

LACTIC ACID PRODUCED FROM TAPIOCA AND SAGO WASTES

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Abstract

Liquid waste as consequences of tapioca and sago starch industries has caused environmental pollution. Mono- and disaccharides are dominant in the waste. These components are easily fermented to produce lactic acid, a potential raw material for polylactic acid (PLA). Objective of this research is to obtain the optimum fermentation condition of tapioca and sago starch industrial wastes in producing lactic acid. Liquid wastes from tapioca and sago starch industries were fermented using pure culture of *Lactobacillus casei*, *Lb. delbruecki* and *Streptococcus faecalis*. Treatments of 2.5, 5 and 10% glucose addition and 5% glucose combined with 5% nitrogen were compared to none addition as a control. The effect of time fermentation was also observed. During the preliminary observation, *Lb. casei* exhibited more suitable as compared *Lb. delbruecki* and *S. faecalis*, hence only *Lb. casei* and sago isolate used at the main study. The results showed that tapioca waste produced lactic acid higher than that of sago starch waste. Treatment by adding 5% nitrogen and 5% glucose indicated the optimum condition for 4 days fermentation. At this condition, *Lb. casei* produce 1.98 % total acid using tapioca waste and 1.85% using sago starch waste, while culture isolated from sago waste produced 2.82% total acid using tapioca waste and 0.63% using sago starch waste.

Key words: Lactic acid, tapioca waste, sago waste

INTRODUCTION

Cassava and sago are carbohydrate -sources that found abundantly in Indonesia. Mostly, cassava cultivated by traditional technology and limited inputs in upland farming area. It is found in all islands of Indonesia as one of secondary food crops. In contrary, sago grows as forest plants, usually found in swampy or tidal area without cultivation. Both of them are used in manufacturing value-added food product such as starch flour, crispy chips and snack food. The starch flour of cassava is obtained from the tuber by series of processing: grating, milking, condensation and separation the solid fraction. In the same processing series, sago starch flour is obtained from the sago trunk. The process will yield high volume of liquid waste, which usually threw out to the drain on river. Liquid waste from starch industry still contains carbohydrates. The liquid waste as consequences of tapioca and sago starch industries has caused environmental pollution, because the carbohydrate component within the waste will be fermented by microorganism presence in the river. The drain as well as the river will produce the putrid odor, and increase both COD and BOD value.

Mono- and disaccharides are carbohydrates that dominant in the waste. These components are easily fermented to produce lactic acid. Lactic acid is widely used in food, pharmaceutical, leather, and textile industries. Although it can be prepared by chemical synthesis, production of lactic acid by fermentation of glucose and other substances is a less expensive method. Recently, there has been an increased interest in lactic acid production because it could be used as a raw material for the production of polylactic acid (PLA). PLA is a polymer used as environmental-friendly biodegradable plastics, which

substitute for synthetic plastics derived from petroleum feedstock (Datta et al, 1995 in Yun et al, 2003). Lactic acid exists as two optical isomers, D- and L-lactic acid. Both isomeric forms can be polymerized and polymers with different properties can be produced depending on the composition (Hofvendahl and Hahn-Hagerdal, 2000).

Fermentative production of lactic acid is preferably made by the group of lactic acid bacteria capable of converting hexose into lactic acid (Sodergard and Stolt, 2002). Lactic acid bacteria are found from the genera *Lactobacillus* (*Lb*), *Lactococcus*, *Enterococcus*, *Carnobacterium*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. The carbohydrates have been transformed via different pathways resulting in homo-, hetero-, or mixed acid fermentation (Stiles and Holzafel, 1997 in Yun et al, 2003). However, only *Lb. casei*, *Lb. bulgaricus*, *Lb. helveticus*, *Lb. amylophilus*, and *Lb. delbrueckii*, could produce optically pure lactic acid. In addition, most lactic acid bacteria have complex nutritional requirements and very low growth rates. Almost of them are facultatively anaerobic, catalase negative, nonmotile and nonspore forming. They have high acid tolerance and survive at pH 5 and lower (Hofvendahl and Hahn-Hagerdal, 2000). The optimal temperature for growth varies between the genera from 20 to 45⁰C (Wood and Holzapfel, 1995 in Hofvendahl and Hahn-Hagerdal, 2000).

