Effect of purple sweet potato levels (Ipomoea batatas L.) carbohydrate sources on fermentation kinetics and lactic acid production by Lactobacillus paracasei

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Abstract. This research aims to determine the effect of purple sweet potato extract level as a carbohydrate source substrate on the kinetics of fermentation and lactic acid production by Lactobacillus paracasei. Research using L. paracasei, which is a lactic acid bacteria (LAB) collection of the Laboratory of Nutrition Biochemistry of the Faculty of Animal Sciences UGM. Fermentation kinetics were observed with a graph of substrate degradation derived from the Michaelis-Menten equation. Purple sweet potato extract concentration (S) used to determine the value of $K_m$ and $q_{max}$ of L. paracasei was 0.01; 0.05 and 0.1%. Purple sweet potato extract concentration (S), which was used for observing the kinetics fermentation and lactic acid production, was $\frac{1}{2} K_m$, $K_m$ and 1.5 $K_m$. Observation of substrate degradation was known by conducting the Antrone test. The results of the fermentation kinetics test in the form of slope values, as a parameter of the speed of substrate degradation L. paracasei. Lactic acid production was measured using the Barker and Summerson methods. Analysis data for the value of $K_m$ and $q_{max}$ observed descriptively. While, the data of the fermentation kinetics test, pH values and production of lactic acid were analyzed using analysis of variance with a Completely Randomized Design (CRD) in a unidirectional pattern. The addition of purple sweet potato extract to liquid fermentation L. paracasei grading 1.5 $K_m$ (3 g/100ml) has shown the optimum pH value and lactic acid production but $\frac{1}{2} K_m$ concentration shows higher substrate efficiency.

Keyword: $K_m$ and $q_{max}$, L. paracasei, Lactic Acid, Purple Sweet Potatoes

1. Introduction
Lactic acid bacteria (LAB) is a microbial that has several benefits, one of which is as a probiotic. BAL is a bacterium that produces lactic acid as its main product. [1] states that lactic acid plays a role in reducing pH so that it can inhibit and even kill pathogenic bacteria and reduce the damage to the chemical composition of the media that is overgrown by the process of respiration. Lactic acid is produced from the utilization of dissolved carbohydrates (water soluble carbohydrate, WSC) as a substrate source by LAB. Because of its ability, the application of the use of BAL as a probiotic has been developed in the field of animal husbandry. Feed processing technology by adding BAL can improve its functional properties.

Probiotics are living microorganisms that can provide various clinical benefits to the host by improving the balance of microflora in the digestive tract. Generally probiotics are available in the form of LAB fermentation, specifically the genera Lactobacillus and Bifidobacterium [2]. The total BAL...
expected to be available in the intestine is $10^8 - 10^{11}$ CFU every gift [3] (Rahayu, 2009) so that the benefits can be felt as a probiotic. 

*Lactobacillus paracasei* is a probiotic lactic acid bacteria, able to survive in acidic conditions and inhibits the growth of pathogens [4]. Probiotic activity can increase when added to prebiotics [5] (Gustaw *et al.*, 2011). *L. paracasei* needs a substrate as a source of nutrients that will be used for the growth process. *L. paracasei* can utilize several carbon sources for growth such as glucose, fructose, lactose, sucrose, arabinose, [6] (Hedberg *et al.*, 2008), rafinosa, inulin and some plant extracts [7] (Palacio *et al.*, 2014). Different types of substrates will produce different growth rates and metabolite production. The use of excess substrate will produce excessive lactic acid so that the pH decreases, but the use of too little substrate can cause a lack of significant changes and no growth occurs. [8] Ghosh and Ray (2010) state that the more substrates, the higher the resulting product.

Prebiotics are generally carbohydrates that cannot be digested by digestive enzymes and cannot be absorbed by the body. Oligosaccharides found in sweet potatoes are carbohydrates that are beneficial for the growth of probiotic bacteria [9] (Haydensah *et al.*, 2012); [10] Irvine and Hekmat (2011) added that sweet potatoes have a fairly complete nutritional content, namely carbohydrates, proteins, vitamins and minerals for the growth of lactic acid bacteria. [11] Apraidji (2006) states that oligosaccharides in sweet potatoes are non-digestible components that are undigested but are beneficial for the growth of probiotic bacteria, so sweet potatoes can function as prebiotics.

