



## Optimization of Fermented White Glutinous Rice Tape for Ethanol Content Using the Gas Chromatography Method

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### ABSTRACT

One of the traditional foods that have health-preserving properties is tape which can prevent an increase in cholesterol and prevent a shortage of red blood cells. Tape products are the result of the fermentation of processed raw materials that contain starch, that is, these food ingredients become simple forms such as sugar with the addition of microbes such as yeast or yeast (*Saccharomyces cerevisiae*) which work to break down carbohydrate foods into alcohol and carbon dioxide by settling to form compounds. namely ethanol. The test was carried out to determine the fermentation time of the ethanol content in white sticky rice tape. The test used the gas chromatography method. The location of the testing carried out at the Pharmacy and Food Analysis Campus was obtained. The results obtained for the system suitability test analysis consisted of precision, column efficiency, and accompanying factors that met the requirements. Meanwhile, the ethanol content in samples carried out continuously for five days on days I, II, III, IV, and V respectively was 2.94%; 3.03%; 3.12%; 3.14%, and 3.28%. The greatest ethanol content obtained on day V was 3.28%, and the smallest content on day I was 2.94%. So it can be concluded that the ethanol content resulting from fermentation in white sticky rice tape increases every day and the quality of the tape decreases

## INTRODUCTION

Indonesia is an agricultural country that has many sources of food, one of which is white sticky rice as one of the main basic needs of humans. The statistics agency states that the employment sector is still dominated by 27.33% compared to trade which is only 18.81% (Fauzin, 2021). White glutinous rice can be used as raw material for tape. Sticky rice tape is a healthy food made from sticky rice that has many benefits in maintaining health, such as lowering cholesterol levels, preventing carcinogens, red blood cell deficiency, heart disease, controlling blood sugar levels, slowing skin aging, and many other benefits (Fauzin, 2021).

White glutinous rice (*Oryza sativa* L. var. *glutinosa*) is a rice variety belonging to the Graminae family, has a carbohydrate content of 100 grams of 79.40 grams so it is quite high, namely consists of a water content of 22.5 grams, a fat 0, 8 gr, protein 7.4 gr and ash content 0.5 gr (Maulana & Muhaji, 2023). The amylose content is 1-2%, and amylopectin 98-99%, the higher the amylopectin, the fluffier the rice (Hartesi et al., 2021). White glutinous rice grains mostly consist of starch, around 78.4 grams in 100 grams. This means that white glutinous rice can act as a raw material for making ethanol (Maulana & Muhaji, 2023).

Tape is a product resulting from the fermentation process, where a simple change of ingredients occurs. The starch in food is converted into a simple form, namely sugar, with the help of a microorganism called yeast or yeast (*Saccharomyces cerevisiae*), yeast or yeast can break down the carbohydrate content in food into alcohol carbon dioxide (Novia et al., 2018).

Ethanol or alcohol has the molecular formula  $C_2H_5OH$ , is a clear, volatile liquid, has a very sharp aroma, and can mix with distilled water, ether, and chloroform (Nurhayati et al., 2024). Ethanol is the most frequently used solution in the pharmaceutical world and food industry because it is non-toxic and is widely used in everyday life. Most chemical industries and laboratories use water and ethanol as solvents. Because ethyl alcohol is easy to make with readily available ingredients and can be fermented with the help of yeast, it is used for the production of beer, wine, bread, and so on (Berlian et al., 2016).

In 1979, Cronk, Mattick, Steinkraus, & Hackler tested the alcohol content in fermented sticky rice tape. Meanwhile, T.C. Cronk proved that during the fermentation process, sticky rice tape produces iso butanol, active amyl, and isoamyl. During the fermentation process, research shows that there is an increase in the alcohol content in the sample with increasing time used.

Based on this description, research will be carried out on the fermentation of white sticky rice tape starting from day 1 to day 5 to determine whether on that day the ethanol content has been produced using gas chromatography. This test uses the gas chromatography method because the gas chromatography tool can quickly analyze volatile organic compounds in samples and carry out precise qualitative and quantitative analysis. From this background, an optimization analysis of fermented white sticky rice tape was carried out to determine the ethanol content using the gas chromatography (KG) method.

## LITERATURE REVIEW

Food comes from biological sources, agricultural products, plantations, forestry, fisheries, animal husbandry, waters, and water, both processed and untreated. Humans in everyday life need food and drink as the main basis that must be fulfilled every time they need it. So the main food is a crop commodity, namely rice which is the most important. The availability of food is necessary to meet the daily needs of communities, individuals, and households to achieve sustainable food security (Farid et al., 2018) (Fauzin, 2021).

