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EFFECT OF COMBINATION SODIUM ALGINATE-GELATIN 1% : 2% CONTENT IN CHARACTERISTIC AND ANTIMICROBIAL ACTIVITY OF PROBIOTIC MICROSPHERES *Lactobacillus acidophilus*

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INTRODUCTION

Probiotics is live micro-organisms which when administered in adequate amounts, confer a health benefit on the host (FAO / WHO, 2002). Probiotics may give therapeutic effect in minimal amounts 10^6-10^7cfu/g daily. Some reports in the last decade have described that probiotic, such as *Lactobacillus acidophilus*, is useful for treating skin disorder such as dermatitis, acne, cellulitis, and psoriasis. This relates to the content of probiotics metabolites that protect the skin from most pathogenic bacteria, such as *Staphylococcus aureus*, and improve skin structure (Cinque et al., 2011).

In topical application, the active ingredient should last a long time on the skin and prolonged release. Meanwhile, *Lactobacillus acidophilus* unstable to environtment factors, such as the high temperature, oxygen, and humidity (Al-Hurr, 2011). Therefore, probiotics encapsulation is needed. In addition, encapsulation also may provide prolonged release so that its effectiveness increased as antimicrobial activity becomes longer.

Encapsulation process that forms microparticles (1-1000 µm) called micro-encapsulation. Spherical microparticles called microspheres (Sahil et al., 2011). The recommended size of microspheres which active ingredient site action is in epidermis layer is 5-300 µm (Chadawar and Shaji, 2007).

Sodium alginate is widely used as a matrix of microencapsulated probiotics. Microspheres made with sodium alginate matrix may improve the viability of probiotics during storage. Gelatin also often used in microencapsulated probiotics (Solanki et al., 2013). Gelatin is biodegradable, non-toxic, and easy to experience a cross-linking so that it can be used in the preparation of colloidal delivery systems, such as microspheres and nanoparticles (Sailaja, et al., 2010).

Type of matrix influence the characteristic of microsphere. Microspheres are only made with sodium alginate tend to provide simultaneous release. Therefore for prolonged release, sodium alginate should be combined with gelatin (Lee *et al*., 2014; Roy *et al*., 2009). Combination of sodium alginate and gelatine in the ratio 1: 2 will provide small particle size that not easily aggregate during the process of mixing and drying (Lee *et al*., 2014).

In this study, microspheres were formed by cross linking between combination of sodium alginate and gelatin (1% : 2%) with $CaCl_2$ as cross linker using ionotropic gelation method.

OBJECTIVE

The aim of this research was to investigaste the effect of combination of sodium alginate and gelatin (1% : 2%) content in characteristics and antimicrobial activity of probiotic microspheres *Lactobacillus acidophilus.*

METHOD

Materials. *Lactobacillus acidophilus* from the Food and Nutrition Studies Center of Gadjah Mada University, *Staphylococcus aureus*, gelatin type B (Pharmaceutical Grade), sodium alginate (Pharmaceutical Grade), calcium chloride (Food Grade), de Man Rogosa and Sharpe (MRS) media, Phosphate buffered saline (PBS) sterile, sodium hydroxide, and Nutrient Agar (NA).

Tools. Hot plate stirrer (Dragon Lab MS pro), pH meter SCHOTT glass mainz type CG 842, FTIR spectrophotometer (Jasco FT-IR/5300), atomizer aerosolization (size noozle 200 µm and a pressure of 40 Psi) , ultra sentrifugator Hermle Z36HK, optical microscope, incubator (Memmert INB 500), colony counter (Suntex 570), vortex (Labinco L46), autoclave (Huxley HL340), thermoshaker (Gerhardl Labshake), an analytical balance, an oven, petri dish, ose, micro pipette, and calipers.

Optimization Growth of Probiotic *Lactobacillus acidophilus*. 500 ml of de Man Rogosa and Sharpe (MRS) sterilized at 121°C for 30 minutes. Further,

cultures of *Lactobacillus acidophilus* taken to be activated 3 times. The 1st activation is 1 ose cultures of Lactobacillus acidophilus put into 10 ml MRS and incubated at 37°C for 24 hours. Continued the 2nd activation is 1 ml of the culture of the 1st activation put in 9 mL of MRS and incubated at 37°C for 24 hours. Furthermore, the 3rd activation is taken 5 mL culture of the 2nd activation into 45 ml MRS and incubated at 37°C for 24 hours. The 3rd activation culture taken 15 mL and put into 135 mL sterile MRS. It incubated at 37°C for 48 hours. Samples taken at 0, 6, 12, 18, 24, and 48 hours. Then determination Lactobacillus acidophilus growth by total plate count (TPC) of each sample (Usmiati and Marwati, 2007).