Fermentation *L. paracasei* as a probiotic with sweet potato extract substrate as a prebiotic has never been done and no known effect on the kinetics of fermentation and lactic acid production. Therefore, further research is needed regarding the potential *L. paracasei* as a probiotic at the optimum level of purple sweet potato extract substrate. Judoamidjojo *et al.* (1992) [12] states that the fermentation process can be described from the fermentation kinetics. Fermentation and growth kinetics studies are needed as a basis for understanding each fermentation process. Fermentation kinetics describe enzymatic reactions in the formation of products by microorganisms. Fermentation kinetics in product formation affects the ability of cell responses, therefore a study of fermentation kinetics in the fermentation process is rational.

Optimizing the use of substrates in the fermentation process *L. paracasei* is an important factor for producing efficient fermentation products. Fermentation kinetics need to be known by finding the value of affinity ($K_m$ and $q_{max}$) bacteria against dissolved substrates. The use of substrates at a certain level based on the value of bacterial affinity, shows the number of substrates needed by bacteria to produce optimal fermentation products. This research was conducted to determine the effect of purple sweet potato extract levels on the kinetics of fermentation and lactic acid production in fermentation. *Lactobacillus paracasei*.

2. Materials and Methods

This research was conducted at the Laboratory of Nutrition Biochemistry, Faculty of Animal Science, Universitas Gadjah Mada. *L. paracasei* comes from the collection of Laboratory of Nutrition Biochemistry then rejuvenated with MRS Broth medium. Each treatment was repeated three times and the data were analyzed with a Completely Randomized Design (CRD) in a unidirectional pattern.

**Flour Making**. Fresh purple sweet potato is cleaned and peeled, then the sweet potato is sliced with a knife. Sweet potato slices are dried in a drying oven at 55 °C for 10 hours until the sweet potato slices can be broken by hand. Sweet potato slices are then ground in a blender and filtered.

**Extraction**. Consider carefully 5 grams of purple sweet potato flour then mixed with 90 ml of boiling water while stirring. The extract is then heated while stirring for 10 minutes. The extract solution is then filtered with filtered paper then the solution is centrifuged and the supernatant is taken.

**Determination of Total Carbohydrate Levels of Purple Sweet Potatoes**. Determination of glucose levels using the Anthrone-Sulfate method is done by adding 1 ml of sample extract solution with a concentration of 0.2 mg / ml with 5 ml of anthrone reagent 0.2%. The solution is homogeneous with vortex so that it is evenly mixed. The solution was then heated over a waterbath at 100° C for 12 minutes,
after which it was cooled. The solution is then read the absorbance in a spectrophotometer at a wavelength of 630 nm.

**Making Define Medium Fermentation Culture.** Define Medium is made with a mixture of mineral 1, mineral 2, yeast extract, distilled water, and purple sweet potato extract with different levels. The composition of defined medium can be seen in Table 1.

**Tabel 1. Define medium composition**

| Reagent            | Amount |
|--------------------|--------|
| Mineral 1          | 15 ml  |
| Mineral 2          | 15 ml  |
| Yeast Extract      | 0,2 gram |
| Aquades            | 70 ml  |

Define the medium mixed in erlenmeyer. Mineral 1 is made using $K_2HPO_4.3H_2O$. Mineral 2 is made using $K_2HPO_4, (NH_4)_2SO_4, NaCl, MgSO_4, dan CaCl_2H_2O$. Then purple sweet potato extract was added from the total medium with different concentrations.

