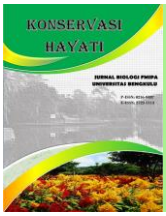

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Quality Improvement of Red Sweet Paste With using *Lactobacillus plantarum*

Dita Isnaini Rambe[1], Titi Candra Sunarti[2], Yohana Caecilia Sulistyaningsih[3], Anja Meryandini [3,4*]

[1] Microbiology Study Program, Biology Department, Faculty of Mathematic and Natural Science; Bogor Agricultural University, Bogor 16680, Indonesia
[2] Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor 16680, Indonesia
[3] Biology Department, Faculty of Mathematic and Natural Science, Bogor Agricultural University, Bogor 16680, Indonesia
[4] Biotech Center, Bogor Agricultural University, Bogor 16680, Indonesia

**Corresponding author: ameryandini@apps.ipb.ac.id*

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ABSTRACT

Red sweet potato (Ipomoea batatas L.), which contains oligosaccharides, is one of carbohydrates source in Indonesia. The oligosaccharides can benefit the growth of probiotic bacteria and increase the number of probiotic bacteria. Different heating processes can affect the physical and chemical properties of oligosaccharides contained in sweet potatoes. This study aims to assess the survival ability of L. plantarum in a suspension containing red sweet potato as a synbiotic formulation. The observed variables included the total LAB, lactic acid level, and pH values. Red sweet potato substrate with different pretreatments increased the total number of lactic acid bacteria (LAB) and lactic acid levels and decreased the synbiotic pH value. The effect of L. plantarum application on red sweet potatoes resulted a total acid of 0.45% greater, pH reaching 3, total LAB of 11 log CFU/mL. The best result was pasta with microwave treatment because it can produce simpler sugars, has high anthocyanin levels, and has antioxidant activity.

Keywords: *functional foods, lactic acid bacteria (LAB), oligosaccharides, probiotics*

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INTRODUCTION

Public awareness of the importance of maintaining a healthy digestive tract has increased the need for functional food. Functional foods are foods that contain one or more compounds, such as probiotics, prebiotics, anthocyanins, and antioxidants, which can provide health benefits for the digestive tract (Terpou *et al*., 2019). Probiotics are live microorganisms in sufficient quantities that, after consumption, can provide health benefits (FAO/WHO, 2002). Lactic acid bacteria (LAB), which produce lactic acid as their main product, are microbes with several benefits, such as a probiotic. The commonly used lactic

acid bacteria (LAB) are Lactobacillus and Bifidobacterium (Quinto *et al*., 2014). The majority microorganisms in the human body are in the intestines (Hajela *et al*., 2015). Changes in gut microbiota have the most significant potential to cause dysbiosis. The health of the digestive tract is influenced by the composition of microorganisms found in the human digestive tract, called gut microbiota. If the composition of the gut microbiota is dominated by pathogenic bacteria (dysbiosis), it can cause damage to intestinal permeability, infection of the intestinal epithelium, and failure to absorb nutrients. Infection of the intestinal epithelium can disrupt the body's immune system response so that it cannot prevent invasion by pathogenic bacteria (Markowiak & Sli˙zewska, 2017). Therefore, it is essential to maintain the balance of the intestinal microbiota. Common probiotics are often produced based on milk or dairy products (Aspri *et al*., 2020).

The development of milk-based health drinks is essentially inaccessible to various groups, such as people with a vegan lifestyle, lactose intolerant, and people on diets (Ziarno & Cicho ´nska., 2021). Therefore, innovations in probiotic products that are not milk-based (non-dairy) are starting to be developed. Vegetables and fruit can be the alternative ingredients as probiotics carrier because they are rich in antioxidants, beta-carotene, vitamins, minerals, and dietary fiber in form of raffinose oligosaccharides. Sweet potato is included as vegetable and give health effects on the body (Aspri *et al*., 2020; Simanjuntak *et al*., 2021). Red sweet potatoes have an indigenous enzyme in the form of an amylase enzyme. There are three types of amylase in sweet potatoes: α-amylase, β-amylase, and starch phosphorylase (Nangin and Sutrisno, 2015). The concentration of β-amylase is higher than α-amylase which is important during cooking. Processing processes that involve heating will activate the β-amylase enzyme (Mensah *et al*., 2016). The active β-amylase enzyme will cut the starch into shorter saccharides, and the probiotics will use it for their life. Tanbiyaskur *et al*. (2015) showed that adding prebiotics to synbiotics has many benefits in maintaining the health compared to treating probiotics and prebiotics separately. The color in sweet potatoes is caused by natural dyes called anthocyanins. Anthocyanins function as free radical-scavenging antioxidants that have antimicrobial, anticarcinogenic, antimutagenic, aging-preventing, and anti-inflammatory effects which play an important role in preventing various degenerative diseases (Liu *et al*., 2021). The anthocyanin content in sweet potatoes is quite large. However, improper processing of red sweet potatoes can reduce the anthocyanin levels in a product, thus measuring anthocyanin levels after the product manufacturing process is essential.