In this study liquid waste from tapioca and sago starch industries were fermented using pure culture of *Lb. casei*, *Lb. delbruecki*, *Streptococcus faecalis*, and an isolate that isolated from sago waste. Treatments of nitrogen and glucose addition were observed. We also

investigated the effect of various fermentation times to lactic acid production.

MATERIALS AND METHODS

Bacterial strain and media preparation

Pure culture of *Lactobacillus casei*, *Lb. delbruecki* and *Streptococcus faecalis*, and isolate from sago waste were used in the preliminary observation. Firstly, pure culture of *Lb. casei*, *Lb. delbruecki* and *S. faecalis* were cultivated into liquid media of MRSB (37⁰C for 2 days) to make stock culture. All stock cultures and sago isolate were cultivated into 10 ml MRSB and incubated at 37⁰C for 2 days, which then cultivated again into 120 ml MRSB at 37⁰C for 24 hours. The yield, called as work culture, was fresh culture ready to use for fermentation process. To determine the bacteria suitable for tapioca/sago wastes, fermentation was done using *Lb. casei*, *Lb. delbruecki* and *S. faecalis* bacteria. In the other hand to determine the suitable isolate, precipitation test on litmus milk and skim milk, gram coloring analyze, growth ability analyze and reducing sugar analyze were carried out. The main media used in this experiment were tapioca industrial waste and sago starch industrial waste obtained from Bogor-West Java. The organic matter of the waste such as total nitrogen, total reducing sugar, total titratable acid (TTA) and starch content were measured by proximate analyses.

Optimizing time and nutrition on fermentation process

To obtain the optimal time on fermentation, waste was fermented by *Lb. casei* and sago isolate for 1 to 6 days fermentation, then followed by measuring TTA value from the yield (filtrate). Nutrition

treatment was done by added glucose and nitrogen in various concentrations. Treatments of 2.5, 5 and 10% glucose addition were compared to none addition as a control, where cassava waste was inoculated by *Lb. casei*, *Lb. delbruecki* and *S. faecalis*. Furthermore, addition of 5% nitrogen and 5% glucose was compared to none addition. In all treatments, the TTA value from the yield was measured. To convince that the TTA is dominating with lactic acid, HPLC analysis was carried out.

RESULTS AND DISCUSSION

Characteristic of liquid waste and waste isolate bacteria

Fermentation process to produce lactic acid from various raw materials has been reported by many researchers. Some of the results have been applied commercially. Over the years Narayanan (2004) have studied a large number of carbohydrates and nitrogenous materials for production of lactic acid. The choice of the raw material to be used depends on the microorganisms studied and also on the product desired. However, it was difficult to obtain report explaining about producing lactic acid from liquid waste. For that reason, characteristic of liquid wastes were characterized in the early activities. Wastes were picked from washing step and condensation step.

As shown in Table 1, both tapioca and sago wastes noted that waste from the washing step higher in organic matters compare to waste from condensation step. The organic matters measured by proxymate analyses consisting of total nitrogen, total acid (TTA), reducing sugar

and starch content. Tapioca wastes exhibited higher in total acid and starch than that of sago wastes, however lower in total nitrogen. Reducing sugar and starch as well as nitrogen are nutrition sources for microorganism to grow, and from their growth the acid will be produced. Although the nutrition source within the waste (Table 1) is very low, however a starch industry will secrete thousands litre liquid wastes per day, which potential to produce lactic acid.

Table 1. Organic matters on tapioca and sago wastes

No.	Proxymate analyses	Tapioca waste		Sago waste	
		From washing activity	From condensation activity	From washing activity	From condensation activity
1.	Total Nitrogen (%)	0.0192	0.032	0.0256	0.0288
2.	Titratable acid (ml NaOH 0.05 M/100 g wastes)	66.22	21.33	18.28	4.56
3.	Reducing sugar (%)	0.0199	0.0191	0.0807	0.0178
4.	Starch content (%)	1.9304	0.0270	1.3998	0.1707

The presence of acid (titratable acid value) within the waste indicated that spontaneous fermentation by *in situ* microorganism likely occurred. To determine the characteristic of microorganism, samples were randomly isolated from both wastes. All isolates demonstrated have similar characteristics with lactic acid bacteria, such as gram-negative (rod form and purple blue color), able to precipitate litmus milk and skim milk, and negative in catalase test (Table 2). Among samples, certain isolate picked from sago waste with A code perform high ability to produce acid. This isolate then called as sago isolate was used in thermal treatment experiment. Table

2 showed that sago isolate could grow in glucose, sucrose or lactose medium in temperature 25 and 37 °C but could not grow in 55 °C. Those result indicated that sago isolate is not thermophilic bacteria.