**Substrate Degradation Test.** *L. paracasei* that has been rejuvenated in MRS Broth grown into a defined medium by adding purple sweet potato extract substrate with concentrations (0,01; 0,05 and 0,1%) observed a degradation in the substrate for several hours with the total carbohydrate test using Antron. 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 degradation of carbohydrates obtained was measured by connecting $1/S$ and $1/Slope$. Equations obtained from carbohydrate reduction curves are calculated using the following formula to obtain the value of $K_m$.

$$\frac{1}{q_i} = \left(\frac{K_m}{q_m}\right)\frac{1}{S} + \frac{1}{q_m}$$

(*1*)

**Test of Slope Values, pH Levels, and Lactic Acid.** After obtaining the $K_m$ bacterial value of *L. paracasei*, it is then regrown in a liquid medium defined medium with different concentrations of purple sweet potato extracts, namely ½ $K_m$ (1%), $K_m$ (2%) and 1,5 $K_m$ (3%). Substrate reduction is observed with test total carbohydrates with Antron. Antron test was carried out at 0, 4, 8, and 12 hours with three repetitions. Samples were then tested for pH and lactic acid levels produced at the 12 hour with three replications.

3. Results and Discussion

3.1. **Liquid Fermentation in Purple Sweet Potato Extract Medium Limited For Growth L. paracasei**

Liquid fermentation *L. paracasei* using medium in the form of purple sweet potato extract limited to know the kinetics of bacterial fermentation by determining the values of $K_m$ and $q_{max}$. The level of purple sweet potato used is 0,01; 0,05 and 0,1%. The total value of carbohydrates obtained during the observation of degradation substrate by bacteria is then converted into the form dry ingredients. The results of observations of the substrate degradation of purple sweet potato extract as the only source of carbohydrates by *L. paracasei* fermentation in the form of dry matter can be seen in Figure 1.
Based on the results of the graph analysis above, the degradation substrate at each level has a different rate of reaction speed. Bachruddin [13] states that the rate at which the substrate changes to product compounds depends on the amount of substrate content undergoing unit time transitions. Okpokwasili and Nweke [14] state that the speed of microbial growth and substrate concentration are important in biotechnology. With the substrate drop curve, logarithmic phase information can be obtained from a microbial growth.

3.2. Determination Values of $K_m$ and $q_{\text{max}}$
Fermentation kinetics $L$. paracasei can be seen from the value of $K_m$ and $\mu_{\text{max}}$. Value of $K_m$ and $\mu_{\text{max}}$ based on equations Monod [15,16]. $K_m$ and $q_{\text{max}}$ values obtained can be shows the efficient use of substrates by bacteria $L$. paracasei. The results of the graph analysis values of $K_m$ and $q_{\text{max}}$ $L$. paracasei are in Figure 2.

Based on the results of the graph analysis above, the $K_m$ is obtained $L$. paracasei to the substrate in the form of purple sweet potato extract as a source of carbohydrate of 2.14 g/100ml with $q_{\text{max}}$ at 4.95x10^{-3} g/L/hour. Bachruddin says that value $K_m$ is the magnitude of $q_i$ at the point where the substrate content is equal to $\frac{1}{2} q_{\text{max}}$.[13]
3.3. Fermentation L. Paracasei with Purple Sweet Potato Levels of ½ Km, Km and 1.5 Km

Value \(K_m\) of bacteria \(L.\ paracasei\) which has been obtained from the stage initial research, then used as a reference in giving carbohydrate source substrate. Bacteria \(L.\ paracasei\) the substrate drop was observed on the liquid medium defined medium with different levels of purple yam extract which is \(\frac{1}{2}K_m\) (1g/100ml), \(K_m\) (2g/100ml) and 1.5 \(K_m\) (3g/100ml). Things this is done to determine the most efficient substrate concentration in the fermentation process \(L.\ paracasei\). Fermentation parameters observed in the form of value slope degradation substrate, final pH, lactic acid production and substrate efficiency of \(L.\ paracasei\). The test results are presented in Table 2.