Probiotic Microspheres Formulation

Table 1. Probiotic Microspheres Formula

Material	Function	F I	F II	F III
L. acidophilus	Active Ingredient	10 ml	10 ml	10 ml
Sodium Alginate	Matrix	1,5 g (1%)	4,5 g (3%)	-
Gelatin type B	Matrix	3 g (2%)	-	4,5 g (3%)
Aquadest	Solvent	140 ml	140 ml	140 ml
$CaCl_2$ 1,5 M	Cross linker	300 ml	300 ml	300 ml

*probiotics taken during a minimal amount of 10^6-10^7 cfu/ml

First, matrix dissolved in 140 ml of distilled water. Sodium alginate sterilized by autoclave at 121°C for 20 minutes. While gelatin sterilized by UV light for 1 hour (Hu et al., 2009). For gelatin solution, NaOH added to pH 7 and heated to 40°C. Then 10 ml of *Lactobacillus acidophilus* (10^6-10^7cfu/ml) that cultured for 24 hours added into the solution matrix. TPC test performed to determine the viability of probiotic. Then the microspheres formed by extrusion techniques. Matrix containing *Lactobacillus acidophilus* sprayed using aerosol sprayer (extrusion), with pressure 40 Psi and spraying length 8 cm into 300 ml 1,5 M $CaCl_2$ solution while stirred continuously for 1 hour at 1000 rpm. Microspheres were collected by centrifugation 2.500 rpm for 6 minutes then washed with sterile water 3 times. Then dried by oven at 40 °C for 30 hours.

Determination of Morphology and Particle Size. Particle size of 300 microspheres measured by an optical microscope.

Entrapment Efficiency (EE). Total plate count (TPC) is performed before and after the extrusion process. Weighed 0.5 grams of microspheres into 50 ml of sterile PBS solution at pH 7.4 (Dinarvand et al., 2003) then shaker for 2 hours and diluted in 9 parts of PBS. Then plating on MRS agar medium and incubated at 37°C for 48 hours.

$$EE = \frac{\log TPC \text{ after extrusion}}{\log TPC \text{ before extrusion}} \times 100\%$$

Antimicrobial Activity Test. Prepared 8 ml (seed layer) and 10 ml (base layer) of nutrient agar media. First, base layer poured in a petri dish and allowed to solidify. Then prepared $1{,}4 \times 10^8$ cfu / g culture of *Staphylococcus aureus* which equivalent to 0.885 of absorbance. Then put 3 µm into seed layer at 40-45°C, shaken with a vortex. Furthermore it poured on the surface of the base layer in a petri dish and allowed to solidify. Weighed 200 milligrams of microspheres and mixed with 10 ml of sterile PBS 7.4 (Dinarvand et al., 2003). Furthermore, made a hole diameter of 8 mm with a pipe. The hole filled by each 50 mL of sample then incubated at 37 ° C for 24 hours. Inhibitory zone observed and measured using calipers (millimeters).

Data Analysis. Data of characteristic and antimicrobial activity of *Lactobacillus acidophilus* performed statistical analysis by one way ANOVA, then analyzed by Honesty Significant Difference (HSD) Tukey with a confidence level of 0.95 (α = 0.05).

RESULT AND DISCUSSION

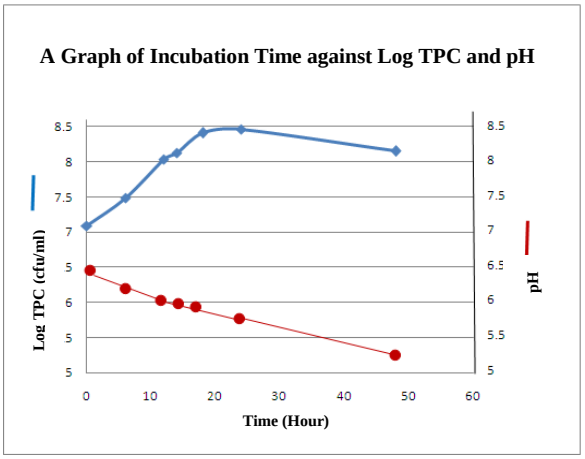
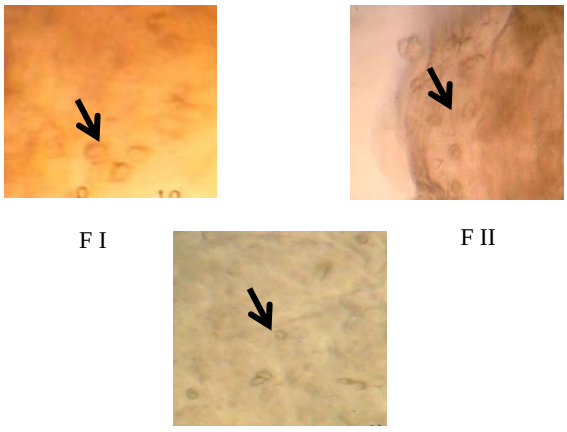


Figure 1: A Graph of Incubation Time against Log TPC and pH

Based on Figure 1, *Lactobacillus acidophilus* optimum growth time is 24 hours. The graph also shows that longer incubation period cause acidic pH. The TPC log increased with increasing incubation time and reached its peak at 24 hours, then the TPC log decreased with increasing incubation time. If the time of growth continued, amount of nutrients decrease and the metabolites

accumulate which showed in decreased pH, lead to death in bacteria (Al-Qadiri et al., 2008). Based on these results, 24 hours as the optimum incubation time will be used in the production of probiotic microspheres.

