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Encapsulation of *Lactobacillus plantarum* Using the Pineapple Peel Extract to Evaluate their Viability Under Gastrointestinal and Storage Conditions

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This study focuses on encapsulating of *Lactobacillus plantarum* using pineapple peel extract prebiotic as wall material to enhance its viability under various conditions. A freshly harvested *L. plantarum* culture was mixed with the encapsulating agent solution to achieve a 1:1.5 (v/v) core-to-wall ratio. The mixture was freeze-dried by first freezing at −40 °C for 24 h in plastic containers, then lyophilising at −52 °C and a condenser pressure of 0.100 mBar for 28 h, after which the dried samples were stored in sterile HDPE bottles at 4 °C. The different combinations of encapsulating agents, such as pineapple peel extract, inulin, and gum Arabic, are used to formulate microcapsules. The encapsulation efficiency of *L. plantarum* is evaluated, and the microcapsules are subjected to simulated gastrointestinal conditions and bile salt treatments to assess their viability. Additionally, the study examines the storage stability of the encapsulated probiotic cells over a 45-day period, as indicative of viability in standard lab settings. The results highlight the protective effects of encapsulating materials in acidic, bile salt, and storage conditions, indicating the potential for utilising pineapple peel extract and gum Arabic to enhance the viability and stability of probiotic microorganisms for potential synbiotics applications.

Keywords: encapsulation; *Lactobacillus plantarum*; microcapsules; prebiotic; synbiotics

I. INTRODUCTION

Pineapple is a tropical fruit extensively consumed because of its exotic aroma and pleasant flavour, as well as health benefit properties. Higher number of byproducts (peel) are produced during processing and generate a lot of residues. However, these byproducts are rich in natural components, such as vitamin C, minerals, enzymes, fibre and phenolic compounds, having the potential to be utilised as ingredients with more nutritional value (Lourenço *et al.*, 2020). The bioactive extracts from pineapple peel have been studied due to their antioxidant and prebiotic potential. It has mainly polysaccharide-based prebiotics with additive and functional properties which can enhance the nutritional value by promoting digestion and absorption (Azam *et al.,* 2020). Moreover, these polysaccharides have potential gel forming abilities to increase the possibility of probiotics while substitute as prebiotics (Pereira *et al.*, 2018; Terpou *et al.*, 2019).

A synbiotics refers to the combined effect of probiotics and prebiotics, working synergistically to enhance the viability and colonisation of beneficial bacteria within the intestinal tract, thus maximising their overall positive impact. In this context, the prebiotic is chosen specifically to promote the growth of existing beneficial microorganisms, while the probiotic is selected to fulfil a specific biological function with a targeted outcome (Terpou *et al.*, 2019). However, for target

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delivery the microorganism must survive during the exposure to environmental stress, being able to colonise and perform the metabolic activity in the gastrointestinal tract (Iravani *et al.*, 2015). Therefore, the cell encapsulation may enhance the resistance of probiotic cell to different stress conditions and reduce the losses of cell viability (Rodrigues *et al.*, 2020).

Microencapsulation is the popular techniques are used to entrap the cell including particles of different properties (Choudhury *et al.*, 2021). Freeze-drying (lyophilisation) is a preferred method due to the ability to enhance the shelf-life of food products and successful preservation effect of starter cultures or probiotic bacteria. The low temperatures during freeze drying process provides a less harmful effect on sensitive microorganisms comparing to drying at room or higher temperatures (Lee *et al.*, 2019). However, dehydration may damage the surface of the cell and decrease the viability of the probiotics (Kiepś & Dembczyński, 2022). Therefore, this method also needs to use of cryoprotectants for adequate cell protection (Lengyel *et al.*, 2019). It is reported that the synergistic effect of cryoprotectants in combination with polysaccharides and proteins provides the best protection of the bacteria cell during long time storage (Basholli *et al.*, 2014; Obradović *et al.*, 2022).

