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Effect of Incubation Time on Amylase Enzyme Activity in Sticky Rice Tape

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Abstract. Indonesia uses fermentation as a common method of food processing. Tape ketan is one of the foods produced using the fermentation process. The enzyme amylase, which hydrolyzes carbohydrates (starch) into sugar, is involved in the fermentation process of sticky rice tape. Many factors affect the activity of the amylase enzyme. Incubation time is one of them. The length of incubation can also impact the tape's sweetness, color, texture, and scent. The 48-hour incubation period had the maximum amylase activity. Organoleptic testing, meanwhile, revealed a range of color, scent, texture, and taste. We'll talk about how the amylase enzyme's activity and incubation period relate to the color, flavor, texture, and aroma of sticky rice tape in this article.

Keyword: Amylase Enzyme, Enzyme Activity, Fermentation, Organoleptic, Sticky Rice Tape,

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1 Introduction

Using traditional biotechnology, local food is processed, one of which uses fermentation. The role that bacteria and fungus play in the fermentation of food cannot be separated. The inclusion of microorganisms in food relies on the primary components and the finished product. Sweets and carbohydrates promote the survival of microbes. In the fermentation process, microorganisms hydrolyze carbohydrates into organic acids [4]. Tape is a typical cuisine that uses the fermentation process.

In Indonesia, tape is a delicacy that is particularly well-liked. The fungus *Endomycopsis*, *Fibuligeria*, *Rhizopus oryzae*, or *Saccharomyces cerevisiae*, which serves as yeast, is added to glutinous rice or cassava to create this dish [6]. A successful tape-making process will result in a

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tape with good colour, flavor, texture, and scent. Amylase enzymes are included in the fermentation process used to make tape.

Amylase is an enzyme that breaks down starch to create glucose. Additionally, it is transformed into acid or alcohol to create fermented foods that have a sweet, alcoholic, or mildly sour flavor [8]. The level and type of sugar as well as the pH of tape typically alter after a given amount of incubation time. The sweetness, color, texture, and scent of the tape are all impacted by this. Given this context, research on the impact of incubation time on the amylase enzyme's activity in tape is required

2. Materials and Methods

This research was conducted with an exploratory method by means of observations carried out in a time span of 48 hours, 96 hours, and 144 hours against the amylase enzyme produced by yeast on sticy rice tape as substrat. Sticky rice tape was the material utilized in this experiment, along with test tubes, a centrifuge, volume and dropper pipettes, beaker glass, a magnetic stirrer, and a UV-Vis spectrophotometer. In this research, supporting substances such phosphate buffer (pH 7), starch, and iodine were used.

2.1 The Production of Sticky Rice Tape

First, 500 grams of sticky rice are weighed, carefully washed, and steeped for three hours. The sticky rice should be thoroughly cleaned before being placed in the steamer pan. After 30 minutes, remove the rice and set it aside. After cooling for about an hour, yeast is added to the cooked sticky rice. Following that, three portions of the sticky rice that has been dusted with yeast are separated and wrapped in banana leaves. Furthermore, the samples were incubated at room temperature for 48 hours, 96 hours, and 144 hours.

2.2 Amylase Enzyme Activity

The liquid formed by the tape is dissolved 1:1 in phosphate buffer (pH 7), followed by centrifugation. Phosphate buffer (pH 7) and starch were then added in a 1:1:2 ratio to the centrifuged supernatant. The solution was then incubated at 270C for 15 minutes. The reaction was stopped by heating the solution to 1000C, followed by the addition of 2 drops of iodine. In addition, the absorbance value was calculated in an iodine-mixed solution. A UV-Vis spectrophotometer operating at a wavelength of 540 nm was used to measure the absorbance value. The resulting absorbance value is then fed into the standard curve regression algorithm (Figure 1). The starch standard curve is created. A variety of starch concentrations, including 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 ppm, are made to create the starch standard curve (Figure 1).

