Lactic acid fermentation of dahlia tuber starch and waste using *Lactobacillus bulgaricus*: A comparative study

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Abstract. Lactic acid fermentation of dahlia tuber starch and waste was performed by means of *Lactobacillus bulgaricus* through enzymatic hydrolysis followed by fermentation process. The effect of pH condition on lactic acid production was investigated during the process. The selected bacteria produced lactic acid after 24 hours of fermentation and the productivity was increased after 24 hours of fermentation. After 120 hours of fermentation, it was found that dahlia tuber starch can produce up to 16.18% of lactic acid, whereas lactic acid produced from dahlia tuber waste was only 0.40% at pH of 4. The lactic acid production increase significantly for pH 3.5 and 4 until 96 hours of fermentation, then slowed down. On the other hand, for pH 4.5 the lactic acid production increase until 48 hours of fermentation and then slowed down. The identification of fermentation product indicated that the lactic acid produced in this study was 16.20%, acidic, yellow and cloudy with pH 3.4 – 4.2. The density of lactic acid produced ranged between 1.21 to 1.25 gr/ml.

1. Introduction
Utilization of excess biomass or waste concept from agricultural and agroindustrial residues to produce energy, feeds or foods, and other useful products is not necessarily new. Though they are rich in carbohydrate, their utilization is very less due to the low content of protein and poor digestibility [1]. Recently, fermentation of biomass has gained considerable attention because of it was found to be able to solve environmental problems associated with their disposal.

Dahlia is an annual ornamental plant and tuber that have not been widely used in Indonesia. Dahlia tubers contain polyfructans, and especially inulin, that can be chemically or enzymatically hydrolyzed to fructose and sucrose. These are fermentable sugars that can be transformed into lactic acid or other substances by fermentation. Lactic acid, a 2-hydroxypropionic acid or 2-hydroxypropanoic acid compound, has many applications in food, pharmaceutical, textile, leather, and other chemical industries as mild acid flavour, pH regulator, or as a preservative. It has attained a high demand as it is the precursor for polylactic acid (PLA), a biodegradable plastic [2]. Biotechnologically, lactic acid can be produced by SHF - separate hydrolysis and fermentation, by SSF – simultaneous saccharification and fermentation, and by CHF – combined hydrolysis and fermentation, using raw materials contain glucose, sucrose, maltose, and lactose [3,4]. Numerous studies on lactic acid fermentation from agricultural residues have been reported, but there have been few studies on lactic acid production.
from dahlia tuber starch and waste. Previous study reported that 1 kg of dahlia tuber would produce 200 grams of dahlia tuber waste (20% of weight) [5]. The technique of lactic acid fermentation is important because it can reduce the time of fermentation or increase the yield of lactic acid. To avoid any contaminants, all processes in fermentation phase are performed aseptically [6]. The present work reports on the investigation results of fermentation time and pH on reduction sugar content and lactic acid yields of dahlia tuber starch and waste.

2. Materials and methods

2.1. Materials
The dahlia tubers used in this study were purchased from West Sumatera area. The starch and waste were taken from post-production of inulin process in Bung Hatta University laboratory.

2.2. Hydrolysis and fermentation
The dahlia tubers starch and waste were subjected to enzymatic hydrolysis using α-amylase and glucoamylase for 2 hours. The resultant hydrolysate contained the reducing sugar necessary for the fermentation process. The hydrolysate pH was adjusted to 3.5, 4 and 4.5, then sterilized and cooled off at 25°C. The hydrolysates was then inoculated with \textit{Lactobacillus bulgaricus} bacteria and immediately incubated at 30°C with agitation at 250 rpm for 120 hours [7]. For pH stabilization, CaCO$_3$ was used as a buffering agent. The analysis of reduction sugar content was performed every 24 hours.

2.3. Analysis
The total reduction sugar concentration in hydrolysates was estimated by Luff-Schoorl. The deviation between initial reduction sugar (after hydrolysis) and residual reduction sugar (after fermentation) was counted as lactic acid content. The identification of lactic acid is performed through measurement of density, pH, odor, color, and esterification process.

3. Results and discussions

3.1. Effect of fermentation time on reducing sugar content from dahlia tuber starch and waste at pH 4
The fermentation of lactic acid from reduction sugar of dahlia tuber starch and waste were performed for 120 hours and the reduction sugar content are presented in Figure 1.

![Figure 1. Effect of fermentation time on reducing sugar content from dahlia tuber starch and waste at pH 4.](image)
After 24 hours, the dahlia tuber starch produced 16.27% of reduction sugar, approximately 12 times higher than those of dahlia tuber waste. The difference in carbohydrates content will affects the reducing sugar content obtained [8]. The residual reducing sugar can be used as an indicator of fermentation process. During the fermentation process, the decrease of reduction sugar was attributed to microbes activity that use reduction sugar as a source of food [9]. After 48 hours of fermentation, dahlia tuber starch show faster decrease of reduction sugar content than dahlia tuber waste and at the end of the fermentation period both of samples showed that the reduction sugar content were approximately reached 0%.

3.2. Effect of fermentation time on lactic acid yield from dahlia tuber starch and waste at pH 4
Lactic acid yields from dahlia tuber starch and waste hydrolysates are presented in Figure 2.

![Figure 2](image)

Figure 2. Effect of fermentation time on lactic acid yield from dahlia tuber starch and waste at pH 4.

After 48 hours of fermentation, the lactic acid produced from dahlia tuber starch was increasing significantly. The maximum 16.18% of lactic acid content was achieved after 120 hours of fermentation. This result is 15 times higher than those produced of dahlia tuber waste. The fermentation proceeded very rapidly at the early stage of fermentation, but slowed down at the later stage producing 16.14% at 96 hours because less reduction sugar is available, hence slow down the fermentation process. At the same time, lactic acid accumulation at the early stage of fermentation process and low pH values have led to inhibition of lactic acid production by the selected bacteria.

3.3. Effect of pH on lactic acid content of dahlia tuber starch fermentation
The effect of pH on lactic acid content of dahlia tuber starch fermentation is shown in Figure 3. After 24 hours of fermentation, the lactic acid started to produced within the three conditions of pH. The lactic acid production increase significantly for pH 3.5 and 4 until 96 hours of fermentation, then slowed down. On the other hand, for pH 4.5 the lactic acid production increase until 48 hours of fermentation and then slowed down. The lactic acid produced indicated that Lactobacillus bulgaricus bacteria can tolerate pH values as low as 3.5. However, low lactic acid yields suggested that the fermentation process were not optimized as Lactobacillus bulgaricus bacteria optimum pH condition range between 5.5-5.8 [10].
4. Conclusion
The carbohydrates derived from dahlia tuber starch and waste could be converted to lactic acid by *Lactobacillus bulgaricus* bacteria through enzymatic hydrolysis followed by fermentation process. The identification through measurement of density, pH, odor, color, and esterification process showed that the combined hydrolysis and fermentation performed on dahlia tuber starch and waste will produce lactic acid that matched the specification. After 120 hours of fermentation, the dahlia tuber starch can produce up to 16.18% of lactic acid content, whereas lactic acid obtained from dahlia tuber waste was only 0.40%, thus suggesting that lactic acid production from dahlia tuber waste is not economically preferable.

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