Profile of Sweet Potato Fermentation using *Leuconostoc Mesenteroides* as a Starter

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Abstract: This study aimed to know the fermentation profile of yellow sweet potato (total lactic acid bacteria, total non-lactic acid bacteria, total lactic acid, pH, total exopolysaccharides, and morphology changing on starch granules) using Leuconostoc mesenteroides as a starter. The sample's withdrawal was performed at 0, 24, 48, and 72 hours. The results showed that during 72 hours fermentation time, there was a linearly decreased of pH (minimum at pH 3.80), a linearly increased of total lactic acid (0.0023% /h), reducing sugars (0.26 mg/ml/h), crude exopolysaccharides (EPS) (0.017 g/l/h), and total Lactic Acid Bacteria (LAB) (maximum at log 8.40 cfu/ml), as well as a decreased of non-Lactic Acid Bacteria. Leuconostoc mesenteroides had significant effect on granule of yellow sweet potato. There was an alteration of starch granules at the end of fermentation time (at 72 hours).

1 INTRODUCTION

Yellow sweet potato is a source of carbohydrates, so that it has good potential to be developed in support of food diversification programs. Yellow sweet potato is also a beta-carotene (provitamin A) sources (Kammona et al., 2015). Some examples of sweet potato-based processed products are baby food, salad dressings, cake mix (Anggraeni & Yuwono, 2014), pickle (Oke & Workneh, 2013; Oloo, 2013; Neti Yuliana et al., 2013), and processing based on sweet potato flour (Sebben et al., 2017). To produce more applicable sweet potato flour, a modification process is required. Modification of sweet potato flour can be done by fermentation of lactic acid (Ajayi et al., 2016, 2018; Liao & Wu, 2017; Yuliana et al., 2018; . Yuliana et al., 2017; Yuliana et al., 2014) The application of lactic fermentation in flour modification will produce flour that is easy to expand and tastes better. Besides, fermentation with the help of specific lactic acid bacteria has the advantage of being able to produce exopolysaccharides (EPS) (Yuliana et al., 2020; Zubaidah et al., 2014) which have many benefits, including improving the properties of flour..

The lactic acid fermentation process can occur with the help of a lactic acid bacteria starter (LAB). One of the LABs that produce EPS is Leuconostoc mesenteroides (Li et al., 2020; Taylan et al., 2019). These bacteria include heterofermentative lactic acid bacteria, which break down glucose and produce 50% lactic acid, ethanol, acetic acid, glycerol, mannitol, and CO2 (Mora-Villalobos et al., 2020). In addition to Leuconostoc mesenteroides, a lactic acid bacterial starter can be obtained from a pickle liquid starter with added salt (Yuliana et al., 2018). Lactic acid bacteria can also be obtained from a spontaneous fermentation process with added salt. In this study, sweet potato fermentation was carried out using the starter *Leuconostoc mesenteroides* from the culture collection unit.

The success of the lactic acid fermentation process is strongly influenced by optimizing the desired LAB growth factors. These factors then provide different conditions according to the LAB environment, which ultimately affects the fermentation process. Each LAB starter will also show different growth patterns, the period needed to grow and adapt, and the resulting metabolites (Yang et al., 2018). Information about growth patterns and metabolites produced is needed to determine the

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fermentation efficiency as the growth and formation of products affect the responsiveness of cells. During growth, microorganisms require a substrate as the raw material used for cell multiplication and the formation of metabolite products (Utami et al., 2012; Zubaidah et al., 2014) Thus, the fermentation profile related to growth patterns, substrate consumption, and metabolite production is needed to determine fermentation's optimum conditions.

Fermentation of sweet potatoes using a starter of lactic acid bacteria to improve the characteristics of sweet potato flour has been reported (El Sheikha & Ray, 2017). Yuliana et al., (2013) examined the fermentation of yellow sweet potato pickles using mixed LAB cultures, which produced the best characteristics of pickles organoleptically with a total lactic acid value of 0.5%, pH 3.39, and a total lactic acid bacteria of 8.46 log CFU / mL. So far, research on the sweet potato fermentation process has been done a lot, but it is still limited to white sweet potatoes. There is no information regarding the growth patterns of lactic acid bacteria, changes in starch granules, and exopolysaccharides during yellow sweet potato fermentation. So that in this study, the fermentation profile of yellow sweet potato with the starter of lactic acid bacteria *Leuconostoc mesenteroides* as a starter was studied

2 MATERIALS AND METHOD

2.1 Materials

The main ingredient used in this study was a yellow sweet potato purchased at the traditional market in Bandar Lampung, Indonesia. *Leuconostoc mesenteroides* FNCC-0023 was from PAU Pangan dan Gizi, University of Gajah Mada, Indonesia. Media used were MRS broth, and MRS agar.

