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Study on the Quality of Fermented Tapioca with Variation of Lactic Acid Bacteria (LAB) Types

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ABSTRACT
Fermentation potentially improves the quality of tapioca. Fermentation of tapioca occurred either spontaneously or with the addition of starters. Lactic acid bacteria widely used as a starter on flour fermentation. This study aimed to determine the effect of the types of Lactic acid bacteria on the characteristic of fermented tapioca. The method used in this study was an experimental method carried out in the laboratory and designed using a Completely Randomized Design (CRD) with one factor, i.e the types of lactic acid bacteria that consisted of 5 treatments (control, *Lactobacillus plantarum, L. acidophilus, L. casei and L. fermentum*). The results showed that the types of lactic acid bacteria significantly affected the pH, A_w, viscosity, and total lactic acid bacteria, but had no significant effect on the yield and degree of whiteness of fermented tapioca. Fermentation using *L. plantarum* as a starter was the best treatment to produced fermented tapioca characterized by pH value of 4.37, water activity 0.54, yield 17.20%, viscosity 27,360 cps, degree of whiteness 95.10 and total lactic acid bacteria 1.96 x 10^{-7} CFU / gram.

Keywords: *Cassava, Fermented tapioca flour, Lactic acid bacteria.*

1. INTRODUCTION

Fermented tapioca has preferable characteristics compared to unfermented tapioca. Fermented tapioca has high swelling power, so this type is widely used in the food industry. However, spontaneous fermentation produces tapioca with low physical quality and food safety [1][2]. According to Rachman [3] spontaneous fermentation significantly increases the protein content, but the product has a high water content that will affect the shelf life of tapioca.

The addition of microbial starter in the fermentation of flours can improve the quality. Lactic acid bacteria is one of the starters that is widely used in the fermentation of flour [4]. Lactic acid bacteria has the ability to accelerate the fermentation process to produce better quality products than spontaneous fermentation [1]. Several types of lactic acid bacteria such as *L. plantarum, L. acidophilus, L. casei*, and *L. fermentum* were reported involved in the fermentation of flour.

As stated by [5], tapioca fermentation using 3% L. plantarum with 9 days fermentation produced the best quality with yield 19.6%, total acid 0.04%, viscosity 1.984 cps, whiteness 97 degree, solubility 18.54% and development volume 276.3%. According to [6] fermented cassava starch using *L. fermentum* with 24 hours fermentation period produced amylose 18.39% with swelling power 8.31%. In addition, [7] reported that, cassava fermentation using 6% *L. casei* with 48 hours fermentation period was the best treatment with swelling power 14.13%. According to [8] fermentation using *L. acidophilus* can reduce the water content of cassava starch.

Lactic acid bacteria improve the characteristic of fermented flour by reducing the water content. Fermentation with the addition of *L. plantarum and L. acidophilus* can reduce the water content of fermented sorghum [9][10]. According to [11], fermentation using *L. casei* can reduce the water content of sweet corn flour to 5.15%. [12], reported that the fermentation using a mixed culture of *L. fermentum, L. plantarum* and *L. acidophilus* can reduce the water content of fermented banana flour.

Lactic acid bacteria also improve the nutritional value by increasing the protein content. [13], stated that cassava fermentation using *L. plantarum* increased the protein content of mocaf. [14] reported the application of 1% *L. plantarum* with 48 hours fermentation time on nagara bean flour resulted in protein content of 25.60%. According to [15], fermentation using a mixed culture of

L. fermentum and *L. plantarum* can increase protein digestibility of corn flour and sorghum flour. This paper reports the effects of lactic acid bacteria types on the quality of fermented tapioca

2. MATERIALS AND METHOD

2.1. Research Design

The method used in this research was an experimental method, which was carried out in the laboratory. The research design used was a completely randomized design (CRD) with four types or lactic acid bacteria as a starter, including *Lactobacillus plantarum*, *L. acidophilus, L. casei* and *L. fermentum*, and the control (without the use of starter). For this control, the fermentation occurred spontaneously. Each treatment was repeated four times.