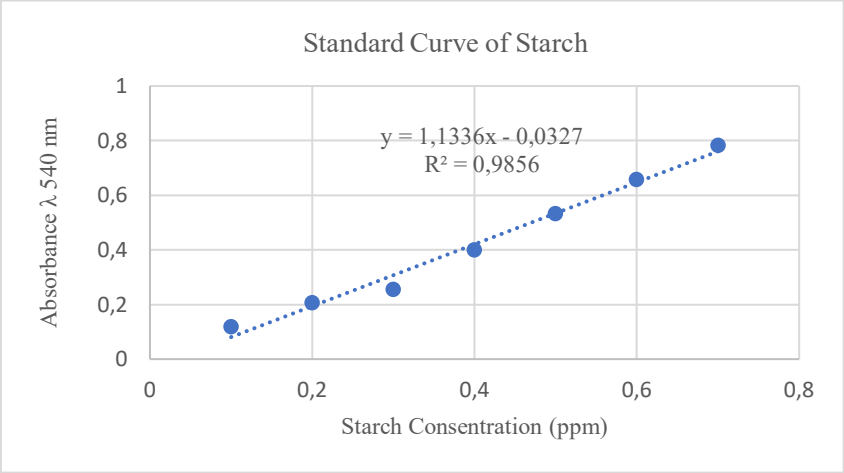

Figure 1. Standard Curve of Starch

3. Result and Discussion

3.1 Substrate Reduction as Enzyme Activity

Table 1. Percentage of substrate reduction with 48 hours, 96 hours, and 144 hours of combined incubation at 27 ºC.

No	Waktu Inkubasi (Hours)	Substrate reduction (%)
1.	48	75,92
2.	96	52,87
3.	144	52,85

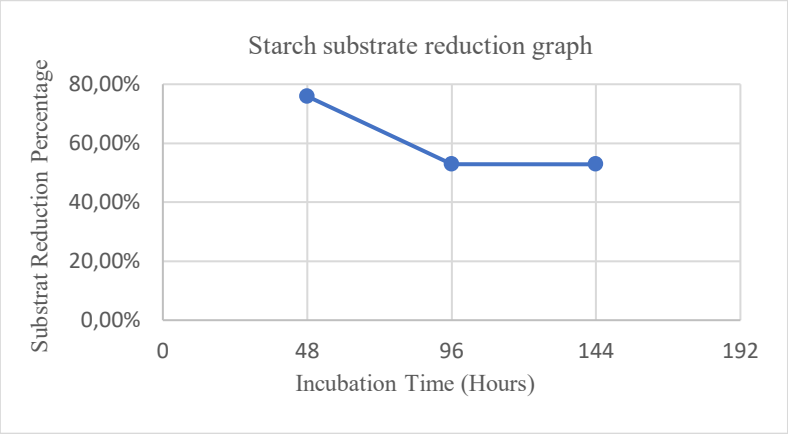

Figure 1. Percentage of substrate reduction with 48, 96, and 144 hours of combined incubation at 27 ºC.

The experiment's findings demonstrated that a 48-hour incubation period for sticky rice tape resulted in a substrate decrease of 75.72%, which was the greatest percentage. The substrate

reduction percentage that was reduced by the least amount 52.85% was at 144 hours. The yeast growth curve and this are equivalent (Figure 2).

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

The results revealed that the largest decrease of substrate was obtained after 48 hours of incubation, indicating that glutinous rice tape has the capacity to manufacture amylase enzymes after 48 hours of incubation. Tape yeast treatment contains four growth phases: lag phase, exponential growth phase, stationary phase, and death phase. The tape yeast treatment was accomplished after 48 hours of incubation during the exponential phase [5]. Enzyme activity is created in synchrony with cell growth and increases towards the stationary phase or at the end of the exponential phase. It occurs as a result of the buildup of metabolites needed for bacterial growth [1].

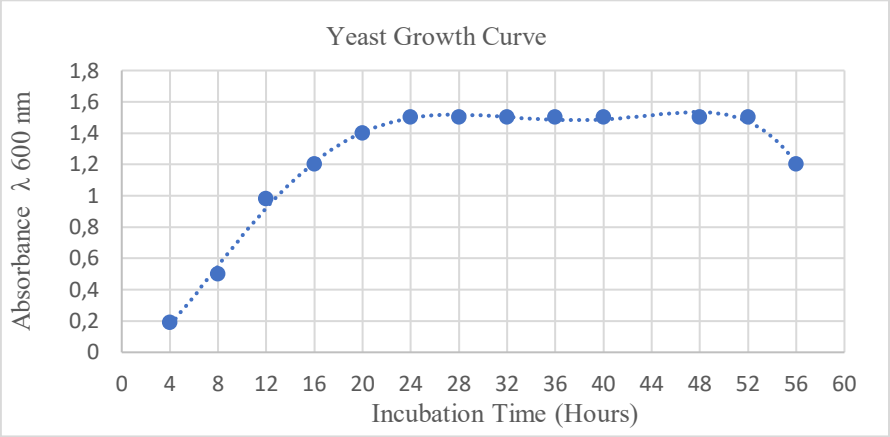

Figure 2. Yeast Growth Curve at 27 ºC.

