

Effect of Incubation Time on Amylase Enzyme Activity in Cassava Tape

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Abstract. Cassava tape is a product made by a fermentation process that requires the help of microorganisms. The tape fermentation process involves the enzyme amylase. Amylase is a glycoside hydrolase enzyme that catalyzes the breakdown of starch into simple sugars. Enzyme activity can be calculated by measuring the amount of product formed, or by calculating the amount of substrate reduction in a given unit of time. This study aims to determine the effect of incubation time on amylase enzyme activity in cassava tape. The research method used was the descriptive experimental method. The results of the experiment showed a reduction in the substrate due to amylase enzyme activity. The highest percentage of substrate reduction occurred at 48 hours incubation time and the lowest percentage at 144 hours incubation time. An organoleptic test was conducted with color, aroma, texture, and taste parameters conducted on 30 panelists. Incubation time affected the percentage of substrate reduction and organoleptic parameters.

Keyword: Cassava Tape, Incubation Time, Enzym Activity, Organoleptic Test

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1 Introduction

Indonesia is one of the countries rich in tubers. Indonesian tubers can be divided into 2 (two) groups, namely: main group tubers such as cassava and sweet potato (sweet potato vines) and a group of smaller tubers, including taro, Gadung, Suweg, Uwi, Gembili, Suweg, Porang, Iles-Iles, Kimpul, and Canna. Indonesia's development process has encouraged the growth of the agricultural products industry (agribusiness) [1].

Products produced by the agricultural industry are tubers such as sweet potatoes (*Ipomea batatas* (L.) Lam), taro (*Colocasia esculenta* (L.) Schott.), and cassava (*Manihot utilisima* Crantz), which

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can be utilized and converted into products to be processed for various purposes including the food industry, textile industry, paper industry, and alternative renewable energy production. One of the processed products, namely cassava tape) [1]. Tape is one of the traditional foods that are widely consumed by the people of Indonesia and is the result of the fermentation of cassava, white sticky rice, black sticky rice, and rice in general [1].

Cassava tape is a product made by a fermentation process that requires the help of microorganisms. The successive fermentation process begins with the hydrolysis of starch by amylase enzymes produced by molds, yeasts, or bacteria that are amylolytic. This cassava tape has a sweet, slightly sour taste, and contains a little alcohol. In addition to its sweet and legit taste, cassava tape is also a food ingredient that has a long shelf life and can also be converted into processed products such as colenak, bluder and sponge tape [2].

The starch in cassava is converted into glucose compounds by microbes. This will cause the formation of total acid in cassava. The more microbes that grow in cassava tape, the more metabolic products in the form of acid will increase [3]. In the process of tape fermentation, sugar is converted into alcohol, organic acids, glycerol, and CO₂. In the esterification process between acids and alcohol produces esters that form the distinctive flavor of tape [4].

One of the enzymes whose use is very large in the food, beverage, and biotechnology industries in Indonesia is the enzyme amylase. The tape fermentation process involves the enzyme amylase. Amylase is a glycoside hydrolase enzyme that catalyzes the breakdown of starch into simple sugars [5]. The process of starch hydrolysis using amylase enzymes can achieve a degree of starch hydrolysis up to 42%-97% depending on the type of substrate and incubation time [6].

The organoleptic test is a test of food ingredients based on the liking of a product. Sensory tests or also called sensory tests are testing methods that use human senses as a tool to test them. This test involves the senses of sight (eyes), smell (nose), taste (tongue), and sense of touch (hands) [7]. Based on this background, it is necessary to conduct research on the effect of incubation time on amylase enzyme activity in cassava tape.

2 Method

Location and Time of Research

The research was conducted at the Microbiology Laboratory of the University of North Sumatra and the tape-making location on Jl. Suka Sari No. 2, Medan Johor. The research time was conducted on December 11, 2022 - December 17, 2022.

Tools and Materials

The tools used in this research are test tubes, centrifuge, volume pipettes, drop pipettes, beaker glass, magnetic stirrer, and UV-Vis spectrophotometer. Material the sample used was cassava tape. Supporting chemicals are phosphate buffer (pH 7), amylum, and iodine. Work Procedure The research method applied is the descriptive experimental method, which is by conducting experiments directly.

