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# Probiotication of Tomato and Carrot Juices for Shelf-life Enhancement using Micro-encapsulation

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ABSTRACT: This study was aimed at probiotication of tomato and carrot juices using *Lactobacillus plantarum, Lb. fermentum*, *Lb. casei, Lysinibacillus sphaericus* and *Saccharomyces boulardii*. To enhance the stability, the probiotic cultures were micro-encapsulated using alginate coated chitosan beads. Tomato and carrot juice samples were pasteurized for 20 min at 63 °C. *Lb. fermentum*, *Lb. plantaram, Lb. casei* and *Lysinibacillus sphaericus, Saccharomyces boulardii* were inoculated and incubated at 37 °C for a period of 72 h. After the probiotication the pH decreased from 6.8 to 4.5 and correspondingly increased the titratable acidity from 0.12 to 0.36% during the period. Among the probiotic strains the viable cell count were increased from 6.5 to 8.9 log CFU/mL in *Lysinibacillus sphaericus* and 5.2 to 7.6 log CFU/mL in *Saccharomyces boulardii,* during 24 to 42 h and later it decreased slowly. Viability of encapsulated cells were higher than free cells in tomato and carrot juices stored at 4 °C over a period of 5 - 6 weeks indicating better cell protection in the former. However, the addition of probiotic beads influenced the sensory quality of the product by increasing the swallowing difficulty and remaining particles of the encapsulated ones increased the turbidity of vegetable juices.

**Keywords**: *Lysinibacillus sphaericus*, *Micro-encapsulation, Probiotication, Saccharomyces boulardii.*

## Introduction

The usage of probiotic products has been increased in the last two decades due to the health awareness of consumers (Menrad, 2002). Probiotics are living microbial supplements, which beneficially affect the host by controlling intestinal infection, serum cholesterol levels, beneficially influencing the immune system, improving lactose utilization in lactose maldigesters, and having anticarcinogenic activity (McNaught & MacFie, 2000; Rafter, 2003).

Regular consumption of high levels of probiotic bacteria is required to confer health benefits. It is important that the organisms remain viable in the food product until the time of consumption and be present in significant numbers (at least $10^7$ CFU/mL) in order to confer benefits to the consumer (Ishibashi & Shimamura, 1993). Despite the importance of viability of these beneficial bacteria, studies conducted have shown poor viability of probiotic bacteria, especially *Bifidobacterium*, in functional foods (Shah & Lankaputhra, 1997). It would be interesting to study the changes in the number of viable probiotic bacteria more extensively during storage of functional foods.

Vegetables and fruits have been showed as appropriate for probiotic products as they do not contain any dairy allergens that might prevent usage by part of the population (Luckow & Delahunty, 2004). Also they have several functional food components such as minerals, vitamins, dietary fibers, and antioxidants. In recent years, studies on non-dairy probiotic beverages such as tomato, cabbage, blackcurrant, orange, beet root and carrot juices have been performed

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in conjuction with different probiotic strains and obtained appealing results.

Protection of probiotics by microencapsulatin in alginate capsules is a method of improving their viability in functional foods. Alginate is often used as an encapsulating material because, it has the benfits of being non-toxic and being readily available (Ding & Shah, 2008). Microencapsulatin technique not only improves the suvival of probiotics in fruit juices (Krasaekoopt *et al.,* 2004), but also may improve the flavor of the product. However, the additon of probiotics encapsulted in alginate beads coated with chitosn may affect the consumer preference and sensory attributes of the product due to the size of the beads (Krasaekoopt & Kamolnate, 2010).

Carrot juice contains carbohydrates, dietary fiber, protein, fat, Vitamins A, C, B1, B2, B3, B6 and E. It also contains traditional antioxidants such as ascorbic acid, phyto-nutrient and beta-carotene (Gopalan *et al.,* 1996).

Tomatoes are one of the most widely used and versatile vegetable crops. Tomatoes are consumed as fresh or as industrically processed. Processed tomatoes include canned and sun dried toamtoes, juices, ketchup, pastes, purees, salads, sauces and soups (Shi & Le Mauger, 2000). Tamaotes contain abundent halth-promoting related components such as lycopene, provitamin A (Beecher, 1998), ascorbic acid (Sahlin *et al.,* 2004), vitamin E, folate, flavonoids and potassium (Leonardi *et al.,* 2000). Reguluar consumption of toamtoes has been associated with a reduced risk of various types of cancer (Weisburger, 1998) and heart diseases (Pandey *et al.,* 1995). These effects are mainly attributed to the presence of antioxidants, especially flavonoids, lycopene and beta-carotene (Lavelli *et al.,* 2000). Among the processed tomatoes, juices may also be conidered as health-promoting beverages (Suzuki *et al.,* 2002). Neverthless, processed fruits and vegetables have lower nutritional and health promoting values than their fresh counterparts due to variable loss of antioxidants during processing (Murcia *et al.,* 2000).