2.2 Method

The sweet potatoes were washed, peeled, sliced, and added to glassware containing a boiled solution of salt-sugar and were left at room temperature. The sweet potato slices were fermented with *Leuconostoc mesenteroides* FNCC-0023 as starters.. Observations were performed on total LAB (Yuliana et al., 2013), and biochemical changes: pH, total acidity as % of lactic acid, total glucose of supernatant (phenol-sulphuric method), and amount of crude exopolysaccharide (Razack et al., 2013) 2013). Sampels were withdrawal at 0 hours (H0), 24 hours (H24), 48 hours (H48), and 72 hours (H72). A 72 hours of fermentation was selected for observation of change in sweet potato starch granule by using scanning electron microscopy.

2.3 Data Analysis

Experimental unit was repeated three times. All data were analyzed to find the average and subjected to polynomial trend line to find either linearly or quadratically pattern in which the rate of the parameter observed was determined.

3 RESULTS AND DISCUSSION

3.1 Change of Lactic Acid Bacteria, Total Lactic Acid and Ph

During fermentation process, LAB utilized starch and sugar in yellow sweet potato as an energy source for cell multiplication and produced metabolites such as lactic acid (Oloo, 2013) and exopolysaccharides (Zubaidah et al., 2014) and resulting in a decrease in pH (Yuliana et al., 2013). LAB activity will degrade and modify starch granules (Liao & Wu, 2017; Yuliana et al., 2014) and leave reducing sugars. (Yuliana et al., 2013) The data recapitulation of biochemical changes during fermentation is presented in Figure 1.

There was a linear increase in total lactic acid from 0.61 to 1.802% during fermentation. On the other hand, there was a linier decreased in pH from point 4.9 to 3.6. An increase of total lactic acid during fermentation occurred at a rate of 0.2%, as *Leuconostoc mesenteroides* FNCC-0023 starter activity.

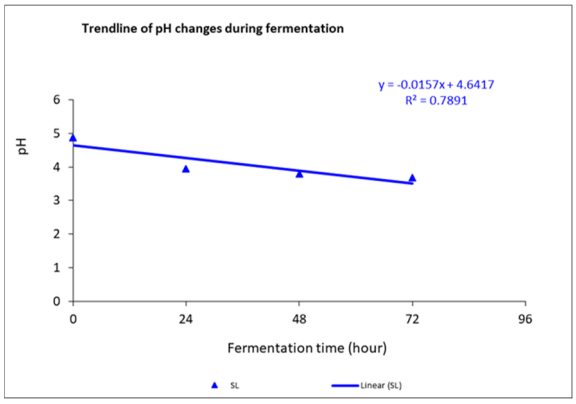

Figure 1: Trend line of pH, Lactic Acid Bacteria, Total Lactic Acid, EPS and Reducing Sugar during Fermentation of Yellow Sweet Potato with *Leuconostoc mesenteroides.*