| Treatment | \(\frac{1}{2} K_m\) | \(K_m\) | 1.5 \(K_m\) |
|-----------|-----------------|--------|------------|
| Slope     | \((48\times10^4\pm1\times10^4)^a\) | \((79\times10^4\pm6.56\times10^4)^b\) | \((79.33\times10^4\pm13.79\times10^4)^b\) |
| pH        | 4.25±0.26\(^a\) | 4.08±0.21\(^b\) | 3.91±0.15\(^a\) |
| Lactate (g/L) | 0.77±0.01\(^a\) | 0.96±0.23\(^b\) | 1.35±0.06\(^c\) |
| Substrate Efficiency | 7.73\(^b\) | 4.81\(^a\) | 4.49\(^a\) |

Note: \(\frac{1}{2} K_m\) : Substrate content of 1g/100ml
\(K_m\) : Substrate content of 2g/100ml
1.5 \(K_m\) : Substrate content of 3g/100ml
Different superscripts on the slope, pH and lactate line show differences (P <0.05)
Different superscripts on the substrate efficiency line show differences (P <0.01)

Test results in substrate degradation rate (slope) in Table 1 shows that at the level of \(\frac{1}{2} K_m\) have value slope which is low while at level \(K_m\) and 1.5 \(K_m\) have value of slope higher and relatively the same. This shows that at level \(K_m\) and 1.5 \(K_m\) does not show a significant change in reaction speed. So that the growth of bacteria at the level of 1.5 \(K_m\) (3g/100 ml) has not been inhibited by the substrate concentration. [17] Widiyanti (2014) states that the growth rate of bacteria is influenced by the substrate concentration. The greater the substrate concentration, the greater the specific growth rate of microorganisms. However, the specific growth rate is relatively the same when the substrate concentration is maximum (2 \(K_m\)).

The results of the pH test at the end of the fermentation of bacteria \(L.\ paracasei\) by purple sweet potato extract substrate showed a decrease in pH at each additional level of purple sweet potato extract substrate as a source of carbohydrates. The pH value shows level of fermented product produced. The lower the pH value, then fermentation products produced are increasingly high. Safitri [18] stated that a decrease in pH and an increase in the total acid value in the fermentation media due to the activity of lactic acid bacteria resulting in the accumulation of organic acids, especially lactic acid. The decrease in pH and an increase in the total acid value in the fermentation media is very beneficial because it can inhibit some pathogenic microbes.

Test results for lactic acid levels at the end of fermentation from bacteria \(L.\ paracasei\) by purple sweet potato extract substrate showed an increase in levels of lactic acid at each additional level of purple sweet potato extract substrate. Lactic acid level values describe the amount of product produced by bacteria during the fermentation process. Nurjannah [19] states that there is a direct correlation between the bacterial growth curve and lactic acid levels. The higher the number of bacterial cells, the higher the levels of lactic acid. Forsythe [20] also states that available substrates will be utilized by BAL to produce lactic acid. The more availability of substrate, the more acid production lactate by BAL is higher, but at substrate concentrations excessive will experience saturation. Afriani [21] said that the success factor of a fermentation is very much determined from the substrate. Microbes need energy from carbohydrates to be able to grow and easily adapt to their environment.

The substrate efficiency value in fermentation by \(L.\ paracasei\) at a concentration of \(\frac{1}{2} K_m\) was higher than \(K_m\) and 1.5 \(K_m\). This shows that the substrate in the form of purple sweet potato extract with a given concentration of \(\frac{1}{2} K_m\) can be utilized by \(L.\ paracasei\) compared to the concentrations of \(K_m\) and 1.5.
Km. Akoetey [22] states that lactic acid bacteria are able to produce 0.83 grams of lactic acid per 1 gram (83%) of fermented starch. The substrate efficiency value obtained from the fermentation of L. paracasei at the level of addition of ½ Km, Km and 1,5 Km purple sweet potato extract showed lower yields.

4. Conclusion
Based on the research that has been done, it can be concluded that the liquid fermentation of L. paracasei with the substrate of purple sweet potato extract as a source of carbohydrates results in Km = 2.14 g/100ml and qmax = 4.95x10^-3 g/L/hour. The treatment of purple sweet potato extract at the level of 1,5 Km (3g/100ml) resulted in optimal pH and lactic acid production, but ½ Km concentration shows higher substrate efficiency.

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