2.2. Preparation of the Lactic Acid Bacteria Starter [16]

Liquid cultures of *L. plantarum*, *L. casei*, *L. acidophilus*, and *L. fermentum* were prepared from the Laboratory of Food Microbiology, Faculty of Food Technology and Agroindustry, University of Mataram. One ml of each culture then subcultured on 9 ml of De Man Rogosa and Sharpe Broth (Oxoid, England) and incubated at 37°C for 24 hours. This step repeated four times. After that, 1 ml of lactic acid bacteria culture has been obtained from MRSB grown on De Man Rogosa and Sharpe Agar (Oxoid, England) and incubated at 37°C for 48 hours. Lactic acid bacteria obtained from this culture were calculated using the Total Plate Count method. If the total number of lactic acid bacteria were 10^8-10^9 CFU/g, the culture is ready to use as a starter.

2.3. Production of Fermented Tapioca [5,17]

Local varieties of white cassava with harvest age of 10-12 months were obtained from Duman Village, Lingsar District, West Lombok Regency. The production of fermented tapioca involved the preparation of cassava with sorted, peeled, washed and grated to get cassava pulp. The cassava pulp was then mixed with 1:2 (w/v) water and allowed to settle for 4 hours to obtain cassava starch. After being precipitated, the water and the sediment were separated to obtain cassava starch. Cassava starch then mixed with water 1: 1 (w / v) and added with 3% starter *L. plantarum*/ *L. acidophilus*/*L. casei*//*L. fermentum* and then fermented at 28°C for 48 hours. After the fermentation process, water and sediment were separated. The sediment was dried with direct sunlight for 12 hours, and coarse tapioca was obtained. Coarse tapioca was then ground and sieved using an 80 mesh sieve to obtain fermented tapioca.

2.4. Determination of Fermented Tapioca Quality

The quality of Fermented tapioca moisture was determined based on the pH measured using a Schott pH meter [18], water activity assessed using Aw meter portable, fermented tapioca yield express in percentage between fermented tapioca weight to the original cassava weight [19], viscosity measured using a viscometer Brookfield [20], color test assessed using colorimeter (MSEZ User Manual) and total lactic acid bacteria calculated using modification of total plate count [21].The data obtained from the observation were analyzed using variance analysis (Analysis of variance) at a 5% significant level using the Costat software. When there was a significant difference, a further test was carried out with the Honest Real Difference Test (HSD) at the same significant level.

3. RESULTS AND DISCUSSION

3.1. Acidity (pH)

As shown in table 1, the types of lactic acid bacteria that gave a significant effect on the pH of fermented tapioca. Low pH value of fermented tapioca with addition of lactic acid bacteria starter associated with the presence of organic acids produced by the lactic acid bacteria starter used during fermentation.

Table 1. Fermented tapioca acidity (pH), water activity (a_w), yield, viscosity, degree of whiteness and total lactic acid bacteria (LAB)

Tapioca with fermentation of	pH	A_w	Yield (%)	Viscosity (cp)	Degree of Whiteness	Total LAB (log CFU/g)
None (Control)	4.48b	0.56^a	16.66	18.83^d	94.48	6.07^c
L. plantarum	4.37c	0.54^b	17.2	27.83^a	95.1	7.29^a
L. casei	4.41^c	0.55^{ab}	16.89	23.16^{ab}	95	7.26^a
L. acidophilus	4.67^a	0.56^{ab}	16.76	22.3^c	94.7	6.61^b
L. fermentum	4.72^a	0.56^{ab}	16.73	23.36^{bc}	94.61	6.57^b

Different letter in the same column indicate data is significantly different at 5% alpha.

Glucose is broken down by lactic acid bacteria into lactic acid, pyruvic acid, acetic acid, ethanol, and CO_2, thereby increasing total acid and lowering pH [21]. This is in line with previous study [22] which states that the accumulation of lactic acid resulting from the metabolism of lactic acid bacteria can lower the pH of the medium.

