Effect of yellow sweet potato extract (*Ipomoea batatas* L.) as a prebiotic source for the kinetics of fermentation and the production of lactic acid by *Lactobacillus paracasei*

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Abstract. This study aims to determine the effect of yellow sweet potato extract as a prebiotic source for fermentation kinetics *L. paracasei*. Bacteria *L. paracasei*, which is used here, is a collection of Laboratory Nutritional Biochemistry, Faculty of Animal Science UGM. Concentrations of yellow sweet potato extract used to determine the *Km* value *L. paracasei* were 0.01, 0.05, 0.1, and 0.2%. Lactic acid production and fermentation pH value of *L. paracasei* with different levels of yellow sweet potato extract (½ *Km*, *Km*, 1.5 *Km*) were measured after 24 hours incubation period. *Km* value bacteria *L. paracasei* obtained 2.1 g/100ml. Different concentrations of yellow sweet potato (½ *Km*, *Km*, 1.5 *Km*) as a treatment had a significant effect (P<0.05) on the increase in lactic acid produced and decrease in pH. Based on this experiment, it can be seen that level substrate with *Km* value have not produced optimal lactic acid.

1. Introduction

Lactic acid bacteria are potential bacteria as probiotics. Probiotics are interpreted as a dietary supplement that contains living microbes such as bacteria, mold, and yeast that can benefit the host by improving the balance of microbes in the digestive tract [9]. The term lactic acid bacteria (LAB) was initially intended only for a group of bacteria that cause acidity in the milk. Lactic acid bacteria is a group of gram positive bacteria, which do not produce spores, are spherical or stem producing lactic acid as the main metabolic end product during carbohydrate fermentation. Lactic acid bacteria is grouped into several general, among others *Streptococcus* (including *Lactococcus*), *Leuconostoc*, *Pediococcus*, *Lactobacillus* [5].

Lactic acid forming organisms consist of two species, namely homofermentative species, which can convert 95% hexose into lactic acid and heterofermentative species, are groups that produce in addition to lactic acid other products in the form of ethyl alcohol, acetic acid, formic acid, and carbon dioxide. Lactic acid bacteria that are classified as homofermentative are *Lactobacillus sp*, *Streptococcus sp*, *Peddiococcus sp*, while classified heterofermentative is *Leuconostoc sp*. Lactic acid bacteria in the carbohydrate fermentation process can produce lactic acid, which can reduce pH. A decrease in pH can inhibit the growth of other microorganisms, especially pathogenic bacteria [9].

Requirements for probiotic bacteria are not pathogenic and toxic, can stick to and colonization of the digestive tract, can utilize nutrients on the existing substrate, can survive during the digestive system, have good viability and intact in the body, have beneficial effects on the host by preventing
infection or disease, improving health or improving nutrition. Another requirement that must be possessed by probiotic bacteria is its ability to produce antimicrobial substances so that it can suppress the growth of pathogenic bacteria [4].

Sweet potato (Ipomoea batatas L.) is an alternative plant that contains carbohydrates that have the potential as a source of prebiotics. The types of oligosaccharides contained in raw and steamed sweet potato extracts include maltose, maltotriose, sucrose, and raffinose. The content of raffinose from raw and steamed sweet potatoes is 48.04 and 39.50 ppm [6]. Sweet potatoes contain oligosaccharides, which are non digestible components that are undigested but are beneficial for the growth of probiotic bacteria so that those sweet potatoes can function as prebiotics [11]. Prebiotics are defined as indigestible foods consisting of inulin, fructooligosaccharides (FOS), galactooligosaccharides, and lactose. Food type requirements can be classified as prebiotics, such as not being hydrolyzed in the digestive tract, selectively stimulating the growth of beneficial bacteria, being able to regulate the composition of microflora in the intestine by increasing the number of beneficial bacteria and reducing the number of beneficial bacteria. Undigested oligosaccharides such as raffinose, fructooligosaccharides, galactosyl lactose, isomaltooligosaccharides, or transgalacto-oligosaccharides can increase the number of indifferent bifidobacteria and lactic acid bacteria. Tubers contain oligosaccharides in the form of raffinose in high amounts [10].

The kinetics of bacterial growth is a relationship between biomass growth, substrate use, and the resulting product [7]. Fermentation and growth kinetics studies are needed as a basis for understanding each fermentation process. Fermentation kinetics describes the growth and formation of products by microorganisms [11]. The success of the fermentation process is strongly influenced by the success in optimizing the desired microbial growth factors. These factors will provide different conditions for each microbe under their respective living environment so that it affects the kinetics of fermentation [12]. Life phases of bacteria generally include adaptation, log or exponential growth, stationary, death. Growth curves are used to determine the speed of cell growth and the effect of the environment on the speed of growth [8].

