ORIGINAL ARTICLE

Characteristics of *Lactobacillus casei* probiotic microparticles in L-type methacrylic acid copolymer matrix

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ABSTRACT

Lactobacillus casei (LC) is a type of lactic acid bacterium that is known for its beneficial probiotic properties. However, it is not typically found in the human intestine because it lacks acid resistance. LC thrives in an optimal pH environment of 6.8 and can be initiated in a more acidic environment at a pH of 3.5. This study purposed to compare the effect of L-type methacrylic acid copolymer (MAC) as a matrix (0.50%, 0.75%, and 1.00%) on the physical characteristics of LC probiotic microparticles made by the spray drying process. Probiotic microparticles were also made from a dry suspension of LC FNCC 0090 bacteria and dispersed in a solution of L-type MAC. The results showed that a rise in matrix content by 1.00% increased particle size (4.47 ± 0.19 μm) and reduced moisture content (7.45 ± 0.11%). The analysis of microparticle morphology also indicated a positive correlation between the level of L-type MAC and the production of smooth, nonporous, and almost spherical shapes. In addition, it was observed that encapsulation efficiency (92.46 ± 0.17%) and protection against stomach acid (98.17% ±1.17%) increased with the level of the matrix.

Key words: *Lactobacillus casei,* L-type methacrylic acid copolymer, microencapsulation, probiotic, spray drying

INTRODUCTION

A probiotic refers to a collection of nonpathogenic living bacteria that offer a wide range of beneficial health effects to humans when administered in appropriate amounts. These microorganisms have been reported to maintain intestinal health through several mechanisms, such as nutrient production, competition with pathogenic bacteria, and stimulation of the immune response of the host.[1,2] Since probiotics are not immune to stomach acid, they must be released in sufficient quantities within the intestines. To ensure optimal efficacy, the use of these microorganisms in oral dosage forms is recommended to contain a minimum of 10^7 CFU/mL or per gram of live bacteria.[3]

Lactobacillus casei (LC) is widely used commercially and has an immunomodulatory and antibacterial effect. It contains lactic acid bacteria that are resistant to acidic conditions, but the viability may decrease in an acidic stomach environment.[4,5] In addition, *Lactobacillus* spp. can withstand temperatures of up to 60°C for 5 min, but the viability may decrease depending on its thermal sensitivity.[6]

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Microparticles have been demonstrated to offer several benefits, such as shielding active ingredients from the surrounding environment, stabilizing sensitive active ingredients, and decreasing the incompatibility of active ingredients. Therefore, this technology can improve bioavailability and mitigate the potential side effects of drugs.[7] Microencapsulation refers to a method where active ingredients are entrapped within a polymer matrix, acting as a carrier. In the context of probiotics, the matrix employed for coating must possess the ability to safeguard them from high temperatures and acidic conditions, leading to a reduction in their viability.[8,9]

L-type methacrylic acid copolymer (MAC) has a 1:1 composition of methacrylic acid and methyl methacrylate. This anionic polymer has gained widespread commercial use as an enteric coating in the domain of oral drug delivery and facilitates the release of drugs within the intestine, specifically in an alkaline environment.[10] It is also resistant to gastric acid and dissolves rapidly in alkaline conditions in the intestine. Subsequently, the carboxylic acid groups of copolymer form hydrogen bonds, intra and inter-molecules, which causes the matrix to protect the material inside. In an alkaline environment of the intestine, the ionized carboxylic acid groups and the polymer dissolve rapidly.[11]

Spray drying is chosen in this research because it is a simple and affordable method. The spray drying technique is known to take a short time, and the process cost is cheaper. High temperatures in the microencapsulation process with spray drying can affect bacterial viability, but previous studies have reported that, with the addition of a protective matrix, bacterial viability is tolerable.[12] The inlet temperature is a critical factor that affects the outcome of spray drying. This study evaluates the impact of varying levels of L-type MAC matrix (0.50%, 0.75%, and 1.00%) on the properties of LC microparticles generated through a spray drying process, using an inlet temperature of 120°C. The LC probiotic microparticles are made to protect active ingredients and reduce degradation against stomach acid. The novelty in this research is the microencapsulation of LC probiotics in an L-type MAC matrix.

