l and 1 g/l, respectively), and samples were incubated again at 37° C for 2 h under agitation (enteric phase 2), achieving 6 h of the assay. Enumeration of Lactobacillus casei was performed in aliquots collected from triplicate samples at initial and after 2 h, 4 h and 6 h, using proper volume. Aliquots of 0.01 ml were pouring plated in LP-MRS and incubated at 37 °C for 2 days under aerobic condition. All results are presented as log cfu/g of mango juice.

2.5. Statistics

The significance of differences between the treatments was established by the ANOVA and t- test procedure of SPSS statistical software (version 20) and using Duncan's multiple range test post hoc. The significance level was set at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Stability of microencapsulated Lactobacillus during the storage in the mango juice

Inoculation by approximately 10^9 cfu mL^{-1} as a commercial representative level of juice supplementation was carried out.

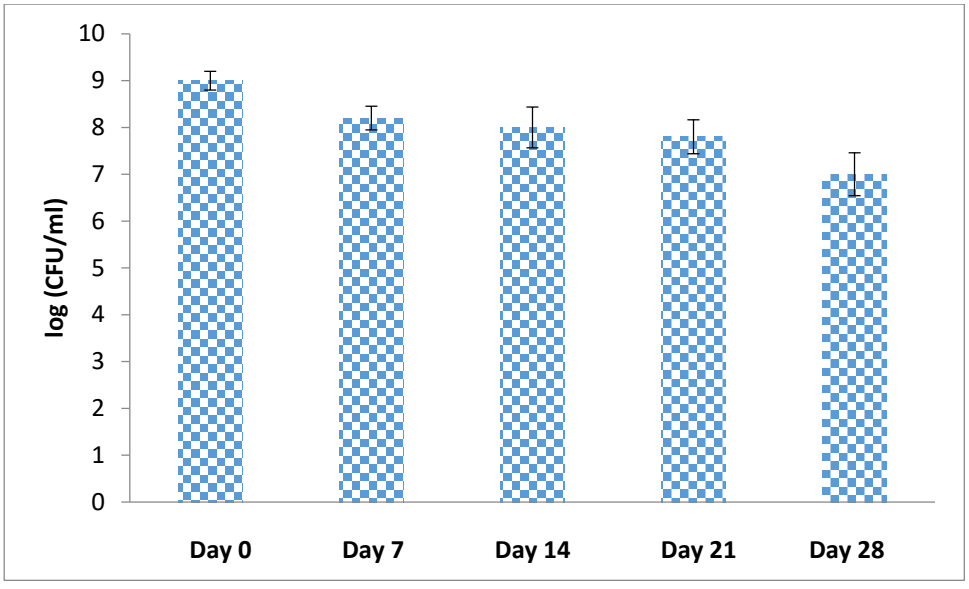


Fig1. Survival of free lactobacillus in mango juice stored at 4 ˚C

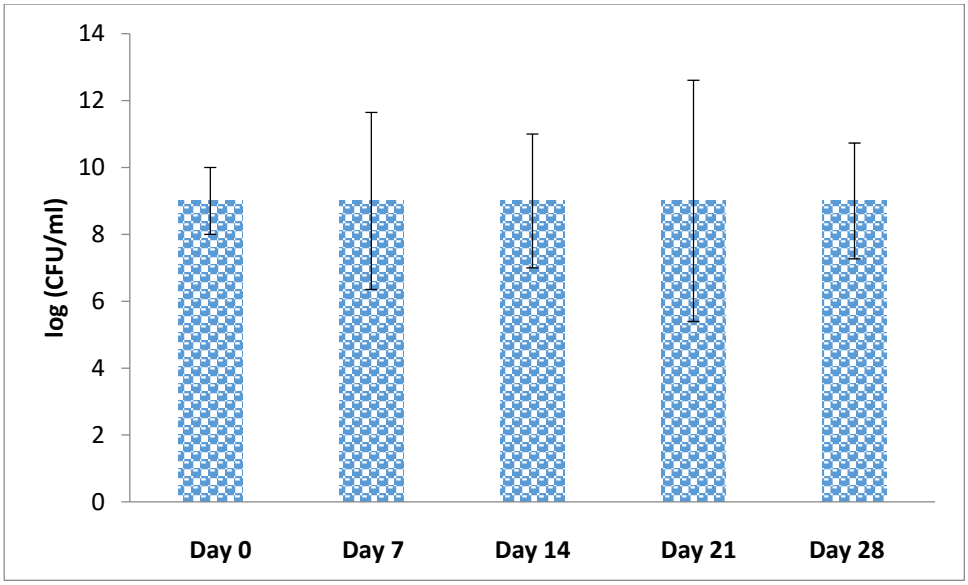