This study was aimed at probiotication of tomato and carrot juices by using free and microencapsulated cells of probiotics such as *Lb fermentum, Lb. plantarum, Lb. casei, Lysinibacillus sphaericus* and *Saccharomyces boulardii.* Preservation and shelf-life of free and encapsulated probotic cells in vegetable juics stored at refrigirator coditions was also studied.

## Materials and Methods

- *Preparation of substrate*

Tomatoes and carrots were purchased from a local vegetable market in Tirupati, India and stored in box at room temperature for further maturation. The tomatoes and carrots were washed with tap water to remove soil and other impurities, air dried at room temperature prior to use, and blanched in water bath for 20 min at 60 ºC. They were cut into pieces and juices were extracted from these pieces by using a laboratory grinder and filtered through a muslin cloth with a sieve (0.8 to 1.1 mm pore size) to get a clear juice.

- *Preparation of probiotic cultures*

*Lactobacillus plantaram, Lb. fermenutm* and *Lb. casei* were obtained from the Microbial Type Culture Collection, Chandigarh (India). *Lysinibacillus sphaericus* was isolated from fish intestine, and they were maintained in MRS (de Man, Rogosa and Sharpe) agar stabs as pure cultures. These were activated with two successive subculturing in MRS broth cultures at 37 ºC for 24 h. The activated cultures were again inoculated into MRS broth incubated at 37 ºC for 24 h and this was used as the initial inoculum (2 log CFU/mL). *Saccharomyces boulardii* was

isolated from the dietary supplement sachet 'Daraorlac' obtained from local drug shop and was maintained as a pure culture on potato dextrose agar (PDA) slants at 4 °C. For co-fermentation, lactic acid bacteria and *S. boulardii* were mixed (1:1 ratio) and used as inoculum for probiotication of tomato and carrot juice.

- *Probiotication of tomato and carrot juices*

Tomato and carrot juices 100 mL in 250 mL Erlenmeyer flasks were taken and autoclaved for 15 min at 121 °C. The juices were inoculated with probiotic cultures either individually or in combination with *Saccharomyces boulardii* and incubated at 37 °C for a period of 72 h.

- *Harvesting of probiotic cultures before inoculation*

The probiotic cells that are grown in respective culture media as indicated above were harvested by centrifuging at 3000 rpm for 15 min at 25 °C and washed twice with sterile saline. The cell suspension of each probiotic bacterium was divided into two parts: One part was used for micro-encapsulation and another was used as free cells for the use in vegetable juices. Initial viable count of both bacteria and yeast was determined and expressed as colony forming units (CFU)/mL.

- *Micro-encapsulation of probiotics*

After washing, the cells were suspended in 5 mL of sterile water and mixed with 20 mL of 2 % sodium alginate solution (SRL Research Chemicals Ltd. Mumbai) that was sterilized at 121 °C for 15 min. Then cell suspension was taken in a sterile syringe and injected through a 0.11 mm needle into sterile 0.05 M $CaCl_2$ containing 0.1 % Tween 80. After 30 min gelification in $CaCl_2$, the beads were rinsed and then kept in, sterile water at 4 °C. Low-molecular-weight chitosan (0.4 g) was dissolved in 90 mL distilled water acidified with 0.4 mL of glacial acetic acid to achieve a final concentration of 0.4 %. The pH was then adjusted to between 5.7 and 6.0 by adding 1 M NaOH. The mixture was filtered through Whatman No.4 filter paper and the volume was adjusted to100 mL before autoclaving at 121 °C for 15 min. Then 15 g of washed beads were immersed in 100 mL of sterile chitosan solution with gentle shaking at 100 rpm for 40 min on an orbital shaker for coating (two-step method). The chitosan-coated beads were washed and kept in sterile water at 4 °C. The beads were then used on the same day.

