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Encapsulation of probiotic strain *Lactobacillus rhamnosus* GG with black bean extract in alginate-pectin microcapsules

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ABSTRACT

Aims: Probiotics are claimed to confer many health effects upon consumption. However, the survivability of probiotic under the harsh conditions in the gastrointestinal tract has been a challenge. This study aimed to improve the survivability of *Lactobacillus rhamnosus* GG under gastrointestinal condition through co-extrusion microencapsulation and the addition of black bean extract.
Methodology and results: Optimization was carried out on wall material formulation, types of pectin (low and high methoxyl pectin) and alginate: pectin ratio (2:1 and 3:1), and black bean extract concentration (0 to 1% w/v) to produce capsules with desired properties. The effect of *L. rhamnosus* GG microencapsulation with and without black bean extract on its survivability under simulated gastrointestinal conditions was also investigated. The optimal formulation that gives the highest microencapsulation efficiency (86.17%) was low methoxyl pectin, alginate: pectin ratio at 3:1, and 0.5% (w/v) of black bean extract. The inclusion of black bean extract into *L. rhamnosus* GG microencapsulation showed no significant effect (p >0.05) on the capsule diameter, with a mean diameter of 715.44 µm and a high microencapsulation efficiency of 97.4%. The viability of encapsulated *L. rhamnosus* GG increased with black bean extract after 6 h of sequential digestion with the final viable cell count of 12.47 $\log_{10}$ CFU/mL, which meet the minimum requirement of 10^6-10^7 $\log_{10}$ CFU/mL viable cells.
Conclusion, significance and impact of study: The high microencapsulation efficiency and survivability through sequential digestion showed that the optimized encapsulated *L. rhamnosus* GG with black bean extract has the potential to be a value-added ingredient in food application.

Keywords: Microencapsulation, *Lactobacillus rhamnosus* GG, black bean extract, sequential digestion, optimization

INTRODUCTION

Among the different probiotics available in the market, *Lactobacillus* and *Bifidobacterium* are the most common probiotics used in the food industry (Ruiz *et al.*, 2013). *Lactobacillus rhamnosus* GG is a widely studied probiotic strain from the *Lactobacillus* genus. *Lactobacillus rhamnosus* GG was isolated from a healthy adult's fecal sample by two scientists, Sherwood Gorbach and Barry Goldin (Segers and Lebeer, 2014). Besides that, *L. rhamnosus* GG has shown to exert various health benefits such as relieving irritable bowel syndrome, preventing and treating diarrhea incidents, and improving the digestive health (Rosenfeldt *et al.*, 2002; Horvath *et al.*, 2011).

The ability of *L. rhamnosus* GG to tolerate high acidic and high bile salt conditions allows it to survive the harsh conditions in the gastrointestinal tract (Ruiz *et al.*, 2013). However, an adequate amount of probiotics are required to survive, adhere, and colonize the gastrointestinal tract to exert therapeutic health benefits to the host (WHO and FAO, 2002). Hence, probiotic has to overcome the challenge of harsh conditions in gastrointestinal tract such as low pH and bile salt and prolong storage in food matrices to maintain its viability. Therefore, microencapsulation was proposed to protect probiotics (Steenson *et al.*, 1987). Recent microencapsulation technology, such as co-extrusion technique has been adopted to shield probiotic from the outer environment and improve its survival under adverse environments (Chan and Pui, 2020; Lai *et al.*, 2020b; Lai *et al.*, 2020c; Yong *et al.*, 2020).

Alginate combined with pectin is the commonly used wall material combination in microencapsulation.

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Microencapsulation of probiotics in alginate and pectin have been reported to improve their viability in food products and gastrointestinal tract (Chew *et al.*, 2019). Moreover, there has been a growing interest in incorporating prebiotics into microencapsulated probiotics to give a synergic effect (Scholz-Ahrens *et al.*, 2016). Prebiotics are short-chain carbohydrates that are non-digestible by humans and serve as food for probiotics. In additional, they help to improve the survivability of probiotics during storage and when exposure to gastrointestinal conditions (Roberfroid, 2007). Common examples of prebiotics are fructooligosaccharides, inulin, or resistant starch, which are often found in plant sources such as beans (Vargas-Torres *et al.*, 2004). *Phaseolus vulgaricus* L., also referred to as black beans are rich in low digestible carbohydrate, protein, and various phytochemicals such as anthocyanin, saponin, tannin, resins, and steroids (Udeozo and Egbe, 2015; Hernandez *et al.*, 2020). As a result, black bean extract is often reported to exhibit health properties such as anti-oxidant, anti-aging, anti-colon cancer activity, and anti-inflammatory (Hangen and Bennink, 2002; Evangelho *et al.*, 2016; Contreras *et al.*, 2020; Hernandez *et al.*, 2020).

Black beans are legumes that is accessible, cost-efficient and highly nutritious, however it is underutilized and underexplored for its prebiotic potential (Maphosa and Jideani, 2017). To date, this is the first study exploring the prebiotic and protective effect of black bean extract on probiotic strain *L. rhamnosus* GG microencapsulation using co-extrusion technique. Therefore, the formulation of wall materials and black bean extract for *L. rhamnosus* GG microencapsulation was optimized in this study. This study investigated the microencapsulation efficiency and diameter size of *L. rhamnosus* GG microbeads using the co-extrusion method with black bean extract as prebiotic and alginate-pectin as wall materials. The survivability of the encapsulated *L. rhamnosus* GG with black bean extract after subjecting into sequential digestion simulation was also determined.

MATERIALS AND METHODS

Culture preparation

Lactobacillus rhamnosus GG capsules (LactoGG, USA) were purchased from local pharmacy. The probiotic (2 g) were propagated in sterilized De Man Rogosa (MRS) broth (Merck, Germany) using 10% (v/v) inoculum and incubated at 37 °C for 24 h. The multiplied probiotic cells at the exponential growth phase were suspended in sterilized phosphate buffer saline (PBS) prior to centrifugation (3200 × *g* for 15 min at 4 °C, 5840 R, Eppendorf, Germany). The cell pellets were resuspended in 50 mL of PBS and diluted to obtain cell counts of approximately 15 $\log_{10}$ CFU/mL (Siang *et al.*, 2019).