3.2 Organoleptic Test

Table 2. Average Organoleptic Sticky Rice Ketan Test Results from 30 Panelists

Among the 30 panelists that performed the organoleptic test on glutinous tape. The color depicted on the tape is closest to yellowish white during 144 hours of incubation, which is when it is at its optimum. At 96 hours after incubation, sticky rice tape's alcohol fragrance peaked. At 144 hours

after incubation, sticky tape has the softest feel. The sticky rice flavor reaches its peak sweetness after 144 hours of incubation.

Color is one of the factors that influences how people perceive a product. Colors that stand out are typically the best choice. However, a beautiful color does not always indicate a high nutritional content [9]. The color of the tape was yellow after 48 and 96 hours of incubation, and it had begun to transition to yellowish white after 144 hours of incubation.

The organoleptic test of sticky rice tape created a little alcoholic odour after 48 hours of incubation, and the scent of sticky rice produced alcohol after 96 and 144 hours of incubation. The study's findings revealed that the alcohol fragrance of sticky rice tape was strongest after 96 hours of incubation. Previous research [2] show that the alcohol content of sticky rice tape increases by more than 2% after incubation for 2 days (48 hours), and by more than 4% after incubation for 4 days (96 hours). These findings reveal that the high alcohol concentration in sticky rice tape was achieved after a 96-hours incubation period.

The organoleptic test on 30 panelists revealed that incubating sticky rice tape for 48 hours resulted in a little soft texture, incubating it for 96 hours resulted in a soft tape texture, and incubating it for 144 hours resulted in the softest tape texture. On the tape, a delicate and slightly wet feel is required. The texture of tape fermentation reflects its success; a soft texture signifies the success of the tape fermentation process; carbohydrates are broken down during the process to give a soft texture (Kino, 2019).

Taste is a product parameter/condition that may be sensed or identified by the flavours in the shape of the tongue. The tongue might respond to the state of the product in the form of sour, salty, sweet, or bitter. The sour flavor that appears can be impacted by the yeast's activity throughout the fermentation process. Tape has a distinct and distinct flavor, mainly a blend of sweet and acidic tastes. The adhesive tape contains sugar, which gives it a sweet flavor. Taste is evaluated by chemical stimulation of the sense of taste (tongue), which involves a unity of interaction between scent and texture features and can impact customer perception of a product. Furthermore, taste is impacted by the preferences of each individual [9]. Tape has a somewhat sweet flavor after 48 hours of incubation and a sweet taste after 96 hours of incubation. At 144 hours of incubation, the tape already had a highly sweet flavor, which not all panelists liked.

The ethanol concentration obtained tends to increase with higher steaming and fermentation duration, although this has no major effect. This is because a longer fermentation time can enhance the activity of organic molecules, resulting in a sequence of events catalyzed by enzymes into another form, such as alcohol. A nice fermented tape is 48 hours in length because the texture is not too soft and has a distinct scent of tape and the aroma is a bit sharper. Most people enjoy 48 hours of tape because it has a flavor that is neither too sweet nor too sour [3].

The sweetness level of sticky rice was not very sweet after 48 hours of incubation due to the high concentration of starch in sticky rice tape, but after 96 and 144 hours of incubation, a significant sweet flavor was formed in sticky rice tape. This assumes that the starch substrate in the sticky rice has been completely hydrolyzed into glucose, which makes the sticky rice sweet. The sweet flavor is produced by the enzyme amylase, which converts sticky rice starch into simple sugars [6].

4. Conclusion

The incubation time of the tape causes changes in chemical properties, especially the level and type of sugar and changes in the pH of the tape. The longer the incubation time, the percentage of substrate reduction will decrease. At 48 hours of incubation the percentage of substrate reduction was 75.92%, at 96 hours of incubation the percentage of substrate reduction was 52.87%, and at 144 hours the percentage of substrate reduction was 52.85%. This condition is in accordance with the yeast growth curve which decreases starting from the 48 hours incubation time. The organoleptic test performed showed that the best color of glutinous tape was at an incubation time of 144 hours. At 144 hours of incubation, the color of the tape was yellowish white, while the alcohol aroma of glutinous tape was highest at 96 hours of incubation. And the softest texture of sticky tape is at 144 hours of incubation. The longer the incubation time, the softer the texture of the tape will be, but the alcohol aroma of glutinous tape will decrease as the incubation time increases.