Riani *et al.* (2020) have isolated *Lactobacillus plantarum* (NHC6) from pineapple juice, which can be developed into probiotics. *L. plantarum* (NHC6) has probiotic characteristics such as acid resistance, bile salt resistance, auto aggregation and coaggregation properties, antioxidant activity, and antimicrobial activity. Ariyanto *et al.* (2021) observed the shelf life of *L. plantarum* (NHC6) in pineapple juice and showed that the number of cells increased in the first to the second week and decreased after that in a storage temperature of 10°C. The temperature at 4°C causes bacteria the decrease of bacteria cell numbers every week. This research uses *L. plantarum* NHC6 as probiotics with red sweet potato to produces paste as a functional food formulation.

METHODS

Red sweet potato (*Ipomoea batatas* L.) obtained from Carangpulang Village, Bogor, and *Lactobacillus plantarum* NHC6 bacteria obtained from the Biotech Research Center Laboratory, IPB University. Lactic acid bacteria were grown on Mann Rogosa Sharpe (MRS) medium (Merck, Germany).

Red sweet potato paste treatment

Proximate analysis was carried out on the raw sweet potatoes first. Red sweet potatoes are washed, peeled, cut into pieces and the pasta is carried out using various treatments, namely (1) steaming for 15 minutes, (2) cooking with a microwave heating for 3 minutes, (3) with Heat Moisture Treatment (HMT) by autoclaving for 1 hour with three autoclaving-cooling cycles and (4) combination of treatments between alpha-amylase enzymes and HMT (EHMT) enzymes (Putri & Mulyani, 2021). For EHMT were adding the enzyme α-amylase at a dose of 1U/g. Sweet potatoes that have been heated using various methods are mashed and mixed with distilled water to form a paste with a concentration of 50% (w/v).

Characterization of red sweet potato paste

Sweet potato paste was characterized as including reducing sugars with dinitrosalycilic acid (DNS) using the method of Julaeha *et al*. (2016), total sugar content with phenol sulfate (Nurjannah *et al*., 2017), degree of polymerization (Marlida *et al*., 2014), total anthocyanins (pH differential method) with the method (Putri *et al.* (2015) and antioxidant activity DPPH method Ruttarattanamongkol *et al.* (2016). Antioxidant activity was determined using the IC50 (Inhibition Concentration 50%) value.

Rejuvenation of *L. plantarum* NHC6 isolate

The *Lactobacillus plantarum* NHC6 were rejuvenating in MRSB media, and incubated at the room temperature (±28°C) for 24 hours (Prasirtsak *et al*., 2013). The isolate NHC6 was purified using the quadrant scratch method by taking one loop from the MRSB tube, scratching it on MRSA media and spotting it on MRSA media + 1% CaCO3, then incubating at room temperature (±28°C) for 48 hours for stock culture.

Growth curve of *L. plantarum* NHC6

L. plantarum was inoculated into 10 mL of MRSB medium. The culture was incubated for 24 hours at room temperature (±28°C) to a density with an OD (optical density) of 0.4 to 0.8. A culture with a density OD of 0.5 was taken at 1 mL and inoculated into an Erlenmeyer flask containing 99 mL MRSB media. The number of bacteria was counted every 2 hours using the total plate count (TPC) method by making serial dilutions in physiological NaCl. A 1 mL culture is diluted to achieve a dilution concentration of 10-1 to 10-10. 100 µL of bacterial culture from each dilution of 10^{-5} to 10^{-10} was taken and spread onto MRSA media in duplicate, then incubated at room temperature (±28°C) for 48 hours, and then the colonies were counted. The growth curve of L. plantarum on steaming pasta media, microwave cooking, HMT, and EHMT was also carried out. The number of colonies was counted every 3 hours.

