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Thermal Characteristics of *Lactobacillus Plantarum* on Various Types of Carrier for Starter Powder Preparation

Zulafa Noor
Student, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia
Muh. Nur Cahyanto
Lecturer, Department of Doctoral Program, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia
Retno Indrati
Lecturer, Department of Doctoral Program, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia
Sardjono
Professor, Department of Doctoral Program, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract:
Mocaf fermentation usually carried out by depping raw cassava in water and added by lactic acid starter. Powder starter for mocaf fermentation prepared by Solid State Fermentation (SSF) has not been provided. Actually this method able for energy saving use for drying the fermented product. in so far there is now starter powder for mocaf fermentation is still no data. Whereas this proses design for starter production is very important for support this kind for fermentation. In relation of starter powder production the higher viability and stability the strain should be found in starter. Thermal characteristics of Lactobacillus plantarum on various carrier and its thermal conductivity is required to design the process starter production with high viability. The aim of the study was to determine the heat resistance of L. plantarum cells on several carriers so determine the best carrier and thermal conductivity of the carrier needed for designing a good starter production. Heat resistance cells were measured by determining the value of D and Z of the cell on different carrier. Three kinds of carrier are tapioca starch, rice flour and grated cassava. Heat resistance measurements using Yamazaki et al. (1997) method. Thermal conductivity of the carrier using Heat Conduction Apparatus Radial Conduction (HCARC). The results show that thermal conductivity of carrier was 0.460, 0.455, and 0.453 W/m^{0}C, for rice-flour, grated cassava and tapioca-flour respectively. Based on the heat resistance test, the highest D value (9.17 h) was obtained when rice flour was heating at 35 ^{0}C and its Z value was 20 ^{0}C, so the rice flour is the best for carrier among others carriers. Based on these data the process for starter powder production could be designed using L. plantarum with rice flour carrier at 55 ^{0}C for 55 minutes to get the cells concentration 10^9 cel/g, if desired only 1 log cycle reduction in cells content. Prolonged heating to 2.75 h would reduce 3 log cycles of cells amount to get the starter powder with cel viability 10^6 cel/g.

Keywords: *heat resistance, thermal conductivity, carrier, process design*

1. Introduction

M*ocaf flour* is a modified flour made from fermented cassava. Fermentation conditions for mocaf production was usually done using submerge fermentation by addition a *mocaf* starter to the water soaking (Leroy *et al.,* 2004). With this method, it was required energy for the drying. Traditional lactic acid fermentation occurs during starch to precipitate stage for approximately 30 days (Sobowale *et al.,*2007). Besides, lactic acid can improve the aroma and flavor (Bertolini *et al.,* 2001). When using Solid State Fermentation (SSF) it will reduce the energy for the drying but it required starter powder of Lactic Acid Bacteria (LAB) to be inoculated on fresh grated cassava without depping in water during fermentation. In order to design the process for starter production, research was conduct to measure the heat resistance of LAB during drying process of starter which prepared on a variety of carrier.
In relation of starter powder production the higher viability and stability the strain should be found in starter (Lian *et al.,*2002). Gilliland (1985) and Yousef, A.E.and Carolyn (2003), reports that the viability starter is depend on strain, high resistant of strain during drying and also thermal caracteristic of carrier used. So in this research selection of bacteria, age of strain, thermal caracteristic of carrier, is important to evaluate in order to meet the good caracteristic of starter powder. From this kind evaluation, the process desain of starter production able to produce. The aim of the study was to determine the best carrier that giving protection for LAB to be survive during heating or drying process, with determine the heat resistance of *L. plantarum* cells on several carrier and thermal

conductivity of the carrier, needed for designing a good starter production. Starter powder was produced by growing the isolate of *L. plantarum*in on 3 kinds of media, namely carrier grated cassava, tapioca starch and rice flour, inoculated with spores or cells of *L. plantarum*. Cell viability is very important to determine the quality of the starter. So, that drying process is a crucial stage in the starter production, in relation to the death of cells during the drying process (Heldman and Singh, 2001).
Heat resistance of Cell *L.plantarum* during drying process need to be evaluated in order to design the process production of starter powder. The use of a starter powder in the mocaf fermentation through solid state fermentation aim to gain dominance of lactic acid bacterial cells so it can produce lactic acid during fermentation which needed to change the character or nature of cassava flour (Huch *et al.,* 2008). Widiatmi (2013) said that the starter prepared using grated raw cassava as the carrier were able to dominate or compete with the indigenous bacteria on grater cassava after 2 days of fermantation. This fermentation results indicated that the mocaf produced had increased flour baking expansion. But in the study used starter was starter wet, no information on cell resistance *L.plantarum* for set up process starter powder. Therefore these study will be conducted by solid state fermentation (SSF).

2. Materials and Methods

2.1. Material

For the carrier materials were used tapioca starch, rice flour (*Rose Brand*), and fresh grated cassava from varieties *Meni* (Manihot esculenta Cranz) in the age 8-10 months. *Lactobacillus plantarum* was from Food and Nutrition Culture Collection, Universitas Gadjah Mada. Media used was De Man, Rogosa und Sharpe (MRS) Broth and MRS Agar.