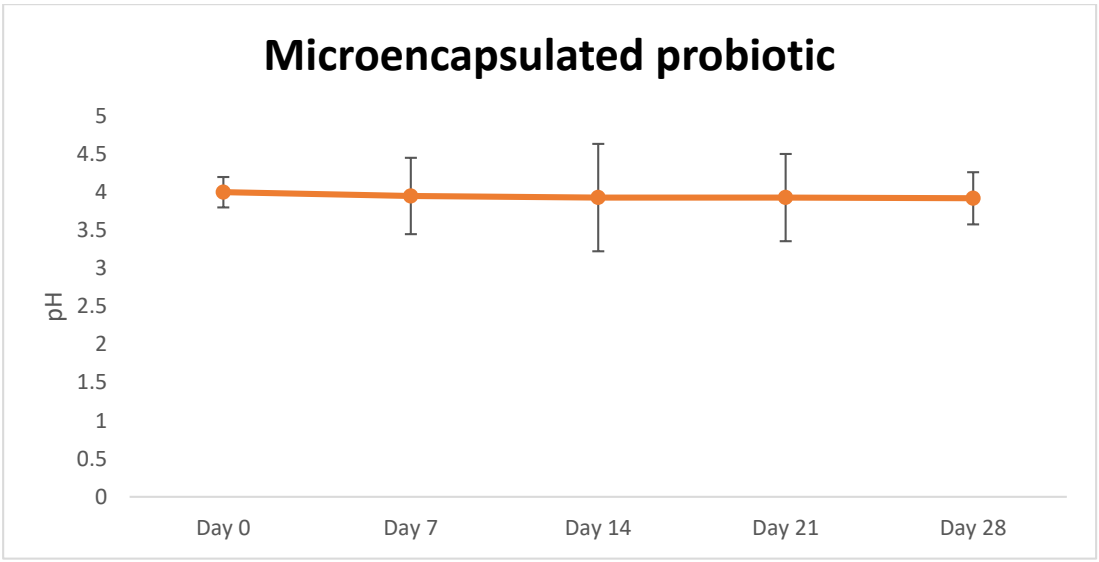


Fig2. Survival of microencapsulated lactobacillus in mango juice stored at 4 ˚C

The survival of microencapsulated probiotic bacteria increased over uncoated cells (100 times more on day 28, Figures 1& 2). The observed difference primarily was due to the protection of cells by microencapsulation. In the same vein, [17] also concluded that encapsulated the Lactobacillus casei and Bifidobacterium lactis in calcium alginate beads, increase the probiotic survival rate up to 30% during the 180 days of storage of ice cream on - 20 °C. In a study by [18], free Lactobacillus casei and Bifidobacterium lactis numbers dropped substantially (about 3 log numbers) by 150 days of storage at -18 °C, while the encapsulated state of the same strains portrayed a decrease of less than 2 log for both of them. In frozen ice milk, 40% more lactobacilli survived when entrapped in calcium alginate beads [19]. Additionally, [20] revealed that microencapsulation promoted the counts of Lactobacillus acidophilus MJLA1 and Bifidobacterium spp. BDBB2 in comparison with free cells in frozen fermented dairy desserts stored for 12 weeks.