Application of *L. plantarum* NHC6 and its storage in red sweet potato paste

The application was carried out by following the method of Ariyanto *et al.* (2021) with temperature modifications. One loop of NHC6 bacteria was grown in 10 mL MRSB medium and incubated for 24 hours. 1 mL of culture was taken and then grown in 100 mL MRSB media. The culture was incubated for 24 hours at room temperature (±28°C). Probiotic starter was taken 1 mL from a culture with a bacterial colony count of 9 log CFU/mL, then put into 99 mL paste which had previously been subjected to thermal pasteurization at 80°C for 15 minutes (Lagnika *et al*., 2017) with various paste treatments, namely steaming, microwave cooking, HMT, EHMT and control (without starter), then storage at three temperatures, namely 4°C, 10°C and room temperature (±28°C) with 3 repetitions each. Observations, including bacterial viability, total acid, and pH were carried out every 3 days for 21 days.

Characterization of the Effect of *L. plantarum* on red sweet potato pasta

Bacterial culture from each dilution of 10^{-5} to 10^{-10} was taken in 100 µL and spread onto MRSA media in duplicate, then incubated at room temperature (±28°C) for 48 hours and the growing colonies were counted. Sample pH testing is carried out using indicator paper. The indicator paper is dipped into the sample for 5 minutes. The results of the indicator color change are matched with the indicator's pH trajectory. The lactic acid contain was calculated according to Suhaeni (2018).

RESULT AND DISCUSSION

Characteristics of Red Sweet Potatoes

Proximate analysis is a chemical test to identify the nutritional content of food ingredients and also an assessment of the quality of food ingredients based on the standards of the food substances contained therein. Proximate analysis of red sweet potato shows that the largest content is water, namely 67.5%, ash content 0.6%, crude protein 1.1%, crude fat 1.6%, crude fiber 0.98%, and carbohydrates (by difference) 29 %. From around 80 types of red sweet potato analyzed, the starch content was 42.4 - 77.3%, crude fiber 1.9-6.4%, protein 1.3-9.5%, ash 1.1 - 4.9 and fat 0.2 - 3.0.(Wang *et al*., 2016). Analysis of red sweet potato flour carried out by (25) obtained a protein composition of 5.28%, fat 1.13%, carbohydrates 87.5%, fiber 2.10% and ash 4.00%. Yaningsih *et al*. (2013) showed that harvest age significantly affects the proximate content of a food ingredient.

Characteristics of Red Sweet Potato Paste After Pretreatment

Preheating sweet potatoes aims to modify the functional properties of starch (Putri & Mulyani, 2021). The total sugar, reducing sugar, degree of polymerization, anthocyanins, and antioxidants in sweet potato paste experienced different changes in each cooking treatment (Table 1).

Table 1. Characteristics of red sweet potato paste with various cooking treatments

Parameter	Treatment			
	Steam	***Microwave***	**HMT**	**EHMT**
Total sugar (mg/mL)	22,15^{b}	51,08^{a}	9,78^{c}	16,84^{c}
Reducing sugar (mg/mL)	1,44^{b}	2,54 ^{a}	0,65 ^{c}	0,76^{c}
Degree of polymerization (glucose units)	20,10^{b}	15,38^{a}	15,04^{a}	22,15^{b}
Anthocyanin (mg/100 g)	0,1^{ab}	0,27^{a}	0,08^{b}	0,04^{b}
Antioxidant IC50 (ppm)	21,22^{d}	24,69^{c}	31,11^{b}	29,79^{a}

Microwave cooking produces 2 times more total sugar than steaming. Total sugar and reducing sugar levels are related to the time required for cooking. Sunarti *et al*. (2012) state that microwave heating can quickly convert starch into simple sugars. Starch becomes glucose using microwaves; microwaves are 100 times faster than conventional heating. On the other hand, the reducing sugar and total sugar content of steamed sweet potato paste was higher than HMT because the HMT treatment is carried out with 3 autoclaving-cooling cycles, which will cause retrogradation. Retrogradation is the recombination of starch when stored at low temperatures (Putri & Mulyani, 2021). Sweet potato pasta treated with EHMT showed a high degree of polymerization (DP) value because many of the glycosidic bonds between the polymer groups have not been broken so the polymer groups are still in long chains and have not been broken down into monomers. A low DP value indicates that more polysaccharides are depolymerized into compounds with shorter chains (Sasongko *et al*., 2018).