2.2. Starter Preparation

L.plantarum (selected bacteria) were grown in MRS broth at 37 °C for 10 h (the best condition to be harvested). The cells were harvested and mixed on a various carrier (100 g). Adda sterile distilled water to the mixtures with a ratio of 2:1 (media: water) then incubated at 37 ° C for 48 h. Heat resistance was measured using the method of Yamazaki (1997) at a temperature of 35, 40 and 45 ^{0}C. Carrier conductivity was measured using *Conduction Radial Heat Conduction Apparatus* (HCARC).

2.3. Scanning Electron Microscopy Analysis

The apparatus used was Inspect Fei with Inspect S50 Type. These analysis for see the granula size of the best carrier in the heating effect , that the cells can still survive.

3. Results and Discussion

3.1. Heat Resistance

The viability starter is depend on heat resistance of strain during drying . Heldman and Singh (2001) reported that heat resistance cells measured by determining the value D and Z of the cells on different types of carrier. The higher the value of D indicates the cell more resistance to heating. Result calculation D-value of *L.plantarum* for rice flour carrier shown in Table 1.

Heating Temperature (T) (°C)	Heating time (t) (h)	Total Bacteria (N) (colony/g)	Log N (Y)	Linear regression y = ax + b	D-value D = (1/a) (h)
45	0	$1.05x\ 10^9$	9.02	y = -0.346x + 9.144	2.89
	4	$1.41x\ 10^8$	8.15	R^2 = 0.917	
	8	$1.0x\ 10^8$	8.00		
	12	$8.0x\ 10^7$	7.90	Where	log D = 0.461
	16	$3.3x\ 10^7$	7.52	slope (a) = - 0.346	
	20	$9.8x\ 10^6$	6.99		
40	0	$6.31x\ 10^8$	8,80	y = -0.234x + 8.751	4.27
	4	$1.23x\ 10^8$	8.09	R^2 = 0.908	
	8	$8.3x\ 10^7$	7.92		
	12	$6.0x\ 10^7$	7.78		log D = 0.631
	16	$3.2x\ 10^7$	7.51	where	
	20	$2.5x\ 10^7$	7.40	slope (a) = - 0.234	
	22	$1.64x\ 10^7$	7.21		
35	0	$1.42\ x\ 10^9$	9.15	y = -0.109x + 9.172	9.17
	4	$8.0x\ 10^8$	8.90	R^2 = 0.97	
	8	$6.4x\ 10^8$	8.81		
	12	$5.1x\ 10^8$	8.71	where	log D = 0.963
	16	$4.0x\ 10^8$	8.60	slope (a) = - 0.109	
	20	$3.6x\ 10^8$	8.56		
	24	$2.52x\ 10^8$	8.40		
	28	$2.1x\ 10^8$	8.32		

Table 1: Results calculation D-values of L.plantarum for rice flour carrier

Highest D-value (Table 1) found at heating temperature of 35^{0}C. The higher the heating temperature, cell viabillity was lower even though at the same heating time. Even though the same degree of death, at the temperature of 35^{0}C required a longer heating time. As shown in above data, *L.plantarum* cells on rice flour dried at 35^{0}C provides the best heat resistance with D-value at 9,17 hours. This means rice flour provides the least number of died cells during the drying process, to reduce 1 log cycle of cells content needs 9,17 h. While the dat of D-value for tapioca and grated cassava could be seen in Table 2. This possibly has to do with the fact that rice flour has smaller particles compared to tapioca and grated cassava, this offers better protection to the cells. D-values of *L. plantarum* grown on various carrier at various drying temperatures were shown in Table 2.

Temperature(^{0}C)	D-Value (Hours) At Various Carrier		
	Grated Cassava	Tapioca Starch	Rice Flour
35	1.29	9.17	9.17
40	0.79	3.51	4.27
45	0.31	2.63	2.89

Table 2: D-value of L. plantarum grown on various carrier at various drying temperatures

In the carrier of rice flour , the D-value at 35^{0}C was 9,17 h > D-value at 40^{0}C was 4,27 h > D-value at 45^{0}C was 2,89 h. This suggest that rice flour drying at35^{0}C has better heat resistance than drying at 40^{0}C and 45^{0}C.
The higher of D and Z value indicates the cells more resistance to heating (Heldman and Singh,2001). As shown in Table 3. the Z-value of grated cassava was 16.13 ° C < the Z-value of tapioca (18.52 °C) < the Z- value of rice flour (20 °C). This means that the cells of *L. plantarum* in the rice flour carrier had the highest heat resistance during the drying process in the production starter.