The selection of suitable wall materials is also crucial for the stability of the particles during encapsulation. These materials must be non-toxic and protect the entrapped cells from any injury caused by environmental stress (Rathore *et al.*, 2013). Furthermore, they must provide the target release during the passage through the gastrointestinal tract (Chen *et al.*, 2017). Although polysaccharides, proteins and lipids have been utilised to immobilise probiotics, natural polysaccharides extracted from plant sources and gums have been increasingly exploited (Rajam & Anandharamakrishnan, 2015; Rodrigues *et al.*, 2020) Gum Arabic (GA) is widely used wall materials for microencapsulation process due to its desirable characteristics such as high solubility, low viscosity and good emulsifying properties (Carneiro *et al.*, 2013). It is considered a tremendous source of dietary fibre including excellent miscibility with water and acceptable tolerance in acidic milieu which make it a suitable matrix for encapsulation purposes (Marcillo *et al.*, 2021). It is either coated alone or in amalgamation with other polysaccharides to impart exceptional protective barrier to the cored probiotic (Ahmad *et al.,* 2021).

II. MATERIALS AND METHODS

A. Materials

Freeze dried culture of *L. plantarum* was used in this study as bacterial strain (probiotic). The encapsulating agents used were freeze dried pineapple peel extract (PPE; prebiotic), inulin (IN), and gum Arabic (GA; Sigma Aldrich, Steinheim, Germany). All other reagents were of analytical grade.

B. Culture Preparation

The strain was reactivated by inoculating it into “de Man Rogosa Sharpe” (MRS) broth from Merck, Darmstadt, Germany. The mixture was then incubated overnight at 37 °C. A portion of the media was used to do further cultured in 50 mL of MRS mixture under the same environment for 24 hours to achieve a cell density of approximately $10^{9\text{-}10}$ CFU/mL. The reactivated media is collected after centrifugation at 2000×g for 10 minutes at 4 °C (Kubota Centrifuge, 5500, Tokyo, Japan). The obtained bacterial pellet was cleaned twice with sterile peptone solution (0.1%).

C. Preparation of Drying Media

The wall materials for the encapsulation process were produced by combining the bacterial pellet with a 30% (w/v) total solid solution of pineapple peel extract (PPE), inulin (IN), and gum Arabic (GA), following the method described by (Gul, 2017; Gul & Atalar, 2019). All wall materials (PPE, IN and GA) were dispersed in distilled water until the complete dissolution. The microcapsules were formulated using different combinations of encapsulating agents: pure PPE, a 1:1 ratio of PPE to GA (50% each), pure IN, and a 1:1 ratio of IN to GA (50% each).

Next, the mixture solution was subjected to heat treatment at 80 °C for 30 minutes and left to cool down. 80 °C/30 min was a pragmatic, literature-consistent choice to fully dissolve IN/GA (Kim *et al.*, 2001). A freshly harvested cell culture of *L. plantarum* was combined with all coating material solution to achieve a desired core-to-wall ratio of 1:1.5 (v/v) (Sun *et al.*, 2023). The mixture solutions were homogenised by a High-Speed Benchtop Laboratory Stirrer (JK RW20DZM.n, IKA

Labor Technik) at 5,000 rpm for 2 minutes and subsequently microencapsulated using freeze-drying methods.

D. Freeze Drying

The freeze-drying method for encapsulation followed the process described by (Moayyedi *et al.*, 2018). The encapsulating material solutions, along with the bacterial strain, were placed in plastic boxes with a 1 cm thick layer. The boxes were then frozen at -40 °C for 24 hours using a freezer (Haier Deep Freezer, DW-40L262, Netherlands). Subsequently, the dried samples were placed into a laboratory-scale freeze dryer (Labconco Freeze Dryer, Fisher Scientific, USA) operating at a condenser pressure of 0.100 mBar at -52 °C for 28 hours. After that the freeze-dried samples were stored in sterile HDPE bottles. They were then kept at 4 °C for further processing.

E. Encapsulation Efficiency

The encapsulation efficiency of *L. plantarum* was assessed following the method described by (Gul, 2017). To release the entrapped bacteria 0.1 g of dry samples were dissolved in 9.9 mL of phosphate buffer solution (0.1 M, pH 7.2). The mixture was then homogenised for 10 minutes. Serial dilutions of the resulting solution were prepared using 0.1% sterile peptone solution on MRS agar media. After incubating at 37 °C for 48 hours, the viable cell counts were observed and the results expressed as log CFU/mL.

