Development and Characterization of Pectin-Based Colon-Targeted Pellets Containing *Lactobacillus Plantarum* FNCC-0461

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Article Info	ABSTRACT
	Lactobacillus plantarum FNCC-0461 is a strain of lactic acid bacteria derived from "*dadih*", a traditional Indonesian food, with significant potential as a probiotic. Particularly, probiotics provide health benefits once a minimum cell viability of 7 log CFU is maintained in the distal ileum and colon. However, most of these live microorganisms are not resistant to harsh conditions of the gastrointestinal tract (GIT). Encapsulating probiotics in the form of pectin-based colon-targeted pellets offers a promising solution to enhance viability in the presence of extreme GIT conditions while facilitating release to a specific site in the colon. In addition, the pellets were produced through the extrusion-spheronization method using microcrystalline cellulose (MCC), lactose, and pectin. Optimizations were conducted by varying the spheronization speed and time, as well as adjusting the total pectin concentration and the type of coating polymer (cellulose acetate phthalate (CAP) or shellac) used for the pellet formulation. Subsequently, the morphology, particle size, moisture content, micromeritic properties, process yield, and viability of pellets were evaluated. The release of probiotics from coated and uncoated pellets was examined under simulated colon fluid conditions at pH 1.2, 6.8, and 7.4 for 24 h. The formulation containing MCC, lactose, and pectin in a ratio of 5:4:1, with a spheronization speed of 1500 rpm for 15 min, showed the best pellet characteristics and cell viability. The prepared pellets were spherical, with a particle size distribution of 0.914 ± 0.008 mm, a process yield of 88.71 ± 1.04 %, and viability of 7.50×10^7 cfu/g. Those coated with CAP had the highest probiotic release in simulated colon fluid, reaching 1.38×10^7 CFU/g at 24 h. This research proved that the CAP-coated pellet formulation indicated promising potential for colon-targeted delivery of *L. plantarum,* offering a means to protect probiotics viability. **Keywords:** *Lactobacillus plantarum* FNCC-0461, colon targeted delivery system, pellets, pectin, extrusion-spheronization

INTRODUCTION

Probiotics are live microorganisms that improve intestinal microflora balance, inhibit pathogen growth, and stimulate the immune system when present in adequate amounts in humans (Qin *et al*., 2021; Zheng *et al*., 2017). For instance, *L. plantarum* FNCC-0461 isolated from "*dadih*", a traditional Indonesian food produced from the fermented milk of buffalo, exhibits probiotic potential (Maghfirotin Marta *et al*., 2019). This strain of lactic acid bacteria prevents the growth of pathogens such as *Enterobacteriaceae*, *E. coli,* and non-*E. coli* coliform (Liwan *et al*., 2020).

Furthermore, it adheres strongly to the intestinal surface of rats, possesses pH resistance, remains relatively stable at pH 3.0 for 3 h, and grows in MRS media (de Man Rogosa Sharpe) containing 0.5% bile salt (oxgall) (Darmastuti *et al*., 2021; Utami *et al*., 2017).

For probiotics to provide health benefits, a cell viability of at least 10^7 CFU must be maintained while traversing the gastrointestinal tract (GIT) before colonizing the distal ileum and colon (Sun & Wicker, 2021). However, most probiotics encounter significant irresistible challenges during processing, storage, distribution, and navigation

through the GIT where there is exposure to stomach acid, digestive enzymes, and intestinal bile salts (Torp *et al.*, 2022). This was evident in a previous result showing that the incubation of *L. plantarum* ZJ2868 in pH 2.5 for 3 h and a bile salt concentration of 0.1% for 1 h reduced the number of viable cells to 61.2% and 59.8%, respectively (Fu *et al.*, 2022). Encapsulation of probiotics serves as a promising solution by enveloping the therapeutic material within a protective matrix to shield against harsh environmental conditions (Raise *et al.*, 2020). In this regard, colon-targeted delivery systems gained prominence as a means to enhance probiotic viability during transport across the GIT and provide a direct release in the colon (Kumar *et al.*, 2020). Various strategies have been employed to achieve this type of delivery through the oral route, including time-dependent systems, osmotic pressure-dependent systems, coatings with pH-sensitive polymers, and enzyme-based biodegradable, each with its inherent limitations (Prudhviraj *et al.*, 2015; Wang *et al.*, 2021).

A particular source recently explored the encapsulation of *L. plantarum* in a colon-targeted microsphere dosage form, primarily using pectin and starch as key excipients. The recorded observations indicated higher viability in encapsulated probiotics after exposure to simulated gastric fluid and bile salt solution, compared to free cells (Dafe *et al.*, 2017). Another investigation, featuring a dissolution process, encapsulated *L. plantarum* in a colon-targeted microemulsion dosage form with alginate and Ca-EDTA as excipients. The results showed that the *L. plantarum* microemulsion shielded probiotic cells from simulated gastric fluid and facilitated direct release in simulated colon fluids (Qin *et al.*, 2021). Both approaches had limitations associated with the complexity of the respective methodologies applied. Furthermore, relying on polymers with one release mechanism may reduce their effectiveness in colon-targeted probiotic delivery, considering the variability in digestive tract conditions influenced by the fasting state, medical history, gender, age, and concurrent drug administration (Shahdadi *et al.*, 2019). Consequently, in this research, a combination of enzyme-based biodegradable polymers (pectin) and pH-sensitive polymers, namely shellac or cellulose acetate phthalate (CAP), was implemented as the optimal strategy for probiotic delivery to specific sites. It is important to note that shellac and CAP dissolve at pH values around 7 and over 5.8, positioning both as suitable candidates for colon-targeting formulations (Gately & Kennedy, 2017; Sampath Udeni Gunathilake *et al.*, 2020). Pectin, a polysaccharide, resists enzymatic degradation in the small intestine but succumbs to the action of colon microbial enzymes, including pectinase, pectate lyase, and polygalacturonase (Noreen *et al.*, 2017; Wang *et al.*, 2021).

