Versi Online: http://journal.ipb.ac.id/index.php/jtip
https://doi.org/10.6066/jtip.2025.36.2.276
Hasil Penelitian

Jurnal Teknologi dan Industri Pangan
Vol. 36(2): 276-287 Th. 2025 ISSN: 1979-7788
Terakreditasi Kemendikbudristek: 164/E/KPT/2021

Production of Probiotic Pineapple Juice: Air Extrusion-Spherification System Development and Juice Storage Stability Assessment

Siti Mariam A Rani[1)], Boon-Beng Lee[1,2)]*, Nor Hidawati Elias[1,2)], Chung-Ming Tan[1)], Shi-Yee Ng[1)], and Khairul Farihan Kasim[1,2)]

[1)] Chemical Engineering Department, Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis, Arau, Malaysia
[2)] Centre of Excellence for Biomass Utilisation, Universiti Malaysia Perlis, Arau, Malaysia

Received July 10th 2025 / Revised November 7th 2025 / Accepted November 14th 2025

ABSTRACT

Consumption of probiotic beverages has been shown to enhance intestinal health and immune function. Encapsulation technology has been introduced to strengthen the stability of the probiotics in beverages. To improve the stability of the probiotics in pineapple juice, a spherification system was developed to encapsulate Lacticaseibacillus paracasei strain Shirota in Ca-alginate gel microbeads using a co-axial air extrusion method. The effect of process variables (diameter and sphericity of microbeads) in the spherification system was investigated using image analysis software. The interrelationship of the process variables of the system was analyzed using dimensional analysis. Subsequently, the stability of probiotic gel microbeads in pasteurized pineapple juice was assessed. The results showed that the spherification system could produce uniform spherical probiotic gel microbeads with a size range of 0.4–2.6 mm. A mathematical model was developed to enable the production of microbeads with the desired diameter by selecting the proper process variables, specifically the Ohnesorge number, Weber number, and the liquid-to-air mass flow rate ratio. During the refrigerated storage period, the microbeads experienced minor shrinkage and shape distortion, but the pH and total soluble solids of the pineapple juice remained stable. The viability of the encapsulated L. paracasei strain Shirota was well retained in the refrigerated pineapple juice until the ninth day of the storage period. Lastly, the encapsulated L. paracasei strain Shirota demonstrated good tolerance to simulated gastric and intestinal juices. The probiotics spherification system enables the production of probiotic pineapple juice with good stability and viability of the prebiotics.

Keywords: *Ca-alginate microbead, Co-axial air extrusion, probiotics encapsulation, probiotic pineapple juice, sphericification*

INTRODUCTION

Functional drinks have grown in popularity recently due to the health benefits they can offer beyond the essential nutrients that the human body needs. They are added with minerals, vitamins, amino acids, dietary fibres, probiotics, and so on. Probiotics are living microorganisms that, when consumed in sufficient amounts, can help to restore the natural balance of bacteria in the gut so that the body's immunity can be boosted, blood cholesterol level can be reduced, and symptoms of certain digestive disorders can be reduced (Olaide *et al.,* 2020; Chen *et al.,* 2017; Manoj *et al.,* 2023; Morsy *et al.,* 2022; Olivares *et al.,* 2019; Tonde *et al.,* 2022). Hence, it is important to ensure the viability of the probiotics in the drinks for successful marketing as a functional food (AdebayoTayo and Akpeji, 2016; Olaide *et al.,* 2020; Barik *et al.,* 2023; Dimitrovski *et al.,* 2015; Giordano *et al.,* 2022; Morsy *et al*., 2022; Sarkar, 2018; Sun *et al.,* 2023; Tan *et al.,* 2023; Yee *et al.,* 2019).

Throughout the drink manufacturing stages, probiotics experience different stress conditions such as heat stress, osmotic pressure, pH stress, and oxidative stress, which can affect their viability and stability and their effectiveness when passing through the gastrointestinal tract (Barik *et al.,* 2023; Olivares *et al.,* 2019; Sarkar, 2018). Moreover, the probiotics in drinks are commonly administrated through the oral route, passing the mouth, stomach, intestine, and colon. Along the gastrointestinal (GI) tract, their survival rate is significantly reduced due to the gastric acid, bile salts, and degrading enzymes (Ayama *et al.,* 2014; Sarkar, 2018). Therefore, spray drying, vacuum drying, freeze-drying, and fluid bed drying are commercially used to produce dry probiotic powders that can preserve the viability and stability of

*Corresponding Author: E-mail: bblee@unimap.edu.my

the probiotic bacteria (Lee *et al.,* 2019). However, these probiotic powders are overly fine for mouth perception. As compared to the mentioned methods, the spherification technique appears to be more attractive because the probiotics can be stabilised via gelation of sodium alginate solution droplets using either calcium chloride or calcium lactate gluconate to produce squishy spheres (i.e., Ca-alginate gel beads), also known as boba pearls which visually and texturally resemble caviar. These boba pearls have become increasingly popular toppings in drinks due to their distinctive appearance and interesting subtle flavours. In addition, they append a unique mouthfeel to the drinks.

The extrusion dripping method has been widely used for spherification to produce boba pearls because of its simple and low-cost setup. Although the extrusion dripping method can produce uniform and size-controllable Ca-alginate gel beads, the production rate of the method is low and not suitable for large-scale production. The rate of droplet detachment from the extrusion nozzle should be increased to increase the gel bead production. Therefore, external forces can be used to increase droplet detachment in the extrusion system, like jet cutting, air shearing, and vibration. Previous studies showed that the low-air shearing extrusion system could produce uniform Ca-alginate gel microbeads (Chan *et al.,* 2012; Herrero *et al.,* 2006). However, there is limited information can be obtained from the studies because the production of Ca-alginate gel microbeads using only one concentration of sodium alginate solution was reported (Chan *et al.,* 2012; Herrero *et al.,* 2006).

Fruit juices are consumed not only for refreshment but also for health benefits because they are rich in vitamins, minerals, and beneficial bioactive compounds. It is speculated that the probiotics-encapsulated squishy Ca-alginate gel microbeads can be incorporated into fruit juices to enhance their functionality, stability, and create a new, unique mouthfeel. Therefore, this study was initiated to construct a spherification system that can encapsulate model probiotics, i.e., *Lacticaseibacillus paracasei* strain Shirota, in Ca-alginate gel microbeads using the co-axial air extrusion method. The capability of the system to produce spherical gel microbeads was evaluated. Fruit juice extracted from locally grown MD2 pineapple was selected as the base of the functional drink. Subsequently, the stability of the gel microbeads and pineapple juice during a refrigerated storage period of 14 days was investigated. Moreover, the viability of the free and encapsulated *L. paracasei* strain Shirota in the refrigerated pineapple juice was measured and compared. Lastly, the survival of the free and encapsulated *L. paracasei* strain Shirota in the simulated gastric and intestinal juice was periodically assessed during the storage period.

MATERIALS AND METHOD

Materials

Sodium alginate was purchased from Kimika Cooperation, Japan. Calcium chloride ($CaCl_2$), sodium chloride (NaCl), and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Bendosen, Malaysia. The Royal Sweet™ MD2 pineapples were provided by a local producer, i.e., Smart KJ Agro (Asia) PLT. *Lacticaseibacillus paracasei* strain Shirota was cultured from a commercial probiotic-fermented milk beverage (Yakult, Malaysia). The peptone, de Man, Rogosa and Sharpe (MRS) broth and agar powder were purchased from Oxoid, United Kingdom. HCl and NaOH were purchased from Merck, Germany. Pepsin was purchased from Sigma-Aldrich, USA.