Wall materials and black bean extract preparation

Alginate with low methoxyl pectin was used and the alginate: pectin ratio was fixed at 3:1 throughout the optimization of black bean extract concentration. Different combinations of pectin types and alginate: pectin ratio were prepared as listed in Table 1. Sodium alginate solution (R&M Chemicals, UK) and low or high methoxyl pectin solution (R&M Chemicals, UK) were mixed thoroughly using a dispersing instrument (T25 digital Ultra-Turax, IKA, China) at 9000 × *g* for 5 min. The final mixture solution was sterilized at 121 °C for 15 min and cooled down to room temperature before microencapsulation (Singh *et al.*, 2018).

The black bean extract solution was prepared at 0.5% (w/v) and 1.0% (w/v), where 2.5 g and 5.0 g of black bean powder were added into 500 mL of sterile distilled water, respectively. The solution was homogenized using an ultrasonic machine (WiseClean Ultrasonic Processor, Elma Schmidbauer GmbH, Germany) and filtered using a filter paper. The final solution was sterilized at 121 °C for 15 min and cooled down to room temperature prior to microencapsulation.

Optimization of alginate and pectin

To optimize the concentration of alginate and pectin in *L. rhamnosus* GG microencapsulation, different types and concentration of pectin (high and low methoxyl pectin), and different alginate: pectin ratio (Table 1) without black bean extract were used for the microencapsulation process. The optimum concentration of the alginate-pectin mixture was determined based on the capsule diameter and microencapsulation efficiency (Yee *et al.*, 2019).

Table 1: Optimization parameters of alginate and pectin solution.

Ratio	Alginate % (w/v)	Low pectin % (w/v)	High pectin % (w/v)	Volume of alginate (mL)	Volume of pectin (mL)
4:1	1.5	0.5	0	240	60
4:1	1.5	0	0.5	240	60
3:1	1.5	0.5	0	150	50
3:1	1.5	0	0.5	150	50
2:1	1.5	0.5	0	120	60
2:1	1.5	0	0.5	120	60

Optimization of black bean extract

The concentration of black bean extract in *L. rhamnosus* GG microencapsulation was optimized by testing different concentrations of black bean extract which were 0, 0.5, and 1% (w/v). The type and concentration of pectin and the alginate: pectin ratio was fixed based on the optimum concentration chosen previously (3:1 alginate: pectin ratio, low methoxyl pectin). Capsule diameter and microencapsulation efficiency were used to determine the optimum concentration of black bean extract (Yee *et al.*, 2019).

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Microencapsulation of *L. rhamnosus* GG using co-extrusion technique

Microencapsulation of *L. rhamnosus* GG was carried out with a co-extrusion method using Büchi Encapsulator B-390 (Büchi Labortechnik AG, Switzerland) (Chew *et al.*, 2015). The core fluid (*L. rhamnosus* GG with or without black bean extract) and the shell fluid (sodium alginate with high/ low methoxyl pectin) were transferred into two separate pressurized bottles connected to the Büchi Encapsulator B-390. The core fluid and shell fluid were pumped simultaneously into the concentric nozzles (inner and shell nozzle's diameter were 150 µm and 300 µm, respectively) during microencapsulation. The microencapsulation parameters were air pressure set at 500 mbar, vibration frequency set at 300 Hz, amplitude set at 3, and voltage set at 1.5 kV. The capsules were formed and hardened in 1% (w/v) calcium chloride solution (R&M Chemicals, UK) for 30 min. The capsules were then collected using a nylon sieve and rinsed with distilled water. The capsules were dried using filter paper and stored at 4 °C before further analysis.

Morphology and diameter of capsules

The morphology and diameter of 20 randomly selected *L. rhamnosus* GG capsules were determined and measured using an optical microscope (CX 31, Olympus, Japan) at 10× magnification attached with a stage micrometer (Lai *et al.*, 2020a).

Enumeration of viable cells and microencapsulation efficiency

The encapsulated viable cells were released before the enumeration. One gram of capsules was dissolved in 9 mL of 10% (w/v) trisodium citrate solution and mixed thoroughly using a vortex. Enumeration of encapsulated and free viable cells was determined using the pour plate method (Ng *et al.*, 2019). One milliliter from the mixture of the encapsulated or free viable cell was serially diluted with 9 mL of PBS solution. The diluents (1 mL) were then plated in MRS agar (Merck, Germany) using the pour plate method and incubated at 37 °C for 48 h. The viability of encapsulated and free *L. rhamnosus* GG cells was calculated using Equation 1 and expressed as $\log_{10}$ CFU/mL. Microencapsulation efficacy was calculated using Equation 2.

$$\text{Colony} - \text{forming units (CFU/mL)} = \frac{\text{number of colonies formed}}{\text{dilution factor} \times \text{volume plated (mL)}} \quad (1)$$

$$\text{Microencapsulation efficiency (\%)} = \frac{\text{Log N}}{\text{Log N}_0} \times 100\% \quad (2)$$

Where, N is the number of viable counts of cells released from microbeads, and N_0 is the number of viable counts in free cells.

Sequential digestion

The sequential digestion was carried out to determine the viability of encapsulated and free *L. rhamnosus* GG cells under the simulated digestive system (Siang *et al.*, 2019). Simulated gastric juice (SGJ) was prepared through the mixture of 3.5 mL of 0.1 M hydrochloric acid (Merck, Germany) and 1 g of sodium chloride (R&M chemicals, UK) in 500 mL of distilled water and the addition of 1.6 g pepsin (HmbG, Germany). While simulated intestinal juice (SIJ) was prepared by dissolving 3.4 g of potassium dihydrogen phosphate (Bendosen, Germany) in 125 mL of distilled water and 95 mL of 0.1 M sodium hydroxide with the addition of 3 g bile salt and 1 g pancreatin (Chemsoln, India).