2. Material and Methods

2.1. Tools and materials
The tools used were waterbath, magnetic stirrer, pH meters, refrigerators, centrifuge, oven, autoclave, vortex, laminar air flow, erlenmeyer, hungate tube, bunsen, tube rack, beaker, spectrophotometer, filter paper, and blender. The materials used were culture L. paracasei, yellow sweet potatoes, aquadest, deMann Rogosa Sharp (MRS) broth, KH2PO4, (NH4)2SO4, NaCl, MgSO4.7H2O, yeast extract, TCA 10%, CuSO4 20%, CuSO4, 4%, H2SO4, p-hydroxybiphenyl solution, Antron reagents, and aquadest.

2.2. Flour making and extraction of yellow sweet potato
Sweet potatoes are peeled, then chopped. It is dried in an oven at 55°C for 24 hours. Chopped sweet potatoes with blender and filtered. Ten grams of sweet potato flour is mixed with 100 ml of boiling water and stirred. Extract solution is filtered with filter paper and then used as prebiotic source on fermentetation L. paracasei.

2.3. Enrichment of L. paracasei isolates
Bacteria were regenerated by growing on solid medium using MRS broth and agar in a petri dish. First, the medium is sterilized using an autoclave at a temperature of 121°C with a pressure of 15 psi for 15 minutes. The bacteria were then inoculated on a sterile medium, then incubated at 37°C for 24 hours. Bacteria that had been regenerated in solid medium were then grown in sterile MRS broth liquid medium. The bacteria were then incubated at 37°C for 24 hours.
2.4. Making define medium fermentation culture
Define medium made with a mixture of mineral I, mineral II, yeast extract, aquades, and yellow sweet potato extract with different levels. Composition of 100 ml defined medium can be seen in Table 1.

Tabel 1. Composition define medium

| Reagent     | Amount |
|-------------|--------|
| Mineral I   | 15 ml  |
| Mineral II  | 15 ml  |
| Yeast extract | 0.2 g |
| Aquadest    | 70 ml  |

Define medium mixed in erlenmeyer. Mineral I is made by 0.0393 g K$_2$HPO$_4$.3H$_2$O. Mineral II is made using 0.3 g KH$_2$PO$_4$, 0.6 g (NH$_4$)$_2$SO$_4$, 0.6 g NaCl, 0.123 g MgSO$_4$, and 0.0795 g CaCl$_2$.3H$_2$O. Mineral I, mineral II, yeast extract and aquadest without the addition of yellow sweet potato were homogenized using a magnetic stirrer and heated to boiling. The medium and yellow sweet potatoes were then sterilized using an autoclave at a temperature of 121°C at a pressure of 15 psi for 15 minutes. Giving yellow sweet potato to the media according to the treatment to be given.

2.5. Determination $K_m$ value of L. paracasei
Bacteria L. paracasei that has been regenerated in MRS Broth liquid medium were then grown in defined medium with a concentration of yellow sweet potato extract 0.01, 0.05, 0.1, and 0.2%. The fermentation process was incubated temperature at 37°C and observed substrate reduction at 0, 6, 18, dan 24 hours. Substrate reduction could be determine by testing carbohydrates with Antrone-Sulfate method. The total value of carbohydrate decrease at various incubation times was then changed through logarithmic calculations to obtain Michaelis constant values from each treatment. The decrease of carbohydrates obtained was graphed 1/V$_i$ and 1/S. $K_m$ value can be calculated by following this formula [1].

$$
\frac{1}{V_i} = (\frac{K_m}{V_m}) \frac{1}{S} + \frac{1}{V_m}
$$

Notes:

$V_i$ = Initial speed
$K_m$ = Michaelis constant
$V_m$ = Maximum speed
$S$ = Substrate

2.6. Liquid fermentation of L. paracasei
After obtaining the $K_m$ value of L. paracasei bacteria, then grows back in a liquid medium defined medium with different concentrations of yellow sweet potato extract were $\frac{1}{2} K_m$, $K_m$, and 1.5 $K_m$. Fermentation with various levels of substrate treatment was then incubated at 37°C for 24 hours. The pH value was measured at each treatment after an incubation period of 24 hours using a pH meter and three replications. Measurement of total lactic acid levels was carried out by sampling at 24 hours of incubation using Barker and Summerson method [3] and samples were tested with three replications. Substrate efficiency is measured by $\frac{\text{lactic acid}}{\text{substrate}} \times 100%$.

2.7. Data analysis
Results of the research data were analyzed using unidirectional pattern variance analysis, if there is a real difference, continue with the Duncan's New Multiple Range Test (DMRT).
3. Results and Discussion

3.1. Determination $K_m$ value of L. paracasei
Fermentation with limited liquid medium of L. paracasei using yellow sweet potato extract substrate were 0.01, 0.05, 0.1, and 0.2%. Observation of substrate reduction was carried out at incubation time of 0, 6, 18 and 24 hours to measure the growth rate of L. paracasei. Sweet potato extract, which is in the medium, will be converted into lactic acid by L. paracasei. Over time, sweet potato extracts that are in the medium will diminish because bacteria consume them. A decrease in sweet potato substrate can be seen in Figure 1.

