BirEx JOURNAL
Budapest International Research in Exact Sciences
Medical, Biological, Argiculture, Engineering
Science and other related areas
e-ISSN : 2655-7827
p-ISSN : 2655-7835
http://www.bircu-journal.com/index.php/birex

Response Surface Methodology in determining the Temperature and Drying Time of Lactobacilus Casei Probiotic Granules

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Abstract: *Lactobacillus casei is a probiotic bacteria that has an important role in maintaining body health, but its use in liquid preparations is still constrained by shelf life. This study aims to determine the effect of temperature and drying time of wet granulation method on the physical and viability of probiotic granules. This research begins with increasing bacterial biomass and then harvesting the bacteria using centrifuge. Bacteria are encapsulated using 2% alginate and mannitol as fillers. It was dried at a temperature and time variation of 13 test formulas that had been designed by central composite design. The optimal temperature and drying time of probiotic granules obtained from Response Surface Methodology is 45 ° C for 2 hours.*
Keywords: *lactobacillus casei; probiotics; wet granulation; temperature and time*

I. Introduction

Probiotics are live microorganisms that can improve the health of the host when consumed in appropriate amounts (Sanders 2000; Schrezenmeir and De Vrese. 2001). Probiotics can improve individual health and probiotics do not cause residues and resistance. Probiotics can contain one or more strains of microorganisms and can be administered in the form of a liquid, flour, tablet or paste either orally or mixed in feed or drinking water. Probiotics cannot maintain a balance of beneficial microorganisms and eliminate pathogenic microorganisms (Lopez 2000; Pascual et al 1999).

*Lactobacillus casei*is an adaptable species, growing at temperatures of 15°-41°C and a pH greater than 3.5 (optimum 6.8). Lactobacillus casei strain shirota grows well at pH above 3.5 (optimum 6.8) (Widodo et al. 2003). Lactobacillus casei will not be active or dormant at cold temperatures or at -18°C, when it reaches body temperature the bacteria will be active again. Each microorganism has a maximum, minimum and optimal growth temperature, namely the temperature that provides the best growth and fastest self-propagation. Most bacteria in lactate culture have an optimum temperature of 30°C, but some cultures can form acid at the same rate at 37°C and 30°C. Bacteria can infect the body if it reaches beyond the normal intestinal (Ramadhianto and Nasution, 2019).

Considering the importance of Lactobacillus casei bacteria, maximum utilization can be done by granulating the bacteria in the form of granules. Generally, granules are made by wet granulation and dry granulation methods. The wet granulation method is the process of mixing the active substance and excipient particles into larger particles by adding the right amount of binder liquid so that a moist mass can be granulated. Commonly used fillers include lactose, mannitol, dextrose, starch, sucrose and microcrystals (Lachman et al. 1994).

Mannitol is generally used as an additive in the manufacture of chewable tablets because it is cold, sweet and pleasant in the mouth. Mannitol is a white crystalline, odorless, water soluble, sweet taste with a relative sweetness level of 0.5 to 0.7 times the sweetness level of sucrose. Mannitol is not hygroscopic, soluble in water, noncariogenic, low calorific value and has a sweet and cold taste (Siregar. 2010). Mannitol is used in the application of direct compressed tablets, dry granules and wet granules.

Encapsulation is a process of wrapping (coating) a core material, in this case probiotic bacteria as the core material by using a certain encapsulation material, which is useful for maintaining its viability and protecting probiotics from damage due to unfavorable environmental conditions. (Salminenet al.1998) explained the importance of the viability of probiotics, namely the preparation of live microbes that are beneficial to health. The most commonly used biopolymer for encapsulation of probiotic bacteria is alginate.

Based on this background, it is necessary to optimize the utilization of Lactobacillus casei bacteria. Optimum operating conditions in the manufacture of Lactobacillus casei granules can be determined by the Response Surface Methodology (RSM) method. With this method, it can be seen how the combination of temperature and time is good enough to get Lactobacillus casei granules with good yields.

II. Research Method

2.1 Tool

The tools used for this research are oven, incubator, analytical balance (Adventurer Ohaus), graded sieve (Bunsekifurui), Granule Flow Tester, homogenizer (Vortex Mixer), autoclave (Hiclave HVE-50), Tapped Density Tester, Laminar Air Flow , Portable Rotary Shaker (Eyela).

2.2 Ingredient

The materials used include Lactobacillus casei (LIPI Cibingong) bacteria, mannitol, medium for Rogosa Shape Agar, DeMan Rogosa Shape Broth, sucrose, sodium alginate.