MATERIALS AND METHODS

Matrix

L-type MAC used as a matrix was obtained from Evonik (Germany).

Culture preparation

Sterile de Man Ragosa Shorpe (MRS) broth media was added with one loop of probiotic starter LC FNCC 0090 from Gadjah Mada University (Pure culture certificate No. PSPG-NCC/24-III//2017) incubated at 37°C. Samples were taken at certain times from 0 to 48 h with observed pH and total plate count (TPC) to determine the optimum growth time for probiotic LC.

Microparticle preparation

Table 1 contains the LC probiotic microparticle formulas studied.

LC was cultured by inoculating it into 50 mL MRS Broth overnight at 37°C. The culture was then poured and mixed into 450 mL MRS Broth and was stored at 37°C for 48 H. Meanwhile, the cells were harvested by centrifuging from MRS Broth and rinsing three times with 0.9% NaCl solution. The bacteria were then reconstituted with a solution containing L-type MAC with a concentration of 0.50, 0.75, or 1.00%. The suspension should also contain 10^9 CFU/mL and the mixture of LC, L-type MAC, and MRS, tested the viscosity. Subsequently, the mixture was heated at 50°C for 30 min as a preadaptation process and then spray dried with spray dryer Buchi B-290 type at 120°C and 60°C as inlet and outlet temperatures, respectively. The preadaptation process may contribute to enhancing LC probiotic viability under adverse conditions.[13] The minimum and maximum inlet temperatures required for the manufacture of probiotic microparticles were 100°C and 170°C, whereas the outlet was between 45°C and 105°C.[14] Each spray drying process was conducted in triplicate, and microparticles were stored in sealed polyethylene bags at 25°C.

Physical characterization of *Lactobacillus casei* probiotic microparticles

Morphological examination

The morphologies of microparticles were evaluated using scanning electron microscopy (SEM) FEI Type: Inspect-S50.

Infra-red spectra test

Samples were measured using the Jasco Fourier transform infrared spectroscopy spectroscopy with a 4000 - 450 cm^{-1} number wave.

Moisture content test

Samples were tested using the Mettler Toledo HB43-S Moisture Analyzer.

Particle size test

Particle size examination was carried out with an Optilab Biological Microscope Novel Model XSZ-107 Series®

Table 1: *Lactobacillus casei* probiotic microparticle formula

Material	FI (0.50%)	FII (0.75%)	FIII (1.00%)
Probiotic suspension (10^9 CFU/mL) (mL)	250	250	250
L-type MAC (g)	5.0	7.5	10.0
MRS broth (g)	5.0	5.0	5.0
Aquadest (g)	750	750	750

MAC: Methacrylic acid copolymer, MRS: Man Ragosa Shorpe

microscope with 10 and 40 times ocular magnification and an objective lens for 300 particles.

Encapsulation efficiency test

Encapsulation efficiency (EE) test was performed by determining the absorbance or transmittance percentage (%) to calculate the bacterial biomass. This was carried out by the ultraviolet-Vis spectrophotometer at λ_{max} 580 nm before the spray drying process, which was then compared with the amount of biomass in microparticles.