![Figure 1. Graph of decrease in yellow sweet potato substrate](image)

Based on the graph above shows the rate of substrate reduction by L. paracasei at various levels of the substrate. Level 0.2% gave the highest substrate reduction rate and level 0.01% gave the lowest substrate reduction rate. From the graph of substrate reduction can be calculated as the value of $K_m$. Fermentation kinetics of L. paracasei can be seen from $K_m$ value. The results of the graph analysis of the $K_m$ value can be seen in Figure 2.

![Figure 2. Graph of $K_m$ values using sweet potato extract](image)

$K_m$ value bacteria L. paracasei obtained 2.1 g/100ml. This shows that L. paracasei bacteria need 2.1 g of yellow sweet potato extract in every 100 ml to reach $\frac{1}{2} V_{max}$. $K_m$ value used as a reference in the administration of sweet potato extract in the medium.
3.2. Liquid fermentation of *L. paracasei* with levels of yellow sweet potato extract ½ *Km, Km and 1.5 Km*

Bacteria *L. paracasei* has grown in a liquid defined medium with sweet potato extract content were ½ *Km* (1 g/100ml), *Km* (2 g/100ml), and 1.5 *Km* (3 g/100ml) as treatments. The thing this is done to determine the most efficient substrate concentration in the fermentation process *L. paracasei*. Fermentation parameters observed were final pH of the fermentation process, amount of lactic acid produced by *L. paracasei*, and substrate efficiency at each level of sweet potato extract used. The results obtained are presented in Table 2.

| Treatments | pH     | Average lactic acid (mg/ml) | substrate efficiency (%) |
|------------|--------|----------------------------|--------------------------|
| P1         | 4.14±0.10<sup>a</sup> | 0.86±0.09<sup>a</sup> | 8.60±0.58<sup>a</sup> |
| P2         | 3.89±0.02<sup>a</sup>  | 1.44±0.17<sup>b</sup> | 6.71±0.86<sup>d</sup> |
| P3         | 3.84±0.03<sup>a</sup>  | 1.61±0.06<sup>c</sup> | 5.36±0.20<sup>d</sup> |

Notes: P1: Levels of substrate ½ *Km* (1g/100ml)  
P2: Levels of substrate *Km* (2 g/100ml)  
P3: Levels of substrate 1.5 *Km* (3 g/100ml)  
<sup>a,b,c</sup>: Different superscripts on the same column show differences (P<0.05)  
<sup>d</sup>: Different superscripts on the same column show differences (P<0.01)

The value pH testing was carried out to determine the quality of fermentation produced by bacteria from different levels of the substrate. The lower pH, then fermentation products produced are increasingly high. Data Table 2 shows that administration sweet potato extract level 1/2 *Km, Km, 1.5 Km* significantly affect the final pH value and amount of lactic acid produced of fermentation *L. paracasei* (P<0.05). The highest pH value is in P1 so that the amount of lactic acid produced is low, while the lowest pH is in P3 so that the highest lactic acid produced. Decrease in pH occurs because lactic acid bacteria produce lactic acid from metabolic processes. The acid formed is influenced by the addition of the substrate. The higher the substrate concentration, the lower the resulting pH [13].

*L. paracasei* bacteria produced the lowest lactic acid at P1, namely 0.86 mg/ml, while the highest lactic acid was produced at P3 namely 1.61 mg/ml. P3 treatment still showed an increase in lactic acid production and did not show saturation. Glucose in the fermentation medium will be converted into lactic acid by bacteria. The greater the substrate concentration, the more lactic acid is produced [2].

The P1, P2 and P3 treatments of *L. paracasei* bacteria were able to change the yellow sweet potato substrate into lactic acid at different rates, so that the total lactic acid produced was significantly different. Based on Table 2, it can be seen that giving levels of yellow sweet potato extract ½ *Km, Km* and 1.5 *Km* has a significant effect on the efficiency value of *L. paracasei* fermentation substrate (P<0.01). Substrate efficiency decreases with increasing substrate level. The highest substrate efficiency was found in P1 with an average of 8.60%. Substrate levels at P2 and P3 were not significantly different (P> 0.01). The efficiency of using the substrate is lower but the amount of lactic acid produced is higher.

4. Conclusions

Based on the research results, it can be concluded that the *Km* values of *L. paracasei* for yellow sweet potato extract were 2.1 g/100 ml. The lowest pH value and the highest lactic acid content were achieved in *L. paracasei* fermentation using a substrate equal to 1.5 *Km*, however there is no significantly different of efficiency substrate utilization at *Km* or 1.5 *Km*.
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