The encapsulation efficiency was measured by following the equation:

$$EE(\%) = 100 \times \left(\frac{N}{N_0}\right) \quad (5.1)$$

In Equation (5.1), the encapsulation efficiency (EE) is expressed as a percentage, N_0 is the initial number of bacteria before the drying process (log CFU/mL), and N is the number of bacteria obtained after the encapsulation (log CFU/mL).

F. Survival of Microencapsulated L. plantarum Under Acidic Condition

The consequence of acidic treatments on the survival of *L. plantarum*, whether in free form or entrapped in microcapsules, was evaluated using the methodology described by (Azam *et al.*, 2020; Gul & Atalar, 2019). In this study, either 1 mL of free cells or 0.1 g of dried samples were placed in a tube containing 10 mL of simulated gastric juice. The simulated gastric juice consisted of 0.2% NaCl and 0.3% pepsin (Sigma) at pH 2.0, which was adjusted using 1 N HCl. The mixture was thoroughly mixed, and 1 mL aliquots were withdrawn at different time intervals of 30, 60, 120, and 180 minutes and added to 9 mL of peptone water (0.1%). The samples were further used for serial dilution to determine the viability, and the results were plated on agar media for incubation at 37 °C for 48 hours.

G. Survival of Microencapsulated L. plantarum in Bile Salt Conditions

The survival of both free and encapsulated *L. plantarum* after bile salt treatments was determined following the methodology described by (Gul, 2017). For this analysis, 0.1 g of microcapsules and 1 mL free cell were incubated at 37 °C for 180 minutes in sterile bile solutions (2 g/L) adjusted to pH 6.8 with 0.1 N NaOH. After specific incubation periods (30, 60, 120, and 180 minutes), samples were collected and serially diluted with 0.1% peptone water for subsequent cell viability assessment.

H. Survival of Microencapsulated L. plantarum During Storage

The storage stability of the free cell and encapsulated cell was evaluated at a temperature of 4 °C over a period of 45 days. The encapsulated powder was dissolved in 0.9 mL of phosphate buffer solution (0.1 M, pH 7.2) and homogenised using a magnetic stirrer for 10 minutes. The viable cell counts were observed at 0, 15, 30, and 45 days of storage period after serially diluted with 0.1% sterile peptone solution (Azam *et al.*, 2020).

I. Statistical Analysis

One- and two-way ANOVA tests were conducted at a 5% significance level (P = 0.05) of 95% confidence level. The effect of various groups of independent variables was examined by conducting the general linear model (GLM) analysis of variance (ANOVA) using IBM SPSS version 27 statistical software.

III. RESULTS AND DISCUSSION

A. Encapsulation Efficiency

The Encapsulation efficiency of the microcapsules after the freeze-drying processes are shown in Table 1. The viability of encapsulated *L. plantarum* after freeze drying was ranged from 88.43 to 91.47%. This result was in agreement with another study that investigated the survival of encapsulated bacteria cell by freeze drying process (Gul & Atalar, 2019; Reyes *et al.*, 2018). According to (Gul, 2017), around 97% survival was observed by *L. plantarum* CFR 2191, *Pediococcus acidilactici* CFR 2193, and *L. salivarius* CFR 2158 with different type of wall materials. In this study the higher viability was observed in PPE+GA, when the cell was encapsulated by using PPE and GA mixture. However, this was probably because of the synergistic action among the biopolymers, which may provide the protective barrier on cell wall giving place to a continuous network structure more tightly than that resulting from either biopolymer on its own (Gul, 2017; Rodrigues *et al.*, 2020). Moreover, study reported by (Reyes *et al.*, 2018) showed that GA act as a highly protective material of probiotics after the microencapsulation by drying methods. GA contain a higher branched of simple sugars and a protein structure covalently linked with the molecular structure and the functional characteristics (Higuita *et al.*, 2015). However, the recommended numbers of viable cells in the entrapped cells were considered around 6–7 log CFU/g to assert the positive effects on the human health.