Preparation of solutions for spherification

Sodium alginate solutions with a concentration in the range of 1.0–3.0% w/v, with a 0.5% w/v increase were prepared using an agitator stirrer (IKA-Werke GmbH & Co, Malaysia). In which, sterilized sodium alginate powder was dissolved in sterilized distilled water under a sterile environment in a laminar flow cabinet. Then, the solution was left overnight at room temperature to disperse the entrapped bubbles. Calcium chloride powder was dissolved in distilled water to made 1.5% w/v $CaCl_2$ solution before it was sterilized and used as the gelation bath.

Determination of solution physical properties

At room temperature, an alginate solution was poured into a 250 mL measuring cylinder. Then, a hydrometer (Zeal, United Kingdom) was carefully placed into the measuring cylinder, and the density of the alginate solution was recorded. The viscosity of the alginate solution was measured using a viscometer (Brookfield Engineering, LV-I Prime, USA) at room temperature according to standard procedures. The surface tension of alginate solutions was estimated from the literature values (Chan *et al.,* 2009).

Production of pineapple juice

The fully ripe pineapples were selected and manually peeled using a kitchen knife. The juice was extracted using a slow juicer (BioChef Synergy, Australia) and then filtered using a 40-mesh filter cloth to separate large pulps. Subsequently, the filtered juice underwent pasteurisation using a custom-made pasteuriser equipped with a peristaltic pump (Easy-load Masterflex 7518-10, Cole-Parmer, USA), a Wort Chiller 304 stainless steel coil (pipe size: 9.52 mm, coil length: 8.8 m), and a thermostatic water bath

(TW12 Julabo, Germany). Prior to pasteurising the juice, boiled water was circulated through the tube and coil to sterilize the equipment. The holding temperature was set at 90 °C at the flow rate of 0.55 L/min to obtain a holding time of 30 s. The pasteurised juice was then dispensed into sterilized pre-cooled 100 mL polyethylene terephthalate screw-capped bottles. All these procedures were conducted in a laminar airflow cabinet (Midilar MD(A) Camfilfarr, Malaysia).

Preparation of probiotic bacteria culture

L. paracasei strain Shirota was cultivated in a 100 mL MRS broth in a shaking incubator (Sartorius, 3-18K, Germany) for 16 h at 37 °C at 110 rpm. Then, the cell culture was centrifuged at 5000 rpm (2800 x g) (Eppendorf, USA) at 25 °C for 10 min. The supernatant was carefully poured away, and the remaining pellet was homogenously dispersed into a 100 mL sterilized alginate solution and 100 mL pasteurized pineapple juice using a vortex mixer (IKA-Werke GmbH & Co, Germany) (Pourjafar *et al.,* 2020) for microbead production and control sample preparation, respectively.

Setup of spherification system

A spherification system using the co-axial air extrusion method was assembled in a laminar flow cabinet to ensure the probiotics gel microbeads were produced in a sterilised environment. A custom-made co-axial air nozzle was used in the system, where the diameter of the inner and outer orifice of the nozzle is 0.8 and 1.2 mm, respectively. In which the sodium alginate–*L. paracasei* strain Shirota mixture was extruded through the inner channel and orifice with the use of a peristaltic pump (Watson Marlow, United Kingdom), as illustrated in Figure 1. The alginate–*L. paracasei* strain Shirota mixture droplets at the orifice tip were sheared using compressed air supplied by a compressor (Airgen, Taiwan). The droplets were collected and cured in a gelation bath (i.e., 500 mL of calcium chloride solution), which was placed 27 cm underneath the co-axial air nozzle. The production time was controlled at 10 min for each batch. The probiotics-encapsulated Ca-alginate gel microbeads were constantly stirred at 250 rpm using a magnetic stirrer (IKA-Werke GmbH & Co, Malaysia) to ensure the microbeads were uniformly and completely gelled after 30 min of gelation time.

Size and shape characterisation of probiotics encapsulated Ca-alginate gel microbead

For every set of experiments, 30 gel microbeads were randomly placed in a glass Petri dish (60x15 mm) containing 0.5% w/v calcium chloride solution (Lee *et al.,* 2013). A digital camera (Oppo CPH2239, China) was used to capture the image of the gel microbeads from a 10 cm distance. The images were analysed using image analysis software (Image J, USA) to determine the maximum diameter passing through the vertical centroid of the microbeads (d_{max}) and the diameter of the centroid that is perpendicular to the d_{max} (d_{min}). The sphericity factor (*SF*) was computed using Equation 1 (Lee *et al.,* 2013).

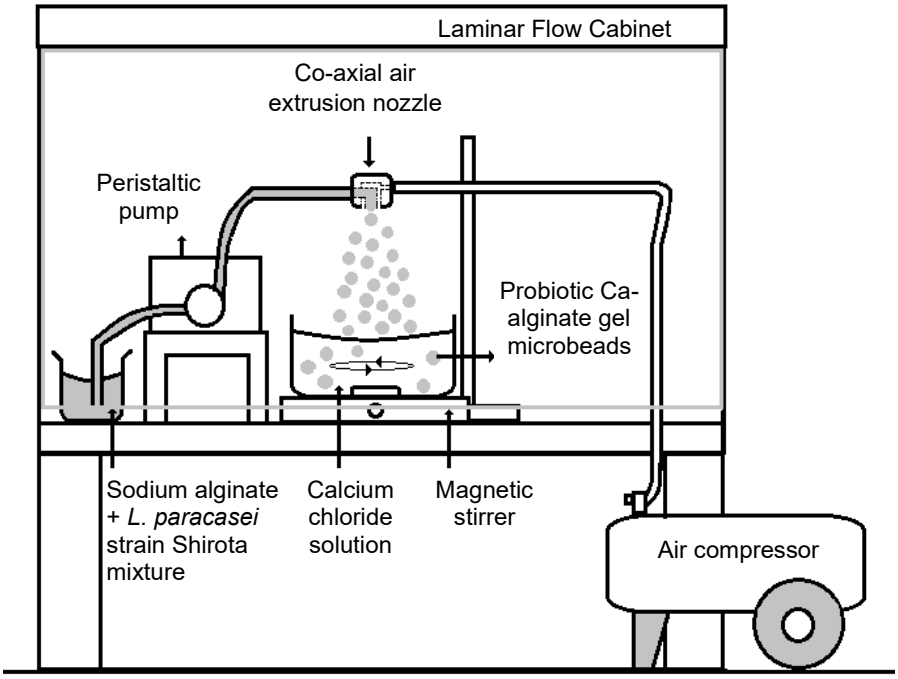

Figure 1. Schematic of a spherification system using the co-axial air extrusion method to produce probiotic Ca-alginate gel microbeads

$$SF=\frac{(d_{max}-d_{min})}{(d_{max}+d_{min})} \quad \text{..... (1)}$$

A perfectly round gel microbead is expected to have *SF* value of 0.05 and below.

Correlation analysis of co-axial air extrusion process variables influence on microbead size and shape using probiotics spherification system

The gel microbeads were produced by varying the alginate concentration, the liquid, and the air mass flow rate of the co-axial air extrusion system to investigate their effect on the diameter and sphericity of the Ca-alginate gel microbeads. It has been reported that the diameter of the Ca-alginate gel microbeads produced using the co-axial air extrusion method may vary; it is caused by the interaction of various process variables (Chan *et al.,* 2012; Herrero *et al.,* 2006; Mansour and Chigier, 1995). The interrelationship between the process variables and microbead diameter (d_B) can be described using Equation 2 (Chan *et al.,* 2012).

$$d_B=d_N\left(1+\frac{\dot{m}_l}{\dot{m}_a}\right)^n X_2\left(\frac{Oh}{We}\right)^{X_1} \quad \text{..... (2)}$$

where, $d_{N=}$ inner orifice tip diameter, $\dot{m}_l$= liquid mass flow rate, $\dot{m}_a$= air mass flow rate, *n*= the exponent of the second term of Equation 2, $X_{1=}$ the exponent of the third term of Equation 2, $X_{2=}$ the coefficient of the third term of Equation 2, *Oh*= Ohnesorge number, and *We*= Weber number.