L. plantarum can be encapsulated into pellets, which offer distinct advantages over conventional solid dosage forms, such as ease of swallowing, size uniformity, free-flowing properties, and seamLess coating and packaging (Aponte *et al.*, 2018). Therefore, this research aimed to formulate pectin-based pellets containing *L. plantarum* FNCC-0461, using CAP or shellac as coating polymers, to achieve colon-targeted probiotic delivery at specific sites for improved cell viability. The pellets were developed through the extrusion-spheronization method, followed by a well-established coating process in a pan (Singh *et al.*, 2021). In the absence of existing literature regarding the selected topic, this research provided the first report on the production of pectin-based colon-targeted pellets encapsulating *L. plantarum* FNCC-0461 as probiotics.

MATERIALS AND METHODS
Materials

The materials used included *L. plantarum* FNCC-0461 obtained from the Food and Nutrition Culture Collection at Gadjah Mada University, pectin (GENU®, CP Kelco Aps, Denmark), lactose (FMC, Philadelphia, USA), Avicel® (PH101) (FMC, Philadelphia, USA), cellulose acetate phthalate (Sisco Research Laboratories, Mumbai, India), flake shellac (AFS, A.F Suter and Co Ltd, United Kingdom), acetone (Brataco, Indonesia), hydrochloric acid, potassium phosphate monobasic, and sodium hydroxide (Merck, Germany), ethanol 97% (Brataco, Indonesia), *deMan Rogosa and Sharpe Agar, as well as* buffered peptone water (Himedia Laboratories Ltd, Mumbai, India), and pectinase (Pectinex Ultra SP-L, Europe). All chemicals applied in this research were of analytical grade.

Optimization of the extrusion-spheronization process

Optimization was conducted by adjusting independent variables, including spheronization speed (500, 1000, and 1500 rpm) and time (5, 10, and 15 min), each with three levels. The dependent variables assessed were shape, particle size, percentage recovery, and cell viability per gram.

Table I. Composition of pellet formulations

Batch Code	Probiotics (cfu/ml)	MCC (%)	Lactose (%)	Pectin (%)
F1	10^9	50	45	5
F2	10^9	50	40	10
F3	10^9	50	35	15
F4	10^9	50	30	20

Specifically, nine experimental conditions were explored to optimize the extrusion-spheronization process for pellet preparation.

Preparation of uncoated pellets

To formulate pellets containing *L. plantarum* FNCC-0461, the extrusion-spheronization method was used. The materials required for this procedure included a 10 g dry mass comprising 10^9 CFU (equivalent to 0.3 g) of lyophilized *L. plantarum*, pectin, MCC, and lactose in the ratio (Table I). These components were thoroughly mixed, and 5.5 mL of distilled water was added to create a suitable dough mass for the pelletization process. Four different formulations (F1 to F4) were developed by varying the concentration of pectin as the enzyme-based biodegradable polymer and lactose as the bulking agent to achieve the desired pellet characteristics. The best formulation was selected for coating with a pH-sensitive polymer, such as CAP or shellac. Moreover, the uncoated pellets were dried at 40°C in an oven (Memmert, Germany) and characterized for morphology and shape, particle size, moisture content, yield, and viability, before being coated.

Pellets coating

CAP and shellac were used as pellet-coating polymers. Each was properly weighed to obtain a 10% concentration in the coating solution. A solvent medium consisting of a mixture of organic solvents (acetone: ethanol, 9:1) was applied, with the addition of 5% glycerol as a plasticizer. Furthermore, the peristaltic pump tube was rinsed using organic solvents before filling it with the coating solution, ensuring the absence of air bubbles, and the spray gun was connected. Pellets from the optimal formulation were conditioned in the coating drum to facilitate drying during the coating process. For every 5 g of pellets, the coating pan parameters were set as follows: drum speed of 20 rpm, blower temperature of 55°C, a flow rate of 1.5 mL/min, and one minute delay to attain complete solvent evaporation (Singh *et al.*, 2021).

This procedure was performed in three repetition cycles to achieve a good coating. CAP-coated pellet formulations were labeled as FC, and those coated with shellac were tagged as FS.

Pellets morphology

Visual observation of pellet shape, texture, and color was conducted under an optical microscope. The diameter of both uncoated and coated pellets was measured using an optical microscope equipped with a camera (Olympus, Japan), and Image J software was deployed for image analysis. Additionally, surface texture and morphology were examined using a scanning electron microscope (SEM, LEO 420i, England).

Micromeritic properties

Micromeritic properties play a crucial role in probiotic release behavior and these can be evaluated as follows:

The angle of repose and flow ability test

Flow rate and angle of repose were determined using a flowmeter (Erweka, Germany). The flow rate was calculated by dividing the sample weight with time, and the angle of repose was estimated by dividing the anti-tangent of the height value using the radius of the pile of preparations formed after the test.

Friability test

Friability was determined using a Roche friabilator (type TAR 10, Erweka, Germany), with 200 rotations at 25 rpm. This was expressed as a percentage of weight reduction in the test sample after the experiment compared to before.

Tapped and bulk density

Bulk and tapped density were measured using a bulk-density tester (Erweka, Germany). Bulk density was calculated by dividing the predetermined mass (50 g) of pellets by the volume formed in the cylindrical glass before turning on the device. Tapped density was estimated by dividing the mass of the pellet by the volume formed in the cylindrical glass after tapping for 10 min.

Hausner's ratio
This ratio was determined by dividing the tapped density value with the bulk density.

Particle size distribution
Particle size was deduced through the sieving method with a shaker (Retsch GmbH, Jerman) by weighing and placing 50 g of pellets on the top sieve. The vibration amplitude was set at 0.25 for 10 min.

Moisture content
The moisture content parameter was evaluated using a moisture analyzer (Adam, USA) by heating one gram of pellets at 115°C.

Process yield
Process yield was calculated by comparing the total weight of raw materials incorporated during pellet preparation to the weight of dry pellets obtained, using the following equation:

$$\text{Process yield (\%)} = \frac{Wm}{Wt} \times 100$$

Where *Wm* represented the weight of dry pellets obtained (g) and *Wt* was the total weight of the raw materials (g).