One gram of encapsulated or 1 mL of free cells were added into 9 mL of sterilized SGJ and both mixtures were separately treated throughout the sequential digestion. The mixtures were mixed gently at 150 rpm and incubated at 37 °C for 0 h, 1 h, and 2 h. The cells were then subjected to centrifugation at 5000 × *g* for 5 min to remove the SGJ. The cells were adjusted to pH 6.8 using 1.0 M sodium hydroxide (Merck, Germany) to inactivate the pepsin. The cells were then added into 9 mL of SIJ and the mixture was incubated in a shaking water bath (150 rpm, 37 °C) for 4 h. The cells were then centrifuged at 5000 × *g* for 5 min to remove the SIJ. The encapsulated cells were then filtered and washed with sterilized PBS solution to further remove SGJ and SIJ. The enumeration of encapsulated or free viable cells after being subjected to the simulated digestive system was determined by pour plate method using MRS agar and incubated at 37 °C for 48 h. The survivability of encapsulated or free viable cells after being subjected to the *in vitro* enzyme digestion was calculated using Equation 3.

$$\text{Survivability (\%)} = \frac{\text{Log N}}{\text{Log N}_0} \times 100\% \quad (3)$$

Where, N is the number of viable cells after the stated time, and N_0 is the number of initial viable cells.

Statistical analysis

The experiments were carried out in triplicate (n = 3) and the data were presented in mean ± standard deviation. The results were analyzed using MINITAB statistical software (version 17) (Minitab Inc., USA). Independent t-test, one-way analysis of variance (ANOVA), and Tukey's post hoc test were performed to compare the significant difference between the mean values, where the data is considered as statistically significant when $p \leq 0.05$.

RESULTS

Optimization of alginate and pectin as wall materials

The formulation for the microencapsulation process of *L. rhamnosus* GG was optimized in order to obtain the

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optimal wall material combination. The effects of different pectin types and ratios of alginate to pectin on the microencapsulation efficiency and capsules diameter of the encapsulated *L. rhamnosus* GG are shown in Table 2.

The concentration of alginate and pectin at 3:1 ratio showed higher in microencapsulation efficiency (86.17% and 61.43%) than 2:1 ratio (52.17% and 49.14%) for low and high pectin, respectively. Low methoxyl pectin showed higher microencapsulation efficiency (86.17% and 52.17%) in comparison to high methoxyl pectin (61.43% and 49.14%) for 3:1 and 2:1 ratio, respectively. In terms of capsules diameter, alginate with low methoxyl pectin at 2:1 ratio showed 15.5-23.5% larger diameter in capsules as compared to the other wall material combination. The wall material combination with low methoxyl pectin produced a larger diameter in capsules than the wall material combination with high methoxyl pectin. As the alginate with low methoxyl pectin at the ratio of 3:1 showed the highest microencapsulation efficiency in encapsulating *L. rhamnosus* GG, this combination was chosen to be used for *L. rhamnosus* GG microencapsulation.

Table 2: Microencapsulation efficiency and average diameter of capsules encapsulated with *L. rhamnosus* GG using different types of pectin and different alginate: pectin ratio.

Wall materials	Alginate: pectin ratio	Microencapsulation efficiency (%)	Diameter of capsules (μm)
Alginate and low pectin	3:1	86.17 ± 0.31[a]	755.00 ± 26.34[b]
Alginate and high pectin	3:1	61.43 ± 0.58[b]	705.83 ± 55.58[b]
Alginate and low pectin	2:1	52.17 ± 0.25[c]	871.67 ± 47.06[a]
Alginate and high pectin	2:1	49.14 ± 0.23[d]	722.50 ± 30.31[b]

Data are expressed as means ± standard deviation (n= 3). Different lowercase letters in the same column represent significantly different ($p \leq 0.05$) via one-way ANOVA and Tukey's test as post-hoc test.

Optimization of black bean extract

The formulation for the microencapsulation of *L. rhamnosus* GG was then optimized with black bean extract combination. The effect of different black bean extract concentration on the microencapsulation efficiency and capsules diameter of encapsulated *L. rhamnosus* GG was shown in Table 3.

The absence of black bean extract 0% (w/v) functions as control. The encapsulated *L. rhamnosus* GG with 0.5% (w/v) and 1.0% (w/v) black bean extract (98.25% and 98.26%, respectively; $p > 0.05$) showed 15.3% higher in microencapsulation efficiency compared to the encapsulated *L. rhamnosus* GG without the black bean extract (85.22%; $p \leq 0.05$). There was also no difference ($p > 0.05$) in the diameter of capsules among encapsulated *L. rhamnosus* GG with 0, 0.5, and 1.0% (w/v) black bean extract. Hence, due to economical consideration, alginate with low methoxyl pectin at 3:1 ratio with 0.5% (w/v) black bean extract was used to microencapsulate *L. rhamnosus* GG for further analysis.

Table 3: Microencapsulation efficiency and average diameter of capsules encapsulated with *L. rhamnosus* GG and different concentration of black bean extract.

Wall material	Black bean extract % (w/v)	Microencapsulation efficiency (%)	Diameter of capsules (μm)
Alginate-low pectin (3:1)	0	85.22 ± 0.43[b]	732.05 ± 23.52[a]
	0.5	98.25 ± 0.32[a]	711.67 ± 16.07[a]
	1.0	98.26 ± 0.76[a]	671.67 ± 18.76[a]

Morphology, diameter, and microencapsulation efficiency of optimized encapsulated *L. rhamnosus* GG

The morphology of the optimized encapsulated *L. rhamnosus* GG are shown in Figure 1. The diameter and microencapsulation efficiency of the optimized encapsulated *L. rhamnosus* GG with and without black bean extract are shown in Table 4. The capsule appeared white in color and surrounded by a membrane layer in a spherical and uniform manner. There was no difference ($p > 0.05$) in diameter between the optimized encapsulated *L. rhamnosus* GG with and without black bean extract. However, the microencapsulation efficiency of encapsulated *L. rhamnosus* GG with black bean extract was higher than the microencapsulation efficiency of encapsulated *L. rhamnosus* GG without black bean extract (97.39% and 86.36% respectively; $p \leq 0.05$).