Table 1. Characterisation of entrapped capsules made by different wall materials

Sample	EE
IN	90.59 ± 1.90[a]
PPE	88.43 ± 2.03[a]
IN+GA	90.31 ± 2.58[a]
PPE+GA	91.47 ± 2.26[a]

IN: inulin; PPE: pineapple peel extract; GA: gum arabic; EE: encapsulation efficiency. Same letters indicate no significant difference between samples at p > .05.

B. Survival of Encapsulated L. plantarum in Acidic Conditions

In general, probiotics are widely used as food components in dairy and other products. It is considered that the first cellular stress for probiotic occurred at pH 2.0 in stomach and loss their viability before reaching to gastrointestinal tract (Gul, 2017). Hence, ensuring the safety of probiotic cells when exposed to various stress conditions through the implementation of encapsulation techniques with a physical barrier is crucial. In this study, the impact of gastrointestinal conditions on the stability of *L. plantarum*, both in free form and microencapsulated via freeze drying, was assessed using different wall materials. The findings of this investigation are illustrated in Figure 1.

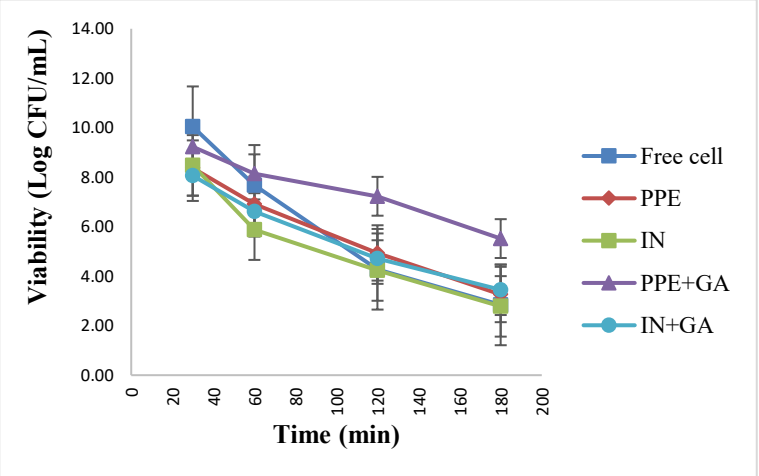

Figure 1. Survival of microencapsulated *L. plantarum* by freeze drying in acidic conditions (PPE: pineapple peel extract, In: inulin, GA: gum arabic)

The viability of probiotic cells exhibited significant variations (p<.05) depending on the wall materials employed for the microencapsulation process. Free cells experienced a loss in survival rate after 60 minutes in acidic conditions at

pH 2.0. The highest survival rate was observed in PPE+GA microcapsules, with a minimal log reduction of 3.71 CFU/mL after 180 minutes. In contrast, the maximum log reduction of 7.2 CFU/mL was observed in the absence of encapsulation (free cells). Notably, the viability of free cells rapidly reduced during the initial 60 minutes under gastric conditions, whereas only slight reduction was found for entrapped cells. In comparison to the free cells, the freeze-dried microcapsules exhibited improved resistance to simulated gastrointestinal digestion in various wall matrices. These findings align with previous studies conducted by (Gul & Atalar, 2019), which reported higher survival rates for freeze-dried microcapsules compared to free cells using different wall materials. Although all entrapped cells demonstrated reduced protection at the end of the exposure period, the PPE+GA capsules exhibited the most effective protective effect with a lower log reduction. This outcome is consistent with other studies that have evaluated the viability of microencapsulated cells using freeze-drying methods (Gul & Atalar, 2019; Reyes *et al.*, 2018).

The viability of free cell reduced as the gastric juice promotes to disrupt their upper surface and reduced the viability. A study conducted by (Azam *et al.*, 2020) stated that the addition of prebiotic fructooligosaccharides (FOS) in high concentration filled the pores of entrapped cell and enhance the viability. Probiotic cell with less protective outer layer is not capable to resist the acidic conditions and resulted in loss of viability. Another study reported by (Arslan-Tontul & Erbas, 2017), showed equivalent result investigated with gum arabic including β-cyclodextrin for entrapping probiotic cell and increased the survival rate with the increased concentration of polymers under acidic conditions. It is also reported that the addition of prebiotic arabinoxylan for encapsulation of *L. plantarum* helped to enhance the viability after treated with gastric juices as compared to the alginate only (Wu & Zhang, 2018). Moreover, inulin improved the survival rate of *L. acidophilus* after using as wall material for encapsulation process and exhibit lower log reduction comparing to the free cell (Wu & Zhang, 2018).