Table 1 shows that the cooking process affects anthocyanin and antioxidant levels. Microwave cooking produces higher anthocyanin levels than steaming, HMT, and EHMT cooking processes. Similar results by Xu *et al.* (2013) show that the steaming process can reduce anthocyanin levels to a greater extent than microwaves. The destruction of anthocyanins at high temperatures will be faster due to the hydrolysis of the 3-glycoside structure, which has a protective effect on unstable anthocyanins. The IC50 antioxidant value during cooking shows the influence of different cooking treatments. Sweet potato paste showed high antioxidant activity (21.22 ppm) in the steaming treatment. These results in accordance to Ilyasoglu and Burnaz (2015), who reported that steaming treatment generally increased the antioxidant activity of fresh samples. Increased antioxidant activity is associated with indirect contact with water, which helps retain water-soluble compounds.

Testing the ability of *L. plantarum* NHC6 to produce lactic acid

Lactic acid bacteria isolates were rejuvenated on MRSA media using the quadrant method. Confirmation of the isolate was carried out by growing NHC6 bacteria in MRSA + $CaCO_3$ media and observing the formation of a clear zone around the isolate (Figure 1).

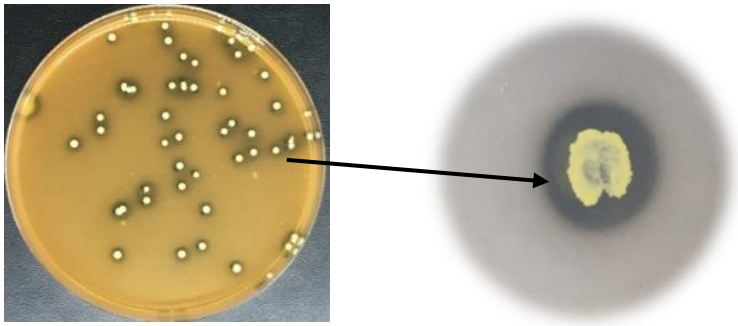

Figure 1. Clear zone formation of NHC6 isolates on MRSA media added with 1% $CaCO^3$ after incubation for 48 hours at room temperature (±28 °C)

The NHC6 isolate showed the formation of a clear zone on MRSA + CaCO3 media, which is in accordance to Yelnetty *et al.* (2020), who stated that the probiotic isolate *L. plantarum* YN1 showed the formation of a clear zone around the colony. The clear zone formed around the lactic acid bacteria colony indicates the presence of water-soluble calcium-lactate compounds. Meryandini *et al.* (2020) stated that the lactic acid produced by LAB will react with CaCO3 in the MRSA medium to form a clear zone around the colony.

Growth Curve of *L. plantarum* NHC6

A growth curve is a graph that shows the growth rate of microorganisms per unit of time. Growth begins with a short adaptation phase from the 0th to the 4th hour. The growth of *L. plantarum* has a long logarithmic phase; this can be seen from the 4th hour to the 22nd hour. In this condition, bacterial cells continue to experience an increase in bacterial cells from 6 log CFU/mL to 11 log CFU/mL. Next, after the logarithmic phase is reached, the stationary phase will follow. The stationary phase of NHC6 is relatively short and then goes into the death phase, which is marked by a decrease in the number of bacterial cells (Figure 2).