2.3 Research Procedure

a. Inoculum Preparation

Inoculum (seedling culture) was prepared by inoculating a suspension of L. casei (10% v/v) into 30 mL of sterile MRSB medium in a 100 mL Erlenmeyer flask carried out at room temperature with agitation of 125 rpm for 48 hours.

b. Bacterial Biomass Production

Bacterial biomass production of L. casei was carried out using a 1000 mL Erlenmeyer flask containing 300 mL of sucrose medium. Inoculation was done by inserting 30 mL of inoculum (seedling culture) into it, followed by incubation at room temperature with 125 rpm agitation for 24 hours. After reaching the incubation time of 24 hours, harvesting and separation of the biomass was carried out using centrifugation at 4000 rpm for 20 minutes.

c. Granule Formulation

Mannitol as filler and 2% Sodium Alginate as binder.

d. Granule Manufacture

The material to be used is weighed first. Sodium alginate is made by heating water to boiling, then adding binder and stirring until mucilage is formed. The harvested bacteria were put into the mucilage and put in the incubator for a few minutes for temperature adjustment, then mannitol was added and stirred until a homogeneous mass was formed that could be clenched (banana breaking). The mass is then sieved through a No. 12 sieve and weighed. After the granules were sieved, the granules were then dried in an oven according to the temperature and time specified in the Central Composite Design (CCD). The dried granules were then weighed again and sieved with sieve no. 18.

e. Calculation of the number of Lactobacillus casei bacteria in granules using the Total Plate Count (TPC) method.

A total of 1.0 grams of probiotic granules were added to 9 ml of sterile distilled water to obtain a dilution of 10-1 . The dilution was continued in the same way to obtain a 10-2 dilution to a 10-6 dilution. A total of 100 L of dilutions 10-4 to 10-6 was pipetted into a petri dish containing MRSA medium and spread using drugalsky.

f. Granule evaluation

Evaluation of granules includes granule size distribution, flow time test, angle of repose test, and compressibility test.

2.4 Data analysis

Three-dimensional curves (Three dimensional response surface and contour plot) were used to test the correctness of the effect of the experimental variables on each response obtained. The coefficients on the empirical model were estimated using multidirectional regression analysis. The suitability of the empirical model with experimental data can be determined from the coefficient of determination (R2). To test the significance or not of the resulting empirical model, an Analysis of Variance (ANOVA) was carried out. The data obtained is the optimal condition of each tested factor. The next step is to make granules with optimal conditions from the RSM results, to test the results of whether the RSM mathematical model which is the optimal point produces the best Lactobacillus casei probiotic granules.

III. Results and Discussion

3.1 Biomass Production of Lactobacillus Casei Bacteria

Bacterial biomass production was carried out by first making a bacterial starter culture by inoculating the bacteria into 30 ml of liquid medium with ManRogosa Shape Broth (MRSB) and incubating at room temperature for 48 hours with 125 rpm agitation. The bacterial starter was then put into 300 ml of 30% sucrose medium. Bacterial harvesting was carried out after 24 hours using a centrifuge, so that bacterial pellets were obtained.

3.2 Optimization of Temperature and Drying Time of Probiotic Granules

The optimization conditions tested were temperature (42.93 , 45, 50, 55, and 57.07°C) and drying time (1.59 , 2, 3, 4, and 4.41 hours). A total of 13 experimental units, consisting of 4 factorial units, 4 starting point units, and 5 center point units have been carried out using the Central Compsite Design (CCD) experimental design. The results of the response in the form of viability and % compressibility are presented in the following table.

Table 1. Results of viability and percent compressibility of optimization of temperature and drying time of probiotic granules

No	Experimental design	Uncoded factor level		Response	
		Temperature (°C)	Time (hour)	viaBAL (cfu/g)	Compressibility (%)
1	Factorial	45	2	7.90	9.67
2		55	2	5.74	10.93
3		45	4	5.70	6.66
4		55	4	4.84	7.93
5	*Starting point*	42.93	3	5.26	14.28
6		50	1.59	7.5	10

7		50	4.41	5.23	9.09
8		57.07	3	5.14	11.94
9	*Center Point*	50	3	5.77	7.93
10		50	3	5.77	7.93
11		50	3	5.77	7.93
12		50	3	5.77	7.93
13		50	3	5.77	7.93

The table above shows that the drying temperature of the granules affects the viability of bacteria as indicated by the decrease in the number of bacteria with increasing temperature. For the physical test of granule compressibility, the drying time and temperature of the granules did not significantly affect the compressibility quality of the granules as indicated by the diversity of compressibility values. These results indicate that the same formula can provide different compressibility based on different treatments.

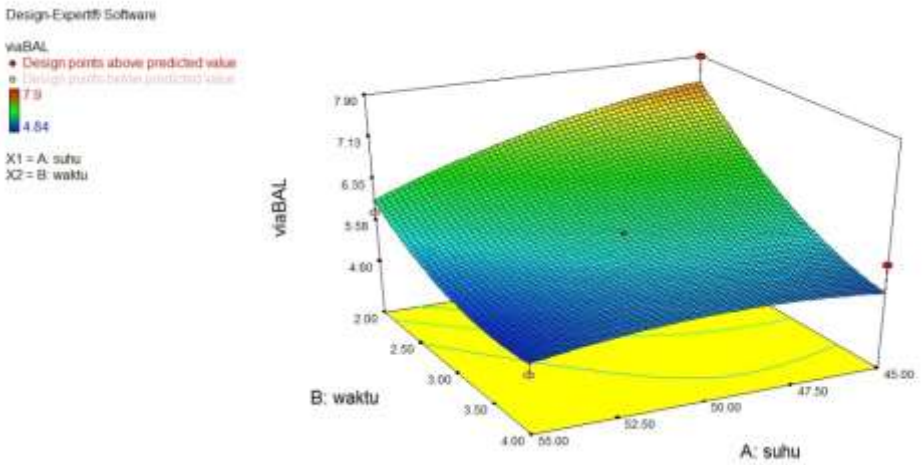

Figure 1. *Plot 3 Dimensions of bacterial viability response of probiotic granules from the relationship between temperature and drying time of probiotic granules*

From Figure 1, it can be seen that the higher the drying temperature of the granules, the lower the viability of the bacteria. This condition is due to the limitations of bacteria to survive at high temperatures, resulting in the presence of bacteria decreasing as the drying temperature of the granules increases.