Based on the results of HSD tests, the pH of fermented tapioca with the addition of *L. casei was* not significantly different from the addition of *L. fermentum*, but significantly different with the addition of *L. plantarum* and *L. acidophilus*. The addition of *L. plantarum* and *L. acidophilus* produced fermented tapioca which had a lower pH compared to tapioca with the addition of *L. casei* and *L. fermentum*. This is due to *L. casei* and *L. fermentum* belonging to the same species, namely the heterofermentative lactic acid bacteria group. Meanwhile, *L. plantarum* and *L. acidophilus* belong to the same species, namely the homofermentative lactic acid group [23]. As stated by [24], the homofermentative lactic acid bacteria produces more lactic acid than the heterofermentative lactic acid bacteria, so the pH value on the product by the homofermentative lactic acid bacteria is lower.

The range of fermented tapioca's pH value in this study was 4.37-4.72. According to *The Tapioca Institute Of America* (TIA), this pH value fulfills the quality standard of fermented tapioca, which determined that the standard pH of fermented tapioca flour ranges from 4-6 [25]. In addition, several industries require a pH value to determine the quality of tapioca flour related to processing processes, for example in the process of forming pasta. Optimum gel formation occurs at pH 4-7 [26].

3.2. Water activity (A_w)

The A_w value is the amount of free water in food that can be used for microbial growth and for chemical and biochemical reactions to take place [27]. The relationship between lactic acid bacteria types and water activity in fermented tapioca can be seen in Table 1.

The A_w of fermented tapioca was significantly affected after being treated with different types of lactic acid bacteria. The data showed that the Aw value of control tapioca was significantly different from fermented tapioca with the addition of lactic acid bacteria. Lower A_w value in the treatment with the addition of lactic acid bacteria starter was caused by the activity of microorganisms in the breakdown of starch, which resulted in the water bound in the material being liberated, so that it was calculated as the A_w value. This is in accordance with the opinion of [28] which states that without fermentation, water molecules will form hydrates with molecules containing oxygen atoms, carbohydrates, proteins and other organic compounds, making them difficult to evaporate. However, during the fermentation process, the enzymes produced by lactic acid bacteria will break down carbohydrates and other compounds, therefore the bound water turns into free water.

Figure 2 shows fermented tapioca with the addition of *L. plantarum* has a lower water activity than tapioca with the addition of *L. acidophilus, L. casei,* and L. *fermentum*. This is related to the high ability of *L. plantarum* to adapt with the environment compared to the other types, resulting in higher growth rate and higher amounts of acid [29]. This condition causes the pH of the environment to be low and causes the condition of the fermentation media to become acidic, consequently the permeability of the cell membrane of cassava tubers is disrupted. This results in the rupture of the cell wall of cassava [30]. *L. plantarum* was also able to produce higher pectinolytic and cellulolytic enzymes than other lactic acid bacteria [31], therefore the ability to lysis the cell wall increased. High number of cell lysis will increase the amount of starch granules that leave the tuber cells. This causes more amount of bound water to be liberated, consequently the water content and water activity in fermented tapioca decreased [32][33].

The A_w value of fermented tapioca in this study ranged from 0.54-0.56. This A_w value also comply the quality requirement for fermented tapioca according to [34], which describes that dry food ingredients such as flour generally have an A_w ranging from 0.60-0.85. In addition, the A_w value in this study is vulnerable below the critical A_w, where the critical water activity of food ingredients is 0.75. Higher water activity value of flour, the higher level of damage that can occur due to microorganisms [35].

3.3. Yield

Determination of the yield is carried out on the yield of flour produced [19]. [5] stated that starch yield is usually influenced by the process of stripping, grating, filtering, depositing and drying starch. The average yield can be seen in Table 1.

The yield of fermented tapioca was not significantly affected after being treated with different types of lactic acid bacteria. The amount of yield produced in the treatment with the addition of starter *L. plantarum, L. acidophilus, L. casei, L. fermentum* and control were 17.20%, 16.89%, 16.75%, 16.73% and 16.65%, respectively. The yield of fermented tapioca in this study did not comply the quality requirements for tapioca based on the SNI No.01-2997-1992 which required the yield of tapioca flour to pass an 80 mesh sieve of at least 90%.