The power of protection against stomach acid test

For each formula, 100 mL LC microparticles were weighed and dissolved in 9 mL sterile phosphate buffer saline (PBS) pH 7. The mixture was then vortexed, and 1 mL solution was taken to count viable bacteria using the TPC method incubated at 37°C for 48 H. Furthermore, the examination of viable bacteria after exposure to an acidic environment was carried out by weighing 100 mg of LC microparticles and placing them in 10 mL of an acidic solution. The solution was shaken using a 150 rpm thermoshaker at 37°C for 2 H, and the precipitate was transferred into 9 mL PBS at pH 7.4. Under an alkaline pH, the solution completely dissolved the matrix to release the bacteria. Subsequently, it was shaken using a 150 rpm thermoshaker at 37°C for 1 h and 1 mL was taken to count viable bacteria. The protective power of LC microparticles against stomach acid was then calculated using the following formula:

$$\text{The protection power (\%)} = \frac{\text{logTPC microparticles after acid exposure}}{\text{logTPC in initial microparticles}} \times 100$$

The data were tested statistically with the one-way ANOVA analysis method of variance followed by Tukey's HSD at a degree of confidence $\alpha = 0.05$.

RESULTS AND DISCUSSION

The optimum growth time of *Lactobacillus casei*

The optimum growth time of LC can be seen by observing changes in the pH and TPC log values. Figure 1 shows the decrease in pH during the growth period from 0 to 12 h because the accumulation of lactic acid LC resulting from fermentation during the process causes a decrease in pH.[15] Furthermore, the pH of the growth medium is stabilized around pH 4 at 12–18 h because LC is subjected to a stationary phase with an equal number of dead and live bacteria. After 18 h, the pH increased to pH 6 because the number of nutrients decreased and LC entered a dead phase.[16]

The life cycle of LC based on the log TPC in Figure 1 shows the value reaching the final exponential phase at the 12th H. Meanwhile, the initial stationary phase occurred at the 12th to 18th H before entering the dead phase. There is also a decrease in the TPC log value because the nutrients have been used up with the accumulation of toxic metabolic products.[7] Therefore, the optimum growth time is selected when the bacteria enter the final exponential or the initial stationary phase. This is because the bacteria are more resistant to drying during the spray drying process and the optimum growth time selected in this study is 12 H.[17]

Microparticles preparation of *Lactobacillus casei*

The successive viscosity test FI, FII, and FIII results of 2.99 ± 0.17, 3.45 ± 0.22, and 3.72 ± 0.23 cP showed that viscosity and the levels of L-type MAC were directly proportional. The next stage was the manufacture of LC probiotic microparticles and characterizes it.

Morphology

The results of the morphological examination can be seen in Figure 2, where the morphology of microparticles in FI and FII shows that the shape had deep and nonspherical-shaped concavities. The morphology of the FIII microparticles showed that the shape had less deep concavity and a more spherical shape. From the results, the three formulas showed a smooth or nonporous surface.

Fourier transform infrared spectroscopy investigation

Figure 3 indicates that the absorption band or spectral peak widening of the carbonyl (C = O) and hydroxyl groups (OH) showed decreasing frequency in microparticles compared to the absorption band of the copolymer. The FI, FII, and FIII wave numbers were 1718.24, 1721.15, and 1714.5 cm^{-1} for the C = O group, and 3437.13, 3418.9, and 3426.6 cm^{-1} for OH group. Meanwhile, the copolymer had an absorption band of 1724.17 cm^{-1} (C = O) and 3511.17 cm^{-1} (OH).

The three formulas decreased the wavelength number of the C = O and-OH groups compared to L-type MAC.