In vitro release research
In vitro drug release from CAP-coated, shellac-coated, and uncoated pellets, was investigated using a USP dissolution test apparatus I (Erweka GmbH, Germany) in a 500 mL medium at 37±0.5°C with a rotational speed of 100 rpm. In this research, approximately 1 g of pellets was placed into the dissolution basket. The dissolution research was conducted for 2 h in Simulated Gastric Fluid (SGF) at pH 1.2, 3 h in the Simulated Intestinal Fluid (SIF) at pH 6.8, and 19 h in Simulated Colon Fluid (SCF) at pH 7.4 with subjection to pectinase enzyme (totalling 24 h). At various time intervals, 5 mL of the sample was collected, and to maintain a constant volume of 500 mL, 5 mL of fresh dissolution medium was added to the dissolution chamber after each sampling.

Viability count of _L. plantarum_ from pellets
The viability test was performed on the dissolution results as well as the uncoated and coated pellet samples obtained. Approximately 1 mL or 1 g of sample was dissolved in 10 mL of phosphate buffer, generating a 10^{-1} stock solution (standard solution). Around 1 mL of the standard solution was mixed with diluent solution (0.1% buffered peptone water + 0.05% Tween 80)

to reach 10 mL, amounting to 10^{-2} dilutions. Serial dilution steps were carried out, producing 10^{-3} and 10^{-6} colony counts, which were used for dissolution research and pellet samples, respectively. Subsequently, 20 μl of the serial dilutions were inoculated into Petri dishes containing 10 - 15 mL of de Man Rogosa Sharpe (MRS) agar medium. These dishes were incubated at 37°C under facultative anaerobic conditions for 48 h. To ensure accuracy, the procedure was conducted three times. *L. plantarum* colonies were counted, and the results were expressed as cfu/g (colony forming units per gram).

Statistical analysis
All data were analyzed for mean and standard deviations. Statistical analyses were conducted using the IBM Statistical Package for the Social Sciences (SPSS) free trial version. Three replicates were performed on each sample, and the results were averaged. Additionally, a one-way analysis of variance (ANOVA) was executed to compare the results of each group, where a p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION
Optimization of the spheronization process
The process variables, namely spheronization speed and time, were optimized. Their effect was observed on shape, particle size, moisture content, percentage yield, and viability of pellets. In the first batch (C1), a spheronization speed of 500 rpm) and time of 5 min were used (Table II). The speed and duration of spheronization were known to affect the pellet shape (Chopra *et al.*, 2013). Therefore, their optimization was crucial for formulating drug-loaded pellets (Kaur *et al.*, 2020). The results of the partial t-test conducted showed that both factors simultaneously exerted a significant negative effect on pellet size (Sig < 0.05). Specifically, a higher spheronization speed led to increased frictional force between the extrudate and the spheronizer lattice plate (crosshatch pattern), making the extrudate more prone to break into smaller pieces upon contact with the rotating spheronizer plate. This phenomenon-initiated collisions between the extrudate and the spheronizer plate, as well as among the extrudate particles during the process (Muley *et al.*, 2016). Increasing the spheronization speed from 500 rpm to 1500 rpm, where the time was constant, led to a decrease in pellet size and an elevation in pellet viability.

Table II. Micromeritic properties of pellets obtained from the optimization process

Batch code	Shape	Particle size (mm)	Yield (%)	Moisture content (%)	Viability (cfu/g)
C1	Rod shape	2.144 ± 0.257	90.32 ± 0.88	2.82 ± 1.72	1.80×10^7
C2	Rod shape	2.045 ± 0.117	79.78 ± 1.67	2.02 ± 0.04	2.06×10^7
C3	Rod shape	1.893 ± 0.254	89.881 ± 1.05	1.96 ± 0.03	2.33×10^7
C4	Rod shape	1.737 ± 0.261	89.82 ± 2.06	2.36 ± 0.05	1.94×10^7
C5	CRE	1.916 ± 0.038	84.29 ± 1.20	1.99 ± 0.04	2.33×10^7
C6	Dumbbell	1.796 ± 0.078	88.75 ± 1.66	3.09 ± 0.09	3.31×10^7
C7	Dumbbell	1.659 ± 0.055	90.68 ± 1.33	2.15 ± 0.17	3.05×10^7
C8	Ellipsoids	1.664 ± 0.047	87.25 ± 1.49	2.15 ± 0.07	8.33×10^7
C9	Spheroids	1.264 ± 0.060	89.66 ± 1.58	1.93 ± 0.05	9.42×10^7

An increase in spheronization time generated rod-shaped pellets at lower speeds, while a spherical shape with a smooth surface was obtained at higher speeds.

Evaluation of the spheronization speed and time showed that only the speed variable had a significant effect on the shape of pellets produced (Sig. < 0.05), while time exerted no significant effect (Sig. > 0.05). These differences in dosage forms might be attributed to variations in the mechanical energy generated from the spheronization plate due to disparities in rotational speed. During spheronization, this mechanical energy was converted into kinetic energy, causing the extrudate to move in a fluidized manner on the plate mechanically, leading to a transformation into a spherical shape (Muley *et al*., 2016). As the extrudate experienced more frictional forces, its roundness increased over time, until an optimum spheronization time was reached, at which point the pellets became compact enough to resist further frictional forces (Sinha *et al*., 2007).

Multiple linear regression analysis revealed that spheronization speed and time did not affect the recovery percentage, as indicated by the significance of the F value (Sig. > 0.05). In contrast, this analysis showed that both variables simultaneously had a significant effect on the cell viability of the pellets (Sig. < 0.05). The conducted partial t-test confirmed that both variables had a positive effect on the pellet viability. This might be attributed to the dosage form generated, as more spherical or near-spherical pellets tended to offer a greater surface area for probiotic cell adsorption compared to other forms such as rods, cylinders with rounded ends, dumbbells, or ellipsoids. Following the attainment of the best results using a spheronization speed of 1500 rpm for 15 min (resulting in spheroid-shaped pellets with roundness of 0.90, circularity of 0.85, moisture content of 1.93 ± 0.05, percentage yield of 89.66 ± 1.58, average particle size of 1.264 ± 0.060 mm, and viability of 9.42×10^7 cfu/g), the process C9 was selected for further formulation (Table II).