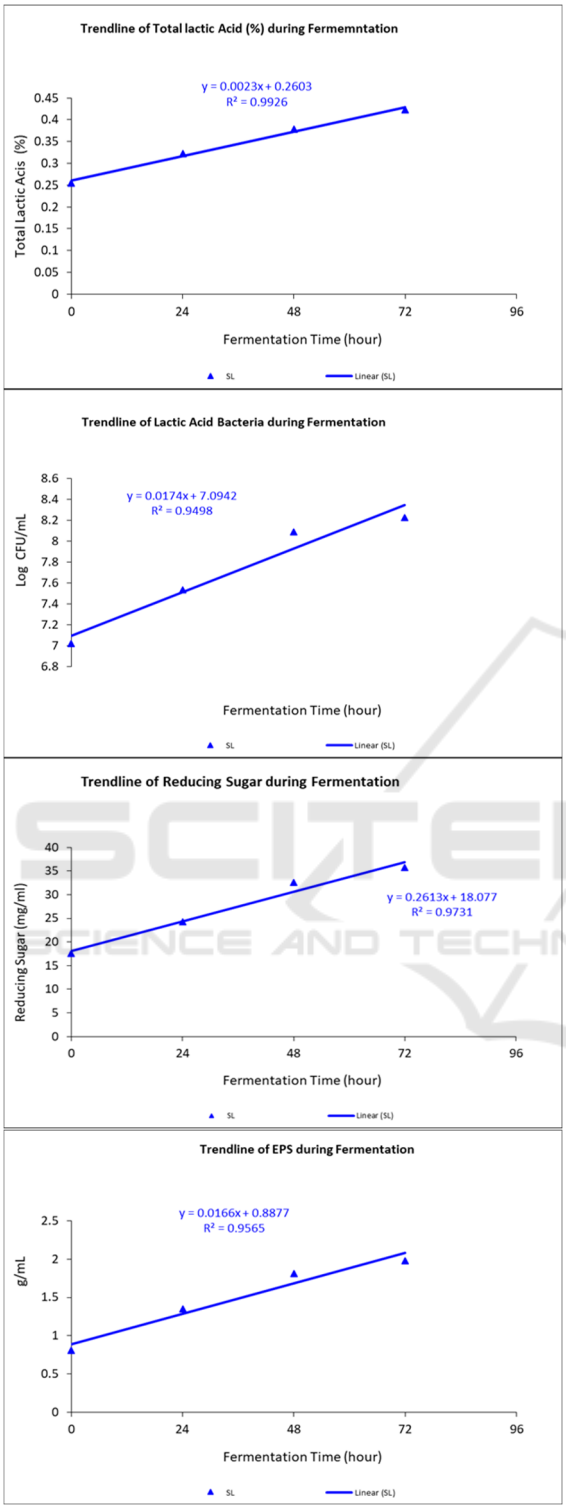

Figure 1: Trend line of pH, Lactic Acid Bacteria, Total Lactic Acid, EPS and Reducing Sugar during Fermentation of Yellow Sweet Potato with *Leuconostoc mesenteroides* (cont.).

The lowest pH value and the highest total acid occurred at 72 hours of fermentation. The same pattern was reported by (Oloo, 2013). In the fermentation of orange sweet potatoes, there was an increase in lactic acid's total content and a decrease in pH during fermentation. The decrease in pH during fermentation is caused by the accumulation of organic acids, especially lactic acid produced by *Leuconostoc mesenteroides* FNCC-0023.

During fermentation, the total LAB also increased quadratically and reduced non-lactic acid bacteria (Table 2). Addition of *Leuconostoc mesenteroides* starter treatment increased the LAB population, and then it was stationary until 72 hours. The growth of LAB in yellow sweet potato fermentation have time incubation dependent. The growth pattern of *Leuconostoc mesenteroides* increased from 0 to 24 hours and afterward tended to be stationary from 24 hours to 72 hours. (Yuliana et al., 2013) study showed that the growth pattern of *Leuconostoc mesenteroides* continues to increase up to 12 days of fermentation.

At the beginning of the yellow sweet potato fermentation (0 hours), besides LAB, non-lactic acid bacteria colonies were also found, namely mold, with an average of 2 log CFU / mL. The addition of starter cultures can cause the desired microbial dominance and suppress the growth of competing microbes. During fermentation, LAB experiences growth by utilizing sugar sources as energy or nutrition. The simple sugars that are used for the development of LAB are partially converted into organic acids such as lactic acid (Oloo, 2013) and then LAB produces crude exopolysaccharides which are secreted outside the cell. In this study, there was an increase in residual reducing sugar and an increase in lactic acid and EPS production during fermentation. Consent ensures that the publisher has the Author's authorization to publish the Contribution.

The length of fermentation has a very significant effect on the value of reducing sugar residual fermentation of yellow sweet potato which increases at a rate of 26.13%. The residual reducing sugar content increased linearly with fermentation time (Table 1). The residual reducing sugar in yellow sweet potato fermentation could come from the starch and sugar contained in the yellow sweet potato tissue. Sanoussi et al., (2016) states that yellow sweet potato contains starch 172.87-326.73 mg/g (DW) and total sugar 24.23-42.64 mg/g (DW). During fermentation, yellow sweet potato starch is degraded by enzymes both from sweet potatoes and LAB into horter chains (simple sugar) (Guo et al., 2019). The simple sugar is then used by *Leuconostoc mesenteroides* FNCC-0023

as a source of energy or nutrition for its growth. Apart from being used for the development of LAB, some of the simple sugars will be converted into organic acids such as lactic acid (Oloo, 2013). During development, LAB produces exopolysaccharides and is secreted outside the cell. The exopolysaccharide level analysis showed the EPS value of yellow sweet potato fermentation increased linearly during fermentation at a rate of 1.6%. The results of this study are in line with previous research which states that EPS production with lactic acid bacteria will increase with the length of incubation time (Onilude et al., 2013; (Zubaidah et al., 2014) .