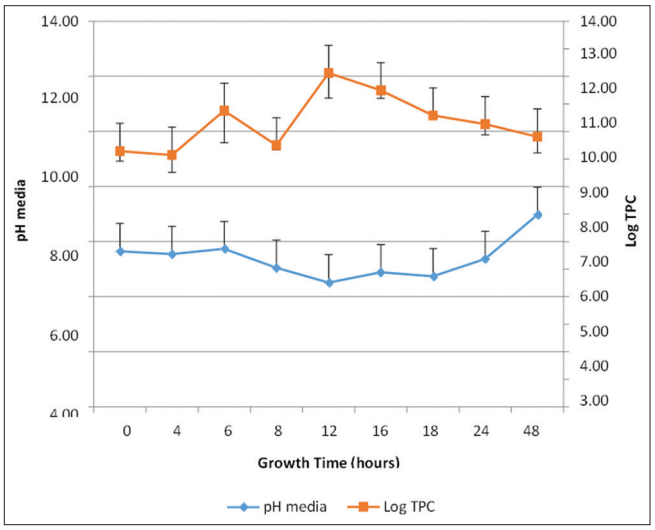

Figure 1: Graph of the relationship between growth time vs. pH media and log TPC. TPC: Total plate count

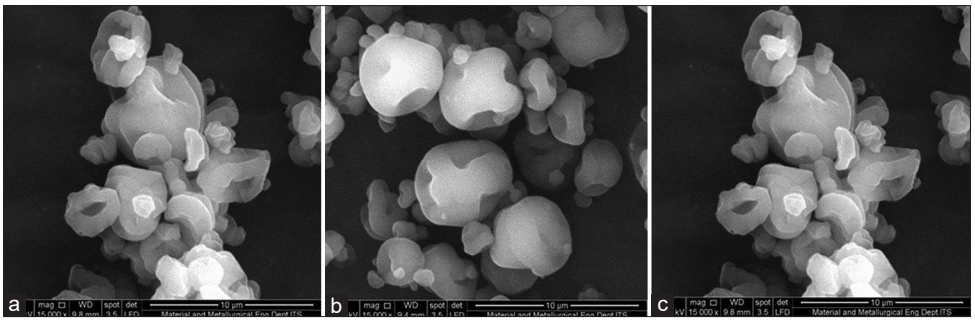

Figure 2: Photograph of *Lactobacillus casei* probiotic microparticles using L-type methacrylic acid copolymer of FI (a), FII (b), and FIII (c) taken with scanning electron microscopy

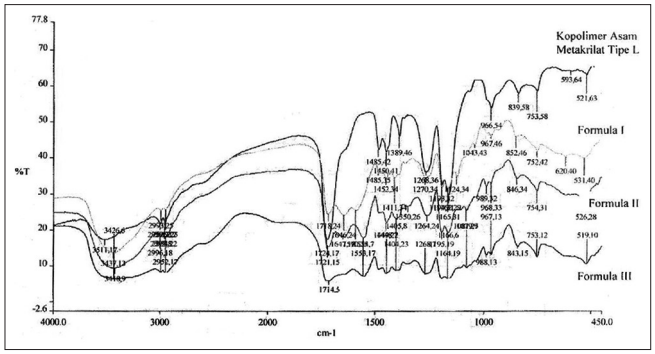

Figure 3: Comparison of the Fourier transform infrared spectroscopy spectra of L-type methacrylic acid copolymer and microparticles FI, FII, and FIII

The carboxyl (CO) and hydroxyl groups (OH) vibration frequency move from 1724.17 to 1714.50 -1721.15 cm^{-1} to and 3511.17 to 3418.90 - 3437.13 cm^{-1}, respectively. The decrease in wave number indicated the formation of intramolecular hydrogen bonds.

Moisture content and particle size

Table 2 shows that the copolymer concentration influenced the microparticles' moisture content (MC), and the minimal MC result was in FIII. This was because the concentration of copolymer increased with the viscosity and decreased the amount of water. Therefore, the water retained in microparticles was reduced according to the statistical analysis. The results showed that there were significant differences between the MC of microparticles FI, FII, and FIII.