- *Enumeration of bacterial and yeast cells*

The enumeration of free probiotic cells was performed using methods described by Shah and Lankaputhra (1997). In brief, for the enumeration of microencapsulated probiotic organisms, the bacteria were released from the capsules by sequestering calcium ions with a phosphate buffer at pH 7.0. Once liberated, the probiotic organisms ere enumerated using the methods of Tharmaraj & Shah, (2003). Enumeration of the probiotic bacteria in fruit juices was performed on a weekly basis over a period of 6 weeks, using MRS agar and incubation at 37 °C for 72 h under anaerobic conditions.

- *Application of probiotics in tomato and carrot juices*

Ten grams of microencapsulated beads or 10 mL of free-cell suspension of each probiotic bacteria were added aseptically into 100 mL of tomato and carrot juices separately. A high proportion of culture to juice was added in order to provide a high number of probiotic cells in the tomato and carrot juices and to increase the sensitivity of the test. The juices were packed in sterile bottles and then kept in the cold room at 4 °C for 6 weeks. Samples were drawn after 1 day (week 0 sample) and then after 1, 2, 3, 4, 5 and 6 weeks from the cold room.

- *Determination of viable count of probiotics*

Viable cell count of bacteria were determined in duplicate by using the pour plate method (David, 2005) using MRS agar medium with 2.5 mg/L Amphotericin B to inhibit the yeast growth, and viable cell count of *S. boulardii* was determined by the spread plate method on potato dextrose agar medium.

Probioticated tomato and carrot juice (10 g of each) samples were added individually to 90 mL of sterile 0.85% saline and vortexed for 30 seconds. The resulting suspension was serially diluted in 9 mL saline and 1 mL of the appropriate dilution was used for selective enumeration by pour plate technique. The cell growth of each organism was assessed by enumerating bacterial population after 12, 24, 48 and 72 h of probiotication of tomato, carrot, and tomato + carrot juice on MRS agar. Plates containing 25 to 250 colonies were counted and recorded as colony forming units (CFU) per gram sample.

- *Chemical analysis*

The pH of the probioticated vegetable juice was measured using a pH meter (Cyberscan–Eutech Instruments). Total soluble solids (TSS) were determined using a hand Refractometer (Erma, Japan) in terms of ºBrix (ºBx). The reducing sugars were determined spectrophotometrically using DNS method. Titratable acidity was determined by titration with 0.1N NaOH solution and expressed as percent oxalic acid (AOAC, 1984).

- *Sensory evaluation*

The sensory characteristics of the vegetable juices were evaluated according to Dias *et al.,* (2007) with a 20-membered panel. The preferences for taste, acidity, mouth feel, aroma, flavour, color and overall acceptability were determined by 9-point hedonic scale. Randomized refrigerated (10 °C) samples (50 mL) were served in clear tulip-shaped glasses coded with a random 3-digit code. The mean intensity scores of all the attributes were calculated and plotted.

- *Statistical analysis*

All the experiments were carried out in triplicate and the mean value and standard deviation were presented. The data were analyzed by one-way analysis of variance (ANOVA) using SPSS, version 16.0.

**Results and Discussion**

- *Effect of microencapsulation on cell viability*

The viability of initial cell of probiotics used before and after encapsulation was approximately 11.2-11.5 CFU/mL or g of beads, which had an average diameter of 1.5-1.8 mm. The viability of free cells and encapsulated cells were examined periodically after 1 day storage at 4 ºC (Table 1). High reductions of viable counts were found in free cells (1.6-5.2 logs) in both vegetable juices, which were significantly higher than encapsulated cells (0.8-2.7 logs) (Tables 2, 4, 6 & 8). *Lysinibacillus sphaericus* showed a better survivability in tomato and carrot juices than other three bacteria and yeast. The cell number of *Lys. sphaericus* reduced 1.3-1.8 and 1.7 2.3 logs for encapsulated and free cells, respectively. Simultaneously, the cell numbers of *Lb. plantarum* (1.4-2.0 and 1.9-2.3 log), *Lb. fermentum* (1.6-2.4 and 2.1-2.6 log), *Lb. casei* (1.8-2.6 and 1.9-2.7 log) and *S. boulardii* (2.0-2.5 and 2.3-2.8 log) were also reduced for encapsulated and free cells, respectively.