In this study, the mathematical model coefficients, *n*, x_1 and x_2, were determined from curve-fitting using regression analysis. The Ohnesorge number (*Oh*) represents the dimensionless ratio of viscous forces to inertial and surface tension forces of the droplet, as expressed in Equation 3 (Chan *et al.,* 2012; Herrero *et al.,* 2006). The Weber number (*We*) represents the dimensionless ratio of inertia forces and the surface tension forces of the droplet (Chan *et al.,* 2012; Herrero *et al.,* 2006; Mansour and Chigier, 1995), as expressed in Equation 4.

$$Oh=\frac{\mu}{\sqrt{\rho\gamma d_N}} \quad \text{..... (3)}$$

$$We=\frac{\rho v^2 d_N}{\gamma} \quad \text{..... (4)}$$

where, μ= viscosity, γ= surface tension, ρ= density, and *v*= velocity of the droplet.

Evaluation of storage stability of MD2 pineapple juice enhanced with free and encapsulated *L. paracasei* strain Shirota

The stability of the MD2 pineapple juice enhanced with free and encapsulated *L. paracasei* strain Shirota was monitored for 14 days of refrigerated storage at 4 °C. At a fixed interval during the storage period, the pH and total soluble solids (*TSS*) content of a bottle of the juice was respectively measured using a portable pH meter (Hanna Instruments pHep H198107, Romania) and a portable refractometer (Atago PAL-1, Japan). The changes in the size and shape of the gel microbeads, as well as the cell viability in the samples, were measured using image analysis. For cell viability measurement, 1 g of the gel microbeads was treated in 235 mM sodium citrate solution (DChemie Chemical Supplies, Malaysia) to dissolve the gel microbeads. Then, 0.1 ml of the dissolved gel microbeads solution was serially diluted and plated on an MRS agar. On the other hand, the free cells were sampled directly from the juice in a bottle. The samples were undergoing a ten-fold serial dilution before being plated onto MRS agar. The MRS agars were incubated in an incubator (Binder, Germany) at 37 °C for 48 h. Subsequently, the colonies were counted to determine the viability of the probiotics.

Measurement of viability of free and encapsulated *L. paracasei* strain Shirota in simulated gastric juice (SGJ)

The tolerance of probiotics in the refrigerated pineapple juice was tested in simulated gastric juice (SGJ). The SGJ was prepared with 0.2% w/v sodium chloride solution and 0.6% w/v pepsin with pH adjusted to 3 using hydrochloric acid (de Oliveira Coelho *et al.,* 2019; Xu *et al.,* 2016). Separately, 1 mL of free *L. paracasei* strain Shirota cell suspension (control) and 1 g of microbeads were collected from the stored pineapple juice and placed into the test tube containing 9 mL of SGJ. Then the sample was incubated at 37 °C with constant agitation at 150 rpm for 120 min. Subsequently, microbeads were dissolved in 235 mM sodium citrate solution (Olivares *et al.,* 2019). Surviving bacteria in the samples were enumerated by spread plate counts in MRS agar incubated at 37 °C for 48 h (Dimitrovski *et al.,* 2015; Olivares *et al.,* 2019).

Measurement of viability of free and encapsulated *L. paracasei* strain Shirota in simulated intestinal juice (SIJ)

Simulated intestinal juice (SIJ) was prepared by dissolving potassium dihydrogen phosphate (KH_2PO_4) in 0.02 M sodium hydroxide and the pH of SIJ was adjusted to pH 6.8 by using hydrochloric acid and continuously monitored using a pH meter (Lavanya *et al.,* 2023; Yee *et al.,* 2019). Similarly to

the test in SGJ, 1 mL of free *L. paracasei* strain Shirota cell suspension (control) and 1 g of microbeads were separately collected from the refrigerated pineapple juice and placed into a test tube containing 9 mL of SIJ solution. Samples were incubated at 37 °C and 120 min. Subsequently, the microbeads were filtered and transferred into a test tube containing 9 mL of SIJ solution and incubated at 37 °C and 120 min. After 120 min, the microbeads were dissolved in 235 mM sodium citrate solution; the surviving *L. paracasei* strain Shirota was enumerated by spread plate counts in MRS agar and incubated at 37 °C for 48 h (Olivares *et al.,* 2019).

Statistical analysis

All experimental measurements were done in triplicate from at least two experimental runs. All statistical analyses were performed using Microsoft Excel (Microsoft Corporation, USA). The data were presented as means with standard deviations (in error bars) in figures. On the other hand, error analysis was conducted to assess microbead diameter prediction model performance, the mean absolute percentage error (E_M) was used to calculate the absolute variances between the predicted microbead diameter (d_{BP}) and the experimental microbead diameter (d_{BE}) in percentage units, as presented in Equation 5. In addition, the root mean square error (E_R) was calculated to determine the standard deviation of microbead diameter prediction errors, as presented in Equation 6.

$$E_M = \frac{1}{N} \frac{\sum \left| d_{BP} - d_{BE} \right|}{d_{BP}} \times 100\% \quad \text{..... (5)}$$

$$E_R = \sqrt{\frac{\sum \left(d_{BP} - d_{BE} \right)^2}{N}} \quad \text{..... (6)}$$

where, N= total number of samples.

RESULTS AND DISCUSSION

Effect of liquid and air mass flow rate ratio of spherification system on microbead size and shape

Ca-alginate gel microbeads were produced using different ratios of mass flow rates of alginate solution ($\dot{m}_l$) and compressed air ($\dot{m}_a$). The diameter of Ca-alginate microbeads produced using the spherification system was presented in Figure 2A. In general, the diameter of the microbeads increased from 0.4 to 2.6 mm as the liquid-to-air mass flow rate ratio of the spherification system increased. A similar data trend was observed for microbeads produced using 1.0, 2.0, and 3.0% w/v sodium alginate solution. This is because the microbead size was significantly increased as the air mass flow rate was greatly reduced in relative to the liquid mass flow rate. The shear force caused by the compressed air at the coaxial nozzle is proportional to the air mass flow rate. At higher air mass flow rate, the shear force creates instabilities on the surface of the alginate solution. The alginate solution was disintegrated into droplets sooner before reaching the maximum size that can be supported by the surface tension of the alginate solution at the orifice of the nozzle, hence it can produce smaller droplets (Chan *et al.,* 2012; Herrero *et al.,* 2006; Lee *et al.,* 2016; Mansour and Chigier, 1995). However, the microbead size increased when the sodium alginate mass flow rate was greatly increased relative to the air mass flow rate. It is expected that a higher mass flow rate of the alginate solution enables more solutions to flow into the nozzle at one time and forms a larger pendant droplet at the nozzle orifice. After the droplet falls off, a larger microbead is produced.

Figure 2B shows the sphericity factor (*SF*) of Ca-alginate gel microbeads produced using the spherification system by varying the liquid and air mass flow rates. Based on the results, the spherification system could produce spherical Ca-alginate gel microbeads because the *SF* of the microbeads was generally scattered around 0.05 and below 0.10. It is interesting to note that the *SF* of the microbeads was well controlled as the liquid-to-air mass flow rate ratio ($\dot{m}_l/\dot{m}_a$) increased. It is speculated that a lower mass flow rate of the compressed air assisted the pendant alginate solution droplet in overcoming the viscous forces and can be axis metrically detached from the nozzle orifice (Mansour and Chigier, 1995). This can be clearly observed in the results of the microbeads produced using viscous alginate solution with high concentrations (i.e., 2.0 and 3.0% w/v alginate solution), which are relatively uniform and spherical in shape. This is expected as the spherification system in this study utilised a mild co-axial air pressure to break up the alginate solution droplet, which is within the shear break-up region as reported in a previous study (Chan *et al.,* 2012).