Optimization and preparation of uncoated pellets containing probiotics

The formulation variables, specifically the weight ratios of pectin in the pellet, were optimized from 5% to 20% (w/w), and their effect was observed on the shape, particle size, yield percentage, water content, and viability. The selection of a maximum concentration of 20% (w/w) was based on prior research indicating that concentrations exceeding 20% could render the formed mass extremely difficult to pelletize (Akhgari *et al*., 2013). The optimal process variable based on previous results was used for the preparation of uncoated pellets. It is important to note that the pelletization process was significantly influenced by the excipient and its quantity in the pellet formulation. The consistency of the wet mass affected the surface and shapes of the pellets (Chopra *et al*., 2013). Formulations F1 to F4 with increasing pectin concentration from 5% to 20% showed a significant effect on pellet shape (Sig. < 0.05). Those comprising lower pectin concentrations produced more spherical pellet shapes compared to the counterparts containing higher concentrations. This phenomenon was attributed to the swelling of pectin in the moist mixed mass, making it challenging to process using the extrusion-spheronization method for pellet production (Martins *et al*., 2017). As the amount of pectin in the formulation increased, the possibility of swelling elevated which reduced the suitability of the mixed mass for pelletization.

Table III. Micromeritic and properties of pellets formulation

Batch code	Shape	Particle size (mm)	Yield (%)	Moisture content (%)	Viability (cfu/g)
F1	Spheroids	1.429 ± 0.954	78.97 ± 1.71	3.10 ± 0.37	8.18×10^7
F2	Spheroids	1.150 ± 0.291	88.71 ± 1.04	2.08 ± 0.31	7.50×10^7
F3	Ellipsoid	1.481 ± 0.163	93.68 ± 3.10	1.71 ± 0.10	4.18×10^7
F4	Dumbbell	1.871 ± 0.155	91.07 ± 1.12	1.77 ± 0.06	2.00×10^7

This change tended to be initiated by numerous hydrogen bonds formed between distilled water and the carboxyl groups in the pectin structure, leading to difficulty in achieving the desired spherical shape during the spheronization process (Tho *et al.*, 2002).

There was a significant correlation between pectin concentration and pellet size (Sig. < 0.05), indicating a positive and moderate relationship. This is proven by increasing the amount of pectin in the formulation which causes larger pellet sizes in F2 to F4 (Table III). The quality of pellets depends greatly on the characteristics of the mixed mass, which must exhibit plasticity and moisture absorption capacity. In this context, the formulation needs to provide sufficient mechanical strength for the production of pellets, influenced by the compatibility properties of the ingredients in the formulation (Palugan *et al.*, 2015). However, pectin is known to have poor compatibility, capable of hindering the formation of a compact mass (Sande, 2005). Pectin swelling in the wet mass could reduce the quality of the resulting pellets when prepared through the extrusion-spheronization method. To address this issue, a granulation liquid, such as a 10% citric acid solution (w/v), could be added. Although, the highest controllable pectin content in formulations is limited to 20% (w/w), as a greater content tended to cause over-expansion (Martins *et al.*, 2017).

The correlation between pectin concentration and recovery percentage (Sig. < 0.05) was also significant, indicating a strong relationship. These results show that elevating pectin concentration from 5% to 20% led to an increase in the percentage of recovery from 78.97% to 91.07%. The effect of pectin concentration on cell viability in the pellet preparations was significant (Sig. <0.05) and negative (Table III). When the pectin content in the formulation increased from 5% to 20%, viability decreased from 8.18×10^7 CFU/g to 2.00 $\times 10^7$ CFU/g. This decrease could be attributed to the resulting dosage forms, as more spherical or near-spherical pellets had greater surface areas for probiotic cell adsorption compared to those with ellipsoid or dumbbell shapes. After achieving the desired results (spheroid shape, moisture content: 2.08 ± 0.31, percentage yield: 88.71 ± 1.04, average particle size: 1.150 ± 0.029 mm, and viability of 7.50 $\times 10^7$ cfu/g), batch F2 was selected for the subsequent coating process.

Pellet coating Process

Following the formulation optimization, batch F2 was coated with either CAP or shellac. Both types of enteric-coated polymers were applied at a 10% concentration, along with a 5% plasticizer (glycerol). At the end of the process, CAP-coated pellets gained an additional weight of 0.496 g (9.92 %) to the initial 5.00 g. In contrast, shellac-coated pellets had an additional weight of 0.411 g (8.21 %). These pellet weights, approaching 0.675 g for a 5 g batch of pellets (equivalent to 13.5%), suggested that the use of 10% material concentration would provide an even coating, leading to a smooth surface.