No	Carrier	D-value (h)			Linear equation Thermal DeadTime (TDT)	Z-value (°C)
		$D_{35°C}$	$D_{40°C}$	$D_{45°C}$		
1.	Grated Cassava	1,29	0,79	0,31	Y= -0,062x+2,315 R^2= 0,969	16,13
2.	Tapioca Starch	9,17	3,51	2,63	Y= -0,054x+2,811 R^2= 0,911	18,52
3.	Rice Flour	9,17	4,27	2,89	Y= -0,050x+2,693 R^2= 0,966	20

Table 3: Summary of D-Values and Z-Values of various Carrier used as bacterial growth media

The Z-values were 16.13; 18.52 and 20^0 C, in the grated cassava, tapioca starch and rice flour respectively. As shown in Table 3, the Z-value of grated cassava was 16.13 ° C < the Z-value of tapioca (18.52 °C) < the Z- value of rice flour (20 °C). Highest the Z-value of rice flour was 20 °C is the best of Z-value. It means that the cells of *L. plantarum* in the rice flour carrier had the highest heat resistance during the drying process and so highest thermal conductivity. It means to decrease 1 log cycle of D-values needs to increase 20^0 C. The D-value of rice flour carrier at 35 °C was 9.17 h>D-value at 40 ° C (4.27 h) >D-value at 45 °C (2.89 h). This suggests that the drying using rice flour as a carrier at temperature of 35 °C has better heat resistance than drying at temperature of 40 °C or 45 °C.

3.2. Thermal conductivity (k)

The thermal caracteristic of carrier used will determine higher or lower conductivity of carrier. Higher conductivity showed he higher heat propagation velocity, which means the cells briefly exposed to heat when drying so that the cells can still survive (Heldman and Singh, 2001).
Halaudin (2006) and Kaban (2009), report that more higher k-values the material indicates the materials has higher heat propagation velocity, which means the cells briefly exposed to heat when drying. The material that has particle with small size, it means has higher capacity of thermal conductivity.

No	Carrier	Particle size (µm)	Conductivity thermal (W/m^0 C)
1.	Grated Cassava	5 – 35	0,455
2.	Tapioca Starch	20	0,453
3.	Rice Flour	3 – 8	0,460

Table 4: Thermal conductivity on various types of carrier before drying process

In the Table 4. shown that thermal conductivity on various type of carrier before drying process. Thermal conductivity of the carrier from high to low were rice flour, grated cassava and tapioca flour, that were 0.460; 0.455; and 0.453 W/m ^{0}C, respectively. Moorthy,S.N.(2004), report that diameter particle size for grated cassava was 5 -35 µm, tapioca starch size was approximately 20 µm, and rice flour size was 3 - 8 µm. SEM analysis shown that rice flour has particle size smaller than tapioca and grated cassava with agregate type. That is to be able the *L,plantarum* cells as safety at drying process. The k-value was also highest, it means the heat propagation velocity,and the cells briefly exposed to heat when drying, so that the cells still survive. Particle type of rice flour as carrier that safety *L.plantarum* cells, analyisis with SEM before and after drying process could be shown in Fig.1.

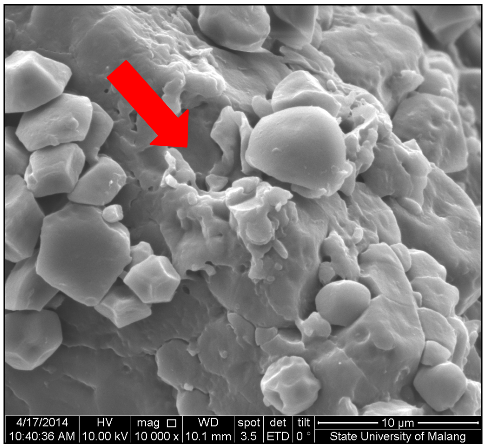

Fig 1.a.
The SEM photo of L.plantarum in rice flour carrier before the drying process

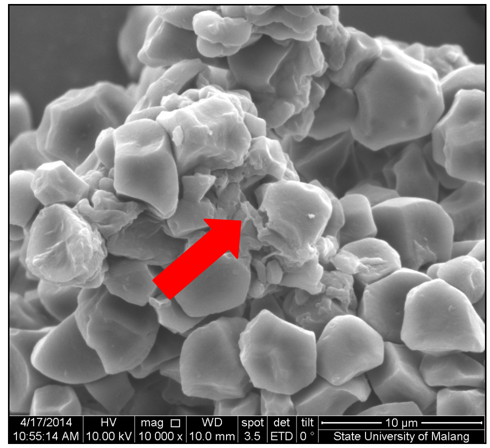

Fig 1.b.
The SEM photo of L.plantarum in rice flour carrier after the drying process

Figure 1

Based on the above data, the highest D-value was found for rice flour that also for conductivity so that the rice flour was selected as the best carrier. Based on the D-value and Z-
value, the drying can be carried out at a temperature of 55 °C for 55 min to reduce 1 log cycle. Utilization of rice flour was able to get the highest viability the cells of L.plantarum. We can desided that the drying process was 55^0 C with D-value 9,17/10, or 0,97 h is 55 min. If we want to get the cel viability after drying is 10^6 cel/g, the time for drying process was 3 x 0,97 h is 2,75 h.

4. Conclusionn

According to this resarch, we purpose to produce starter powder was recommended to use *L.plantarum* at 10 h age with rice flour as a carrier and dried at 55^0 C for 2,75 h to get the cel viability 10^6 cel/g.

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