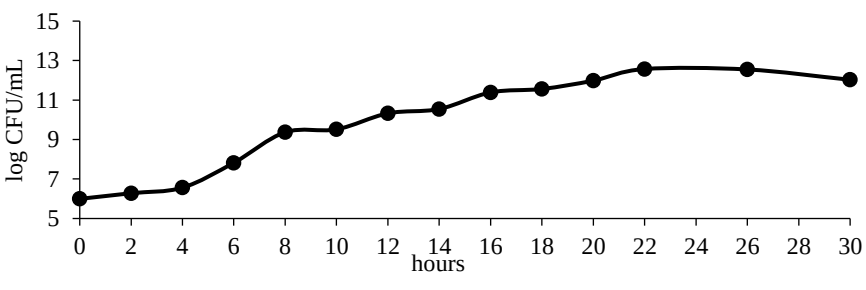

Figure 2. Growth curve of *L. plantarum* NHC6 isolate in MRS broth after incubation for 30 hours at room temperature (±28°C)

The maximum growth rate (μmax) reached 0.23 h-1 at the 8th hour. The minimum number of lactic acid bacteria that must be present in a probiotic drink product that is suitable for consumption is 106 CFU/mL so that it can meet SNI standards as a probiotic product that has the potential to improve health (Alemneh *et al*., 2021). The growth of bacteria is a reference in determining the right time to harvest bacterial cells for the production process, so this treatment is carried out at the 8th hour.

Growth Curve of *L. plantarum* NHC6 on Red Sweet Potato Paste

L. plantarum is also grown on red sweet potato paste media with various physical modification treatments such as steaming, microwave, HMT, and EHMT (Figure 3).

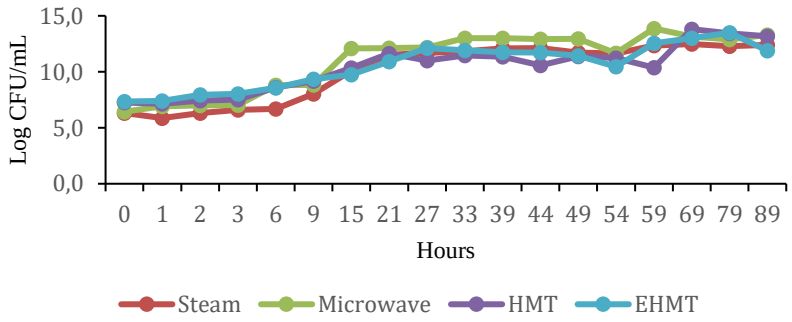

Figure 3. Growth curve of *L. plantarum* NHC6 isolates in paste media with several treatments incubated at room temperature (±28°C)

Red sweet potatoes with different treatments showed differences in carbon sources. The available carbon source influences the bacterial growth curve. The growth curve shows a diauxic phenomenon in the *L. plantarum* curve with HMT and EHMT treatment. The diauxic phenomenon that occurs shows the complexity of the available carbon sources. When one type of carbon is used up (glucose), bacteria can still carry out metabolic processes using the next carbon source (Chen *et al*., 2018). Before bacteria use other carbon sources to grow, they use glucose to produce energy. Steaming and microwaving treatment showed that no diauxic was formed because there is still much glucose in red sweet potato paste.

Total Lactic Acid Bacteria in paste

The minimum amount of probiotics in probiotic drink products is 10^6 CFU/mL (Alemneh *et al*., 2021). The total BAL value of probiotic sweet potato paste before inoculation on sweet potatoes was 7 log CFU/mL. The total increase in BAL occurred on days 3 to 21, and there was also a decrease (Figure 4).

The increase is due to the longer storage time for lactic acid bacteria to grow and develop. The number of lactic acid bacteria cells during the storage period between the 15th and 21st days was relatively constant because the storage period on the 15th day had entered the stationary phase. In this phase, NHC6 experienced growth but was not optimal because

the longer the period causes BAL to experience nutritional deficiencies, and bacterial activity also decreases due to inhibition by the acid produced.