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Sticky Rice Tape Organoleptic Data on Day 2

No	Respondent Name	Color	Scent	Texture	Taste
1	Respondent 1	1	1	1	1
2	Respondent 2	1	1	1	1
3	Respondent3	1	1	1	1
4	Respondent 4	1	2	2	1
5	Respondent 5	1	1	1	1
6	Respondent 6	1	1	1	1
7	Respondent 7	1	1	2	1
8	Respondent 8	1	1	2	1
9	Respondent 9	1	1	2	1
10	Respondent 10	1	2	2	2
11	Respondent 11	1	1	2	2

12	Respondent 12	1	1	2	2
13	Respondent 13	1	1	2	2
14	Respondent 14	1	1	2	2
15	Respondent 15	1	2	2	2
16	Respondent 16	1	2	2	2
17	Respondent 17	1	2	2	2
18	Respondent 18	1	2	2	1
19	Respondent 19	1	2	2	2
20	Respondent 20	1	1	2	2
21	Respondent 21	1	2	2	2
22	Respondent 22	1	2	2	1
23	Respondent 23	1	2	2	2
24	Respondent 24	1	1	2	2
25	Respondent 25	1	2	2	2
26	Respondent 26	1	1	2	2
27	Respondent 27	1	2	2	2
28	Respondent 28	1	2	2	2
29	Respondent 29	1	2	2	2
30	Respondent 30	1	2	2	2

Sticky Rice Tape Organoleptic Data on Day 4

No.	Respondent Name	Color	Scent	Texture	Taste
1	Respondent 1	1	1	3	2
2	Respondent 2	1	2	3	2
3	Respondent 3	1	2	3	3
4	Respondent 4	1	2	3	3
5	Respondent 5	1	2	3	2
6	Respondent 6	1	2	3	2
7	Respondent 7	1	2	3	2
8	Respondent 8	1	2	3	2
9	Respondent 9	1	2	3	2
10	Respondent10	1	1	1	2

11	Respondent 11	1	2	3	1
12	Respondent 12	1	1	3	2
13	Respondent 13	1	2	3	1
14	Respondent 14	1	2	3	2
15	Respondent 15	1	1	3	1
16	Respondent 16	1	1	3	1
17	Respondent 17	1	1	3	2
18	Respondent 18	1	2	3	2
19	Respondent 19	1	1	3	2
20	Respondent 20	1	2	3	2
21	Respondent 21	1	2	3	2
22	Respondent 22	1	1	1	2
23	Respondent 23	1	1	3	1
24	Respondent 24	1	1	3	1
25	Respondent 25	1	1	3	1
26	Respondent 26	1	1	3	1
27	Respondent 27	1	1	3	1
28	Respondent 28	1	1	1	2
29	Respondent 29	1	2	3	1
30	Respondent 30	1	2	3	2

Sticky Rice Tape Organoleptic Data on Day 6

No.	Respondent Name	Color	Scent	Texture	Taste
1	Respondent 1	1	1	3	2
2	Respondent 2	1	2	3	2
3	Respondent 3	1	2	3	3
4	Respondent 4	1	2	3	2
5	Respondent 5	1	1	3	2
6	Respondent 6	1	1	3	2
7	Respondent 7	1	1	3	2
8	Respondent 8	1	1	3	2
9	Respondent 9	1	2	3	2

10	Respondent 10	1	2	3	2
11	Respondent 11	1	2	3	2
12	Respondent 12	1	2	3	1
13	Respondent 13	1	1	3	2
14	Respondent 14	1	2	3	2
15	Respondent 15	1	2	2	3
16	Respondent 16	2	1	3	2
17	Respondent 17	1	2	3	3
18	Respondent 18	2	2	3	2
19	Respondent 19	2	1	3	1
20	Respondent 20	2	1	3	2
21	Respondent 21	2	2	3	2
22	Respondent 22	1	1	1	2
23	Respondent 23	1	2	3	2
24	Respondent 24	1	1	3	2
25	Respondent 25	1	1	3	2
26	Respondent 26	1	1	3	1
27	Respondent 27	1	1	3	2
28	Respondent 28	1	1	3	2
29	Respondent 29	1	2	3	2
30	Respondent 30	1	2	3	2