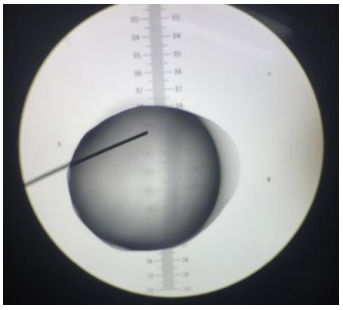

Figure 1: Optical microscope view of encapsulated *L. rhamnosus* GG with alginate- low pectin and black bean extract.

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Table 4: Microencapsulation efficiency (%) and average diameter of capsules encapsulated with *L. rhamnosus* GG with and without black bean extract.

Wall material	Prebiotic	Microencapsulation efficiency (%)	Diameter of capsules (μm)
Alginate – low pectin (3:1)	Without black bean extract	86.36 ± 0.37[b]	726.13 ± 25.31[a]
Alginate – low pectin (3:1)	With black bean extract	97.39 ± 0.24[a]	715.44 ± 13.05[a]

Data are expressed as means ± standard deviation (n= 3).
[ab] in the same column are significantly different ($p \leq 0.05$) via independent T-test.

Viability and survivability of free cells, encapsulated *L. rhamnosus* GG with and without black bean extract under the simulated gastrointestinal digestion

The viability and survivability of free and encapsulated cells after being subjected to SGJ (pH 2.0) and SIJ (pH 7.4) are shown in Table 5. The incubation time in both SGJ and SIJ (2 and 4 h, respectively) was fixed according to estimated human physiology's gastrointestinal emptying time, which is largely dependent on the type of food intake.

Although the viability of free and encapsulated cells remained at least 14 $\log_{10}$ CFU/mL after being incubated in SGJ for 2 h, both were found to decrease due to exposure to digestive enzymes *in vitro* (Table 5). The former decreased 1.6 $\log_{10}$ CFU/mL, whereas the latter showed a lesser reduction of 0.9 and 0.8 $\log_{10}$ CFU/mL for *L. rhamnosus* GG cells with and without black bean extract ($p \leq 0.05$), respectively. Thus, encapsulation with and without black bean extract resulted in higher survivability than unencapsulated cells.

Incubation in SIJ for 4 h caused a reduction in viable cells from approximately 14 to 10-12 $\log_{10}$ CFU/mL. Unencapsulated *L. rhamnosus* GG cells was reduced by 4.4 $\log_{10}$ CFU/mL, whereas encapsulated *L. rhamnosus* GG with and without black bean extract was reduced by 2.2 and 4.2 $\log_{10}$ CFU/mL, respectively. This equates to 22.9-24.3% higher viability in encapsulated cells than that in unencapsulated cells after being incubated in intestinal digestive juice *in vitro*. Furthermore, encapsulated *L. rhamnosus* GG with black bean extract showed higher survivability in comparison to both free cells and encapsulated *L. rhamnosus* GG without black bean extract after incubated in SIJ for 4 h by 17.4% and 13.1%, respectively ($p \leq 0.05$). Despite being exposed to the sequential *in vitro* enzyme digestion, the viable cell counts of unencapsulated and encapsulated *L. rhamnosus* GG cells with and without black bean extract were at least 10 $\log_{10}$ CFU/mL.

Table 5: Viable count and survivability of free and encapsulated *L. rhamnosus* GG cells with or without black bean extract under sequential digestion.

Sequential digestion	Time (h)	Viable count of *L. rhamnosus* GG ($\log_{10}$ CFU/mL)			Survivability of *L. rhamnosus* GG (%)		
		Free cell	Encapsulated *L. rhamnosus* GG	Encapsulated *L. rhamnosus* GG with black bean extract	Free cell	Encapsulated *L. rhamnosus* GG	Encapsulated *L. rhamnosus* GG with black bean extract
SGJ (pH 2.0)	0th	16.05 ± 0.01[a]	15.20 ± 0.09[a]	15.61 ± 0.05[a]	100.00 ± 0.00[A]	100.00 ± 0.00[A]	100.00 ± 0.00[A]
	2nd	14.47 ± 0.05[b]	14.39 ± 0.10[b]	14.70 ± 0.11[b]	90.16 ± 0.38[B]	94.68 ± 1.17[A]	94.14 ± 0.80[A]
SIJ (pH 7.4)	6th	10.03 ± 0.09[c]	10.15 ± 0.15[c]	12.47 ± 0.07[c]	62.49 ± 0.51[C]	66.77 ± 0.75[B]	79.88 ± 0.71[A]

Data are expressed as means ± standard deviation.
[abc] in the same column for viable count of LGG followed by different lowercase letters are significantly different ($p \leq 0.05$) via one-way ANOVA and Tukey's test as post-hoc test.
[ABC] in the same row for survivability of LGG followed by different uppercase letters are significantly different ($p \leq 0.05$) via one-way ANOVA and Tukey's test as post-hoc test.

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DISCUSSION

Initial studies were carried out to find optimum encapsulation (alginate and pectin) and prebiotic (black bean extract) materials for high cell microencapsulation efficiency. Alginate is often incorporated in higher amount in alginate and pectin mixture because the former consists of more galacturonate blocks than pectin. The addition of pectin to alginate formulation provides advantages such as improving the core substance's retention and nutritional value to the capsules (Chew and Nyam, 2016).

High microencapsulation efficiency when alginate is used in high concentration is in agreement with the results reported by Shi *et al.* (2013). Microencapsulation of *Lactobacillus acidophilus* in varying proportion of alginate and calcium carbonate concentration also showed that alginate had a positive effect on the encapsulation efficiency (Lotfipour *et al.*, 2012). The high number of guluronate and galacturonate blocks in alginate presents a high number of binding sites for calcium ions which resulted in a denser gel structure form which provides better protection for the probiotic through better entrapment (Narsaiah *et al.*, 2014).