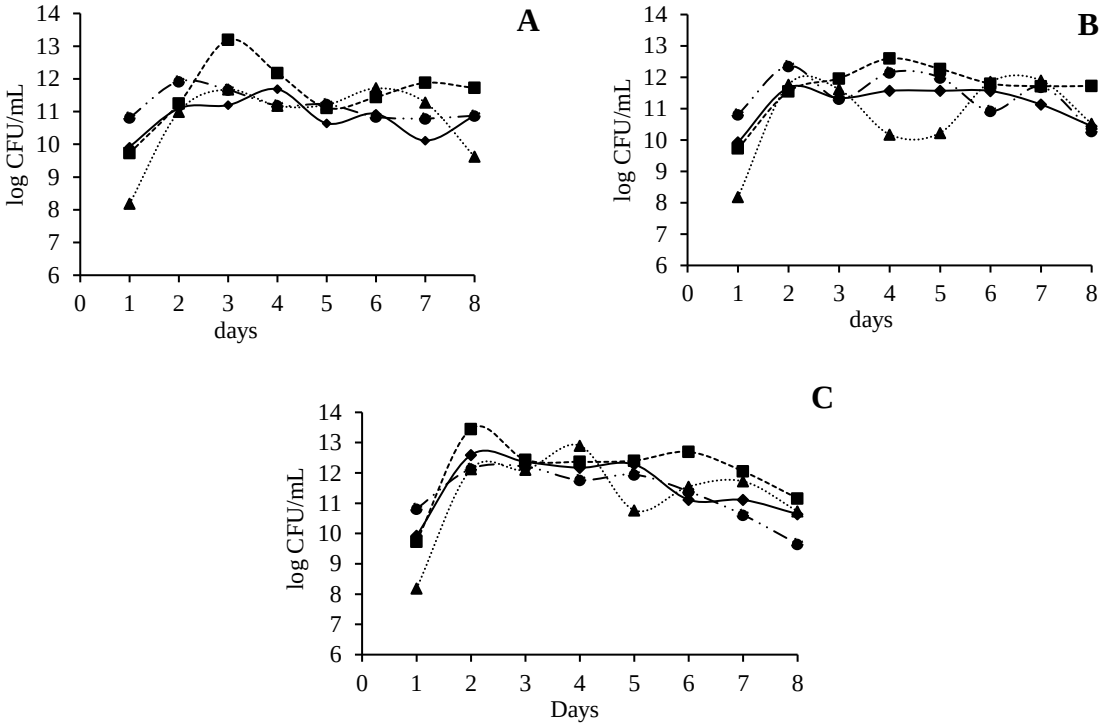

Figure 4. Effect of application of *L. plantarum* NHC6 on total lactic acid bacteria at various temperatures in red sweet potato pasta (A) at 4°C (B) at 10°C and (C) at 27°C, steaming ( ), cooking with microwave ( ), HMT ( ), EHMT (--------)

pH and Total Acid on the paste

The Growth observed for 21 days caused a change in pH in the substrate stored at 4°C, control (without starter) 4°C, 10°C, control (without starter) 10°C and 27°C, control (without starter) 27°C with steaming, microwave, HMT and EHMT treatments (Figure 5).