Cell viability of pellets coated with either CAP or shellac decreased significantly compared to uncoated forms. The viability reduced from 7.5 x 10^7 CFU/g to 4.67 x 10^7 CFU/g for FC, while FS had the lowest viability at 1.48 x 10^7 CFU/g. This decrease might be attributed to the extreme conditions during the coating process, including a temperature of 50 °C and contact between probiotic cells and organic solvents in the form of an acetone and ethanol mixture, with a tendency to cause cell death. In FS, the lowest cell viability results obtained might be due to the presence of seven sesquiterpene acids in shellac, which possessed antibacterial activity (Lu *et al.*, 2014). Five of these seven metabolites are known to have significant antibacterial activity against *S. aureus*, *E. coli,* and *B. subtilis* (Lu *et al.*, 2018).
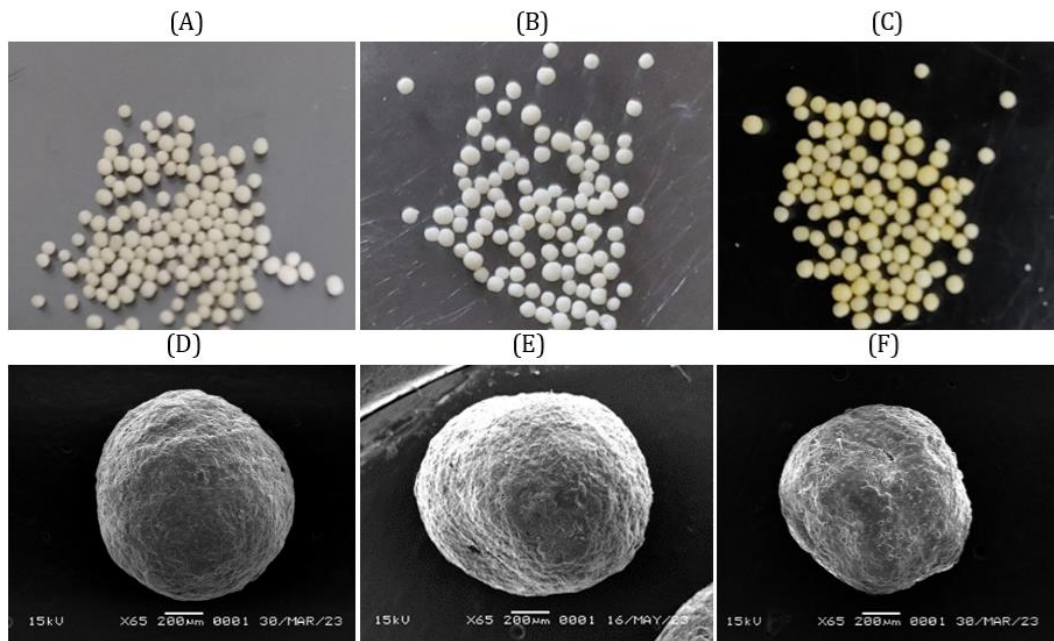
Figure 1. The morphology of uncoated pellets (A), CAP coated pellets (B), and shellac coated pellets (C), and Scanning Electron Micrographs of uncoated pellets (65x magnification) (D), CAP coated pellets (65x magnification) (E), and shellac coated pellets (65x magnification) (F).
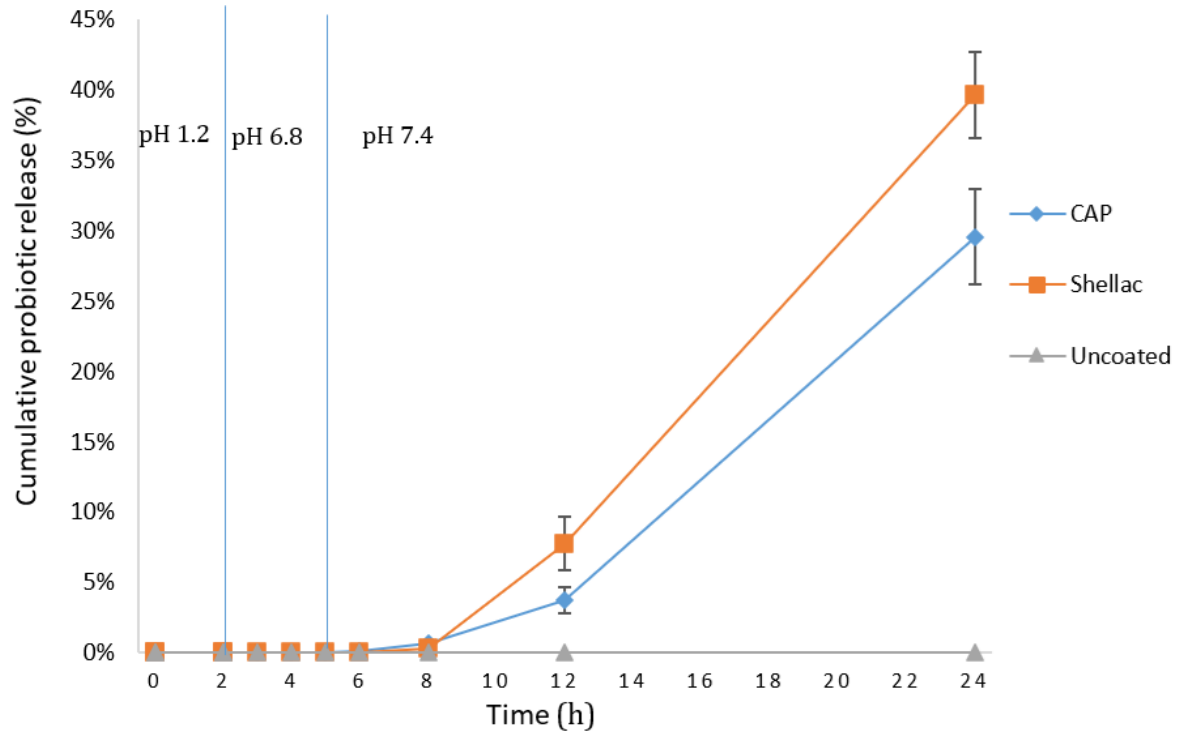

Figure 2. Cumulative probiotic release of *L. plantarum* from uncoated pellets, CAP-coated pellets, and shellac coated-pellets at pH 1.2, 6.8, and 7.4, with data shown as mean ±SD.

Pellet Morphology

Uncoated pellets were odorless, with a rigid and spherical shape, and appeared yellowish white. The application of pH-sensitive coating polymers such as CAP produced spherical, whitish, and odorless pellets. Meanwhile, coating with shellac led to transformation into spherical shapes, yellow to orange coloration, and odorless smell (Figure 1). The surface morphologies of FP, FC, and FS, (Figure 1(D,EF)) showed a predominance of spherical shapes, as revealed by SEM analysis. The FP was smooth, spherical, compact, and uniform on the outer surface.