Low methoxyl pectin showed higher microencapsulation efficiency as compared to high methoxyl pectin (Table 2). Mamet *et al.* (2017) suggested that high methoxyl pectin was only able to form a gel with the presence of a high concentration of sugar at approximately 60% in an acidic medium of pH 2.5–3.4. On the other hand, low methoxyl pectin was able to form a gel when the calcium ions are present as calcium ions were able to form a bond between carboxyl groups of pectin molecules. Hence, low methoxyl pectin was able to give high efficiency in encapsulating probiotic which was in line with the results of the present study. In addition, other components in the medium could also affect the pectin properties such as alginate and black bean extract in the present study as suggested by Evageliou *et al.* (2000) and Mamet *et al.* (2017).

The diameter of capsules is crucial in the microencapsulation process because large microcapsules (> 1000 μm) may have a negative impact on the texture or mouthfeel of the food product upon consumption (Champagne and Fustier, 2007; Nag *et al.*, 2011). The diameter of capsules in the present study is acceptable as it is within the general range of microcapsules between 1 to 1000 μm (Lengyel *et al.*, 2019) (Table 4). Correâ-Filho *et al.* (2019) used arabic gum ranging from 5 to 35% for the microencapsulation of β-carotene and found that the concentration of wall material affected the diameter of capsules. Carneiro *et al.* (2013) also utilizes different combination of wall materials (maltodextrin, Arabic gum, whey protein concentrate, and modified starch) for the encapsulation of flaxseed oil and resulted in different microcapsule's sizes. These suggested that different wall material concentrations and types could result in different capsule diameter which was in line with the finding of the present study (Table 2).

The type and concentration of prebiotic had no impact on the capsules' diameter (Krasaekoopt and Watcharapoka 2014) and this is consistent with the findings of the present study. However, the capsule's diameter reported by Krasaekoopt and Watcharapoka (2014) was 1.90-1.92 mm, which was larger than the capsule diameter in the present study (0.67-0.73 mm). This could be due to the use of different microencapsulation technique (Shi *et al.*, 2013). Capsules that are uniform with an intact surface is desirable. The surface of the capsules is crucial in determining the viability for the encapsulated cells as intact capsules surface protect probiotic from the outer environment, while broken capsules surface usually resulted in low probiotic survivability (Yee *et al.*, 2019). Hence, the spherical and uniform capsules produced in the present study suggests that the wall materials and co-extrusion technique were able to protect *L. rhamnosus* GG cells from the external environment.

The addition of black bean extract in the encapsulation of *L. rhamnosus* GG had improved the microencapsulation efficiency in the present study. This is in agreement with the findings by Khorasani and Shojaosadati (2017) and Sathyabama *et al.* (2014), and Vasile *et al.* (2020) who reported increase in microencapsulation efficiency by incorporating various prebiotics such as nanochitin, nanolignocellulose, sugarbeet, chicory, and black bean into probiotic microencapsulation through freeze-drying or extrusion. Although many commercial and emerging prebiotics have shown to improve viability of encapsulated probiotics, the effect of prebiotic on probiotic is strain-specific (Succi *et al.*, 2017). Prebiotic such as chitin has been reported to inhibit probiotic *L. rhamnosus* GG due to its antimicrobial properties (Selenius *et al.*, 2018). Hence, this shows the symbiotic relationship between black bean extract and *L. rhamnosus* GG strain in the present study. Furthermore, the low glycemic index from black beans allows individual with type 2 diabetes or insulin resistance to have a better control with their glycemic response (Winham *et al.*, 2017); while other prebiotics such as pectin from sugarbeet or potato pulp has proved to have no impact (Schwab *et al.*, 2006; Lærke *et al.*, 2007). Therefore, this allows black bean extracts to be a value-added ingredient to the probiotic microbeads.

Sequential digestion is defined as a continuous incubation process of bacteria in gastric and intestinal environments (Podsędek *et al.*, 2014). In order for probiotics to exert health effects on the host, probiotics must survive through the gastrointestinal tract and maintained viability at high levels of more than 10^7 CFU/g in the gastrointestinal (Kailasapathy, 2002). Chávarri *et al.* (2010) reported that free cells of *Bifidobacterium bifidum* and *Lactobacillus gasseri* showed a significant reduction to less than 1 log CFU/mL at the 1st and 2nd h of SGJ incubation; while encapsulated *B. bifidum* and *L. gasseri* remained viable count of at least 6.85 log CFU/mL at the end of the SGJ incubation. Besides, Gandomi *et al.* (2016) also found that more than 80% of encapsulated *L. rhamnosus* GG survived after SGJ incubation, while less

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than 10% of free *L. rhamnosus* GG cells survived after SGJ incubation, which supports the findings of the present study.

The addition of black bean extract into *L. rhamnosus* GG microencapsulation had no effect on *L. rhamnosus* GG survivability during SGJ incubation. This result was in line with Gandomi *et al.* (2016) where the encapsulated *L. rhamnosus* GG with and without prebiotic (inulin) showed no significant difference (p >0.05) during SGJ incubation. Besides, Zhao *et al.* (2019) subjected black bean extract to low pH incubation (pH 1 to 3) for 8 h and evaluated lectin protein using SDS-PAGE. They found that extracted protein was still present at the end of the incubation, although the intensity of the band on SDS-PAGE decreased over incubation time. This shows that black bean extract is sensitive under a low pH environment which could have resulted in reduced potency as a prebiotic under SGJ incubation.