Step I. Cassava Tape-Making Process

First, cassava (*Manihot utilisima*) was peeled clean. Weighed as much as ± 1000 grams and then cut into pieces according to the desired size and washed thoroughly. Then the sweet potato is steamed until cooked. The cooked sweet potatoes were cooled for ± 1 hour and sprinkled with yeast. Then, the cassava that has been sprinkled with yeast is divided into three parts and packed with banana leaves. Furthermore, the cassava packaging was stored (incubation) for 48 hours, 96 hours, and 144 hours at room temperature.

Step II. Amylase Enzyme Activity Test

The substrate used in this study was a tape solution dissolved in phosphate buffer (pH 7) in a ratio of 1:1, then centrifuged. The supernatant from the centrifugation was taken, then phosphate buffer (pH 7) and amylum were added in the ratio of 1:1:2 respectively. The solution was then incubated for 15 minutes at 27°C . The reaction was stopped by heating the solution at 100°C , then after the heating process, 2 drops of iodine were added to the solution. Then the absorbance value was measured on the solution that had been mixed with iodine. The absorbance value was measured using a UV-Vis spectrophotometer at a wavelength of 540 nm. The amylum standard curve was made by making different concentrations of amylum, namely: 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; and 0.7 ppm (Figure 1).

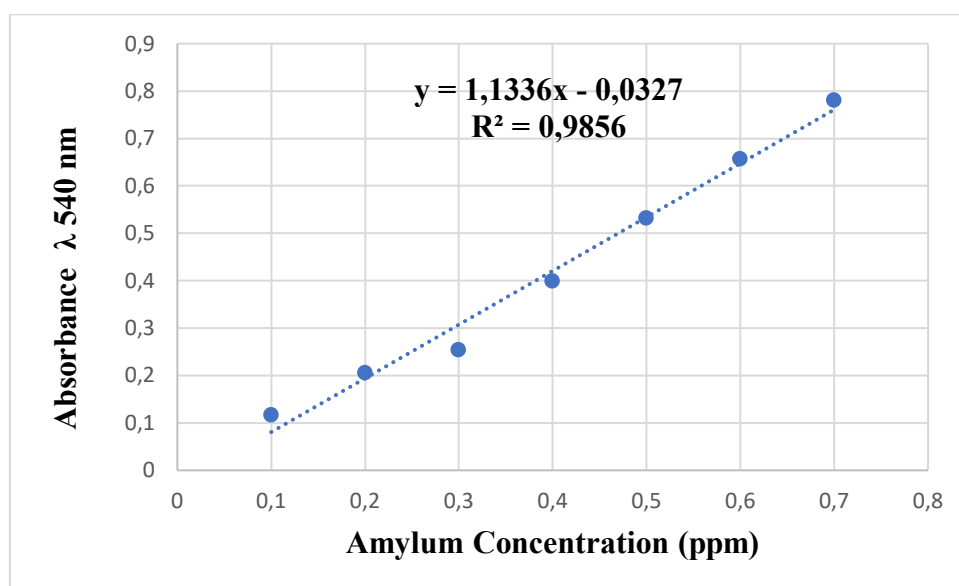


Figure 1. Amylum Standard Curve

Step III. Organoleptic Test

This study used an organoleptic test with the parameters of color, aroma, texture, and taste, with the method of testing preference in the form of scoring (**Table 1**). The number of panelists needed for this test was 30 people. The panelists were Biology students at the University of North Sumatra.

Table 1. Organoleptic Scoring Test Data

Parameter	Scoring		
	1	2	3
Color	Yellow	Very yellow	-
Aroma	Slightly alcoholic	Alcohol	-
Texture	Slightly Hard	Hard	Soft
Taste	Slightly sweet	Sweet	Acid

3 Result and Discussion

Effect of Incubation Time on Substrate Reduction

The ability of an enzyme to catalyze a chemical reaction is called enzyme activity. Enzyme activity can be calculated by measuring the amount of product formed, or by calculating the amount of substrate reduction in a given unit of time [8]. In this study, enzyme activity was calculated based on the percentage of substrate reduction by varying the incubation time of cassava tape (**Table 2**). The incubation time variations were 48, 96, and 144 hours. Based on the results obtained, the cassava tape incubation time at 48 hours showed the highest percentage of substrate reduction, namely 76.91%, while the lowest percentage of the substrate, namely 54.77% at 144 hours incubation time. This is in accordance with the growth curve of yeast (**Figure 3**).