Micromeritic property

The particle size distribution presented in Table IV, showed the sizes of FP, FC, and FS to be 0.913 mm, 0.984 mm, and 0.934 mm, respectively. It was evident that the coating process increased the diameter of FC and FS compared to FP. The size difference was in accordance with the results of the pellet coating thickness test, which showed a 9.9% and 8.8% increase for FC and FS, respectively, after the coating process. Moreover, the results of the flow characteristics, including tapped density, bulk density, angle of repose, Hausner ratio, and Carr's index values, did not indicate significant differences among the three pellet samples (Sig. > 0.05).

The angle of repose, Hausner ratio, and Carr's index values for all three groups showed that the preparations had very good flow properties. This was attributed to the circularity of the pellets, as increased circularity enhanced their flowability. The minor differences in density (tapped or bulk) might be due to the similarities in particle size and size distribution, as these properties were consistent (Sinha *et al.*, 2007). According to Table IV, the friability test results from three sample groups were below 1%, indicating strong mechanical strength and compatibility in the pellets (Jubie *et al.*, 2020). This phenomenon could be due to optimum drying temperature and time, rendering the pellets less friable and more hardened by reducing porosities and shrinkage. The moisture content of each pellet batch remained below 5 %, and the drying process, consistently performed at 40 ˚C for 24 h, ensured complete moisture removal after this time frame.

In vitro probiotic release and viability count

For FP, no bacterial colonies were observed in the viability test conducted during the dissolution research, spanning from SGF to SCF medium (over a period of 30 min to 24 h). This was attributed to the death of all probiotic cells contained in the pellet preparation due to the highly acidic conditions in the SGF medium at pH 1.5 for 2 h. These results were in line with research performed by Fu *et al.*, where *L. plantarum* was found to be nonresistant to very low pH conditions, as probiotic cells reduce to 61.2 % viability at pH 2.5 (Fu *et al.*, 2022). Colon-targeted pellet samples coated with CAP or shellac did not experience complete probiotic cell death as observed in FP (Figure 1). This showed that the combination of enzyme- and pH-based enteric polymers effectively preserved probiotic viability against a pH of 1.2 for 2 h, compared to pellet preparations relying solely on one release mechanism (enzyme-based), such as the uncoated pellets.

The mechanism of retaining probiotic release with pH-sensitive polymers is based on the relationship between their pKa values and the pH of the medium. In general, polyanion polymers are ideal excipients for inhibiting drug release at low pH and promoting release at higher pH (Ghaffar *et al.*, 2020). According to the Henderson-Hasselbalch equation, the ionization of groups in the polymers is affected by the pH of the system and pKa. When the pH of the system is above the pKa value, ionization occurs. Conversely, once the pH of the system falls below the pKa value, the polymer remains un-ionized. Shellac is known to have a fairly high dissolution pH of around 7, making it a suitable candidate for use in colon-targeted formulations (Gately & Kennedy, 2017). Additionally, Cellulose Acetate Phthalate (Cellulose Acetate Phthalate, CAP) dissolves at a pH > 5.8 (Sampath Udeni Gunathilake *et al.*, 2020). For the FC formulation, no probiotics were released within the first 2 h in SGF at pH 1.2 as well as between the 2nd and 5th h in SIF at pH 6.8, Cumulative probiotic release (%) (Figure 2). At the 6th hour in SCF at pH 7.4, only 0.125 % of the probiotics were released due to the dissolution of the coating polymer in the SCF medium, enabling the degradation of pectin by the pectinase enzyme. By the 6th hour, most of the coating had degraded from the pellet surface, facilitating a more rapid release of the active substance compared to shellac-coated pellets. The influence of coating on active substance release can be explained by the Noyes-Whitney/Nerst-Brunner equation. Higher coating concentrations result in thicker diffusion membranes, decreasing the dissolution rate of the active substance. After 24 h, 29.551% of the probiotic was released from 1 g of pellets.

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Table IV. Micromeritic and other properties of uncoated and coated pellets

Batch code	Angle of repose	Bulk density (g/cm³)	Tapped density (g/cm³)	Hausner's ratio	Carr's index (%)	Friability (%)	Yield (%)	Moisture content (%)	Particle size (mm)	Viability (CFU/g)
FP	25.94±1.30	0.83±0.01	0.86±0.01	1.03±0.02	3.32± 1.65	0.08±0.01	88.71±1.04	2.08±0.31	0.913±0.008	7.50×10^7
FC	25.35±1.02	0.85±0.01	0.87±0.02	1.03±0.01	2.83± 0.89	0.19±0.23	87.14±0.70	4.56±0.28	0.984±0.052	4.67×10^7
FS	25.10±1.29	0.83±0.01	0.86±0.05	1.03±0.01	2.81± 1.06	0.02±0.02	85.99±0.27	4.40±0.35	0.934±0.014	1.48×10^7

Note: FP (uncoated pellets), FC (CAP-coated pellets), and FS (shellac-coated pellets)

In the case of FS, there was no probiotic release within the first 2 h in SGF at pH 1.2, between the 2nd and 5th h in SIF at pH 6.8, as well as at the 6th hour in SCF at pH 7,4, similar to the behavior of CAP coated pellets. At the 8th hour and pH 7.4, only 0.337 % of the probiotics were released due to the dissolution of the shellac coating in the SCF medium, followed by pectin degradation by pectinase. After 24 h, 39.616 % was liberated, and the results showed that the type of polymer used significantly influenced the cumulative percent or number of cells discharged into the CSF medium (Sig. < 0.05).

Regarding cumulative probiotic cell release, FS formulation outperformed FP formulation. However, in terms of viable probiotic cell count, FP had the highest number of probiotic cells released within 24 h in the SCF medium (1.38×10^7 cfu/g), compared to FS (5.88×10^6 cfu/g). The difference between cumulative release results and the number of liberated cells was initiated by the variation in viability of pellets formulation, where FC and FC had a viability of 4.67×10^7 cfu/g and 1.48×10^7 cfu/g, respectively. Moreover, a significant increase in viable probiotic cell count was observed in the release of coated compared to uncoated pellets, confirming that the coated formulation could release probiotics at specific colon sites. FC formulations fulfilled the minimum cell viability requirements for colony formation in the colon (1.00×10^7 cfu/g).