Fermentation alcohol formation biological process that converts sugar to produce ethanol and carbon dioxide as byproducts, a natural process when yeast and bacteria convert carbohydrates into acids. In this fermentation, glucose is converted into alcohol and CO<sub>2</sub> gas with the following reaction:



The reaction that occurs is anaerobic. Ethanol is the main product of fermentation besides lactic acid, acetaldehyde, glycerol, and acetic acid (Salsabila et al., n.d.).

Ethanol (also called ethyl-alcohol or just alcohol), is a type of liquid that is volatile, flammable, colorless, and is the alcohol most often used in everyday life. Ethanol is a single-chain alcohol, with the chemical formula C<sub>2</sub>H<sub>5</sub>OH, and is one of the most commonly used types of alcohol. This compound is produced by fermentation of sugar by certain microorganisms such as yeast or bacteria. It is a constitutional isomer of dimethyl ether

Because it is universal, polar, and easy to obtain. 96% ethanol was chosen because it is selective, non-toxic, has good absorption, and has high filtering capacity so that it can filter out non-polar, semi-polar, and polar compounds. Carbohydrates or sugars are fermented with the addition of yeast to produce ethanol. The type of yeast that is good for producing ethanol is used in the genus *Saccharomyces*. (Anggraini et al., 2017).

Distillation is the process of separating a mixture into two or more products, a method of separating solutions using heat as a separator (separating agent). Generally, the distillation method is used to separate the components of a solution with two or more compounds in the solution with different boiling points. Apart from that, the volatile level is due to the possibility that the substance will change to gas, and is carried out at normal atmospheric pressure (Wahyudi et al., 2017).

Gas Chromatography (KG) is a dynamic method for the separation and detection of organic compounds and organic gases in a volatile mixture. Gas chromatography remains one of the most frequently used analytical techniques despite new developments in the field of analytical chemistry. No other analysis method can combine resolution power with analysis speed and sensitivity (KA & Yusriyani, 2021).

A separation technique that is based on differences in the distribution of compounds in the presence of a mobile phase and a stationary phase to separate compounds in solution is called gas chromatography, where the mobile phase is a gas that is volatile but stable at hot temperatures. and the stationary phase is

the column (Ruwindya, 2019). Gas chromatography is a method that has a fast retention time used for testing volatile compounds (Kartika, 2022). Separation with gas chromatography has the advantage of analyzing very small particles because it has column efficiency and high resolution. Gas that flows at a steady, controlled speed. Fast analysis process, samples are not damaged, able to separate mixed compounds, detect compounds with small concentrations, very sensitive (Surani et al., 2023).

## **METHODOLOGY**

The initial procedure for sample preparation is to weigh 500 grams of white glutinous rice, then wash it until clean, steam it for 15 minutes until half cooked, and stir it so it doesn't clump. Steam again for 60 minutes until cooked. Cool and add approximately 4 grams of yeast, stir until evenly divided into 5 containers then cover tightly. Wrap tightly then store at room temperature for 1 to 5 days (Wahyuningsih et al., 2023).

Then make a standard solution, pipette 25.0 mL of Ethanol P. Put it in a 50.0 mL flask. Adjust the volume with Aquadest. Series standard solutions were made in concentrations of 1%, 5%, 10%, 15%, and 20%. The determination was carried out using a Gas Chromatograph.

Next, the test solution, weighing 25.0 grams of sample, is put into the distillation flask. Add 25.0 mL of distilled water, and put it in the distillation flask. Added some boiling stones into the heart flask. The sample was distilled, and the distillate was collected in a 25.0 mL volumetric flask. Dilute with distilled water to the mark, and homogenize. Injected into a Gas Chromatograph.

Before testing the sample, a System Conformity Test is carried out, namely by pipetting 1.0 mL of the 50% mother standard solution, put into a 10.0 mL volumetric flask. Dilute with distilled water until homogenized. Injected into the gas chromatography apparatus 5 times, 1  $\mu$ L each. The LOD & LOQ test injects each series of standards 1, 5, 10, 15, and 20% into a gas chromatograph with a volume of 1  $\mu$ L.

Data analysis was carried out, and all data displayed were presented in the form of average  $\pm$  standard deviation (SB). Data collection was carried out in triplicate ( $n = 3$ ). Statistical analysis was carried out using the SD RSD test.