Table 2. Percentage of Substrate Reduction with Incubation Time Combinations of 48, 96, and 144 hours at 27°C.

No	Incubation Time (Hour)	Percentage Substrate Reduction
1.	48	76,91%
2.	96	54,89%
3.	144	54,77%

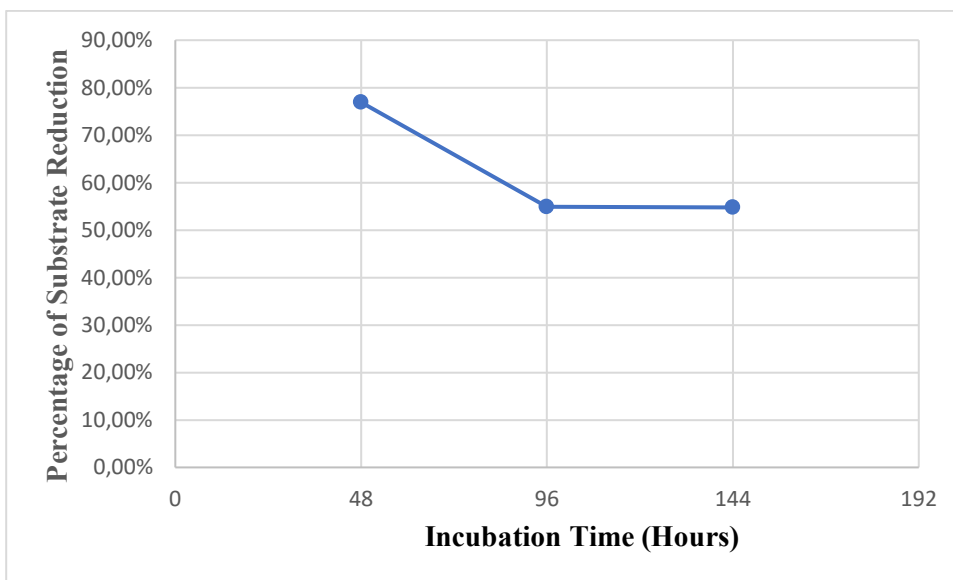


Figure 2. Graph of Percentage of Substrate Reduction with Incubation Time Combination of 48, 96, and 144 Hours at 27⁰C.

The data obtained is in accordance with research conducted [9] which tested the activity of amylase enzymes with variations in incubation time of 24, 48, 72, 96, 120, and 144 hours. The maximum activity of the enzyme was found at 48 hours incubation time. After 48 hours of incubation, there was a decrease in enzyme activity. Research [10] also showed the amylase enzyme reached its maximum at 48 hours after incubation. However, increasing the incubation time did not show a significant increase.

Based on research [11], microbial cell growth occurs at an incubation time of 24 to 48 hours. This indicates the entry of the exponential growth phase (log phase). Tape fermentation entered the stationary phase at 43-168 hours. In this phase, the number of living microbes is proportional to the number of dead microbes. It can be concluded, the increasing time amount of nutrients *Saccharomyces cerevisiae* and substrate decreases [12].