3.2. pH and probiotics changes

The pH of the mango juice remained the same after 28 days of storage illustrating weak metabolic activity of the probiotics at 4 °C regardless of whether they were in a free or encapsulated state (Fig 3). [21] have also shown that pH values below 4.5 were destructive to storage stability of probiotic bacteria, since lower pH conditions led to lower max values. It can be postulated that the pH of the medium is one of the factors which accounts for the stability of different strains during storage in mango juice. As a result, the long term stability of many strains in mango fruit juice was partly associated with the selection of a pH = 4.2 blend integrating dairy ingredients. L. casei showed good viability during storage. Furthermore, results highlighted greater losses in viability of L. casei than of L. Rhamnosus to storage in acid foods, which is in agreement with those reported the literature [22, 23].



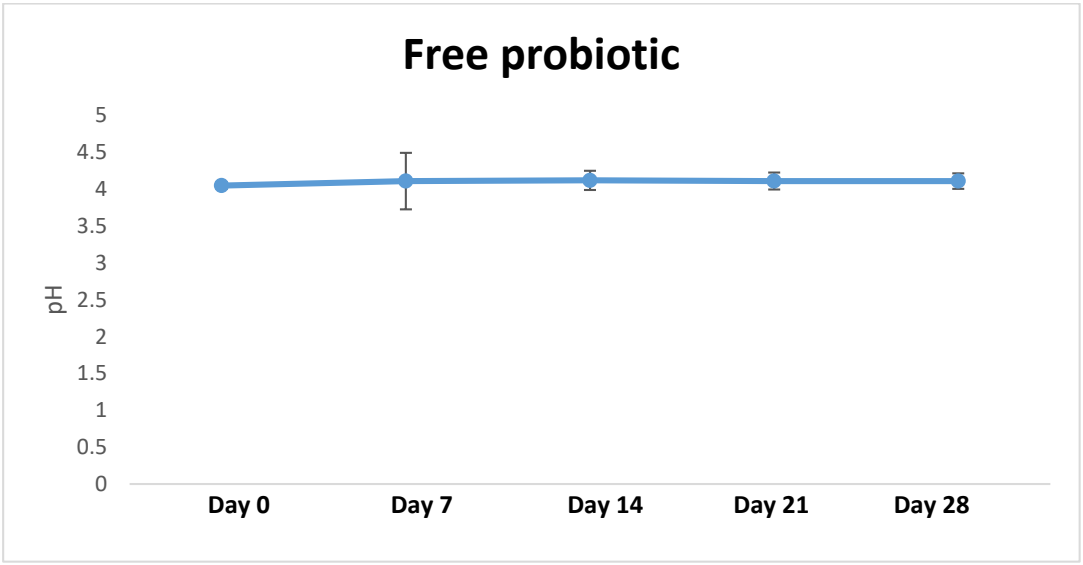


Fig 3. pH changes of mango juice with free and microencapsulated probiotics stored at 4 ˚C

It is widely accepted that probiotics loose viability during storage in acid environments [24,21] or in the presence of oxygen [25]. The plating procedure for the viable counts was aerobically conducted through the methodology employed in this study. Microencapsulated lactobacillus yielded useful results in this situation. However, it is probable that viable, but acid or oxygen-stressed cells reached at the end point of storage period may not have produced colonies and that the methodology employed underrated viable counts. Additionally, the food formulation heavily impacts the viability of probiotics during storage [26, 27]. Mango juices may be composed of natural microbial growth inhibitors or additives, including coloring and flavoring agents, which can be linked to the loss of viability [28]. Studies have shown that encapsulated probiotic bacteria make more stable, functional food products [29, 30].

3.3. Viability during simulated gastro-intestinal stresses

The stability of the Lactobacillus Casei populations, maintaining levels above 9×10 9 cfu/g in the encapsulated samples over the whole storage period studied was the key factor for the results obtained for probiotic survival through the in vitro assay up to 28 days, in comparison with free samples.