Microspheres that have been dried in oven for 30 hours observed using an optical microscope. The results showed, all formula are spherical.



F I F II F III

Figure 2: Morphology of F I, F II, and F III using 400x Magnification Optical Microscope

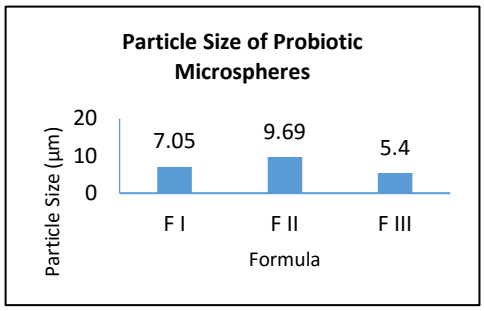


Figure 3: Particle Size of F I, F II, and F III

The particle size of F I, F II, and F III are 7.05 µm; 9.69 µm; and 5.40 µm. FII > FI > FIII, this can be attributed to their mannuronic groups on sodium alginate cannot form the "egg box" so that the particle size of the formula with sodium alginate composition was bigger than gelatin. Additionally, gelatin is thermally reversible. At 40°C gelatin solution will be more dilute. When gelatin cross linked with $CaCl_2$ at room temperature, gelatin solidifies quickly, thereby providing small particle size (Elzogby, 2013).

To determine entrapment efficiency of the microspheres, viability of *Lactobacillus acidophilus* tested by calculating the total plate count (TPC) test.

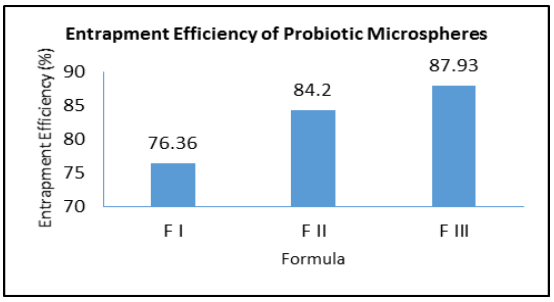


Figure 4: Entrapment Efficiency of F I, F II, and F III

The entrapment efficiency of F I, F II, and F III are 76.36%; 84.20%; and 87.93%. Based on analysis by One Way ANOVA with Tukey HSD, FIII, the highest entrapment efficiency, has significant differences with F I and F II. It correspond to the particle size of F III is the smallest compared to F I and F II. Meanwhile, F I should have greater entrapment efficiency than F II. However, the entrapment efficiency of F I less than F II. This can be caused by the relese of active ingredient in F1 is more difficult than F II. Based on transmittance test, the transmittance values for F I, F II, and F III are 87,7%; 85,6%; and 89,7%. This shows number of bacteria that are released from F I for 2 hours less than F II. Thus, in 2 hours, the possibility of bacteria in F I have not been release completely because of their composition of the gelatin in F I can close the pores formed from sodium alginate matrix thus increase the density of matrix. To know more release mechanism, it is necessary to determine the swelling index (Sahil et al., 2011). While F II, because of the composition of sodium alginate make is porous, then it is likely the bacteria do not trapped perfectly during the process so that the entrapment efficiency becomes smaller than F III.

The antimicrobial activity test was conducted before and after the extrusion. The results of the activity test of FI, F II and F III before extrusion process are 12, 85 mm; 10.98 mm; and 9.68 mm. Meanwhile, the activity test of FI, F II and FIII after the extrusion process are 11.22 mm; 9.80 mm; and 9.27 mm. F I has the greatest antimicrobial activity. Its inhibition zone similar to 3.60 ppm of gentamicin concentration. F I has the greatest antimicrobial activity because it has the highest TPC value after extrusion compared to F II and F III. TPC also showed that the viability of F I, F II, and F III decrease during the process. Based on statistically tested One-Way ANOVA with Tukey HSD test, there is no significant difference between F II and F III. Meanwhile, the antimicrobial activity of F I is significantly differ to F II and F III.

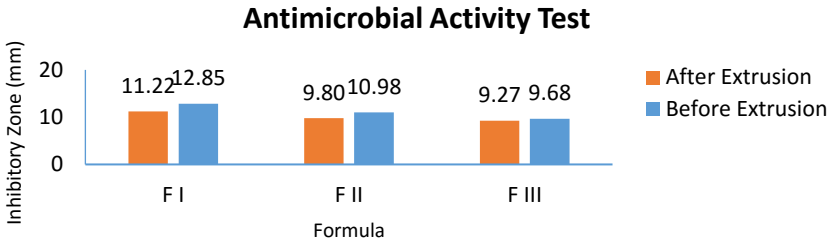


Figure 5: Antimicrobial Activity of F I, F II, and F III

CONCLUSION

From this study, it can be concluded that:

1. The morphology of F I, F II, and F III are spherical.
2. The particle size of F I is smaller than F II but greater than F III.
3. Entrapment efficiency of F I is the smallest.
4. Antimicrobial activity of F I is the highest.

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