CONCLUSION

In conclusion, the optimal formulation for pellets containing *L. plantarum* FNCC-0461 was achieved using a ratio of MCC, lactose, and pectin at 5:4:1, processed through the extrusion-spheronization method at a speed of 1500 rpm for 15 min. Furthermore, the use of cellulose acetate phthalate (CAP) as a coating material proved to be the most effective strategy for targeted probiotic delivery to specific colon sites.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Akhgari, A., Abbaspour, M., & Moradkhanizadeh, M. (2013). Combination of pectin and eudargit rs and eudragit rl in the matrix of pellets prepared by extrusion-spheronization for possible colonic delivery of 5-amino salicylic acid. *Jundishapur Journal of Natural Pharmaceutical Products*, 8(2), 86–92. https://doi.org/10.17795/jjnpp-8350

Aponte, M., Ungaro, F., d'Angelo, I., De Caro, C., Russo, R., Blaiotta, G., Dal Piaz, F., Calignano, A., & Miro, A. (2018). Improving in vivo conversion of oleuropein into hydroxytyrosol by oral granules containing probiotic Lactobacillus plantarum 299v and an Olea europaea standardized extract. *International Journal of Pharmaceutics*, *543*(1–2), 73–82. https://doi.org/10.1016/j.ijpharm.2018.03.013

Bron, P. A., Van Baarlen, P., & Kleerebezem, M. (2012). Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. *Nature Reviews Microbiology*, *10*(1), 66–78. https://doi.org/10.1038/nrmicro2690

Chopra, S., Venkatesan, N., & Betageri, G. V. (2013). Formulation of lipid-bearing pellets as a

delivery system for poorly soluble drugs. *International Journal of Pharmaceutics*, *446*(1–2), 136–144. https://doi.org/10.1016/j.ijpharm.2013.02.029

Dafe, A., Etemadi, H., Dilmaghani, A., & Mahdavinia, G. R. (2017). Investigation of pectin/starch hydrogel as a carrier for oral delivery of probiotic bacteria. *International Journal of Biological Macromolecules*, 97, 536–543. https://doi.org/10.1016/j.ijbiomac.2017.01.060

Darmastuti, A., Hasan, P. N., Wikandari, R., Utami, T., Rahayu, E. S., & Suroto, D. A. (2021). Adhesion properties of lactobacillus plantarum dad-13 and lactobacillus plantarum mut-7 on sprague dawley rat intestine. *Microorganisms*, 9(11), 2336. https://doi.org/10.3390/microorganisms9112336

Fu, B., Huang, X., Ma, J., Chen, Q., Zhang, Q., & Yu, P. (2022). Characterization of an inositol-producing Lactobacillus plantarum strain and the assessment of its probiotic potential and antibacterial activity. *Lwt*, 153, 112553. https://doi.org/10.1016/j.lwt.2021.112553

Gately, N. M., & Kennedy, J. E. (2017). The development of a melt-extruded shellac carrier for the targeted delivery of probiotics to the colon. *Pharmaceutics*, *9*(4), 1–12. https://doi.org/10.3390/pharmaceutics9040038

Jubie, S., Jawahar, N., Arigo, A., Prabha, T., & Anjali, P. B. (2020). Stability enhancement and formulation development of L-Carnitine fast-dissolving pellets through pro-drug strategy. *Journal of Drug Delivery Science and Technology*, 55, 101474. https://doi.org/10.1016/j.jddst.2019.101474

Kaur, V., Goyal, A. K., Ghosh, G., Chandra Si, S., & Rath, G. (2020). Development and characterization of pellets for targeted delivery of 5-fluorouracil and phytic acid for treatment of colon cancer in Wistar rat. *Heliyon*, 6(1), e03125. https://doi.org/10.1016/j.heliyon.2019.e03125

Kumar, A., Gulati, M., Singh, S. K., Gowthamarajan, K., Prashar, R., Mankotia, D., Gupta, J. P., Banerjee, M., Sinha, S., Awasthi, A., Corrie, L., Kumar, R., Patni, P., Kumar, B., Pandey, N. K., Sadotra, M., Kumar, P., Kumar, R., Wadhwa, S., & Khursheed, R. (2020). Effect of co-administration of probiotics with guar gum, pectin and eudragit S100 based colon targeted mini tablets containing 5-Fluorouracil for site specific release. *Journal of Drug Delivery Science and Technology*, 60, 102004. https://doi.org/10.1016/j.jddst.2020.102004

Kurakula, M., Gorityala, S., & Moharir, K. (2021). Recent trends in design and evaluation of chitosan-based colon targeted drug delivery systems: Update 2020. *Journal of Drug Delivery Science and Technology*, 64, 102579. https://doi.org/10.1016/j.jddst.2021.102579

Liwan, S. Y., Utami, T., Murdiati, A., Triwitono, P., & Rahayu, E. S. (2020). Dietary patterns and effect of consumption of probiotic powder containing indigenous bacteria Lactobacillus plantarum Dad-13 on Streptococcus, Enterococcus, Escherichia coli and Klebsiella pneumoniae in the gut of students at Junior High School Pangururan. *International Food Research Journal*, 27(5), 790–797.

