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The formulation of probiotic *Lactobacillus acidophilus* granule with acacia and sodium alginate as binding agents

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ABSTRACT

Probiotics are living microorganisms that can have a positive impact on health when consumed in adequate amounts. This research aimed to determine the effect of different binders on the viability of *Lactobacillus acidophilus*, a member of Lactic Acid Bacteria (LAB). The probiotic granules were prepared with wet granulation method using mannitol as the filling ingredient. Formulas I-III used acacia as a binder (3%, 4%, and 5%), while Formulas IV-VI used sodium alginate (1%, 1.5%, and 2%). The probiotic granules produced from all formulas were evaluated for flow time, the angle of repose, compressibility, and LAB viability. The LAB viability test results showed that all of the six formulas met the probiotic requirements, i.e., at least 10^7 CFUs (Colony Forming Units) per gram. The viable LAB in Formulas I-VI were 3.94×10^7, 4.4×10^7, 2.7×10^7, 2.6×10^7, 3.5×10^7, and 2.3×10^7 CFU/g, respectively. The data were analyzed by comparing the average values of the One-Sample T-Test results, followed by Mann-Whitney test. The results revealed that each formula had different capacity in maintaining the viability of *Lactobacillus acidophilus* with different binders (i.e., acacia and sodium alginate) as a probiotic product. Formula II (4% of acacia) produced probiotic granules that met the requirements of compressibility and the angle of repose. However, its granular flow time exceeded the standard.

Keywords: *Lactobacillus acidophilus*, acacia, sodium alginate, lactic acid bacteria viability.

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INTRODUCTION

Probiotics are defined as living microbes that have beneficial effects on the health and life of the host (Tamime *et al*., 2005). Some probiotics that are part of normal flora, including Lactic Acid Bacteria (LAB), have been successfully isolated. Dosage forms and foods containing probiotics have been widely circulated in the market and used for various purposes, such as anti-diarrhea (Allen *et al*., 2011) and immune system booster.

Probiotics are from a group of lactic acid bacteria, i.e., species in the genera *Lactobacillus, Bifidobacterium,* and *Enterococcus*. Lactic Acid Bacteria (LAB) ferment sugars into lactic acid in the colon. Some species of *Lactobacillus* are known to have an essential role in maintaining health by, for instance, controlling the immune system. These bacteria help to deal with lactose intolerance and prevent diarrhea, constipation, cancer, and hypertension. They can also reduce cholesterol level, normalize bacterial composition in the gastrointestinal tract, and improve the immune system (Allen *et al*., 2011). Probiotics in a dosage form have to be resistant and survive food processing and storage.

Bacteria are groups of organisms that have no nuclear membrane and are one of the microbes that can be isolated and grown in a medium. Successful growth of bacteria in a medium is achieved by adjusting the type of bacteria to the selected medium (Radji 2008). *Lactobacillus* is a probiotic resistant to stomach acid and bile that can attach to the gastrointestinal wall; therefore, it protects the gastrointestinal mucosa and produces potentially anti-microbial substances. *Lactobacillus acidophilus* can thrive in aerobic and anaerobic environments. These bacteria can live in highly acidic environments with pH in the range of 4.0-5.0 (Hidayat *et al*., 2006).

Probiotic products are prepared in the form of granule through granulation. The most widely used agglomeration process in pharmaceutical industries is wet granulation. Wet granulation is the process of adding liquids to a powder or powder mixture in a container equipped with a stirrer to produce granules (Siregar and Wikarsa, 2008).

This research focused on *Lactobacillus acidophilus* in the form of granule made using wet granulation technique. It used binding agents from natural class with good solubility in distilled water, namely acacia and sodium alginate. These two additives have a proper viscosity in a dosage form. The filling ingredient used in this research was mannitol because it is favorably tasty, sweet, smooth, cool, and not hygroscopic. This research used different concentrations of binders. Each formula determined whether *Lactobacillus acidophilus* could remain active and effective as a probiotic product and confer positive impact on human health.

MATERIALS AND METHODS

The materials used in this research included a culture of probiotic bacteria, i.e., *Lactobacillus acidophilus* (LIPI Culture Collection, industrial Strain), sodium alginate, acacia, mannitol, medium deMan Rogosa Sharpe Agar (MRSA), calcium dichloride, and sodium chloride.