## **RESULTS**

Testing the ethanol content in white glutinous rice was first carried out by fermentation. The results and data are described every day as in table 1, then after the observations are made, the sample is weighed for preparation, and the ethanol content is tested by looking at the area data, as in table 2. Before the test is carried out, the system suitability test is first carried out on the gas chromatograph. as in table 3, and the standard area results in table 5 are as follows:

Table 1. Results of Daily Sample Fermentation Description

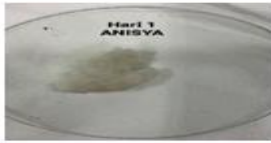
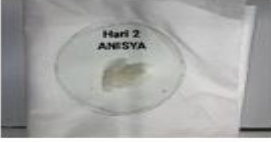



Day Fermentation	Information	Result
Day I		Shape: solid Taste: slightly sour and slightly sweet Smell: typical tape White color
Day II		Form: solid, slightly runny Taste: slightly sour and slightly sweet Smell: typical tape White color
Day III		Form: solid, slightly runny Taste: sweet, slightly sour Smell: typical tape White color
Day IV		Form: watery solid Taste: slightly sour and sweet Smell: typical tape White color
Day V		Form: watery solid Taste: slightly sour and sweet Smell: typical tape White color

Table 2. Area Data and Standard Retention Time Series

No	Concentration	Area	(xi - X)	I	Retention Time
1	1%	1512.11401	-78860.369	6218957798.816	2.730
2	5%	5648.07080	-74724.413	5583737898.194	2.727
3	10%	9902.23438	-70470.249	4966055994.122	2.727
4	15%	169683.0000	89310.516	7976368303.910	2.726
5	20%	215117.0000	134744.516	18156084592.074	2.728
Σ	51	401862.4192	-	42901204587.116	-
X	10,2	80372.4838	-	8580240917.423	-

Table 3. Data on Weighing, Area, and Sample Retention Time

Information	Sample	Weighing Weight (gram)	Area	Retention Time
Day 1 of Fermentation	1	25.3902	525.7192	2.745
	2	25.4092	547.3931	2.746
	3	25.3566	521.7377	2.746
Day 2 of Fermentation	1	25.1470	1483.8380	2.746
	2	25.1416	1413.5094	2.745
	3	25.1404	1408.1617	2.744
Day 3 of Fermentation	1	25.0667	2805.3515	2.747
	2	25.0698	2891.5012	2.749
	3	25.0622	2846.2331	2.748
Day 4 of Fermentation	1	25.2092	3293.9716	2.746
	2	25.3002	3395.6401	2.748
	3	25.2084	3286.0866	2.748
Day 5 of Fermentation	1	25.0896	5369.7724	2.747
	2	25.0888	5263.7841	2.747
	3	25.0892	5285.7929	2.747

Table 4. System Conformity Test Data Based on Area

No	Ethanol area	(xi - X)	I
1	3353.49390	-20.405	416.364
2	3150.77100	-223.127	49785.658
3	3519.93164	146.033	21325.637
4	3331.68652	-42.212	1781.853
5	3513.60962	139.711	19519.163
Σ	16869.49268	-	92828.675
X	3373.898536	-	18565.735

The results of the ethanol content calculation that was carried out on the results of fermentation of white sticky rice carried out for 5 consecutive days starting from the first day to the fifth day obtained the following data:

Table 5. Results of Sample Content Calculation and Data Analysis

Fermentation Time (Days)	RSD (%)	Average Level
1	0,13	2,94%
2	0	3,03%
3	0,13	3,12%
4	0,12	3,14%
5	0	3,28%

## DISCUSSION

When carrying out the fermentation process of white sticky rice it must be optimal. Apart from using good quality sticky rice and yeast, clean the basic ingredients and pay attention to the equipment used. This aims to ensure that the influence of impurities does not occur and the fermentation process can take place optimally (Kristian et al., 2021).

First, white sticky rice tape is made (before testing to determine the ethanol content). Weigh five hundred grams of rice then use running water to wash it until it is clean so that no impurities stick to the white sticky rice. Added water. Steam for 15 minutes until half cooked, stir so that there are no lumps. Steamed again for 60 minutes until cooked. White sticky rice that has been cooked is cooled and aired to room temperature. After it cools, white sticky rice is fermented, which functions to ferment the rice into tape. During fermentation the water content increases, this is due to the hydrolysis of the starch being changed by the yeast, so that it develops more. White sticky rice that has been mixed with yeast is divided into five different containers. Then for the fermentation process by microbes to obtain an anaerobic atmosphere to get the optimal results possible, the white sticky rice is wrapped in banana leaves and stored in a tightly closed container to minimize the occurrence of impurities. Stored at room temperature, tightly closed containers will be stored for one, two, three, four, and five days respectively (Yuliantoro et al., n.d.).