Effect of sodium alginate solution concentration on the microbead size and shape

Several cases with different liquid-to-air mass flow rate ratios were selected to investigate the effect of sodium alginate concentration on the size and shape of Ca-alginate gel microbeads produced using the spherification system. As illustrated in Figure 3A, the diameter of the microbeads is not apparently influenced by the alginate solution concentration. The reason could be because of the influence of viscous forces in the droplet break-up process is weakened by the shear forces generated by the co-axial air and

liquid flow at the nozzle office (Chan *et al.,* 2012; Mansour and Chigier, 1995).

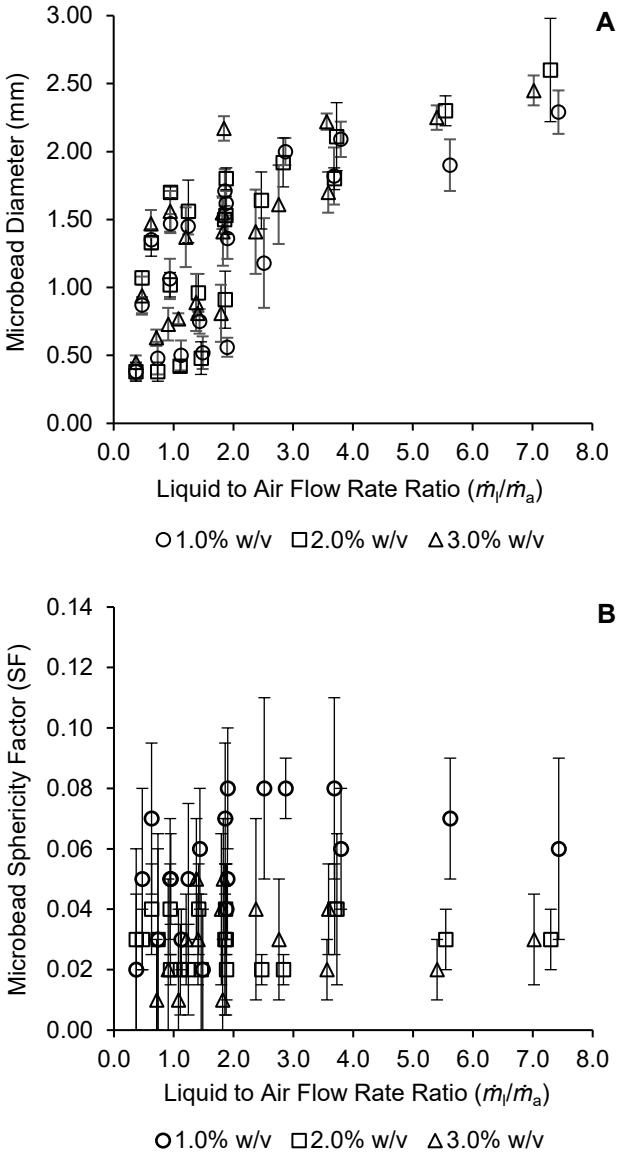

Figure 2. Diameter (A) and sphericity factor (*SF*) (B) of Ca-alginate gel microbeads produced by different liquid and air mass flow rate ratios of the spherification system using 1.0%, 2.0%, and 3.0% w/v alginate solution [Experimental conditions: 1.5% w/v $CaCl_2$ and 30 min gelation time]. Error bars in figures are means with standard deviations

However, the size distribution of the microbeads is narrower when the alginate solution concentration is increased using the same liquid-to-air mass flow rate ratio. This could be explained by the viscosity of the alginate solution. It is increased when the concentration of the alginate solution is increased hence the degree of droplet disintegration during the break-up process can be minimised. At the same time, the amount of alginate available for cross-linking with calcium cations increases when the droplet penetrates the gelation bath. Therefore, there are more cross-linking points within the network, leading to a denser and more compact gel structure that can withstand the shear forces due to impact on the gelation bath surface and agitation in the gelation bath.

Figure 3B shows the *SF* of the microbeads produced by manipulating sodium alginate concentration under the selected process conditions. The *SF* value of the microbeads decreased as the sodium alginate increased. It has been reported that the viscosity of the alginate solution plays an important role in retaining the spherical shape of the alginate solution droplet when the droplet impacts and penetrates through the gelation bath surface (Lee *et al.,* 2016). The results have shown that the *SF* of the microbeads produced using high concentrations of alginate solutions (i.e., 2.5 and 3.0% w/v) is in a narrow range and less than 0.05. In contrast, the alginate solution droplet was distorted by the shear forces during falling, impact and penetrating the gelation bath when the alginate solution concentration as well as the liquid-to-air mass flow rate ratio was low (Lee *et al.,* 2016). The resultant microbeads are deformed and less uniform, with low *SF* values.

Correlation analysis of spherification process variables for probiotics-encapsulated Ca-alginate gel microbeads diameter production

Since the size of the gel microbeads plays an important role in determining the mouthfeel quality of the functional juice, a good prediction of the size of the gel microbeads that can be produced by the spherification system is desirable. The relationship between the process variables of the co-axial air extrusion system and the diameter of microbeads was analysed based on a mathematical model suggested in previous studies, as expressed by Equation 2. Figure 4A displays the regression analysis of the experimental data to determine the model coefficients of Equation 2. The natural logarithm of microbead diameter ($\mathrm{Ln}(d_B)$) data were plotted in the function of $\mathrm{Ln}(1+\dot{m}_l/\dot{m}_a)$. The power dependency term or the coefficient n was obtained as 0.7628 from the logarithmic plot, as shown in Figure 4 (A). On the other hand, the other model coefficients, x_1 and x_2, were obtained respectively as 0.1004 and 1.1024 from the logarithmic plot of d_B/d_N $(1+\dot{m}_l/\dot{m}_a)^n$ versus Oh/We, as shown in Figure 4B. Finally, a mathematical model to predict the diameter of the microbeads produced using the probiotics spherification system can be expressed using Equation 7.

$$d_B = d_N\left(1+\frac{\dot{m}_l}{\dot{m}_a}\right)^{0.7628}\left[1.1024\left(\frac{Oh}{We}\right)^{0.1004}\right] \quad \text{..... (7)}$$

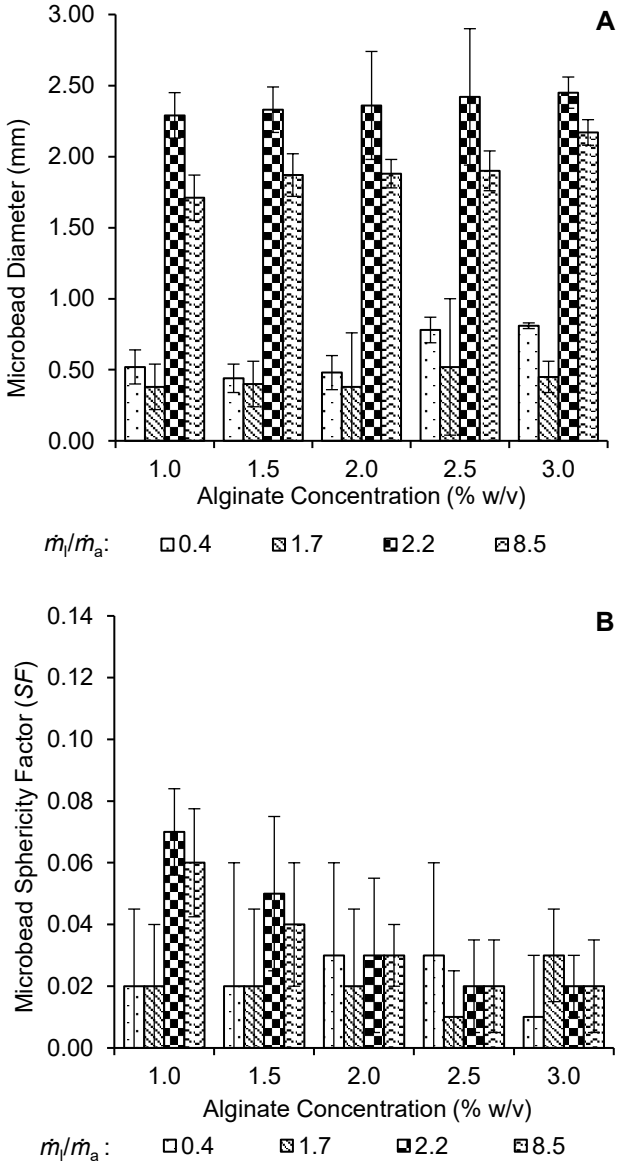

Figure 3. Diameter (A) and sphericity factor (*SF*) (B) of Ca-alginate gel microbeads produced by different alginate concentrations [Experimental conditions: 1.5% w/v $CaCl_2$ and 30 minutes of gelation time]. Error bars in figures are means with standard deviations