Preparation of biomass medium

Mannitol was dissolved in water and then sterilized using an autoclave at a temperature of 121°C for 15 minutes.

Preparation of inoculum

The inoculum (culture seed) was prepared by inoculating 30 mL of sterile MRSA medium in a 100 mL Erlenmeyer flask with 3 mL of *Lactobacillus acidophilus* suspended in 10% v/v mannitol solution. It was incubated at room temperature in a portable shaker at 120 rpm for 48 hours.

Biomass production

The production of *Lactobacillus acidophilus* biomass used 1 L Erlenmeyer flask containing 300 mL of MRSA medium. The inoculation involved the insertion of 30 mL of two-day-old seed, which then incubated in a portable shaker at room temperature at 120 rpm for 48 hours (Goderska *et al*., 2008).

Biomass harvesting

After 48-hour incubation, the biomass was harvested and separated using a centrifuge at 6,000 rpm for 20 minutes (Hwang *et al*., 2015).

Encapsulation of bacteria with sodium alginate

The harvested bacteria were mixed with 100 mL of 4% sodium alginate suspension. The cell suspension was dripped into 500 mL of 0.1 M calcium dichloride solution with a syringe and a dripping space of ± 1 cm to prevent the formation of large beads. Afterward, it was submerged in physiological NaCl. The CaCl element was removed with sterile distilled water, stirred, and filtered (Sirithanyalug *et al*., 2010). The resulted beads were dried in an oven at 40ºC for 6 hours (Krasaekoopt *et al*., 2003).

Granulation

After the encapsulation, the wet granulation process was performed using different binding agents, i.e., acacia for Formulas I-III (3%, 4%, and 5%) and sodium alginate for Formulas IV-VI (1%, 1.5%, and 2%). In this granulation technique, the granule mass was prepared until a shape of banana-like breakage was formed. The formulas of the probiotic *Lactobacillus acidophilus* granules are presented in Table I.

Table I. The formulas of probiotic *Lactobacillus acidophilus* granules

Components	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
Alginate-encapsulated *Lactobacillus acidophilus*	33	33	33	33	33	33
Acacia	3	4	5	-	-	-
Sodium alginate	-	-	-	1	1.5	2
Mannitol ad	100	100	100	100	100	100

Evaluation of granule mass

Following the granulation process was physical evaluations, which included flow rate, the angle of repose, compressibility, and LAB viability tests.

Data analysis

This research relied on a comparative study of the granular properties, i.e., flow time, compressibility, and LAB viability, using One-Sample T-Test to determine any significant difference between the formulas.

RESULTS AND DISCUSSION

The encapsulated *Lactobacillus acidophilus* granules contained LAB that met the requirements set in the Indonesian National Standard (SNI), i.e., 9.0x 10^7 CFU/g. This figure is in line with the previous research (Haghshenas *et al*., 2015) that proves alginate can maintain the immobilization of LAB. The evaluation of the granular properties, namely flow time, the angle of repose, and compressibility, showed different results from one formula to another (Table II).

Table II. The results of *Lactobacillus acidophilus* granule evaluation

Evaluations	F1	F II	F III	F IV	F V	F VI	Standards
Flow time (s)	25.67 ± 1.15	25.67 ± 1.15	30 ± 5.40	22.33 ± 4.12	25.33 ± 1.22	37 ± 13. 91	< 10 s
Angle of repose (°)	28. 67 ± 1.07	26.13 ± 3. 16	25.52 ± 3.88	28. 26 ±2.39	28.04 ± 1.01	28.95 ± 0.76	25^0 - 45^0
Compressibility (%)	2.33 ± 0.57	3.33 ± 1.00	4.67 ± 2.52	8.67 ± 7.57	5.33 ± 3.32	9.33 ± 8.19	< 20%

The probiotic granules produced in this research had favorable characteristics that met the standards except for the flow time. The different granular properties were caused by the variety of the binding agents' concentration in each formula. The amount of acacia and sodium alginate added to each formula greatly affects the granular compactness. The addition of these non-homogeneous binding agents produced granules with poor distribution, which makes them moist and results in the non-uniform concentration of *Lactobacillus acidophilus* product.