Table 2. Characterization of isolates from tapioca and sago wastes

Isolate	Growth in media		Gram coloring	Bacteria form	Catalase test
	Litmus milk	Skim milk			
Tapioca waste A	+	+	(+)	Rod	-
Tapioca waste B	+	+	(+)	Rod	-
Tapioca waste C	+	+	(+)	Rod	-
Sago waste A *)	+	+	(+)	Rod	-
Sago waste B	+	+	(+)	Rod	-
Sago waste C	+	+	(+)	Rod	-

Note: + : precipitation on media occurred
 (+) : purple blue color
 - : no oxygen gas was occurred
 *) : the best performance on yielded total acid

Table 3. Growth ability of sago isolate on thermal treatments

Reducing sugar	Thermal treatments		
	25°C	37 °C	55 °C
Glucose	+	+	-
Sucrose	+	+	-
Lactose	+	+	-

Note: +: grow, -: not grow

Suitable bacteria use for waste fermentation

As has been mentioned above, pure culture of *Lb.casei*, *Lb. delbrueckii* and *Streptococcus faecalis* were used in the preliminary observation. To select the suitable bacteria used on lactic acid producing, a comparison of ability to grow in tapioca wastes with and without nutrition addition (glucose and nitrogen) was done. The parameter measured in this experiment was TTA value as representation of lactic acid.

Figure 1 demonstrated that all bacteria could produce lactic acid even though varies in quantity. *Lb. casei* produced higher total acid than others from day 1 until day 4 fermentation. *Lb. delbrueckii* slightly similar to *Lb. casei* even noted same total acid value in day 3 fermentation, which then decreased in day 4. While *Lb. casei* still remained its TTA value in day 4. *S. faecalis* noted lowest total acid values compare to others since day 1 until day 4. This result indicates that *S. faecalis* not fit to ferment tapioca wastes.

Nutrition-glucose addition as displayed in Figure 2 exhibited the same trend like Figure 1. *Lb. casei* indicates the best one in acid producing followed by *Lb. delbrueckii*, while *S. faecalis* did not obtain good results. Glucose addition likely did not affect the ability of *S. faecalis*. Figure 2 also explained that addition of 2.5% glucose has performed highly increasing on TTA values in tapioca waste fermented by *Lb. casei*. Furthermore, addition of 5% and 10% glucose did not demonstrate high effect anymore. This result indicates that 2.5% is the optimum nutrition-glucose addition in tapioca waste to obtain the high yields.