Examination of the three formulas' microparticle sizes showed that the L-type MAC levels were directly proportional to the particle sizes. This observation was attributed to the low viscosity of the solution in FI, which had the highest water content. As a result, the solidification process took longer due to the slow compaction. The water content trapped in the droplets diffused out during this process, causing the particle size to shrink. In contrast, FIII had the highest viscosity of the three formulas, which led to a faster compaction process and the formation of rigid particles. All

Table 2: Moisture content and microparticle size of probiotic *Lactobacillus casei*

Formulas (matrix level) (%)	MC (%)*	Microparticles size (μm)*
FI (0.50)	10.05±0.49	3.36±0.13
FII (0.75)	9.77±0.19	4.02±0.25
FIII (1.00)	7.45±0.11	4.47±0.19

*Data are the mean of 3 replicates±SD. MC: Moisture content, SD: Standard deviation

three microparticle formulas exhibited particle sizes ranging from 3.36 to 4.47 μm, within microparticles size of 1–1000 μm. These sizes were larger than LC bacteria, which were 0.6–1.1 × 1.5–4.0 μm.[18] Therefore, LC bacteria were assumed to encapsulate well in microparticles.

Encapsulation efficiency

EE of bacteria into the polymer by spray drying process was influenced by the time taken for evaporating the water from the droplet to solidify and dry the matrices as presented in Table 3. The data showed that the lowest and highest EE was found in FI and FIII, respectively. Therefore, the concentration of matrices was directly proportional to the bacteria encapsulation, and FIII produced microparticles with EE of 92.46%.

The statistical analysis showed significant differences between EE of FI, FII, and FIII. The result indicated that the concentration of copolymer significantly influenced EE. The higher concentration resulted in faster polymer solidification and limited the bacteria diffusion across the phase boundary to increase EE in the polymer.

Power protection against stomach acid

The protective power of LC microparticles against gastric acid in the three levels of the L-type MAC matrix can be seen in Table 4. From these data, the level of the L-type MAC matrix was directly proportional to the percentage of acid protection. From the statistical analysis, the protection power of LC microparticles against acidic conditions from FI to FIII had a significant difference.

Table 3: Encapsulation efficiency of *Lactobacillus casei* probiotic microparticles in L-type methacrylic acid copolymer matrix

Formulas (%)	Bacteria biomass in the bacteria suspension (before the spray drying process) (mg)*	Bacteria biomass in the microparticles after the spray drying process (mg)*	Efficiency encapsulation (%)*
FI (0.50)	1.163±0.002	0.306±0.016	26.33±1.33
FII (0.75)	0.941±0.002	0.597±0.043	63.39±4.53
FIII (1.00)	0.805±0.002	0.745±0.001	92.46±0.17

*Data are the mean of 3 replicates±SD. SD: Standard deviation

Table 4: Protection power of *Lactobacillus casei* probiotic microparticles against acidic (%) after the spray drying process

Formulas (%)	Average TPC Log after spray drying*	Average Log TPC after exposure to acid*	Protection power of microparticles against acidic (%)*
FI (0.50)	6.85±0.35	6.30±0.14	91.97±2.07
FII (0.75)	6.91±0.58	6.62±0.15	95.80±2.11
FIII (1.00)	7.27±0.45	7.14±0.09	98.17±1.17

*Data are the mean of 3 replicates±SD. SD: Standard deviation, TPC: Total plate count

The level of the matrix was also directly proportional to the thickness and density of the walls. The increased level of the L-type MAC matrix also affected the formation of carboxylic groups. Therefore, more hydrogen bonds were formed to increase the density of microparticle walls.[19]

CONCLUSION

L-type MAC has the potential to be used as an LC probiotic matrix made by spray drying method with an inlet temperature of 120°C. Increasing L-type MAC levels in LC microparticles can also increase the viscosity of the solution. This is because the copolymer level is directly proportional to the formation of intramolecular hydrogen bonds by the carboxylic group. Meanwhile, increasing the concentration of L-type MAC until level 1.00% improved the morphology of microparticles observed by SEM. This is evident in the smooth, nonporous surface of microparticles, which has a shape close to spherical. It reduces MC, increases particle size, improves EE, and provides better protection against stomach acid.

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Conflicts of interest

There are no conflicts of interest.

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