C. Survival of Microencapsulated L. plantarum in Bile Salt Conditions

The change of viability of *L. plantarum* was observed with and without encapsulating materials in bile salt conditions. PPE+GA showed the maximum protection for survival of probiotic cell with a lower log reduction of 3.91 log CFU/mL. The encapsulating materials showed remarkable effect on the survival of *L. plantarum* in bile salt condition at different time intervals. The isolate showed increased durability with the addition of prebiotic extract (PPE) and GA as compared to single wall materials only. Similarly, the free cell was incompetent to retain their stability to a significant level at different incubation times with a log reduction of 7.06 log CFU/mL.

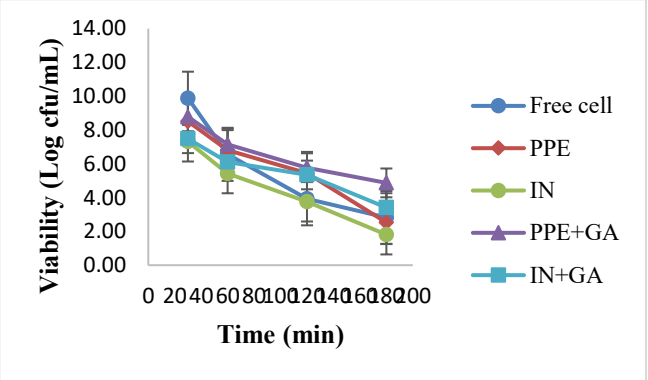

Figure 2. Survival of microencapsulated *L. plantarum* by freeze drying in bile salt conditions (PPE: pineapple peel extract, In: inulin, GA: gum arabic)

The reduced viability of entrapped cells in formulations using only prebiotic pineapple peel extract (PPE) may be attributed to its limited structural support, whereas the highest viability was observed with the synergistic interaction between gum arabic (GA) and PPE, which likely formed a more robust and protective encapsulation matrix. The highest survival was accomplished at 30 min and the lowest was noticed at 180 min incubation period. The longer incubation times in bile salt evolved in low survival and the interconnection between incubation period and the arrangements of wall materials considerably affected the viability of probiotic strain. Consequently, incubation time was considered a critical factor for enhancing probiotic viability.

Similarly, the outcome from another research work found that the usage of FOS as a natural polymer increased the

development of *L. casei* in simulated acidic conditions than the single use of alginate component. In contrast, free (unencapsulated) cells were unprotected when exposed to bile salts and exhibited significantly lower viability compared to encapsulated cells (Rodrigues *et al.,* 2017). However, as a single wall material FOS loss their ability to withstand in gastrointestinal conditions. The inclusion of FOS together with alginate decreased the possibility of structural degeneration and increase the protective effect during encapsulation.

The result from this work is resembling the outcome of another study conducted by (Azam *et al.*, 2020), in which the survival of encapsulated probiotics was evaluated in bile salt conditions with the addition of prebiotics collected from sugar beet and the chicory sources. The results showed that prebiotics increased the viability in 2 and 4% of bile salt solution at maximum level comparing to the alginate beads. Moreover, addition of inulin improved the survival rate 19–32% than the single use of alginate component. The crosslinking of alginate and inulin can prevent the dispersion of bile salt and increase the protective effect. Relatively, encapsulation of probiotics with different biopolymers can prevent the reduction of survival rate comparing to the free cell.

D. Survival of Encapsulated L. Plantarum at Different Storage Periods

The survival of *L. plantarum* (Figure 3) was assessed over a 45-day storage period under standard laboratory conditions to indicate baseline viability trends. The encapsulating materials remarkably influenced the survival of *L. plantarum* and retained viability up to 7 log CFU/mL. The inclusion of GA with prebiotic (pineapple peel extract) significantly improved the viability of the entrapped cell. The free cell was more susceptible to the stress conditions and lost viability during the whole storage period. PPE+GA has the highest storage stability (7.93 log CFU/mL) with the minimum loss followed by PPE and IN+GA, respectively. The production of different substances formed during metabolism can affected the survival of probiotic strains and eventually decrease their viability. In this case high temperature during storage condition could reduce the stability. However, storage at 4 °C can help to maintain the viability and reduce the risk of lower shelf-life.