The flow test revealed that the flow time of all formulas was more than 10 s. Theoretically, the use of acacia and sodium alginate in the preparation of probiotic granules did not meet the specified requirements for flow time, i.e., less than 10 s. The higher the amount of the binding agents, the longer the flow time. The reasons behind this positive relationship include the size and shape of acacia granules, particle density in each formula, granular porosity, and pouring technique. A higher amount of binders in a formula makes the granules more difficult to flow. As a binding agent, sodium alginate strongly influences the granular compactness. This effect is reflected in the flow rate. An excellent granular flow is essential to ensure efficient mixing of materials and uniformly distributed weight (Lieberman, 1994).

In addition to the flow test, this results also measured the angle of repose and compressibility. The test results also showed that the angle of repose of the granules met the specified standard, i.e., 25°-45°. It was caused by the high viscosity of acacia and sodium alginate. A smaller quantity of acacia used in the formula decreases its binding capacity towards the probiotic granule. Consequently, the granules have poor spreadability. A small amount of acacia and sodium alginate does not guarantee the best angle of repose. However, their ability to bind particles represents the adhesive strength of a formula. A stronger adhesion can increase granular density and result in a flat surface or smaller slope (Danjo, 1995). Regarding compressibility, a larger amount of acacia and sodium alginate in a formula increases granular compressibility due to the influential role of bulk density.

The viability of *Lactobacillus* was calculated indirectly by sample dilution. The result of the dilution was placed in a cup, and the number of colonies in each cup was observed. Per the statistical requirements, only the cups containing 30-300 colonies were selected for the base of colony enumeration. Because the amount of *Lactobacillus acidophilus* was unknown, a series of dilutions were performed to obtain at least one cup of the colony. The total bacteria were calculated by multiplying the number of observed colonies in each cup from each formula with the dilution factor (Hwang *et al*., 2015).

The viability test results of the LAB (from bacteria to granule form) are presented in Table III. The figures show that all of the six formulas can maintain the viability of *Lactobacillus* *acidophilus* and meet the standards set in Indonesian National Standard, i.e., ≥ 10^7 CFU/g (SNI, 2009). Furthermore, they are also in line with the results of the previous research (Goderska *et al*., 2008). Formula II (using 4% of acacia) produced the highest viability. The LAB viability of the six formulas proves that acacia and sodium alginate as binding agents can create a good immobilization effect on the LAB. As for the physical properties that have not met the requirements, increased concentration of binding agent is recommended.

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Table III. The viability test results of *Lactobacillus acidophilus* granules

Samples		∑ Colony			Average of Colony	SPC (*Standard Plate Count)*
Bacterial Culture	10^{-4}	291	288	295	291.3	
	10^{-5}	272	281	278	277.0	10.2 x 10^7
	10^{-6}	299	275	256	276.7	
Encapsulation	10^{-4}	252	NC*	NC*	252	
	10^{-5}	241	271	281	264.3	9.0x 10^7
	10^{-6}	217	253	259	243.0	
Formula I	10^{-4}	198	102	115	138.3	
	10^{-5}	118	97	141	118.6	3.94 x10^7
	10^{-6}	98	102	115	105.0	
Formula II	10^{-4}	236	180	218	211.3	
	10^{-5}	148	136	-	142.0	4.4 x 10^7
	10^{-6}	131	120	97	116.0	
Formula III	10^{-4}	198	180	224	200.7	
	10^{-5}	78	121	95	98.0	2.7 x 10^7
	10^{-6}	83	92	37	70.7	
Formula IV	10^{-4}	-	127	236	181.5	
	10^{-5}	211	97	195	167.6	2.6 x 10^7
	10^{-6}	52	72	-	62.0	
Formula V	10^{-4}	216	236	178	210.0	
	10^{-5}	236	119	118	157.7	3.5x 10^7
	10^{-6}	64	81	117	87.3	
Formula VI	10^{-4}	208	118	176	167.3	
	10^{-5}	104	128	57	96.3	2.3x 10^7
	10^{-6}	111	57	78	58.7	

*NC = Not calculable

CONCLUSIONS

Acacia, as a binding agent, produces better probiotic *Lactobacillus acidophilus* granule than sodium alginate. Formula with 4% of acacia creates physically qualified probiotic granule.

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