In this study, the viability of Lactobacillus Casei during 6 h of the in vitro assay at the end of 28 days of storage reaches 1.5 log cfu/g for free cells but for encapsulated trials these data were around 3 log cfu/g. Encapsulated group showed more viable cells at 2h, 4h and 6h in comparison with free probiotic group ($p < 0.05$).

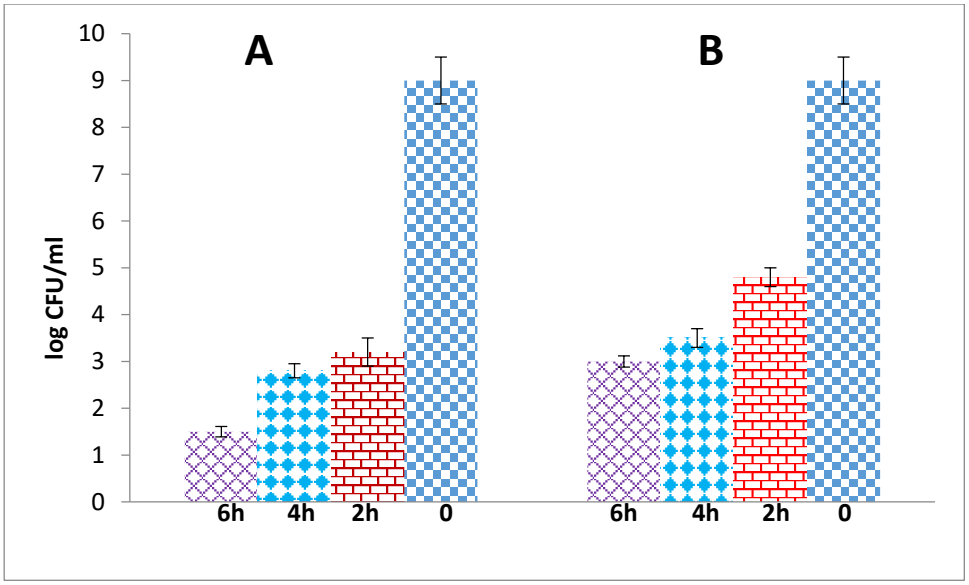


Fig 4. Survival of free and microencapsulated lactobacillus Casei of mango juice at 28 days of storage in simulated gastro-intestinal conditions. A: Free probiotic B: Microencapsulated probiotic

[31] reported that free cells of L. acidophilus La-5 reduced only 1 log cycle after 2 h of exposure to pH 2, but were fully destroyed after 1 h at pH 1. In the present study, an intermediate condition of pH was employed in the gastric phase (1.4– 1.9), probably contributing for a relatively lower survival, when compared with the one observed by the other authors at pH 2 or above. Along the same lines, the survival of Lactobacillus casei during the gastric phase was integral to determining the populations by the end of assays, as the main reductions in the viability of this microorganism take place in this period. In this study, Lactobacillus casei was also vulnerable to the assay phases containing bile. Bile has the potential to influence the phospholipids and proteins of cell membranes and distort cellular homeostasis besides undermining macromolecule stability [32,33] evidenced a 2 log cycle decline in the survival of L. acidophilus La-5, upon exposure to acid and bile in a simulated gastrointestinal model in the presence of a commercial infant formula. The components of the food matrix were integral to protecting the probiotic microorganism throughout the simulated enteric conditions.

4. CONCLUSION

In conclusion, this study introduces a new possibility to make an appropriate probiotic fruit juice that can ensure high cell viability during cold storage. The results of this study indicate that encapsulation can significantly enhance the survival of probiotic bacteria during storage at refrigerator and under simulated gastrointestinal conditions. In addition, these beverages combine interesting nutritional qualities and probiotic characteristics. Finally, this study can shed light on the development of new, non-dairy, probiotic food products.

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