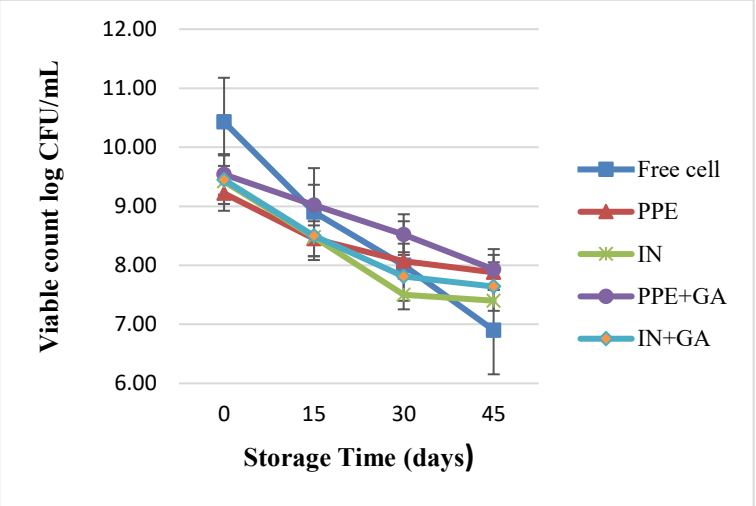

Figure 3. Survival of encapsulated *L. plantarum* by freeze drying in various wall materials at 40 °C storage period (PPE: pineapple peel extract, In: inulin, GA: gum arabic).

Similar results were observed in a research work by (Silva *et al.,* 2018) during the storage of *L. acidophilus* which was encapsulated with gelatine and FOS (prebiotic). The result reported that the inclusion of FOS with gelatine enhanced the viability of the entrapped cell. This was due to an improved interconnected network provided by FOS which helped to enhance the viability more than the free cell. The free cell of *L. acidophilus* showed a considerable reduction in survival rate after 30 days of storage than the entrapped cell. Encapsulation of *L. acidophilus* and *B. lactis* with different concentration of sodium alginate and gum arabic decreased the viability whenever stored for longer time and the desired viability of the cell was retained after storing at 4 °C. However, the encapsulated probiotics showed significantly less reduction of viability than the unentrapped cells (Azam *et al.*, 2020).

As a result, the recent study suggested the addition of prebiotic as protective wall barrier of probiotic to improve the viability during the longer storage time. The synbiotic products obtained from freeze dried encapsulation process need to examine for sensory evaluation by consumer acceptance before commercialisation (Mituniewicz–Małek *et al.*, 2019).

IV. CONCLUSION

In this study, prebiotics and gum arabic biopolymer-based microcapsules were prepared using freeze drying for the encapsulation and further characterisation of synbiotic powder were done. The maximum efficiency was accomplished owing to the consistency of encapsulating wall materials and *L. plantarum*. The inclusion of prebiotic and GA enhanced the structural integrity and the stability of entrapped cell. SEM represent the effect of wall materials incorporation to ameliorate the structural defects in encapsulated cell. Both wall materials showed protective effect in acidic and bile salt conditions comparing to the free cells. Comparatively, single use of wall material (PPE and IN) was more prone to both simulated gastrointestinal conditions than the composite capsules (PPE+GA and IN+GA). Moreover, encapsulated *L. plantarum* was well-conditioned in acidic, bile salt and storage period as compared to free cells. The survival of the probiotic strain was well maintained at an effective level compared to the recommended dosage required for providing health benefits. The current research allowed us to acquire further information on the synbiotic potential for target delivery against different environmental stress. The synbiotic powder (PPE+GA encapsulated *L. plantarum*) can be employed to generate new value-added products and pineapple peel can be utilised as a potential encapsulating element for probiotic strain.

V. ACKNOWLEDGMENT

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