During the storage at 4 °C for 6 weeks, the viability of microencapsulated cells and free cells of *Lys. sphaericus* in vegetable juices are shown in Tables 3 and 5. Micro-encapsulated cells survived better than free cells in both tomato and carrot juices. The viable counts of encapsulated cells slightly declined; whereas, the viable counts of free cells were remarkably dropped during the

**Table 1.** Effect of encapsulation on cell viability of probiotic organisms in vegetable juices.

| Probiotic organism | Type of juice | Type of cell | Before probiotication | After probiotication |
|---|---|---|---|---|
| *Lactobacillus casei* | Tomato | Free | 10±0.4 | 6.9±0.2 |
| | | Encapsulated | 10±0.3 | 8.8±0.2 |
| | Carrot | Free | 10±0.3 | 7.9±0.3 |
| | | Encapsulated | 10±0.3 | 9.0±0.3 |
| *Lactobacillus plantarum* | Tomato | Free | 10±0.2 | 7.8±1.2 |
| | | Encapsulated | 10±0.3 | 8.7±0.2 |
| | Carrot | Free | 10±0.2 | 8.8±0.2 |
| | | Encapsulated | 10±0.3 | 9.2±0.3 |
| *Lactobacillus fermentum* | Tomato | Free | 10±0.3 | 8.0±0.3 |
| | | Encapsulated | 10±0.3 | 8.6±0.2 |
| | Carrot | Free | 10±0.2 | 6.8±0.3 |
| | | Encapsulated | 10±0.1 | 7.9±0.3 |
| *Lysinibacillus sphaericus* | Tomato | Free | 11±1.3 | 8.6±0.2 |
| | | Encapsulated | 11±0.2 | 9.8±0.1 |
| | Carrot | Free | 11±0.3 | 8.8±0.3 |
| | | Encapsulated | 11±0.3 | 10.0±0.2 |
| *Saccharomyces boulardii* | Tomato | Free | 09±0.3 | 7.5±0.3 |
| | | Encapsulated | 09±0.3 | 9.1±0.2 |
| | Carrot | Free | 09±0.2 | 7.7±0.2 |
| | | Encapsulated | 09±0.2 | 8.6±0.2 |

**Table 2.** Stability of free probiotic organisms in tomato juice stored at low temperature.

| Time (weeks) | Cell viability at 4 °C (CFU/mL) | | | | |
|---|---|---|---|---|---|
| | ***Lb. fermentum*** | ***Lb. casei*** | ***Lb. plantarum*** | ***Lb. sphaericus*** | ***S. boulardii*** |
| 0 | $4.2\pm0.6\times10^{7}$ | $4.5\pm0.4\times10^{9}$ | $4.2\pm0.8\times10^{6}$ | $4.7\pm0.5\times10^{7}$ | $3.8\pm0.2\times10^{6}$ |
| 1 | $3.9\pm0.9\times10^{7}$ | $4.0\pm0.2\times10^{9}$ | $3.7\pm0.6\times10^{6}$ | $4.2\pm0.1\times10^{7}$ | $3.3\pm0.2\times10^{6}$ |
| 2 | $3.3\pm0.5\times10^{7}$ | $3.6\pm3.4\times10^{9}$ | $3.2\pm0.7\times10^{6}$ | $3.9\pm0.6\times10^{7}$ | $2.7\pm0.2\times10^{6}$ |
| 3 | $2.6\pm0.3\times10^{7}$ | $3.0\pm3.4\times10^{9}$ | $2.3\pm0.3\times10^{6}$ | $3.4\pm1.3\times10^{7}$ | $1.8\pm0.2\times10^{6}$ |
| 4 | $1.8\pm0.4\times10^{7}$ | $2.5\pm0.3\times10^{9}$ | $1.4\pm0.6\times10^{6}$ | $2.8\pm0.9\times10^{7}$ | $1.3\pm0.2\times10^{6}$ |
| 5 | $1.0\pm0.2\times10^{7}$ | $1.3\pm0.2\times10^{9}$ | $0.9\pm0.6\times10^{6}$ | $1.5\pm0.6\times10^{7}$ | $0.4\pm0.6\times10^{6}$ |