The model coefficients (n, x_1, and x_2) in Equation 7 are relatively lower than those reported for the co-axial air extrusion systems in previous studies. This is because the operating conditions of the probiotics spherification system, the liquid-to-air mass flow rate ratio ($\dot{m}_l/\dot{m}_a$) is less than 8.0, which are lower than those of the co-axial air extrusion systems in previous studies (Chan *et al.,* 2012; Herrero *et al.,* 2006; Mansour and Chigier, 1995). Figure 4C presents the measured diameter of probiotics-encapsulated Ca-alginate gel microbeads produced using the co-axial air extrusion method and the predicted diameter of the microbeads using Equation 7.

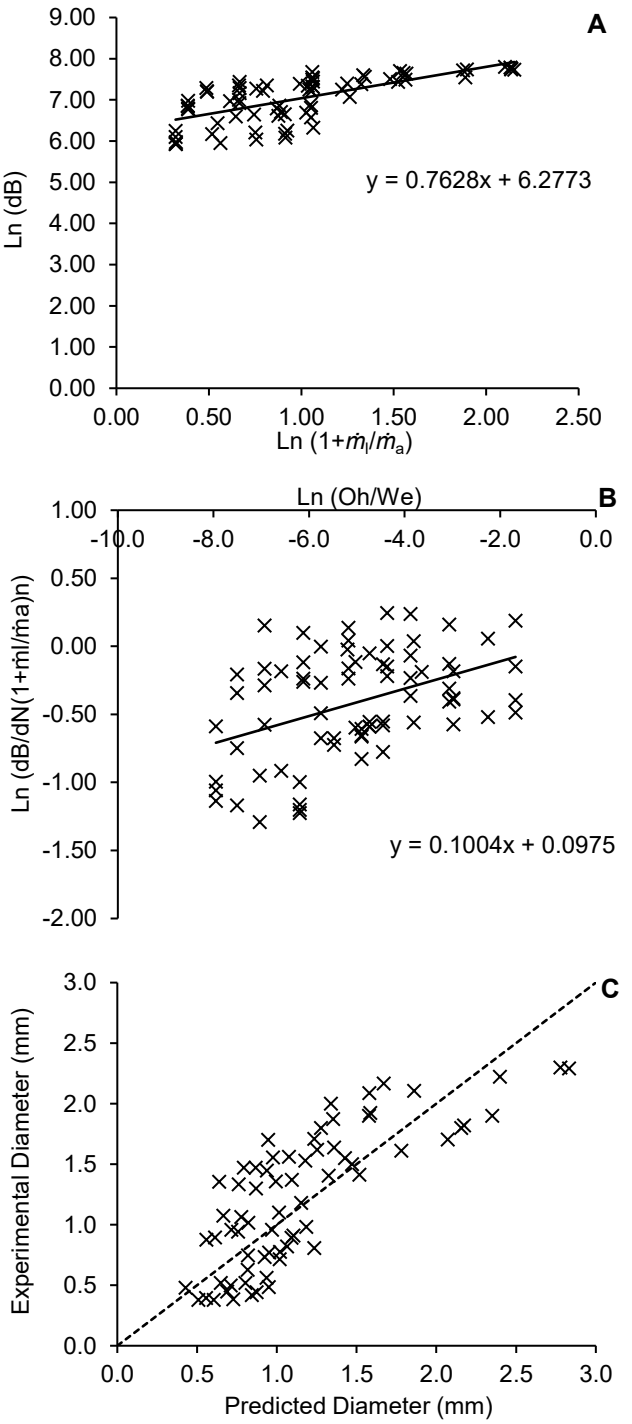

Figure 4. (A) Logarithmic plot of microbead diameter (d_B) versus 1+$\dot{m}_l/\dot{m}_a$, (B) Logarithmic plot of $d_B/d_N(1+\dot{m}_l/\dot{m}_a)n$ versus *Oh*/*We*, and (c) diameter of Ca-alginate gel microbeads produced using the spherification system obtained from experimental data and predicted using the developed mathematical model (Equation 7)

In general, experimental and calculated data were in good agreement because the mean absolute percentage error (E_M) and the root mean square error (E_R) between the predicted microbead diameter (d_{BP}) and the experimental microbead diameter (d_{BE}) was 27.3% and 0.41 mm, respectively. Since the liquid-to-air mass flow rate ratio ($\dot{m}_l/\dot{m}_a$) range of the spherification system is between 0.1 and 7.6, the mathematical models reported in the previous studies which are developed for higher $\dot{m}_l/\dot{m}_a$ ratio, they may underpredict the diameter of the probiotics encapsulated Ca-alginate gel microbeads (Chan *et al.,* 2012; Herrero *et al.,* 2006; Mansour and Chigier, 1995).

Stability of size and shape of *L. paracasei* strain Shirota encapsulated Ca-alginate gel microbeads in the refrigerated pineapple juice over a storage period

The *L. paracasei* strain Shirota encapsulated Ca-alginate gel microbeads were used to produce the functional pineapple juice, and the exterior changes (i.e., size and shape) of the microbeads were quantified during the storage period of 14 days. As shown in Figure 5A, the diameter of the microbeads gradually decreased as the time of the microbeads immersed in the pineapple juice elapsed. The decrease in microbead size could be due to the osmosis process, where the water in the microbeads was moved through the hydrogel membrane into the pineapple juice. On the other hand, the *SF* of the gel microbeads during the storage period was within the spherical range of 0.05, as shown in Figure 5B. Even so, the *SF* of the gel microbeads increased as the storage period increased. This could be due to the shrinkage of the microbeads, which causes uneven changes to the dimensions of the microbeads (i.e., d_{max} and/or d_{min}). On top of that, the mechanical strength of microbeads decreased due to the presence of bromelain, ascorbic acid and citric acid in pineapple juice. In addition, lactic acid produced by *L. casei* also weaks the mechanical strength of microbeads. Distortion of the sphericity of the microbeads could be due to the weakened gel structure of the microbeads.

Storage stability of the pineapple juice enhanced with free and encapsulated *L. paracasei* strain Shirota

As shown in Figure 6, the viable cell concentration, pH and total soluble solids (*TSS*) of the pineapple juice samples during the storage period of 14 days at 4 °C. The encapsulation efficiency (*EE*) of *L. paracasei* strain Shirota in the gel microbeads using the spherification system was determined to be 88.7% (data not shown). The concentration of *L. paracasei* strain Shirota in the pineapple juice of both samples was stable and maintained at 8–9 $\log_{10}$ CFU/mL during the first 9 days of storage. However, the concentration of the encapsulated *L. paracasei* strain Shirota decreased after the 9th day of storage. This is due to the leakage of *L. paracasei* strain Shirota from the microbeads into the pineapple juice. At the end of the studied storage period, the concentration of the leaked *L. paracasei* strain Shirota in the pineapple juice was 5.05x10^3 CFU/mL.