Lu, J., Shang, L., Wen, H., Huang, J., Li, G., & Wang, J. (2018). Structural identification and biological activity of six new Shellolic esters from Lac. *Fitoterapia*, 125, 221–226. https://doi.org/10.1016/j.fitote.2017.11.021

Lu, J., Wang, H., Huang, J., Li, G., Wang, Q., Xu, W., Chen, Y., Zhang, K., & Wang, J. (2014). Sesquiterpene acids from Shellac and their bioactivities evaluation. *Fitoterapia*, 97, 64–70. https://doi.org/10.1016/j.fitote.2014.05.014

Maghfirotin Marta, B., Tyas, U., Muhammad Nur, C., Jaka, W., & Endang Sutriswati, R. (2019). Effects of consumption of probiotic powder containing Lactobacillus Plantarum Dad-13 on fecal bacterial population in school-age children in indonesia. *International Journal of Probiotics and Prebiotics*, 14(1), 1–8. https://doi.org/10.37290/ijpp2641-7197.14:1-8

Martins, A. L. L., de Oliveira, A. C., do Nascimento, C. M. O. L., Silva, L. A. D., Gaeti, M. P. N., Lima, E. M., Taveira, S. F., Fernandes, K. F., & Marreto, R. N. (2017). mucoadhesive properties of thiolated pectin-based pellets prepared by extrusion-spheronization technique. *Journal of Pharmaceutical Sciences*, 106(5), 1363–

1370. https://doi.org/10.1016/j.xphs.2017.01.02

Noreen, A., Nazli, Z. I. H., Akram, J., Rasul, I., Mansha, A., Yaqoob, N., Iqbal, R., Tabasum, S., Zuber, M., & Zia, K. M. (2017). Pectins functionalized biomaterials; a new viable approach for biomedical applications: A review. *International Journal of Biological Macromolecules*, 101, 254–272. https://doi.org/10.1016/j.ijbiomac.2017.03.029

Palugan, L., Cerea, M., Zema, L., Gazzaniga, A., & Maroni, A. (2015). Coated pellets for oral colon delivery. *Journal of Drug Delivery Science and Technology*, 25, 1-15. https://doi.org/10.1016/j.jddst.2014.12.003

Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., & Gil, A. (2019). Mechanisms of Action of Probiotics. *Advances in Nutrition*, 10, S49–S66. https://doi.org/10.1093/advances/nmy063

Prudhviraj, G., Vaidya, Y., Singh, S. K., Yadav, A. K., Kaur, P., Gulati, M., & Gowthamarajan, K. (2015). Effect of co-administration of probiotics with polysaccharide based colon targeted delivery systems to optimize site specific drug release. *European Journal of Pharmaceutics and Biopharmaceutics*, 97, 164–172. https://doi.org/10.1016/j.ejpb.2015.09.012

Qin, X. S., Luo, Z. G., & Li, X. L. (2021). An enhanced pH-sensitive carrier based on alginate-Ca-EDTA in a set-type W1/O/W2 double emulsion model stabilized with WPI-EGCG covalent conjugates for probiotics colon-targeted release. *Food Hydrocolloids*, 113, 106460. https://doi.org/10.1016/j.foodhyd.2020.106460

Raise, A., Dupont, S., Iaconelli, C., Caliri, C., Charriau, A., Gervais, P., Chambin, O., & Beney, L. (2020). Comparison of two encapsulation processes to protect the commensal gut probiotic bacterium Faecalibacterium prausnitzii from the digestive tract. *Journal of Drug Delivery Science and Technology*, 56, 101608. https://doi.org/10.1016/j.jddst.2020.101608

Sampath Udeni Gunathilake, T. M., Ching, Y. C., Chuah, C. H., Rahman, N. A., & Liou, N. S. (2020). Recent advances in celluloses and their hybrids for stimuli-responsive drug delivery. *International Journal of Biological Macromolecules*, 158, 670–688. https://doi.org/10.1016/j.ijbiomac.2020.05.010

Shahdadi Sardo, H., Saremnejad, F., Bagheri, S., Akhgari, A., Afrasiabi Garekani, H., & Sadeghi, F. (2019). A review on 5-aminosalicylic acid colon-targeted oral drug delivery systems. In *International Journal of Pharmaceutics*, 558, 367–379. https://doi.org/10.1016/j.ijpharm.2019.01.022

Singh, A., Mandal, U. K., & Narang, R. K. (2021). Development and characterization of enteric coated pectin pellets containing mesalamine and Saccharomyces boulardii for specific inflamed colon: In vitro and in vivo evaluation. *Journal of Drug Delivery Science and Technology*, 62, 102393. https://doi.org/10.1016/j.jddst.2021.102393

Sinha, V. R., Agrawal, M. K., Kumria, R., & Bhinge, J. R. (2007). Influence of operational variables on properties of piroxicam pellets prepared by extrusion-spheronization: A technical note. *AAPS PharmSciTech*, 8(1), E137–E141. https://doi.org/10.1208/pt0801020

Sun, Q., & Wicker, L. (2021). Hydrogel encapsulation of Lactobacillus casei by block charge modified pectin and improved gastric and storage stability. *Foods*, 10(6), 1–10. https://doi.org/10.3390/foods10061337

Tho, I., Anderssen, E., Dyrstad, K., Kleinebudde, P., & Sande, S. A. (2002). Quantum chemical descriptors in the formulation of pectin pellets produced by extrusion/spheronisation. *European Journal of Pharmaceutical Sciences*, 16(3), 143-9. https://doi.org/10.1016/s0928-0987(02)00084-2

Torp, A. M., Bahl, M. I., Boisen, A., & Licht, T. R. (2022). Optimizing oral delivery of next generation probiotics. *Trends in Food Science & Technology*, 119, 101–109. https://doi.org/10.1016/j.tifs.2021.11.034

Utami, T., Wardani, S. K., Cahyanto, M. N., & Rahayu, E. S. (2017). The effect of inoculum size and incubation temperature on cell growth, acid production and curd formation during milk fermentation by Lactobacillus plantarum Dad 13. *International Food Research Journal*, 24(3), 921–926.

Wang, M., Hao, W., Zhang, L., Zhu, Y., Chen, K., Ma, S., Cheng, X., & Zhao, J. (2021). Lipid-polymer nano core-shell type hybrid system for colon specific drug delivery. *Journal of Drug Delivery Science and Technology*, 63, 102540. https://doi.org/10.1016/j.jddst.2021.1025 40

Zheng, H., Gao, M., Ren, Y., Lou, R., Xie, H., Yu, W., Liu, X., & Ma, X. (2017). An improved pH-responsive carrier based on EDTA-Ca-alginate for oral delivery of Lactobacillus rhamnosus ATCC 53103. *Carbohydrate Polymers*, 155, 329–335. https://doi.org/10.1016/j.carbpol.2016.08. 096