Mean and standard deviation for n=3

**Table 3.** Stability of encapsulated probiotic organisms in tomato juice stored at low temperature.

| Time weeks | Cell viability at 4 °C (CFU/mL) | | | | |
|---|---|---|---|---|---|
| | ***Lb. fermentum*** | ***Lb. casei*** | ***Lb. plantarum*** | ***Lb. sphaericus*** | ***S. boulardii*** |
| 0 | $4.2\pm0.6\times10^{8}$ | $4.5\pm0.4\times10^{9}$ | $4.2\pm0.8\times10^{8}$ | $4.8\pm0.5\times10^{9}$ | $3.8\pm0.2\times10^{6}$ |
| 1 | $3.9\pm0.9\times10^{7}$ | $4.0\pm0.2\times10^{6}$ | $3.7\pm0.6\times10^{6}$ | $4.4\pm0.1\times10^{6}$ | $3.1\pm0.2\times10^{6}$ |
| 2 | $3.3\pm0.5\times10^{6}$ | $3.7\pm3.4\times10^{6}$ | $3.2\pm0.7\times10^{6}$ | $3.9\pm0.6\times10^{6}$ | $2.7\pm0.2\times10^{6}$ |
| 3 | $2.7\pm0.3\times10^{6}$ | $3.1\pm3.4\times10^{6}$ | $2.6\pm0.3\times10^{6}$ | $3.4\pm1.3\times10^{6}$ | $2.2\pm0.2\times10^{6}$ |
| 4 | $2.0\pm0.4\times10^{6}$ | $2.8\pm0.3\times10^{6}$ | $2.3\pm0.6\times10^{6}$ | $3.0\pm0.9\times10^{6}$ | $1.9\pm0.2\times10^{6}$ |
| 5 | $1.7\pm0.2\times10^{6}$ | $2.1\pm0.3\times10^{6}$ | $1.4\pm0.6\times10^{6}$ | $2.8\pm0.2\times10^{6}$ | $1.1\pm0.6\times10^{6}$ |
| 6 | $0.9\pm0.2\times10^{6}$ | $1.2\pm0.2\times10^{6}$ | $0.7\pm0.2\times10^{6}$ | $1.9\pm0.6\times10^{6}$ | $0.4\pm0.2\times10^{6}$ |

Mean and standard deviation for n=3

storage. At the end of the test, the number of encapsulated cells reduced approximately 0.10 -1.9 log, concurrently the number of free cells dropped about 3.2 - 4.8 logs. Parallel to *Lys. sphaericus*, the survival of *Lb. casei* in the form of encapsulated cells was better than that of free cells in all vegetable juices (Tables 7 and 9). The viable counts dropped 0.8-1.7 and 4.4-4.8 log CFU/mL for encapsulated and free cells, respectively. In addition, no change of pH was observed in all treatments in this experiment. It was observed that tomato and carrot juices probioticated with *Lys. sphaericus and Lb. caseai* exhibited better physico-chemical properties like TSS, pH, titratable acidity (Tables 8 and 9). The total sugars, reducing sugars and TSS were high in tomato juice when compared with carrot juice. However, carrot juice had lower titratable acidity than tomato juice.

**Table 4.** Stability of free probiotic organisms in carrot juice stored at low temperature.

| Time weeks | Cell viability at 4 °C (CFU/mL) | | | | |
|---|---|---|---|---|---|
| | ***Lb. fermentum*** | ***Lb. casei*** | ***Lb. plantarum*** | ***Lb. sphaericus*** | ***S. boulardii*** |
| 0 | $4.1\pm0.6\times10^{8}$ | $4.4\pm0.4\times10^{9}$ | $4.0\pm0.8\times10^{8}$ | $4.7\pm0.5\times10^{9}$ | $3.8\pm0.2\times10^{6}$ |
| 1 | $3.8\pm0.9\times10^{7}$ | $4.0\pm0.2\times10^{6}$ | $3.7\pm0.6\times10^{6}$ | $4.2\pm0.1\times10^{6}$ | $3.1\pm0.2\times10^{6}$ |
| 2 | $3.2\pm0.5\times10^{6}$ | $3.7\pm0.4\times10^{6}$ | $3.1\pm0.7\times10^{6}$ | $3.9\pm0.6\times10^{6}$ | $2.7\pm0.2\times10^{6}$ |
| 3 | $2.8\pm0.3\times10^{6}$ | $3.2\pm3.4\times10^{6}$ | $2.6\pm0.3\times10^{6}$ | $3.4\pm1.3\times10^{6}$ | $2.2\pm0.2\times10^{6}$ |
| 4 | $2.0\pm0.4\times10^{6}$ | $2.5\pm0.3\times10^{6}$ | $1.9\pm0.6\times10^{6}$ | $2.9\pm0.9\times0$ | $1.9\pm0.2\times10^{6}$ |
| 5 | $0.6\pm0.2\times10^{6}$ | $1.0\pm0.3\times10^{6}$ | $0.7\pm0.6\times10^{6}$ | $1.5\pm0.6\times10^{6}$ | $0.4\pm0.6\times10^{6}$ |