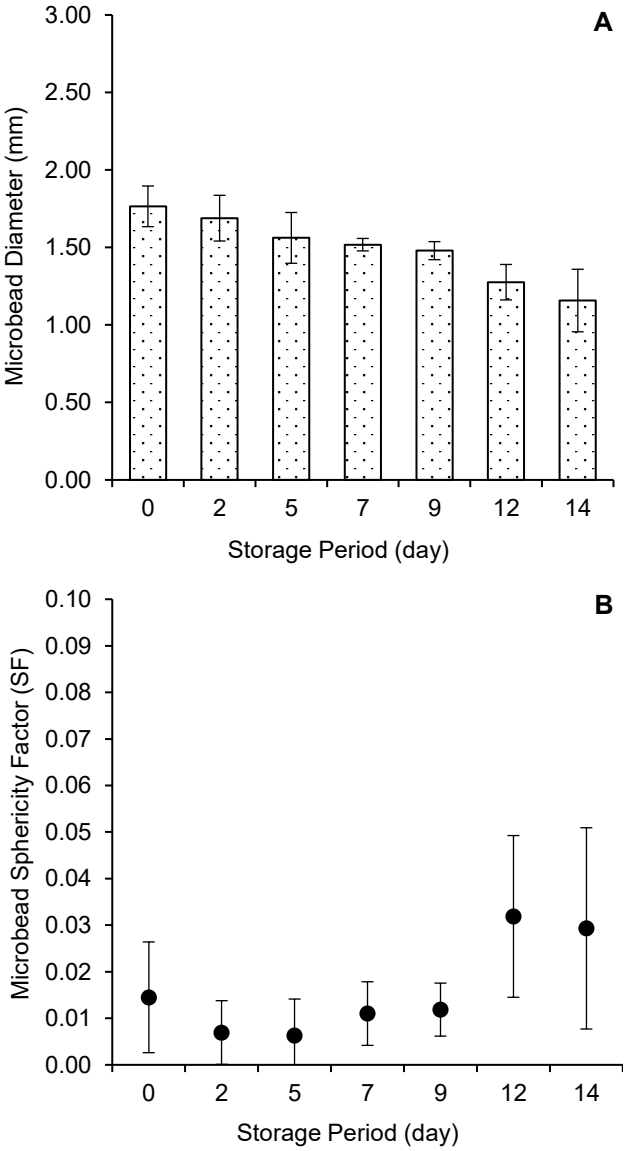

Figure 5. Diameter (A) and sphericity factor (B) of the *L. paracasei* strain Shirota encapsulated Ca-alginate gel microbeads during the refrigerated storage period at 4 °C [Experimental conditions: microbeads were produced using 2.0% w/v alginate concentration, $\dot{m}_l/\dot{m}_a$ = 2.2]. Error bars in figures are means with standard deviations

In general, the viability of free and encapsulated *L. paracasei* strain Shirota was well preserved in the pineapple juice because of the high sugar content in

the juice, as observed in a previous study (Olivares *et al.,* 2019). The leakage of encapsulated *Lactobacillus* cells from Ca-alginate beads into fruit juices was reported in previous studies (Dimitrovski *et al.,* 2015; Olivares *et al.,* 2019). The leakage of cells from the microbeads could be due to the stress experienced by the cells within the restricted space inside the compact gel structure, and the average diameter of *Lactobacillus* cells is smaller than the average pore diameter of the Ca-alginate gels pore (Dimitrovski *et al.,* 2015; Klein *et al.,* 1983). The degradation of the Ca-alginate gel due to the presence of a chelating agent in the fruit juice accelerated the leakage of the cells into the fruit juice (Dimitrovski *et al.,* 2015).

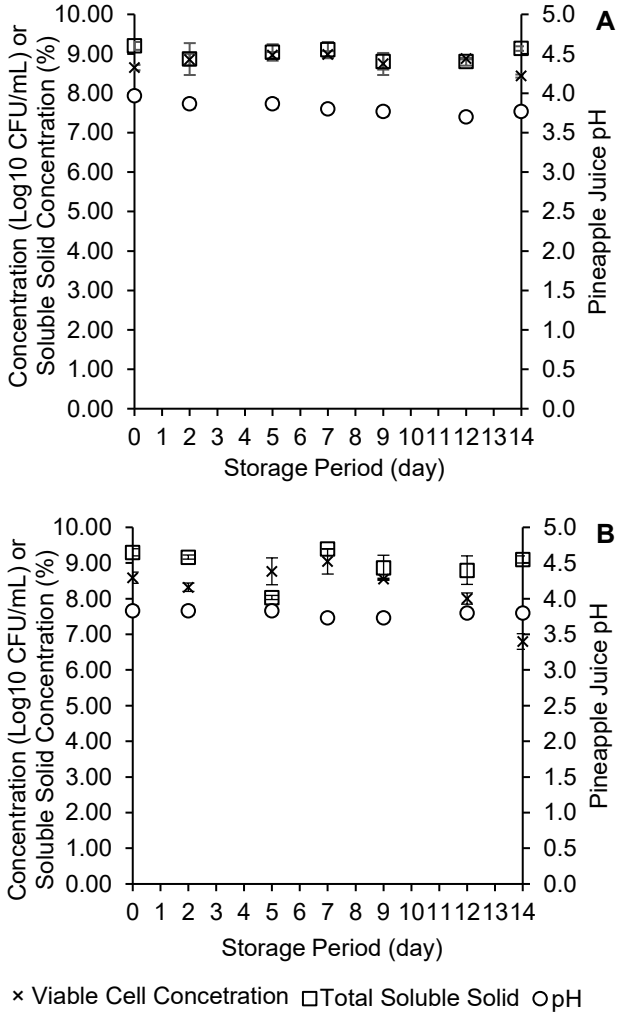

Figure 6. Viable cell concentration, total soluble solids (*TSS*), and pH of pineapple juice that suspended with free (A) and encapsulated *L. paracasei* strain Shirota (B) during the refrigerated storage period of 14 days at 4 °C [Experimental conditions: microbeads were produced using 2.0% w/v alginate concentration, $\dot{m}_l/\dot{m}_a$ = 2.2]. Error bars in figures are means with standard deviations

As shown in Figure 6, the pH of the control samples (raw pineapple juice) remained stable throughout the storage period. In contrast, the pH of the samples enhanced with probiotics bacteria was generally lower than that of the control samples. The pH of all samples enhanced with probiotics bacteria was reduced from the first day until the ninth day and remained constant until the 14th day. The samples with lower pH values indicated that *L. paracasei* strain Shirota in the juice produced lactic acid during their metabolism and increased the acidity of the juice. However, the pH in samples with encapsulated *L. paracasei* strain Shirota was comparable with that of the free *L. paracasei* strain Shirota. The result was in good agreement with the result of a previous study where the pH of the pineapple juice enhanced with probiotics bacteria was lower than that of the raw pineapple juice due to the increase of lactic acid content in the juice (AdebayoTayo and Akpeji, 2016). On the other hand, Figure 6 shows that the *TSS* value of all pineapple juice samples was stable throughout two weeks of storage. In general, the *TSS* of the samples enhanced with free and encapsulated *L. paracasei* strain Shirota was lower than that of the control sample. The results were in good agreement with those reported by a previous study where the *TSS* of the probiotics bacteria-enhanced pineapple juice was lower than that of the control sample (AdebayoTayo and Akpeji, 2016).