The encapsulated *L. rhamnosus* GG showed high survivability than free *L. rhamnosus* GG cells in SIJ incubation. This result was in line with Wang *et al.* (2015) which demonstrated that the encapsulation of *Bifidobacterium adolescentis* improved cells viability in SIJ conditions as compared to free cells. We showed black bean extract resulted in higher survivability in encapsulated cells (Table 5) than in free cells after SGJ incubation. The black bean extract may thus impart crucial prebiotic properties either physically or nutritionally or both to enhance the survivability of *L. rhamnosus* GG under a harsh pH and enzymatic environment. This was also noted in previous studies using prebiotic Hi-Maize starch, mannitol and oligofructose (Iyer and Kailasapathy, 2005; Ng *et al.*, 2019; Yee *et al.*, 2019), which are consistent with our findings.

Free *L. rhamnosus* GG cells retained high viability after sequential in vitro gastrointestinal enzyme digestion and met the minimum required cell count of more than 10^7 CFU/g (Table 5) to qualify as probiotic (Iyer and Kailasapathy, 2005) at the end of the digestion. This is in agreement with Gandomi *et al.* (2016), where the *L. rhamnosus* GG was able to survive under physiological stressful environments such as low pH and high bile salt. The present study has demonstrated black bean extract may be used as a prebiotic additive to increase cells viability. Black bean extracts are high in resistant starch, dietary fiber, phytochemicals, and oligosaccharides (Hernández-Salazar *et al.*, 2010; Udeozo and Egbe, 2015; Monk *et al.*, 2017; Hernandez *et al.*, 2020) that could be utilized by *L. rhamnosus* GG as carbon sources for growth (Silva-Cristobal *et al.*, 2010; Fabbri *et al.*, 2016). This is in line with the findings by Rengadu *et al.* (2020), where resistant starch extracted from cowpea beans stimulated growth of various probiotic strains (*Bifidobacteria animalis* and *Lactobacillus casei*). Thus, *L. rhamnosus* GG microencapsulation may be further enhanced using substances such as black bean extract with prebiotic properties throughout production, storage, and finally during the digestive tract transit.

CONCLUSION

The best formulation to microencapsulate *L. rhamnosus* GG that gives the highest microencapsulation efficiency is 3:1 alginate: low methoxyl pectin ratio with 0.5% (w/v) black bean extract. The encapsulated *L. rhamnosus* GG with black bean extract had a viable cell count of 12.47 $\log_{10}$ CFU/mL after sequential digestion, hence meet the minimum requirement of 10^6-10^7 log10 CFU/mL probiotic cell count to exert health benefits to the host. This study shows that black bean extract has the potential to be used as prebiotic for encapsulation of *L. rhamnosus* GG. Future studies should be carried out to explore the stability of encapsulated *L. rhamnosus* GG with black bean extract during production, storage, and in food product.

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REFERENCES

Carneiro, H. C. F., Tonon, R. V., Grosso, C. R. F. and Hubinger, M. D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering* **115(4), 443-451.**

Champagne, C. P. and Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. *Current Opinion in Biotechnology* **18(2), 184-190.**

Chan, L. Y. and Pui, L. P. (2020). Microencapsulation of *Lactobacillus acidophilus* 5 with isomalto-oligosaccharides. *Carpathian Journal of Food Science and Technology* **12(2), 26-36.**

Chávarri, M., Marañón, I., Ares, R., Ibáñez, F. C., Marzo, F. and Villarán, M. del C. (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *International Journal of Food Microbiology* **142(1-2), 185-189.**

Chew, S. C. and Nyam, K. L. (2016). Microencapsulation of kenaf seed oil by co-extrusion technology. *Journal of Food Engineering* **175, 43-50.**

Chew, S. C., Tan, C. H., Pui, L. P., Chong, P. N., Gunasekaran, B. and Lin, N. K. (2019). Encapsulation technologies: A tool for functional foods development. *International Journal of Innovative Technology and Exploring Engineering* **8(5s), 154-160.**

Chew, S. C., Tan, C. P., Long, K. and Nyam, K. L. (2015). *In-vitro* evaluation of kenaf seed oil in chitosan coated-high methoxyl pectin-alginate microcapsules. *Industrial Crops and Products* **76, 230-236.**

Contreras, J., Herrera-González, A., Arrizon, J., Lugo-Cervantes, E. and Mojica, L. (2020). Mexican

endemic black bean phenolic extract antioxidant and anti-inflammatory potential. *Current Developments in Nutrition* **4(Supplement_2), 382-382.**

Correâ-Filho, L. C., Lourenço, M. M., Moldaõ-Martins, M. and Alves, V. D. (2019). Microencapsulation of β-carotene by spray drying: Effect of wall material concentration and drying inlet temperature. *International Journal of Food Science* **2019, Article ID 8914852.**

Evageliou, V., Richardson, R. K. and Morris, E. R. (2000). Effect of pH, sugar type and thermal annealing on high-methoxy pectin gels. *Carbohydrate Polymers* **42(3), 245-259.**

Evangelho, J. A. do, Berrios, J. de J., Pinto, V. Z., Antunes, M. D., Vanier, N. L. and Zavareze, E. da R. (2016). Antioxidant activity of black bean (*Phaseolus vulgaris* L.) protein hydrolysates. *Food Science and Technology* **36, 23-27.**

Fabbri, A. D. T., Schacht, R. W. and Crosby, G. A. (2016). Evaluation of resistant starch content of cooked black beans, pinto beans, and chickpeas. *NFS Journal* **3, 8-12.**

Gandomi, H., Abbaszadeh, S., Misaghi, A., Bokaie, S. and Noori, N. (2016). Effect of chitosan-alginate encapsulation with inulin on survival of *Lactobacillus rhamnosus* GG during apple juice storage and under simulated gastrointestinal conditions. *LWT - Food Science and Technology* **69, 365-371.**

Hangen, L. and Bennink, M. R. (2002). Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. *Nutrition and Cancer* **44(1), 60-65.**

Hernández-Salazar, M., Osorio-Diaz, P., Loarca-Piña, G., Reynoso-Camacho, R., Tovar, J. and Bello-Pérez, L. A. (2010). *In vitro* fermentability and antioxidant capacity of the indigestible fraction of cooked black beans (*Phaseolus vulgaris* L.), lentils (*Lens culinaris* L.) and chickpeas (*Cicer arietinum* L.). *Journal of the Science of Food and Agriculture* **90(9), 1417-1422.**