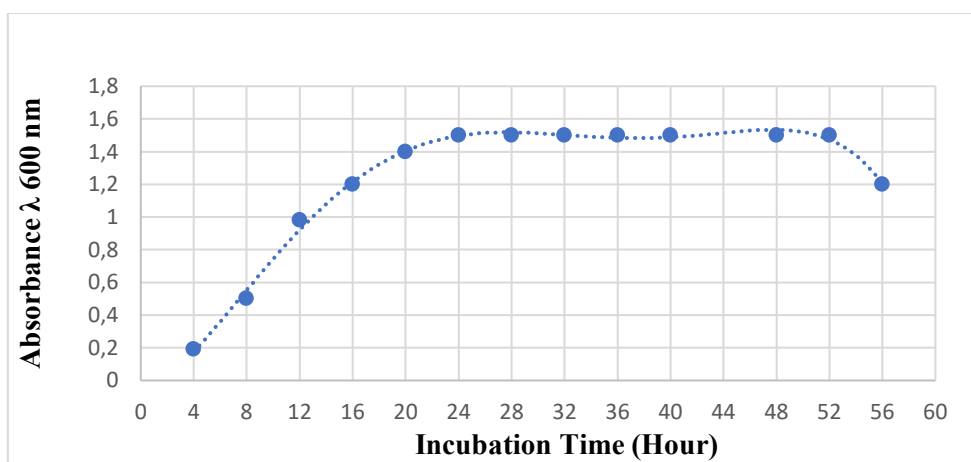


Figure 3. Yeast Growth Curve

Organoleptic Test

Organoleptic tests on cassava tape samples were conducted on 30 different panelists (respondents). The following average results of the organoleptic assessment by panelists on cassava tape can be seen in (Table 3).

Table 3. Average Results of Organoleptic Test of Cassava Tape from 30 Panelists

No	Incubation Time (Hour)	Color	Average		
			Aroma	Texture	Taste
1	48	1,1	1,3	2,3	1,6
2	96	1,5	1,5	2,7	1,7
3	144	1,7	1,4	3	1,8

Based on (Table 2), of the 30 panelists who conducted the organoleptic test, the best color of cassava tape was at 144 hours of incubation time, where the color shown was very yellow. The highest alcohol aroma in cassava tape at 96 hours incubation time. The softest texture of cassava tape was at 144 hours incubation time, and the sweetest flavor of cassava tape was at 144 hours incubation time.

Color

The results of the organoleptic test with color parameters that are widely preferred by panelists are very yellow. This is because the sweet potato used in making tape is yellow cassava. In [12], states that color changes can be caused by the food itself or the addition of ingredients during the processing process. During fermentation, cassava undergoes physical and biochemical changes through the activity of microorganisms.

Aroma

Aroma is an indicator that determines the presence of liking and also supports the taste and determines the quality of a product [12]. From the results of the study, the aroma of cassava tape was very alcoholic at the incubation time of 96 hours. The activity of microorganisms contained in yeast can help give the aroma of alcohol to cassava tape [4]. This is in accordance with [13] which states that the strong aroma of tape is caused by a number of aroma-forming compounds in large quantities. These aroma-forming compounds are formed during the fermentation process, namely from glucose hydrolysis and oxidation of alcohol on tape, and have volatile properties.

Taste

The activity of microorganisms contained in yeast can also help provide flavor to the tape [4]. Organoleptic test results showed that cassava tape had a sweet taste at 144 hours of incubation. The sweet taste is caused by the presence of glucose as a product of enzyme activity. The longer the incubation time, the sweeter the taste of the tape will be. The sweet taste of black glutinous rice tape is obtained due to the amylase enzyme converting the starch in black glutinous rice into simple sugars. The type of tape that sometimes causes a rather pungent sour taste is usually caused

by a lack of accuracy when making tapes such as the addition of yeast that is too excessive, less perfect at the time of storage, and storage that is too long [12].

Texture

The texture is the condition of softness or hardness of the fermentation product. At an incubation time of 114 hours, a soft texture was obtained. The difference in texture is due to the amount of yeast used and the level of maturity at the time of steaming. The steaming process and the length of fermentation time are factors that affect the texture of the tape. The results showed that in the organoleptic test in the study respondents preferred cassava tape with a hard texture [4].

4 Conclusion

Based on the results of the study, it can be concluded that the incubation time treatment on cassava tape affects the percentage of substrate reduction as enzyme activity. Substrate reduction is in accordance with the yeast growth curve, where the 48-hour incubation time shows the highest percentage of substrate reduction, namely 76.91%, while the lowest percentage of substrate, namely 54.77% at the 144-hour incubation time. Incubation time can also affect the organoleptic test parameters of cassava tape in the form of color, aroma, taste and texture. Tape with 144 hours incubation time produces a very yellow color, soft texture and sweet taste.

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