Preparation of the test solution means weighing approximately 25 grams of the fermented sample using a glass beaker in triplicate. The meaning of weighing is carried out carefully according to the Indonesian Pharmacopoeia Edition VI, namely, when the scale is to be used, it is ensured that it has been calibrated by measuring the uncertainty figure of no more than 0.1%. Expressed as "more or less" the size value is shown within the limit of 10% or the number of

samples weighed must not be less than 90%, namely 22.5 grams, and must not be more than 110%, namely 27.5 grams of the amount to be weighed as stated. on testing procedures (Ministry of Health of the Republic of Indonesia, 2020). Then add 25 mL of distilled water as a solvent which was previously measured quantitatively in a measuring cup, then pour it into the distillation heart flask, then add 3-5 boiling stones so that there are no explosions during the distillation process. The sample is distilled first to separate the components from the solid compound to obtain a pure liquid compound at a temperature of 78°C - 100°C because at this temperature the compounds that evaporate are ethanol and water, waiting until 1 hour after the first drop is present. container for holding the distillate, then the distillate is analyzed using gas chromatography (Arwizet, 2017).

System Conformity Test (UKS) before testing on samples, UKS is carried out first to ensure that the equipment that will be used during testing is in good condition and that the data obtained is more accurate. So that the gas chromatography system used meets the requirements according to established regulations. The resulting analytical data is sufficient to be used to conclude a test result. Before testing, to prove that the resolution and repeatability results of a chromatography system meet the requirements, the first thing to do is a system suitability test. These parameters include precision, follow-up factors, and column efficiency (Ruwindya, 2019). By using these precision parameters to test the repeatability of the area and retention time as a result of repeated injections (5-6 times) of the solution containing the analyte, it is expressed as Relative Standard Deviation (% RSD), if  $\leq 2\%$  then the RSD calculation used is data Re-injection was carried out 5 times, the test result was 1.23%, which means it met the test requirements because it was carried out with 5 repeated injections with the RSD limit value  $\leq 2\%$ . Then the column efficiency parameter is a requirement for system suitability testing, especially if there is only one peak of the test substance when carrying out chromatography, the sharpness of a peak is very useful for detecting a substance that is in residual form, the general requirement is that the number of theoretical plates is  $>1000$ , the test results carried out on the column efficiency parameter, namely 3016.2064, which means it meets the requirements because there are no less than 1000 theoretical plates so it can be ascertained that the column performance is good. Finally, the co-factor parameters are carried out, this parameter is a measure of the symmetry of a chromatogram peak, the accuracy of the results obtained will decrease if the co-factor is greater, the co-factor value is considered good if  $< 2$ , the test results that have been carried out are 0.83, co-factor is not greater than 2 so that it meets the requirements for the co-factor parameters and the resulting peak data is accurate, showing that the peak size in the standard is symmetrical (Suseno & Qomariyah, 2021) (Ruwindya, 2020).

LOD and LOQ tests to determine the smallest amount of analyte that can be measured and calculation of levels. In the measurements carried out, the LOD results were obtained, namely 0.00013945729 (detection limit value), and the LOQ value was 0.00046485763 (quantity limit value). Usually, the detection limit for alcohol is 2.8 mg/L, if the sample is tested for alcohol content that is not

detected, the value below the LOD may have a small concentration. This assessment is useful for verifying the chromatography system that the tool is adequate for analysis (Suseno & Qomariyah, 2021).

Data Analysis from the test results carried out statistical data analysis by calculating SD and RSD. In the test, the RSD results for samples on days one, two, three, four, and five were 0.13% respectively; 0%; 0.13%; 0.12%; 0% meets the requirements because it does not exceed 2% so that the data obtained from this test is precise and the data can be processed (Kusumaningtyas & Sumarno, 2017).

## **CONCLUSIONS AND RECOMMENDATIONS**

Based on experiments that have been carried out on the ethanol content in white sticky rice tape on the effect of fermentation time carried out for five consecutive days, the data on the ethanol content obtained is that the highest content of fermentation on day 5 was 3.28%, while the smallest content on day 1 of fermentation it was 2.94%. So it can be concluded that with increasing days or fermentation time, the ethanol content increases.

The advice that can be conveyed is that the public is expected to be more careful and alert and limit the amount of consumption of fermented white sticky rice tape, namely the maximum consumption per day is 3x in each pack of 10 grams of white sticky rice tape considering that this food product contains ethanol which has side effects on the body. It is hoped that the public will pay attention to the fermentation storage time when making white sticky rice tape because the storage time greatly influences the ethanol content in the fermented white sticky rice tape.

## **FURTHER STUDY**

This research still has limitations, so it is necessary to carry out further research related to the topic of Optimization of Fermented White Glutinous Rice Tape for Ethanol Content Using the Gas Chromatography Method in order to improve this research and add insight to readers.

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