Mean and standard deviation for n=3

**Table 5.** Stability of encapsulated probiotic organisms in carrot juice stored at low temperature.

| Time (week) | Cell viability at 4 °C (CFU/mL) | | | | |
|---|---|---|---|---|---|
| | ***Lb. fermentum*** | ***Lb. casei*** | ***Lb. plantarum*** | ***Lb. sphaericus*** | ***S. boulardii*** |
| 0 | $4.1\pm0.6\times10^{8}$ | $4.5\pm0.4\times10^{9}$ | $4.2\pm0.8\times10^{8}$ | $4.7\pm0.5\times10^{9}$ | $3.8\pm0.1\times10^{6}$ |
| 1 | $3.8\pm0.9\times10^{7}$ | $4.0\pm0.2\times10^{6}$ | $3.7\pm0.6\times10^{6}$ | $4.2\pm0.1\times10^{6}$ | $3.4\pm0.2\times10^{6}$ |
| 2 | $3.1\pm0.5\times10^{6}$ | $3.3\pm3.4\times10^{6}$ | $3.0\pm0.7\times10^{6}$ | $3.8\pm0.6\times10^{6}$ | $2.7\pm0.5\times10^{6}$ |
| 3 | $2.7\pm0.3\times10^{6}$ | $3.0\pm3.4\times10^{6}$ | $2.9\pm0.3\times10^{6}$ | $3.3\pm1.3\times10^{6}$ | $2.2\pm0.2\times10^{6}$ |
| 4 | $2.2\pm0.4\times10^{6}$ | $2.6\pm0.3\times10^{6}$ | $2.1\pm0.6\times10^{6}$ | $2.8\pm0.9\times10^{6}$ | $1.9\pm0.3\times10^{6}$ |
| 5 | $1.4\pm0.2\times10^{6}$ | $1.7\pm0.2\times10^{6}$ | $1.4\pm0.6\times10^{6}$ | $2.1\pm0.6\times10^{6}$ | $0.9\pm0.2\times10^{6}$ |
| 6 | $0.6\pm0.2\times10^{6}$ | $0.8\pm0.2\times10^{6}$ | $0.5\pm0.2\times10^{6}$ | $1.3\pm0.6\times10^{6}$ | $0.3\pm0.1\times10^{6}$ |

Mean and standard deviation for n=3

**Table 6.** Stability of free probiotic organisms in tomato + carrot juice stored at low temperature.

| Time (weeks) | Cell viability at 4 °C (CFU/mL) | | | | |
|---|---|---|---|---|---|
| | ***Lb. fermentum*** | ***Lb. casei*** | ***Lb. plantarum*** | ***Lb. sphaericus*** | ***S. boulardii*** |
| 0 | $4.1\pm0.6\times10^{8}$ | $4.6\pm0.4\times10^{8}$ | $4.2\pm0.8\times10^{8}$ | $4.9\pm0.5\times10^{6}$ | $3.8\pm0.2\times10^{6}$ |
| 1 | $3.9\pm0.9\times10^{7}$ | $4.0\pm0.2\times10^{8}$ | $3.7\pm0.6\times10^{8}$ | $4.3\pm0.1\times10^{6}$ | $3.3\pm0.2\times10^{6}$ |
| 2 | $3.2\pm0.5\times10^{6}$ | $3.6\pm3.4\times10^{8}$ | $3.4\pm0.7\times10^{8}$ | $3.9\pm0.6\times10^{6}$ | $2.7\pm0.2\times10^{6}$ |
| 3 | $2.5\pm0.3\times10^{7}$ | $2.7\pm3.4\times10^{8}$ | $2.2\pm0.3\times10^{8}$ | $3.1\pm1.3\times10^{6}$ | $2.1\pm0.2\times10^{6}$ |
| 4 | $2.1\pm0.4\times10^{7}$ | $2.3\pm0.3\times10^{8}$ | $1.9\pm0.6\times10^{8}$ | $2.6\pm0.9\times10^{6}$ | $1.8\pm0.2\times10^{6}$ |
| 5 | $1.0\pm0.2\times10^{7}$ | $1.3\pm0.3\times10^{8}$ | $1.1\pm0.6\times10^{8}$ | $1.7\pm0.6\times10^{6}$ | $0.7\pm0.6\times10^{6}$ |