Viability of free and encapsulated *L. paracasei* strain Shirota in simulated gastrointestinal juice (SGJ) and simulated intestinal juice (SIJ)

The storage condition and period may affect the functionality of the probiotics bacteria immersed in the pineapple juice. The tolerance of the free and encapsulated *L. paracasei* strain Shirota to the acidic juice of the stomach and intestinal environment was periodically investigated during the 14 days of storage. Figure 7 shows the viable cell concentration of free and encapsulated *L. paracasei* strain Shirota after they were inoculated from the refrigerated pineapple juice and subsequently exposed to the simulated gastrointestinal juice (SGJ) and simulated intestinal juice (SIJ). The results suggest the free and encapsulated *L. paracasei* strain Shirota have a lower decline in viability cell concentration in SIJ as compared to that in SGJ. A similar data pattern was reported for the survival of probiotics inoculated from the refrigerated apple and tomato juices in SGJ and SIJ (Ajlouni and Bhoi, 2024). This could be attributed to the low pH of SGJ and the pepsin activity in the juice that can damage the cell walls of probiotics (Ajlouni and Bhoi, 2024). On the other hand, the encapsulated *L. paracasei* strain Shirota has better tolerance to both SGF and SIJ environments than that of the free cell, especially the refrigerated stored samples in the early 9 days.

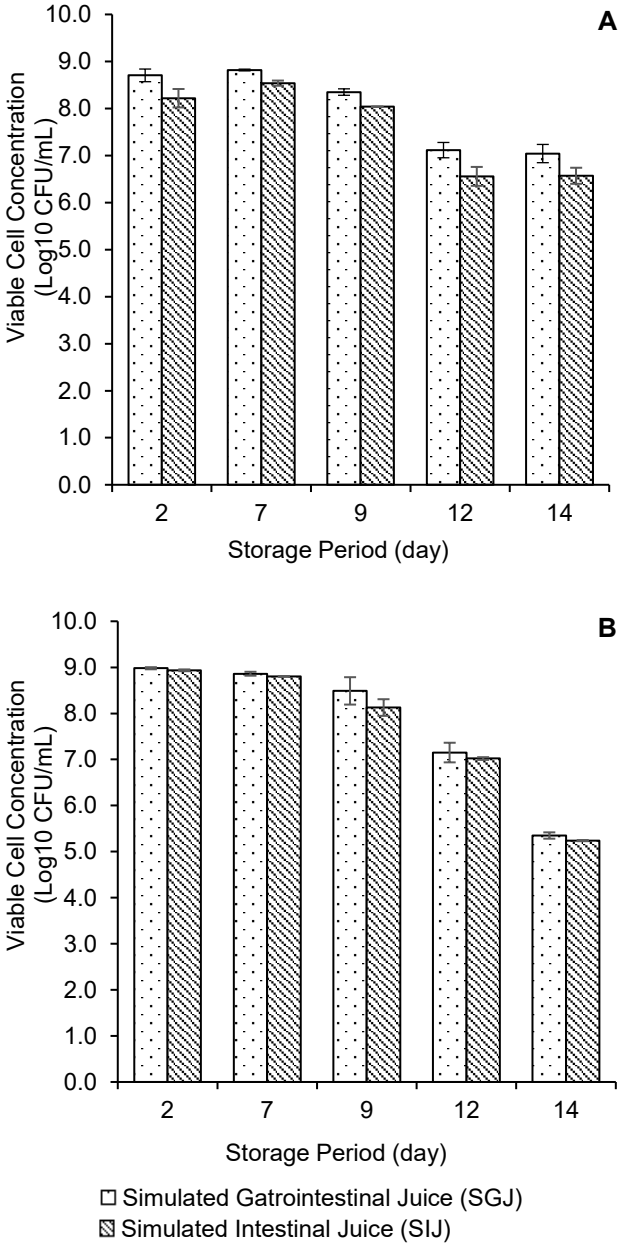

Figure 7. Concentration of viable free (a) and encapsulated *L. paracasei* strain Shirota (b) from the refrigerated pineapple juice in simulated gastric juice (SGJ) and simulated intestinal juice (SIJ) during the storage period [Experimental conditions: SGJ and SIJ exposure time = 120 min, microbeads were produced using 2.0% w/v alginate concentration, $\dot{m}_l/\dot{m}_a$ = 2.2]. Error bars in figures are means with standard deviations

However, the viable cell concentration of free and encapsulated *L. paracasei* strain Shirota in both SGJ and SIJ environments was decreased after the storage period exceeded 9 days. The data trend was associated decreased viable cell concentration of the free and encapsulated *L. paracasei* strain Shirota in the pineapple juice after 9 days of storage (Figure 6). The initial cell loading in the samples was low and consequently, the concentration of *Lactobacillus* cells after exposure to both SGJ and SIJ was also low. It is notable that the viable cell concentration of the encapsulated *L. paracasei* strain Shirota was significantly lower than that of free cells in both SGJ and SIJ. This is because the degree of cell leakage from the microbeads into the pineapple juice increased as the storage period is prolonged.

CONCLUSION

A spherification system has been developed to encapsulate probiotics bacteria, i.e., *L. paracasei* strain Shirota, in Ca-alginate gel microbeads using a co-axial air extrusion method. The system produces the microbeads with diameters between 0.4 and 2.6 mm when the liquid-to-air mass flow rate ratio ($\dot{m}_l/\dot{m}_a$) increases from 0.4 to 7.4. The majority of the microbeads are uniform and spherical when a low $\dot{m}_l/\dot{m}_a$ is preset because the extruded droplets experience less shear force during the gelation process. The correlation analysis suggests a mathematical model that is capable of giving a good prediction of the microbead diameter based on the Ohnesorge number (*Oh*), Weber number (*We*), and $\dot{m}_l/\dot{m}_a$. On the other hand, the probiotics encapsulated Ca-alginate gel microbeads are incorporated into a locally produced MD2 pineapple juice to enhance its functionality and attractiveness. The probiotic pineapple juice is refrigerated at 4 °C and stored for 14 days. The microbeads experience minor shrinkage and shape distortion as the storage period elapses. Even so, the pH and total soluble solids (*TSS*) of the probiotic pineapple juice remain stable throughout the storage period. Moreover, the viability of the free and encapsulated *L. paracasei* strain Shirota in the refrigerated pineapple juice is retained until the 9th day of the storage period. Lastly, the encapsulated *L. paracasei* strain Shirota exhibits good tolerance to the simulated gastric and intestinal juice. In short, the spherification system enables the production of a trendy and highly demanded probiotic fruit juice that offers health improvements and a unique mouthfeel. Future studies focus on solving the cell leakage issues by exploring more efficient encapsulation techniques that can provide ample spaces in the microbeads for cell growth and/or suitable coating materials that reduce the porosity of Ca-alginate gel and consequently improve the mechanical strength of the microbeads.

ACKNOWLEDGEMENT

The authors thank Universiti Malaysia Perlis (UniMAP) for providing the facilities, technical support and financial support (University – Private Matching Fund (UniPRIMA) (9001-00637 and 9002-00114) to this work. The authors also thank the Education Sponsorship Division, Ministry of Education Malaysia for providing a Ph.D scholarship to Madam Siti Mariam A Rani.

REFERENCES

AdebayoTayo, B., & Akpeji, S. (2016). Probiotic viability, physicochemical and sensory properties of probiotic pineapple juice. *Fermentation*, *2*(4), 20. https://doi.org/10.3390/fermentation2040020

Ajlouni, S., & Bhoi, Y. (2024). Survival of encapsulated vs. free probiotics in fruit juices during refrigerated storage and in vitro gastrointestinal digestion. *Journal of Dairy Research and Technology*, *6*(028), 1–10. https://doi.org/10.24966/DRT-9315/100028

Ayama, H., Sumpavapol, P., & Chanthachum, S. (2014). Effect of encapsulation of selected probiotic cell on survival in simulated gastrointestinal tract condition. *Songklanakarin Journal of Science and Technology*, *36*(3), 291–299.