3.4. Viscosity

Measurement of viscosity in fermented tapioca flour will make it easier to determine the level of starch viscosity during the gelatinization process. In addition, the viscosity test on fermented tapioca aims to determine the level of viscosity of the flour, consequently it can be applied to products that match the required level of viscosity. Lactic acid bacteria types gave a significant effect on the viscosity of fermented tapioca (Table 1). The viscosity value of fermented tapioca flour was higher than control tapioca

Fermented tapioca with the addition of *L. casei* and *L. fermentum* had a lower viscosity than fermented tapioca with the addition of *L. plantarum* and *L. acidophilus*. According to [36] the increase in viscosity is due to the fact that during the fermentation process, the growing microbes produce pectinolytic and cellulolytic enzymes that can destroy cell walls, resulting in starch consisting of amylose and amylopectin fractions easily leaving the granules. In addition to breaking down cellulose, lactic acid bacteria also modifies fine starch granules into holes. These holes strengthen the bond between the grains, consequently the dough is difficult to separate and more sticky.

The relationship between viscosity and pH value is directly proportional, the higher acidity level results in higher viscosity (Table 1). Fermented tapioca with the addition of *L. plantarum* and *L. acidophilus* had lower pH values compared to fermented tapioca with the addition of *L. casei* and *L. fermentum*. This is in accordance with the opinion of [35], one of the factors that affect the stickiness of flour is acid, the higher acidity level or lower pH will increase the level of starch hydrolysis.

3.5. Total Lactic Acid Bacteria

Total lactic acid bacteria of fermented tapioca were significantly affected after being treated with different types of lactic acid bacteria (Table 1). The data showed that total Lactic acid bacteria of control tapioca was significantly different from fermented tapioca with the addition of lactic acid bacteria. High total lactic acid bacteria in fermented tapioca flour were caused by the ability of lactic acid bacteria to utilize the nutrients available in the medium to grow during fermentation.

Fermented tapioca with the addition of *L. casei* and *L. fermentum* had lower total lactic acid bacteria compared to fermented tapioca with the addition of *L. plantarum* and *L. acidophilus*. High number of lactic acid bacteria in the treatment with the addition of *L. plantarum* and *L. acidophilus* associated with characteristic of these two bacteria as a homofermentative bacteria, that have the ability to adapt rapidly, hydrolyze complex compounds into smaller compounds such as lactic acid and are tolerant to acidic conditions with a growth speed of 50% Dornic or 0.5% lactic acid after 48 hours [29].

In addition, according to [37], homofermentative bacteria such as *L. plantarum* has high viability to be used as a starter in fermented tapioca. *L. plantarum is* different from other bacteria because this bacterium adapts more quickly to a new environment. According to [38] the logarithmic phase of *L. plantarum* is 15 hours.

In contrast to *L. plantarum,* other types of starter like *L. acidophilus* have a longer logarithmic phase for 8-32 hours. According to [38], L. *acidophilus* has a longer logarithmic phase than *L. plantarum,* which is 21 hours. The long logarithmic phase indicates that *L. acidophilus* takes a long time to adapt to a new environment. Likewise with starter *L. acidophilus*, according to [39] *L. casei* takes a long time to adapt to a new environment, where the logarithmic phase is up to 18 hours.

4. CONCLUSION

Based on the results obtained in this research, it can be concluded that the types of lactic acid bacteria significantly affected the pH, A_w, viscosity, and total lactic acid bacteria, but had no significant effect on the yield and degree of whiteness of fermented tapioca. Fermentation using *L. plantarum* as a starter was the best treatment to produced fermented tapioca characterized by pH value of 4.37, water activity 0.54, yield 17.20%, viscosity 27,360 cps, degree of whiteness 95.10 and total lactic acid bacteria 1.96×10^{-7} CFU / gram.

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