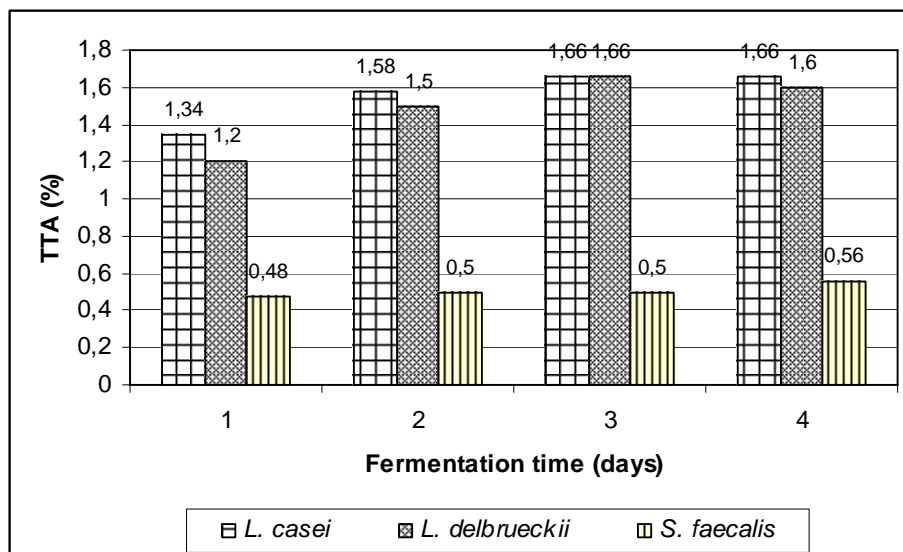


Figure1. Ability of selected bacterias on lactic acid (TTA) producing

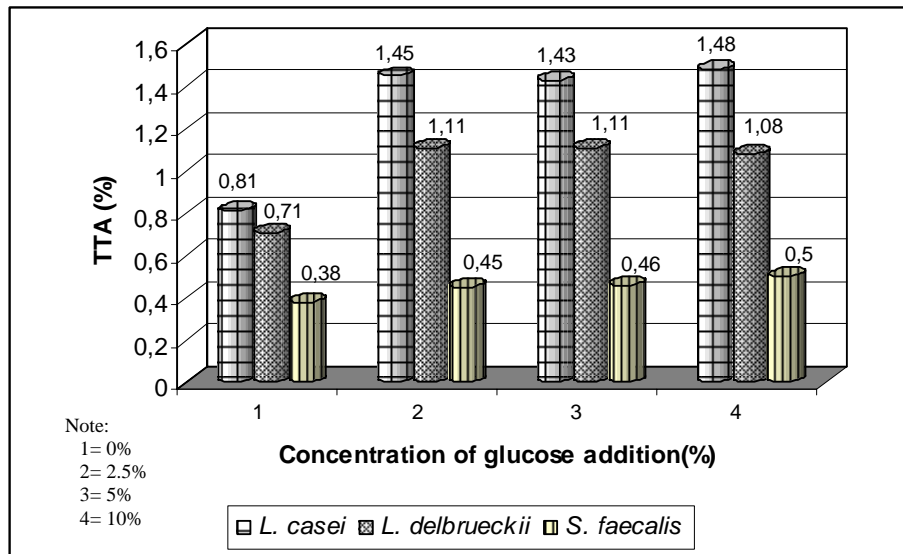


Figure 2. Effect of glucose concentration on TTA value

Nitrogen is an important nutrition for bacteria to stimulate their growth, deficiency of this nutrition will inhibit bacteria to multiply. In this research combination of 5% glucose – 5% nitrogen addition was compared with none. The result as seen in Figure 3 showed that addition glucose-nitrogen has increased the total acid highly even on fermentation with *S. faecalis*. In this experiment, *Lb. delbrueckii* exhibited lowest total acid after added with combine of glucose – nitrogen. This phenomenon indicates that *S. faecalis* needs a certain concentration of nitrogen to grow. At nitrogen concentration around 0.02 % (Table 1), *S. faecalis* only produced not more than 0.56 % total acid even though 10% glucose has been added. In contrast when nitrogen (combine with glucose) was added, it could produce more than 1.2 % total acid. However, *L. casei* still the best one that performed a stable behaviour.