Hernandez, D. F., Orozco-Avila, I., Lugo-Cervantes, E. and Mojica, L. (2020). Black bean (*Phaseolus vulgaris* L.) phenolic extract exhibits antioxidant and anti-aging potential. *Current Developments in Nutrition* **4(Supplement_2), 24.**

Horvath, A., Dziechciarz, P. and Szajewska, H. (2011). Meta-analysis: *Lactobacillus rhamnosus* GG for abdominal pain-related functional gastrointestinal disorders in childhood. *Alimentary Pharmacology and Therapeutics* **33(12), 1302-1310.**

Iyer, C. and Kailasapathy, K. (2005). Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria under *in vitro* acidic and bile salt conditions and in yogurt. *Journal of Food Science* **70(1), M18-M23.**

Kailasapathy, K. (2002). Microencapsulation of probiotic bacteria: Technology and potential applications. *Current Issues in Intestinal Microbiology* **3, 39-48.**

Khorasani, A. C. and Shojaosadati, S. A. (2017). Improvement of probiotic survival in fruit juice and under gastrointestinal conditions using pectin-nanochitin-nanolignocellulose as a novel prebiotic gastrointestinal-resistant matrix. *Applied Food Biotechnology* **4(3), 179-191.**

Krasaekoopt, W. and Watcharapoka, S. (2014). Effect of addition of inulin and galactooligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. *LWT- Food Science and Technology* **57(2), 761-766.**

Lærke, H. N., Meyer, A. S., Kaack, K. V. and Larsen, T. (2007). Soluble fiber extracted from potato pulp is highly fermentable but has no effect on risk markers of diabetes and cardiovascular disease in Goto-Kakizaki rats. *Nutrition Research* **27(3), 152-160.**

Lai, J. T., Lai, K. W., Zhu, L. Y., Nyam, K. L. and Pui, L. P. (2020a). Microencapsulation of *Lactobacillus plantarum* 299v and its storage in kuini juice. *Malaysian Journal of Microbiology* **16(4), 235-244.**

Lai, K. W., How, Y. H. and Pui, L. P. (2020b). Storage stability of microencapsulated *Lactobacillus rhamnosus* GG in hawthorn berry tea with flaxseed mucilage. *Journal of Food Processing and Preservation* **44(12), e14965.**

Lai, K., How, Y. and Pui, L. (2020c). Microencapsulation of *Lactobacillus rhamnosus* GG with flaxseed mucilage using co-extrusion technique. *Journal of Microencapsulation* **(2020), 1-45.**

Lengyel, M., Kállai-Szabó, N., Antal, V., Laki, A. J. and Antal, I. (2019). Microparticles, microspheres, and microcapsules for advanced drug delivery. *Scientia Pharmaceutica* **87(20), 1-31.**

Lotfipour, F., Mirzaeei, S. and Maghsoodi, M. (2012). Evaluation of the effect of $CaCl_2$ and alginate concentrations and hardening time on the characteristics of *Lactobacillus acidophilus* loaded alginate beads using response surface analysis. *Advanced Pharmaceutical Bulletin* **2(1), 71-78.**

Mamet, T., Yao, F., Li, K.-k. and Li, C.-m. (2017). Persimmon tannins enhance the gel properties of high and low methoxyl pectin. *LWT- Food Science and Technology* **86, 594-602.**

Maphosa, Y. and Jideani, V. A. (2017). The role of legumes in human nutrition. *In:* Functional Food - Improve Health through Adequate Food. Heuda M. C. (ed.). Intech, London, United Kingdom. **pp. 103-121.**

Monk, J. M., Lepp, D., Wu, W., Pauls, K. P., Robinson, L. E. and Power, K. A. (2017). Navy and black bean supplementation primes the colonic mucosal microenvironment to improve gut health. *Journal of Nutritional Biochemistry* **49, 89-100.**

Nag, A., Han, K. S. and Singh, H. (2011). Microencapsulation of probiotic bacteria using pH-induced gelation of sodium caseinate and gellan gum. *International Dairy Journal* **21(4), 247-253.**

Narsaiah, K., Jha, S. N., Wilson, R. A., Mandge, H. M. and Manikantan, M. R. (2014). Optimizing microencapsulation of nisin with sodium alginate and guar gum. *Journal of Food Science and Technology* **51(12), 4054-4059.**

Ng, S. L., Lai, K. W., Nyam, K. L. and Pui, L. P. (2019). Microencapsulation of *Lactobacillus plantarum* 299v incorporated with oligofructose in chitosan coated-alginate beads and its storage stability in ambarella juice. *Malaysian Journal of Microbiology* **15(5), 408-418.**

Podsędek, A., Redzynia, M., Klewicka, E. and Koziołkiewicz, M. (2014). Matrix effects on the stability and antioxidant activity of red cabbage anthocyanins under simulated gastrointestinal digestion. *BioMed Research International* **2014, Article ID 365738.**

Rengadu, D., Gerrano, A. S. and Mellem, J. J. (2020). Prebiotic effect of resistant starch from *Vigna unguiculata* (L.) Walp. (cowpea) using an *in vitro* simulated digestion model. *International Journal of Food Science and Technology* **55(1), 332-339.**

Roberfroid, M. (2007). Prebiotics: The concept revisited. *The Journal of Nutrition* **137(3), 830S-837S.**

Rosenfeldt, V., Michaelsen, K. F., Jakobsen, M., Larsen, C. N., Møller, P. L., Pedersen, P., Tvede, M., Weyrehter, H., Valerius, N. H. and Pærregaard, A. (2002). Effect of probiotic *Lactobacillus* strains in young children hospitalized with acute diarrhea. *Pediatric Infectious Disease Journal* **21(5), 411-416.**