Barik, A., Pallavi, P., Sen, S. K., Rajhans, G., Bose, A., & Raut, S. (2023). Fortification of orange juice with microencapsulated *Kocuria flava* Y4 towards a novel functional beverage: Biological and quality aspects. *Heliyon*, *9*(7), e17509. https://doi.org/10.1016/j.heliyon.2023.e17509

Chan, E.-S., Lee, B.-B., Ravindra, P., & Poncelet, D. (2009). Prediction models for shape and size of ca-alginate macrobeads produced through extrusion–dripping method. *Journal of Colloid and Interface Science*, *338*(1), 63–72. https://doi.org/10.1016/j.jcis.2009.05.027

Chan, E.-S., Lim, T.-K., Ravindra, P., Mansa, R. F., & Islam, A. (2012). The effect of low air-to-liquid mass flow rate ratios on the size, size distribution and shape of calcium alginate particles produced using the atomization method. *Journal of Food Engineering*, *108*(2), 297–303. https://doi.org/10.1016/j.jfoodeng.2011.08.010

Chen, H., Ma, D., Li, Y., Liu, Y., & Wang, Y. (2017). Application of box-behnken design for optimizing the process of microencapsulation of *Bifidobacterium bifidum* Bb28. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI - Food Technology*, *41*(1), 118–130.

de Oliveira Coelho, B., Fiorda-Mello, F., de Melo Pereira, G. V., Thomaz-Soccol, V., Rakshit, S. K., de Carvalho, J. C., & Soccol, C. R. (2019). In vitro probiotic properties and DNA protection activity of yeast and lactic acid bacteria isolated from a honey-based kefir beverage. *Foods*, *8*(10), 485. https://doi.org/10.3390/foods8100485

Dimitrovski, D., Velickova, E., Langerholc, T., & Winkelhausen, E. (2015). Apple juice as a medium for fermentation by the probiotic *Lactobacillus plantarum* PCS 26 strain. *Annals of Microbiology*, *65*(4), 2161–2170. https://doi.org/10.1007/s13213-015-1056-7

Giordano, I., Abuqwider, J., Altamimi, M., Di Monaco, R., Puleo, S., & Mauriello, G. (2022). Application of ultrasound and microencapsulation on *Limosilactobacillus reuteri* DSM 17938 as a metabolic attenuation strategy for tomato juice probiotication. *Heliyon*, *8*(10), e10969. https://doi.org/10.1016/j.heliyon.2022.e10969

Herrero, E. P., Del Valle, E. M. M., & Galán, M. A. (2006). Modelling prediction of the microcapsule size of polyelectrolyte complexes produced by atomization. *Chemical Engineering Journal*, *121*(1), 1–8. https://doi.org/10.1016/j.cej.2006.04.003

Klein, J., Stock, J., & Vorlop, K.-D. (1983). Pore size and properties of spherical Ca-alginate biocatalysts. *European Journal of Applied Microbiology and Biotechnology*, *18*(2), 86–91. https://doi.org/10.1007/BF00500829

Lavanya, B. S., Sreejit, V., & Preetha, R. (2023). Lactobacillus plantarum J9, a potential probiotic isolated from cereal/pulses based fermented batter for traditional Indian food and its microencapsulation. *Journal of Food Science and Technology*, *60*(3), 906–915. https://doi.org/10.1007/s13197-021-05258-3

Lee, B.-B., Bhandari, B. R., & Howes, T. (2016). Air extrusion system for ionotropic alginate microgel particle formation: A review. *Chemical Engineering & Technology*, *39*(12), 2355–2369. https://doi.org/10.1002/ceat.201600088

Lee, B.-B., Ravindra, P., & Chan, E.-S. (2013). Size and Shape of calcium alginate beads produced by extrusion dripping. *Chemical Engineering & Technology*, *36*(10), 1627–1642. https://doi.org/10.1002/ceat.201300230

Lee, Y., Ji, Y. R., Lee, S., Cho, M.-J., & Cho, Y. (2019). Microencapsulation of probiotic *Lactobacillus acidophilus* KBL409 by extrusion technology to enhance survival under simulated intestinal and freeze-drying conditions. *Journal of Microbiology and Biotechnology*, *29*(5), 721–730. https://doi.org/10.4014/jmb.1903.03018

Manoj, P. M., Mohan, J. R., Khasherao, B. Y., Shams, R., & Dash, K. K. (2023). Fruit based probiotic functional beverages: A review. *Journal of Agriculture and Food Research*, *14*, 100729. https://doi.org/10.1016/j.jafr.2023.100729

Mansour, A., & Chigier, N. (1995). Air-blast atomization of non-Newtonian liquids. *Journal of Non-Newtonian Fluid Mechanics*, *58*(2–3), 161–194. https://doi.org/10.1016/0377-0257(95)01356-Z

Morsy, M. K., Morsy, O. M., Abdelmonem, M. A., & Elsabagh, R. (2022). Anthocyanin-colored microencapsulation effects on survival rate of *Lactobacillus rhamnosus* GG, color stability, and sensory parameters in strawberry nectar model. *Food and Bioprocess Technology*, *15*(2), 352–367. https://doi.org/10.1007/s11947-022-02758-8

Olaide, O. A., Samuel, L., Ahmad, H., Rosmawati, S., Fazia, M. S., Idris, A. A., & Wahab, A. A. (2020). Use of the *Lactococcus lactis* IO-1 for developing a novel functional beverage from coconut water. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI - Food Technology*, *44*(1), 118–131. https://doi.org/10.35219/foodtechnology.2020.1.07

Olivares, A., Soto, C., Caballero, E., & Altamirano, C. (2019). Survival of microencapsulated *Lactobacillus casei* (prepared by vibration technology) in fruit juice during cold storage. *Electronic Journal of Biotechnology*, *42*, 42–48. https://doi.org/10.1016/j.ejbt.2019.10.002

Pourjafar, H., Noori, N., Gandomi, H., Basti, A. A., & Ansari, F. (2020). Viability of microencapsulated and non-microencapsulated Lactobacilli in a commercial beverage. *Biotechnology Reports*, *25*, e00432. https://doi.org/10.1016/j.btre.2020.e00432

Sarkar, S. (2018). Whether viable and dead probiotic are equally efficacious? *Nutrition & Food Science*, *48*(2), 285–300. https://doi.org/10.1108/NFS-07-2017-0151

Sun, W., Nguyen, Q. D., Sipiczki, G., Ziane, S. R., Hristovski, K., Friedrich, L., Visy, A., Hitka, G., Gere, A., & Bujna, E. (2023). Microencapsulation of *Lactobacillus plantarum* 299v Strain with whey proteins by Lyophilization and its application in production of probiotic apple juices. *Applied Sciences*, *13*(1), 318. https://doi.org/10.3390/app13010318

Tan, L. L., Ang, K. L., & Loo, S. C. J. (2023). Alginate encapsulation improves probiotics survival in carbonated sodas and beers. *PLOS ONE*, *18*(3), e0283745. https://doi.org/10.1371/journal.pone.0283745

Tonde, A. B., Bhoite, A., & Gaikwad, N. N. (2022). Studies on microencapsulation of Lactobacillus acidophilus NCIM 5306 and evaluation of matrix material efficiency in pomegranate juice. *Food Research*, *6*(2), 255–264. https://doi.org/10.26656/fr.2017.6(2).147

Xu, M., Gagné-Bourque, F., Dumont, M.-J., & Jabaji, S. (2016). Encapsulation of *Lactobacillus casei* ATCC 393 cells and evaluation of their survival after freeze-drying, storage and under gastrointestinal conditions. *Journal of Food Engineering*, *168*, 52–59. https://doi.org/10.1016/j.jfoodeng.2015.07.021

Yee, W. L., Yee, C. L., Lin, N. K., & Phing, P. L. (2019). Microencapsulation of *Lactobacillus acidophilus* NCFM incorporated with mannitol and its storage stability in mulberry tea. *Ciência e Agrotecnologia*, *43*, e005819. https://doi.org/10.1590/1413-7054201943005819