



Effect of sodium alginate concentration on characteristic, viability, and antibacterial activity of probiotic-alginate microparticles

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ABSTRACT

Probiotic (*Lactobacillus acidophilus*) has been known as an effective antibacterial activity, however storage, high oxygen levels, and gastric acid condition was generally decrease the probiotic viability. To maintain the probiotics' viability, extrusion technique was selected to produce microparticles. This technique used sodium alginate polymer and calcium chloride cross linking solution. This research investigated effect of sodium alginate concentration on the physical characteristics, viability and antibacterial activity. Results showed that increasing alginate concentration from 2.5% to 3.5% increased microparticle size (from 15.40 μ m to 37.53 μ m). Viability of *Lactobacillus acidophilus* of all formulas were above 94%, however an increased of alginate concentration did not significantly affect antibacterial activity. The extrusion method demonstrated its potential in encapsulation of *Lactobacillus acidophilus* for probiotics delivery.

Keywords : *Lactobacillus acidophilus*, microparticles, sodium alginate, extrusion, antibacterial activity



INTRODUCTION

Probiotics *Lactobacillus acidophilus* has been widely used to overcome problems in GI tract such as infections. Some advantages of probiotics as antibacterial including reduce antibiotic resistance (1). Generally, probiotics viability was 10⁶ to 10⁷ cfu/mL daily (2), however some reduction was found during storage caused by high oxygen concentration and acid pH exposure (3,4). Microencapsulation was selected as delivery system for protecting probiotics from extreme environment during storage (5). Extrusion technique has advantages such as simple, cheap, produce stable probiotics and high viability (2). Polymer was the important material to form microparticles (6). Alginate polymer has been investigated thoroughly because of its biocompatibility, non toxic and cheap. Sodium alginate formed gel microparticles by crosslinking with Ca²⁺ ion. These microparticles were used to protect active compound which sensitive to acid pH and release active in the intestine (7). The ratio of drug and polymer affected microparticles characteristics (8). The aim of this research was to investigate effect of alginate concentration on the characteristics, viability and antibacterial activity of

Lactobacillus acidophilus probiotics-loaded alginate microparticles.

MATERIALS AND METHODS

Materials: *Lactobacillus acidophilus* (from PAU Universitas Gajah Mada). Natrium Alginate (Wako Pure chemical Industry Ltd.), Natrium citrate pharmaceutical grade, sterile PBS, HCl (p.a) Natrium Chloride (p.a), sterile water, AA agar (Food Grade), Media de Man Ragosa Shorpe (MRS) Broth Steril, Nutrien Agar medium (NA), and *Salmonella thypimurium*.

Methods

Formulation of probiotics-loaded alginate microparticles: Gram staining method was used for qualitative analysis of *Lactobacillus acidophilus*. For characterization, FTIR and DTA were used for alginate and CaCl₂. Microparticles were prepared using extrusion method. Initial probiotics were dispersed into concentrated alginate polymer in sterile water as shown in Table 1. Mixture of probiotics-alginate was sprayed into CaCl₂ solution and stirred at 1000 rpm for 2 hours. Nozzle diameter size was 200 μ m and distance between solution and nozzle was 8 cm. Microparticles was washed of by centrifugation at 2500 rpm for 6

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minutes twice and was then dried using freeze dryer at -80°C for 20 hours. Microparticles were then characterized in terms of morphology, size and distribution, viability and antibacterial activity of probiotics by measuring MIC using *Salmonella thypimurium*.

Viability measurement: Viability study was conducted by measuring total plate count (TPC) and Log TPC of *Lactobacillus acidophilus* in microparticles. 1 gram of microparticles was weighted and mixed into 99 mL of sterile sodium citrate solution (1%) at pH 6.0. The mixture was then mixed homogenously for 1 hour followed by plating in the MRS agar and agar was incubated at 37°C for 48 hours. Viable count was determined by colony forming units (cfu)/gram unit.

Measurement of Minimum Inhibitory Concentration: Inhibitory activity was conducted using diffusion method Kirby-bauer. Two tubes of sterile sodium alginate (15 mL) (*Seedlayer*) and 35 mL (*Base Layer*) were prepared. Base layer was poured into sterile plate until solidified. 2 μL inoculum of *Salmonella thypimurium* was poured in the *seed layer* tube at temperature $40-45^{\circ}\text{C}$, mixed and vortexed it. The mixture was then poured into *base layer* and let it solidified. 100 μL of microparticles sample (200 mg microparticles dissolved in 10 mL sodium citrate). Plates were then incubated at 37°C for 24 hours. Zone diameter was measured and MIC was evaluated. Ciprofloxacin antibiotic was used as positive control and blank microparticles was used as negative control.