Ruiz, L., Margolles, A. and Sánchez, B. (2013). Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. *Frontiers in Microbiology* **4, 1-8.**

Sathyabama, S., Ranjith kumar, M., Bruntha devi, P., Vijayabharathi, R. and Brindha priyadharisini, V. (2014). Co-encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment. *LWT- Food Science and Technology* **57(1), 419-425.**

Scholz-Ahrens, K. E., Adolphi, B., Rochat, F., Barclay, D. V., de Vrese, M., Açil, Y. and Schrezenmeir, J. (2016). Effects of probiotics, prebiotics, and synbiotics on mineral metabolism in ovariectomized rats- impact of bacterial mass, intestinal absorptive area and reduction of bone turn-over. *Journal of the Society of Nutrition and Food Science* **3, 41-50.**

Schwab, U., Louheranta, A., Törrönen, A. and Uusitupa, M. (2006). Impact of sugar beet pectin and polydextrose on fasting and postprandial glycemia and fasting concentrations of serum total and lipoprotein lipids in middle-aged subjects with abnormal glucose metabolism. *European Journal of Clinical Nutrition* **60(9), 1073-1080.**

Segers, M. E. and Lebeer, S. (2014). Towards a better understanding of *Lactobacillus rhamnosus* GG - host interactions. *Microbial Cell Factories* **13(Suppl 1), S7.**

Selenius, O. V. O., Korpela, J., Salminen, S. and Gallego, C. G. (2018). Effect of chitin and chitooligosaccharide on in vitro growth of *Lactobacillus rhamnosus* GG and *Escherichia coli* TG. *Applied Food Biotechnology* **5(3), 163-172.**

Shi, L., Li, Z., Li, D., Xu, M., Chen, H., Zhang, Z. and Tang, Z. (2013). Encapsulation of probiotic *Lactobacillus bulgaricus* in alginate – milk microspheres and evaluation of the survival in simulated gastrointestinal conditions. *Journal of Food Engineering* **117(1), 99-104.**

Siang, S. C., Wai, L. K., Lin, N. K. and Phing, P. L. (2019). Effect of added prebiotic (isomalto-oligosaccharide) and coating of beads on the survival of microencapsulated *Lactobacillus rhamnosus* GG. *Food Science and Technology* **39, 601-609.**

Silva-Cristobal, L., Osorio-Díaz, P., Tovar, J. and Bello-Pérez, L. A. (2010). Chemical composition, carbohydrate digestibility, and antioxidant capacity of cooked black bean, chickpea, and lentil Mexican varieties. *CYTA - Journal of Food* **8(1), 7-14.**

Singh, J., Kaur, K. and Kumar, P. (2018). Optimizing microencapsulation of α-tocopherol with pectin and sodium alginate. *Journal of Food Science and Technology* **55(9), 3625-3631.**

Steenson, L. R., Klaenhammer, T. R. and Swaisgood, H. E. (1987). Calcium alginate-immobilized cultures of lactic *Streptococci* are protected from bacteriophages. *Journal of Dairy Science* **70(6), 1121-1127.**

Succi, M., Tremonte, P., Pannella, G., Tipaldi, L., Cozzolino, A., Romaniello, R., Sorrentino, E. and Coppola, R. (2017). Pre-cultivation with selected prebiotics enhances the survival and the stress response of *Lactobacillus rhamnosus* strains in simulated gastrointestinal transit. *Frontiers in Microbiology* **8, 1-11.**

Udeozo, I. P. and Egbe, D. E. (2015). Phytochemical and proximate analysis of black turtle beans (*Phaseolus vulgaris*). *African Journal of Basic and Applied Sciences* **7(2), 88-90.**

Vargas-Torres, A., Osorio-Díaz, P., Islas-Hernández, J. J., Tovar, J., Paredes-Lopez, O. and Bello-Perez, L. A. (2004). Starch digestibility of five cooked black bean (*Phaseolus vulgaris* L.) varieties. *Journal of Food Composition and Analysis* **17(5), 605-612.**

Vasile, M. A., Milea, Ş. A., Enachi, E., Barbu, V., Cîrciumaru, A., Bahrim, G. E., Râpeanu, G. and Stănciuc, N. (2020). Functional enhancement of bioactives from black beans and lactic acid bacteria into an innovative food ingredient by comicroencapsulation. *Food and Bioprocess Technology* **13(6), 978-987.**

Wang, J., Korber, D. R., Low, N. H. and Nickerson, M. T. (2015). Encapsulation of *Bifidobacterium adolescentis* cells with legume proteins and survival under stimulated gastric conditions and during storage in commercial fruit juices. *Food Science and Biotechnology* **24(2), 383-391.**

Winham, D. M., Hutchins, A. M. and Thompson, S. V. (2017). Glycemic response to black beans and chickpeas as part of a rice meal: A randomized cross-over trial. *Nutrients* **9(10), 1-12.**

World Health Organization (WHO) and Food and Agriculture Organization of the United Nation (FAO) (2002). Guidelines for the evaluation of probiotics in food. London Ontario, Canada. **pp. 1-11.**

Yee, W. L., Yee, C. L., Lin, N. K. and Phing, P. L. (2019). Microencapsulation of *Lactobacillus acidophilus NCFM* incorporated with mannitol and its

storage stability in mulberry tea. *Ciência e Agrotecnologia* **43, e005819.**

Yong, A. K. L., Lai, K. W., Ghazali, H. M., Chang, L. S. and Pui, L. P. (2020). Microencapsulation of *Bifidobacterium animalis* subsp. *lactis BB-12* with mannitol. *Asia-Pacific Journal of Molecular Biology and Biotechnology* **28(2), 32-42.**

Zhao, J., He, S., Tang, M., Sun, X., Zhang, Z., Ye, Y., Cao, X. and Sun, H. (2019). Low-pH induced structural changes, allergenicity and *in vitro* digestibility of lectin from black turtle bean (*Phaseolus vulgaris* L.). *Food Chemistry* **283, 183-190.**

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