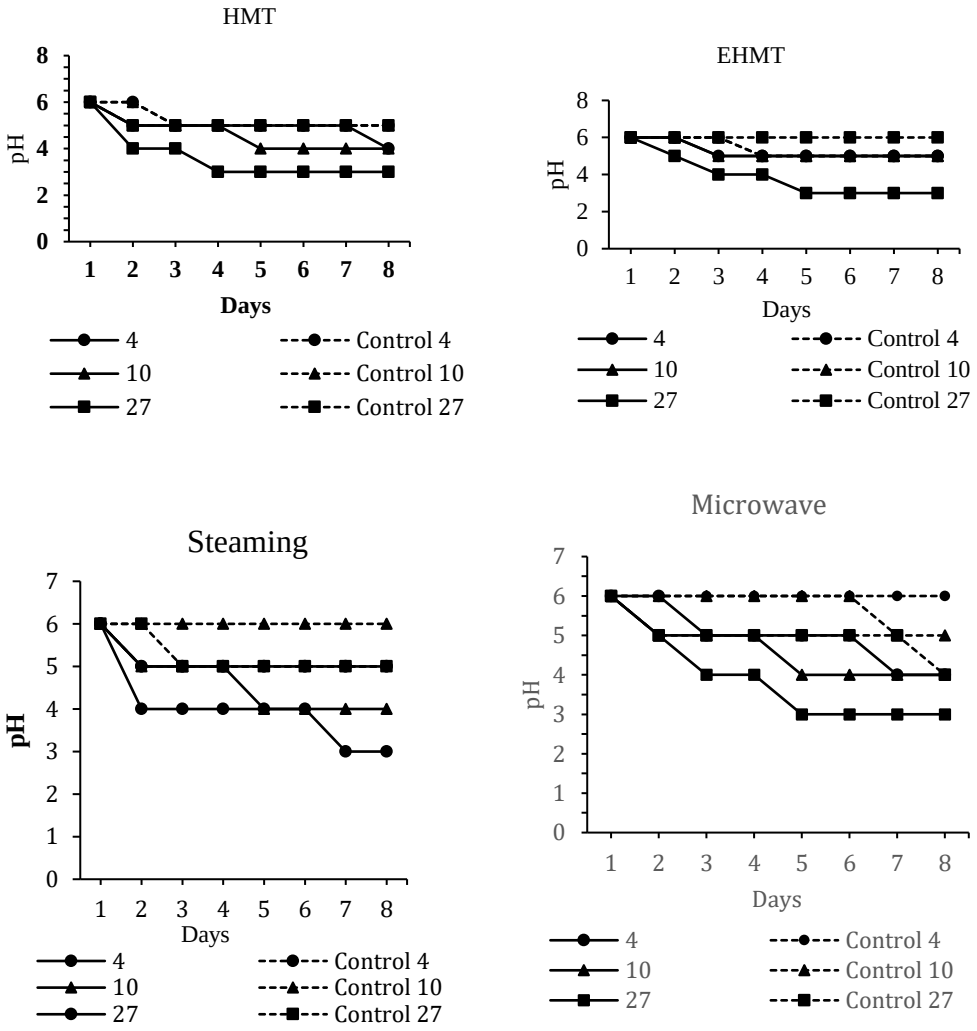

Figure 5. Effect of storage time on changes in pH of red sweet potato pasta

The storage temperature has a significant effect on the resulting pH. The higher storage temperature, the lower the pH value tends to decrease because NHC6 uses red sweet potato as a substrate, which will produce acid with more extended storage. The fermentation process, which produces a lactic acid product, causes a decrease in pH because *L. plantarum* carries out homo-fermentation in converting the substrate into lactate (Zheng *et al*., 2015). A temperature of 27°C close to the optimum temperature will cause metabolism to occur quickly, increasing lactic acid production. It will cause the pH of the pasta to decrease. The storage time on days 9 to 21 caused a slight decrease in pH and did not decrease as much as at the beginning of the storage period, namely on days 0 to 9. It is possibly because the viability of probiotics is decreasing, thereby reducing the metabolic ability of LAB to produce lactic acid. Storage at a temperature of 27ºC experienced a significant decrease until it reached pH 3 on day 15 until the end of storage.

Storage also affects the total amount of acid in red sweet potato paste. The longer storage is, the more acid will increase and lower the pH. Storage affects the total amount of acid in red sweet potato paste. The total acid obtained ranged from 0.2% to 4%. This percentage meets the SNI requirements for the acidity level of probiotic drinks, namely a minimum of 0.45% (Febricia *et al*., 2020). Based on the data obtained, all treatments increased the total acid concentration from days 3 to 18 (Figure 6). It is comparable to a

decrease in the pH value due to the presence of organic acids resulting from bacterial metabolism during storage. Harahap *et al.* (2018) stated that the total level of lactic acid aligns with the number of LAB that grow. An addition, without a starter produces lower total acid compared to the addition of a starter. Adding sweet potatoes with various treatments will increase the faster growth, more reducing sugars will be used both for growth and to form lactic acid so that the levels of reducing sugars will decrease. According to Pranayanti and Sutrisno (2015), the availability of nutrients will increase the number of cells. It will have an impact on the maximum breakdown of sugar, which will cause total acid to increase and pH to decrease.

CONCLUSION

Sweet potato paste treatment increase the total sugar, reducing sugar, anthocyanin, and antioxidants at different levels. Microwave cooking treatment produces simpler sugars and high anthocyanins, but antioxidant activity decreases. The addition of LAB showed diauxic growth and decrease in pH. Pasta cooked by microwave is a good treatment due to the produces high LAB growth, lactic acid levels > 0.45%, and pH reaches 4 at a storage temperature of 4^{o}C.

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