RESULTS AND DISCUSSION

Formulation of probiotics microparticles by extrusion method has been successfully produced by crosslinking alginate polymer and Ca^{2+} resulting entrapped probiotics in the microparticles. Particle sizes were found 15.40 μm , 32.17 μm and 37.53 μm for formula FI, FII and FIII respectively (Figure 1). For formula FII using 3% alginate concentration, distribution of particle size showed normal distribution compare to other formulas. The differences were found between FI and FII and between FII and FIII. By increasing concentration of alginate from 3% to 3.5%, no significant differences of size were resulted. The increase of size was due to an increase in the alginate viscosity which contributed to the bigger droplet size of microparticles. In the form of morphology, optical microscopy of formulas microparticles showed spherical form of microparticles as shown in figure 2 as well as dried morphology conducted by SEM (Figure 3). Some factors that influence characteristics of microparticles were cross linker's

contact time. The longer contact time, the more fragile and easily breakable of microparticles, however this may caused the irregular microparticles. The distance between nozzle and crosslinker solution affected diameter of microparticles (9). Stirring speed was also an important factor in resulting stable microparticles contribute to form spherical microparticles and avoid aggregation. Lower speed may caused agglomerated particles, whereas faster speed formed irregular particles (10). *Lactobacillus acidophilus* probiotics have many advantages such as having antibacterial activity from its bactericides (11), hydrogen peroxide, lactic acid (12). Probiotics bacteria's viability influences activity. Probiotics viability was 10^6-10^7 cfu/mL daily (2), however viability of *Lactobacillus acidophilus* can reduced during storage, high level of oxygen and acid pH (3,4).

Microparticles have been widely used for protecting active substance from extreme exposure, including maintains viability. Sodium alginate was used as matrix in the range of 0.5 to 4% and calcium chloride as crosslinker at 0.05-1.5 M (2). Krasaekoopt et al (2) investigated comparison between viability of *Lactobacillus acidophilus* before and after microparticles preparation. They found that the minimum viability was more than 10^7 cfu/g. Viability of *Lactobacillus acidophilus* before extrusion was found fulfilled the requirements, however after formulation a small decrease of viability was found, but not significant as shown in Table 2. In terms of activity of *Salmonella thypimurium*, minimum inhibitory concentration (MIC) was shown in table 3. We can see that negative control had MIC of 11.05 ± 0.13 mm. This was occurred because of existence of sodium citrate inside the negative control which able to inhibit bacteria growth. Significant difference of antibacterial activity between negative control and formula I, II and III were analyzed statistically using one way ANOVA followed by post hoc Tukey test. This concluded that an increase of alginate concentration did not significantly influenced antibacterial activity. When compared to MIC of ciprofloxacin, all formulas I, II and III produced as same as MIC of ciprofloxacin, therefore it concluded that all formula I, II and III were potential in producing antibacterial activity.

CONCLUSION

Probiotics-loaded alginate microparticles have been successfully produced using extrusion method by aerosolisation. Spherical, almost smooth and small size of microparticles of 15.4 μm ; 32.2 μm and 37.5 μm were produced using alginate 2.5%; 3%

and 3.5% respectively. An increased of alginate concentration from 2.5% to 3.5% did not significantly affect viability and antibacterial activity of *Lactobacillus acidophilus* to *Salmonella thypimurium*.

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Table 1. Formula of Probiotics Microparticles

Compounds	Function	FI	FII	FIII
<i>Lactobacillus acidophilus</i> (10 ⁸ cfu/mL)	Active compound	5mL	5 mL	5 mL
Sterile aquadest	Solvent	20 mL	20 mL	20 mL
Na Alginate	Matrix	2.5%	3.0%	3.5%
Aquadest	Solvent	50 mL	50 mL	50 mL
CaCl ₂ 1.5 M	Crosslinker	150 mL	150 mL	150 mL

Table 2. Viabilitas *Lactobacillus acidophilus*

Group	Viability (%)
Formula I	94,59 ± 1,38
Formula II	97,29 ± 3,15
Formula III	99,76 ± 0,35

Table 3. MIC of bacteria *Salmonella thypimurium*

Group	MIC zone (mm)
F I	16,04± 0,11
F II	14,30± 0,08
F III	13,46± 0,25
Positive control	17,75± 0,10
Negative control	11,05± 0,13

Note:

Data was gathered from two replicates

Positive control = Ciprofloxacin

Negative control = Blank Microparticles

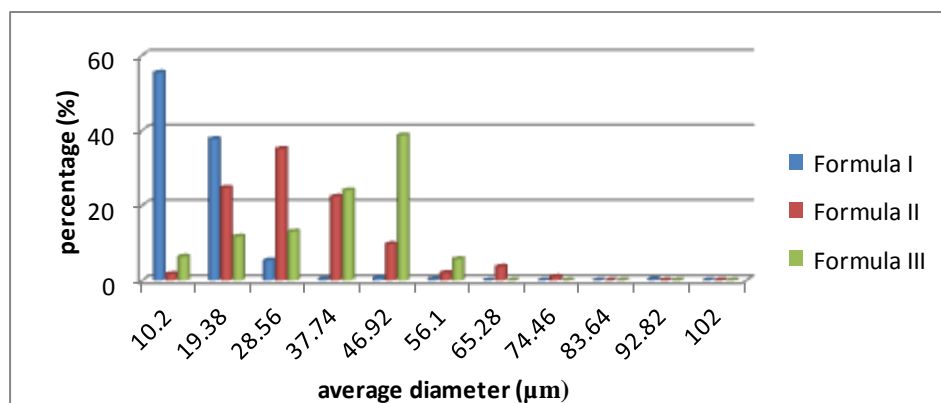


Figure 1. Size distribution of all formulas

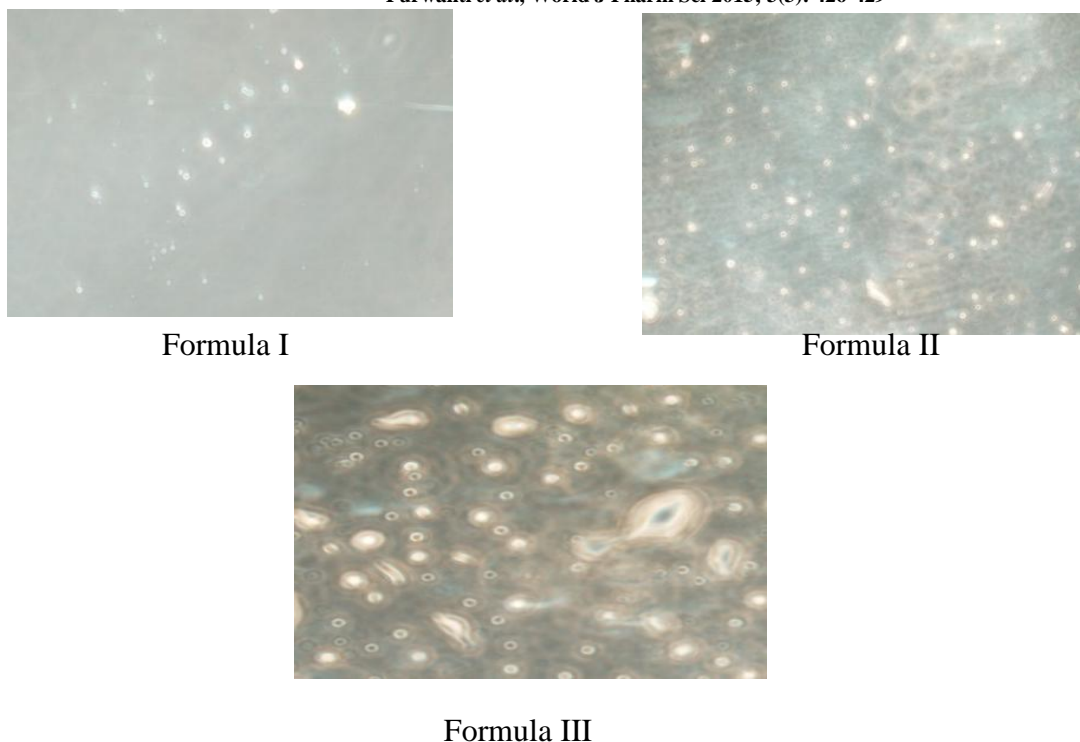


Figure 2. Optical microscope of microparticles formulas (at magnification 400x)

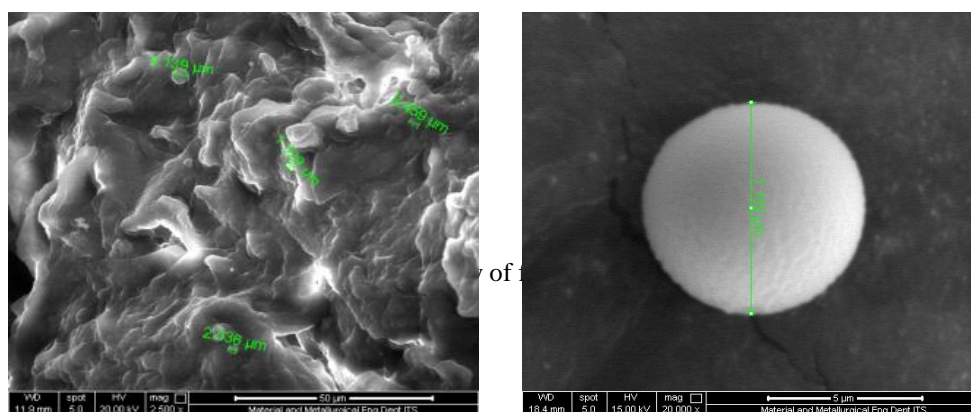


Figure 3. SEM morphology of formula II

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