Mean and standard deviation for n=3

**Table 7.** Stability of encapsulated probiotic organisms in tomato + carrot juice stored at low temperature.

| Time (weeks) | Cell viability at 4 °C (CFU/mL) | | | | |
|---|---|---|---|---|---|
| | ***Lb. fermentum*** | ***Lb. casei*** | ***Lb. plantarum*** | ***Lb. sphaericus*** | ***S. boulardii*** |
| 0 | $4.4\pm0.6\times10^{7}$ | $4.7\pm0.4\times10^{8}$ | $4.3\pm0.8\times10^{8}$ | $4.9\pm0.5\times10^{9}$ | $3.8\pm0.2\times10^{6}$ |
| 1 | $3.4\pm0.9\times10^{7}$ | $3.6\pm0.2\times10^{8}$ | $3.1\pm0.6\times10^{8}$ | $3.8\pm0.1\times10^{9}$ | $3.1\pm0.2\times10^{6}$ |
| 2 | $3.0\pm0.5\times10^{7}$ | $3.3\pm3.4\times10^{8}$ | $3.0\pm0.7\times10^{8}$ | $3.9\pm0.6\times10^{9}$ | $2.7\pm0.2\times10^{6}$ |
| 3 | $2.6\pm0.3\times10^{7}$ | $2.7\pm3.4\times10^{8}$ | $2.1\pm0.3\times10^{8}$ | $3.1\pm1.3\times10^{9}$ | $2.1\pm0.2\times10^{6}$ |
| 4 | $2.0\pm0.4\times10^{7}$ | $2.2\pm0.3\times10^{8}$ | $1.9\pm0.6\times10^{8}$ | $2.7\pm0.9\times10^{9}$ | $1.9\pm0.2\times10^{6}$ |
| 5 | $1.5\pm0.2\times10^{7}$ | $1.8\pm0.5\times10^{8}$ | $1.4\pm0.6\times10^{8}$ | $2.2\pm0.6\times10^{9}$ | $1.3\pm0.6\times10^{6}$ |
| 6 | $0.5\pm0.5\times10^{7}$ | $0.9\pm0.5\times10^{8}$ | $0.4\pm0.5\times10^{8}$ | $1.1\pm0.5\times10^{9}$ | $0.4\pm0.6\times10^{6}$ |

Mean and standard deviation for n=3

**Table 8.** Physico-chemical analyses of probioticated juices fermented with *Lys. sphaericus* at 37 °C.

| Vegetable juice | Incubation time (h) | TSS (Brix) | Titratable acidity (% lactic acid) | pH |
|---|---|---|---|---|
| Tomato | 24 | 19±0.8 | 0.28±0.08 | 6.6±0.04 |
| | 48 | 17±1.0 | 0.38±0.03 | 5.5±0.04 |
| | 72 | 12±0.81 | 0.31±0.02 | 4.7±0.08 |
| Carrot | 24 | 18±0.47 | 0.22±0.016 | 6.5±0.04 |
| | 48 | 16±0.81 | 0.36±0.08 | 5.7±0.04 |
| | 72 | 12±0.47 | 0.29±0.004 | 4.3±0.04 |

**Table 9.** Physico-chemical analyses of probioticated juices fermented with *Lb. casei* at 37 °C.

| Vegetable juice | Incubation time (h) | TSS (Brix) | Titratable acidity (% Lactic acid) | pH |
|---|---|---|---|---|
| Tomato | 24 | 16±0.8 | 0.23±0.08 | 6.2±0.04 |
| | 48 | 14±1.0 | 0.28 ±0.03 | 5.8±0.04 |
| | 72 | 12±0.81 | 0.21±0.02 | 4.8±0.08 |
| Carrot | 24 | 15±0.47 | 0.26±0.016 | 6.3±0.04 |
| | 48 | 13±0.81 | 0.30±0.08 | 5.2±0.04 |
| | 72 | 11±0.47 | 0.25±0.004 | 4.6±0.04 |

- *Sensory evaluation*

Sensory evaluation results indicated good sensory scores for probioticated tomato, carrot and tomato+carrot juices (Figure 1). It was realized that the addition of probiotic beads had a remarkable influence on the texture profile of the product. The presence of the beads created the swallowing difficulty and the beads sometimes remained in the mouth. The scores of swallow ability of vegetable juices with probiotic beads were 3.2 and 3.6, and without probiotic beads were 7.2 and 7.0 for tomato and carrot juices, respectively.