3.2 Morphology of Starch Granula

The results of Scanning Electron Microscopy can be seen in Figure 2.

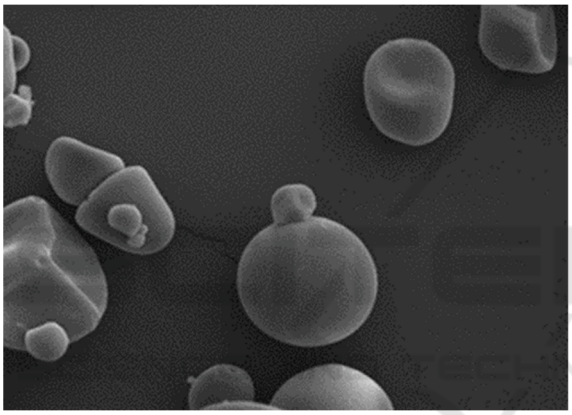

Figure 2a: Morphology of starch granule of yellow sweet potatoes before fermentation (Magnification 2000 X).

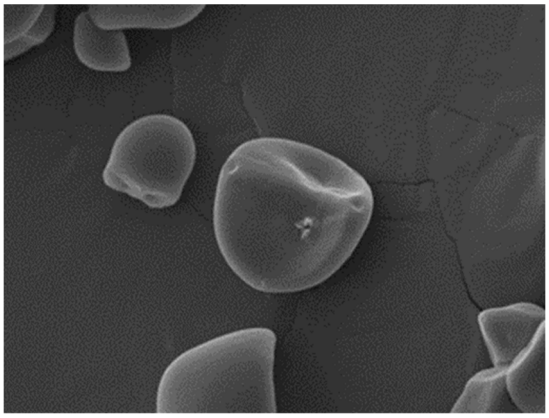

Figure 2b: Morphology of starch granule of yellow sweet potatoes after 72 hours fermentation (Magnification 2000 X).

Figure 1a shows the appearance of yellow sweet potato starch granules (control), which do not look hollow. Meanwhile, the yellow sweet potato starch granules changed shape at the end of the fermentation time (t = 72 hours), which was degraded by *Leuconostoc mesenteroides* (2b).

The granule structure of control yellow sweet potato starch and fermented starch resulted in a significant difference in appearance and shape when identified by Scanning Electron Microscopy. Figure 2b confirmed that there was a change in the starch granule structure. Similar results were reported by (Liao & Wu, 2017) on *Lactobacillus plantarum* fermented yellow sweet potato starch granules. (Yuliana et al., 2014) also reported spontaneous fermentation of white sweet potato, causing starch granules changes. According to (Liao & Wu, 2017), the prolonged treatment of fermentation destroys the crystal structure of yellow sweet potato starch and significantly affects the crystalline and amorphous parts. This change is thought to be caused by the activity of lactic acid bacteria. Yuliana et al., (2014). stated that the size of the starch granules in the fermentation process of white sweet potato changes after the fermentation process, which causes changes in the amorphous structure of starch granules, size of starch granules, chemical composition, and also modifies the physical and rheological characteristics of white sweet potato starch.

The form should be completed and signed by one author on behalf of all the other authors. Figure 2. Morphology of starch granule of yellow sweet potatoes (magnification 2000 X).

4 CONCLUSIONS

The fermentation profile of yellow sweet potato with starter *Leuconostoc mesenteroides* is as follows: during fermentation, there was a linear increase in total lactic acid (at a rate of 0.1%), residual reducing sugar (at a rate of 26.13%), crude EPS (at a rate of 1.6%), and quadratically lowering the pH (with the lowest point at pH 3.80) with total LAB (optimum at 8.63 log CFU / mL) and a decrease in non-LAB. The morphology of yellow sweet potato starch granules fermented with Leuconostoc mesenteroides starter during 72 hours of fermentation caused starch granules changes.

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