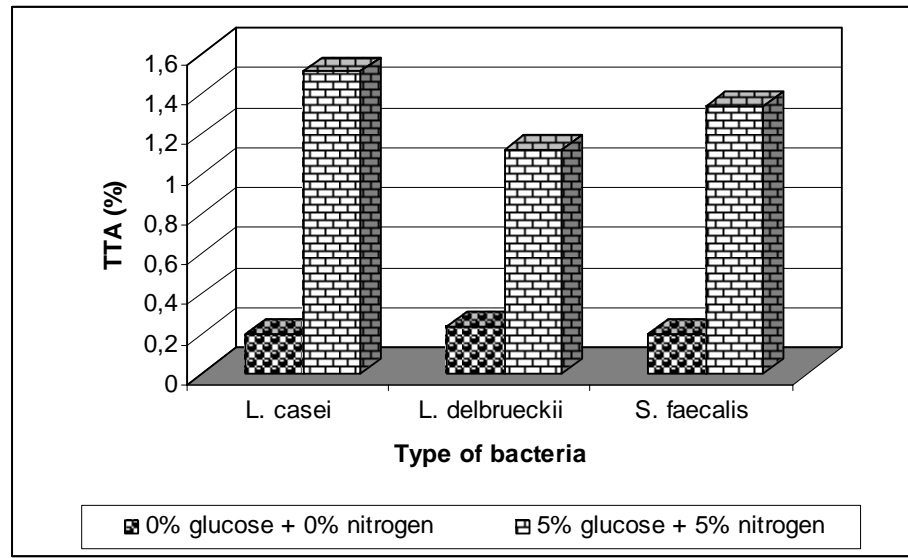


Figure 3. Effect of nitrogen and glucose addition on TTA value

Optimizing fermentation time

In order to obtain an efficient fermentation, running time should be determined. To determine the optimal time, *L. casei* and sago isolate were used in fermentation process without nutrition added. TTA values were measured each day during fermentation. In this experiment, fermentation was stopped when the total acid showed trend to decrease. Figure 4 showed that in day 5 TTA has decreased for *L. casei* and stagnant for sago isolate. It meant that optimal time to conduct fermentation should be at 4 days. Figure 4 also showed different pattern on increasing TTA values between *L. casei* and sago isolate. Since the first day, *L. casei* has showed high value on TTA, which then the increase was slightly flat until day 4. In contrast, sago isolate exhibited linear increase from day 1 to day 4.

Figure 4 noted that without nutrition addition *L. casei* exhibited better than sago isolate with regard to the ability to ferment tapioca

waste. However, addition of 5% glucose combined with 5% nitrogen demonstrated different results. *Lb. casei* produce 1.98 % total acid from tapioca waste, on the other hand sago isolate produce 2.82%. This result indicates that similar to *S. faecalis*, nitrogen is critical growth factor of sago isolate. When fermentation was carried out on sago starch waste, the TTA yields of *Lb. casei* and sago isolate were 1.85% and 0.63% respectively. To convince that lactic acid was present dominantly in fermented waste, the HPLC analysis was carried out. The yield of tapioca waste fermented with *Lb. casei* performed single peak of lactic acid, and the value is 17.58 ppm.

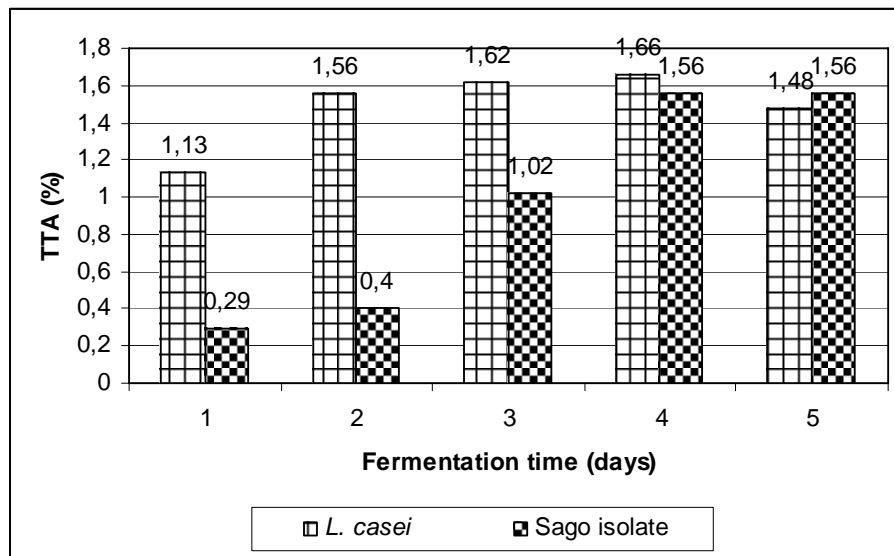


Figure 4. Effect of fermentation time on TTA value

CONCLUSION

Tapioca waste contained higher organic-nutrition matter than sago starch waste, however both could be fermented to produce lactic acid. *Lb. casei* showed the best performance on producing acid from

tapioca wastes compare with *Lb. delbrueckii*, *S. faecalis* and sago isolate. However, addition of 5% glucose – 5% nitrogen has changed the behaviour of sago isolate, which was able to produce total acid more than *Lb. casei* could.

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