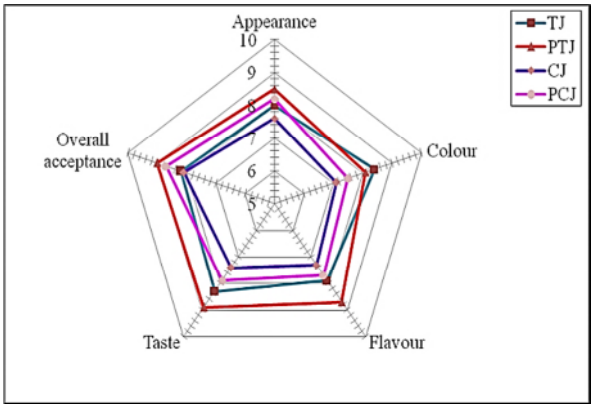


**Fig. 1.** Sensory evaluation of probioticated tomato and carrot juices.
CJ-carrot juice, PCJ-probioticated carrot juice, TJ-tomato juice, PTJ- probioticated tomato juice with *Lys. sphaericus*.

The addition of probiotic beads significantly affected the turbidity of vegetable juices. The intensity of turbidity increased from 6.8 to 7.6 in the presence of probiotic beads. This might be caused by the white color of the beads contrasting with the deep purple color of tomato juice, resulting in an increase of the turbidity of the juice. The probioticated juices had a good acceptance among the consumers than the control because of increasing taste profile. The marginal difference was identified between the sensory scores of probioticated and control vegetable juices. The taste, acidity, mouth feel, aroma, flavour, color and overall acceptance were changed in probioticated juices. The results were in agreement with the earlier report of sensory evaluation of probiotication of mango and sapota juices using *Lactobacillus* (Vijaya Kumar *et al.,* 2015).

The microencapsulation of probiotics in alginate beads coated with chitosan can protect the cells inside from the inhibiting compounds, for example acidity, pH, and flavonoids in vegetable juices. Chitosan forms a semi-permeable membrane around alginate and provided micro-porous structure, resulting in denser membrane that can slow down the diffusion rate of inhibiting compounds from vegetable fruit juices (Sezer & Akbuga, 1999).

Additionally, chitosan coated alginate beads were almost totally inert to the gut hydrolytic enzymes, such as pepsin, lysozyme, chotosanase, trypsin, and chymotrypsin. Less than 2% of the

membrane weight was hydrolyzed (Quong *et al*., 1999; Koo *et al*., 2001). This might be ensured that the probiotics in alginate beads coated with chitosan survive the digestive system of the host and colonize at the place where they can provide the benefits to the host. The level of microencapsulated probiotics in fruit juices was also above the therapeutic level ($10^7$ CFU/mL) throughout the storage. This is probably guaranteed that the consumers will derive the highest benefits from the consumption of probiotic vegetable juices.

Moreover, no growth of probiotics in alginate beads was observed like in yogurt (Krasaekoopt *et al.,* 2004). It is indicated that although the condition of the vegetable juices is not favorable to their growth, the probiotics can survive when they are well protected in alginate beads coated with chitosan. *Lysinibacillus sphaericus* showed a better survival in vegetable juices followed by *Lb. casei*, due to its adaptive properties to the presence of inhibiting compounds in vegetable juices. The presence of probiotics in fruit juices also did not change any composition of vegetable juices. Therefore, vegetable juices containing probiotic beads may be a new choice for the consumers. The encapsulation method also increases the survival of probiotics in vegetable juices. The effect of probiotic beads on the sensory characteristic and consumer acceptability should be further studied.

## Conclusion

The micro-encapsulation of probiotic cultures in alginate beads coated with chitosan increased the survival ability of probiotics by way of protecting the cells inside the small intestine from the inhibiting factors namely pH, acidity in probioticated tomato and carrot juices. *Lys. sphaericus* was found to survive better and exhibited stable viable counts during storage in refrigerator for 6weeks than the remaining micro-encapsulated cultures. The presence of probiotics in vegetable juices did not change the composition of juices. Therefore vegetable juices containing probiotic beads might be a new choice for the consumers.

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