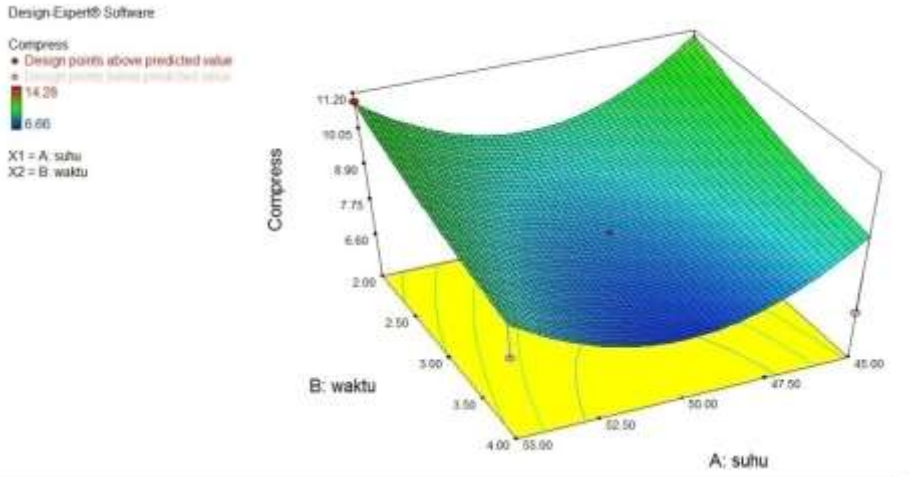

Figure 2. *3-dimensional plot of the compressibility response of probiotic granules from the relationship between temperature and drying time of probiotic granules*

The picture above shows that the drying temperature and time do not really affect the compressibility of the resulting granules. This is indicated by not achieving the red zone which is a marker of the achievement of the optimization point of a given treatment.

3.3 Optimization of Granule Drying Time and Temperature with Modeling Results

The experiments that have been carried out have shown that the recommended model to see the effect of two variables (temperature and drying time of the granules) on the response of bacterial viability and granule compressibility is a quadratic model recommended. The time factor affects the viability and the temperature factor affects the compressibility. This shows that the length of time for drying certain granules causes bacteria to decrease, so that the viability of bacteria at certain drying times can give bacteria the opportunity to survive or die due to the drying time of certain granules.

The drying temperature factor of the granules has a quadratic effect on the compressibility response of probiotic granules. This shows that increasing the drying temperature of the granules to a certain extent will produce very good quality granules and good quality granules. This is because the temperature can affect the binder in binding the fine grains of the filler used, so that the diversity of granules obtained at various temperatures is also different.

The optimal conditions of temperature and drying time of granules suggested by RSM as well as predictions of their viability and compressibility are presented in Table 2.

Table 2. Recommended optimal conditions for RSM and prediction of viability and compressibility

No	Temperature (°C)	Time (hour)	viaBAL (log cfu/g)	Compressibility (%)	Desirability	
1	**45**	**2**	**7.43**	**11.07**	**0.845**	***Selected***

Numerical optimization is carried out to obtain optimal conditions that produce good viability and compressibility. By setting the granule drying conditions (temperature and time) within the experimental range, the optimal conditions can be determined. The optimal conditions suggested by numerical optimization are temperature of 45°C and time of 2 hours. Maximum yield prediction for viability is 2.7x107cfu/g and for granule compressibility is 11.07% with Desirabillity 84.5%.

Table 3. Summary of results of viability and compressibility of probiotic granules based on optimization of CCD experimental design, numerical prediction and model validation

Condition	Average (response)	
	Viability	Compressibility
Initial experimental variable conditions	5.86 ± 0.84 cfu/g	9.24 ± 2.02%
RSM result numerical model	7.43 cfu/g	11.07%
Confirm optimal condition of RSM	8.47 cfu/g	8.06%
Percent Error	14%	27.19%
Percent increase in yield	44.53%	12.77%

IV. Conclusion

From the results of the study, it can be concluded that the optimal drying temperature and time for the wet granulation of the probiotic Lactobacillus casei using the Response Surface Methodology method and supported by the results of the evaluation of the compressibility of the granules at 45°C for 2 hours.

Suggestion

From the results obtained, it is recommended not to dry the granules in an ordinary oven in the manufacture of probiotic granules, because it causes a fairly high decrease in the presence of bacteria. Drying using a vacuum oven is highly recommended to prevent high pressure which can result in a decrease in the presence of bacteria.

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