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Analysis Types and Functions of Microbes and Duration of Fermentation in the Process of Reducing Levels of Concentration Oxalate Levels in Taro Kimpul

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ABSTRACT
Kimpul tuber is a source of carbohydrates that have high levels of oxalate, therefore it is necessary to reduce oxalate levels, several methods have been used to reduce oxalate levels, high levels of oxalate are caused by the sap or mucus in the taro. The process of decreasing oxalate levels by physical and chemical means has been carried out but has not yet obtained optimal results for low oxalate levels, therefore a fermentation process is carried out to obtain optimal results, which is recommended for 71mg / 100gr. Use of taro is one of the foods used to replace rice or other types of carbohydrates, but taro has a low-calorie level so it is safe for those who have diabetes. In this study, the fermentation process was carried out for 48 and 72 hours to get a perfect process by involving the microbes saccharomyces cerevisiae and rhizopus oryzae. The results of this study indicated that the levels of oxalate produced were 54 mg/100gr in the 72-hour fermentation process using 0.2% / liter rhizopus orizae.

Keywords: Kimpul, taro, oxalate, tuber, fermentation, rhizopus oryzae, saccharomyces cerevisiae

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INTRODUCTION

The need for carbohydrates as a basic material for consumption is one of the benchmarks in the use of foodstuffs, especially in the food industry. Based on 2019 BPS data(1). It shows that the amount of rice consumption reaches 29 million tons or 111 kg per capita per year. This figure shows that it is very high, so it is necessary to diversify the foodstuffs to reduce dependence on rice as one of the main staple foods in Asian countries. The use of local tubers that contain high carbohydrates is an alternative as food additives(2). One of the high carbohydrate tubers is the Colocasia and Xanthomas spp, which are tropical plants known as cocoyams which are also used in tropical and sub-tropic regions in Africa and Australia(3) (4).

There are many varieties of the taro that are cultivated in Indonesia, Africa, and Australia, one of which is kimpul tubers or known as Xanthosoma sagitifolium, this type is mostly in Indonesia, especially in Aceh-Indonesia (5). The very high carbohydrate content in taro reaches 23.7 g / 100 g, protein content 1.9 g / 100 g tubers, and contains fat, minerals, and vitamins even in small amounts. Taro tubers also contain vitamin A, B1, and vitamin C (6–8).

The use of tubers is currently very much, almost 10% of the components are used as material for food diversification, but taro has a high content of calcium oxalate which has an itchy feeling in the tuber (9). This concentration will cause itching in the hands and throat, this is caused by needle-shaped oxalate compounds which can irritate (10). These high levels of calcium oxalate are fed by various methods such as physical, chemical, and fermentation methods. The fermentation method used microbes lactobacillus plantarum, lactobacillus brevis, rhizopus aspergillus spp, and yeast candida albians (11). This fermentation process can be carried out for 48 hours and will reduce 65% of the oxalate content(12). The fermentation process with the help of bacteria, fungi, and yeast can reduce oxalate calcium by 70.8% with a fermentation time of 96 hours (13). Taro fermentation with the help of saccharomyces cerevisiae for 72 hours reduced the calcium oxalate content from 35.75 mg / 100 g to 6.93 mg / 100 g (14). In the fermentation process that involves molds such as rhizopus oryzae in tempeh inoculation, it can reduce calcium oxalate levels by 91.31% during the 48-hour fermentation time (12). Therefore, this study will reduce the levels of calcium oxalate. The fermentation method with a time difference of 48 hours and 72 hours and the types of microbes used were of two types, namely saccharomyces cerevisiae and rhizopus oryzae.

RESEARCH METHODS

Preparation sample

The material used in this study is taro tubers from Aceh Besar. This research was conducted at the Laboratory of Food and Agricultural Product Analysis, Syiah Kuala University. The chemicals and microbe used are saccharomyces cerevisiae, rhizopus Orizae, PDA, MEA, Pepton, and chemicals for proximate analysis.

Activation microbe

The microbes used were saccharomyces cerevisiae and rhizopus oryzae. These microbes are heated as much as 2 grams in 1 liter of water at 40 Celsius, then 14 grams of sugar are added and stirred until dissolved. Then the sugar solution is allowed to stand for 15 minutes before using it on the sample to be tested.

Taro preparation for research

The process of reducing oxalate levels is carried out by several treatments, namely tubers that have been harvested and then cleaned and sorted and washed using clean water. Taro tubers were cut to a thickness of 1 cm and weighed the sample and carried out the fermentation process in a closed bottle with a capacity of 500 grams of taro in a 1.5 L bottle containing a solution of activated saccharomyces cerevisiae and rhizopus inoculums.

Fermentation of tubers

Fermentation was performed using saccharomyces cerevisiae and rhizopus inoculums.

for 48 hours and 72 hours. After completion of fermentation, the drying process is carried out in an oven for 24 hours at a temperature of 60 Celsius.

Processing into kimpul flour

After the kimpul is finished drying it is ground using a grinder with a size of 80 mesh and then stored in an airtight package before analysis.

Observed parameters

The parameters observed were the levels of calcium oxalate (15), crude fiber fiber (SNI 01-2891-1992), moisture content, ash content, pH, total microbes, and total starch (16).

Analysis of total oxalate levels

Analysis of total calcium oxalate levels used volumetric permanganatometric titration method (15). The sample was weighed as much as 1 gram and suspended in 95 ml of distilled water which was put into 250 ml Erlenmeyer. Then the 6 M HCl solution was added as much as 5 ml. The suspension was heated for 1 hour at 100^{o}C, followed by cooling. Add water to 125 ml before filtering. The total 62.5 ml filtrate produced from the heating stage was diluted to 150 ml. 62.5 ml is taken to be heated until almost boiling. Followed by titration using $KMnO^4$ until it turns pink, it almost disappears, which lasts for 30 seconds. The total oxalate content (mg/100 g) is calculated by the following equation.

$$\text{Calcium oxalate} = \frac{volume\ KMnO4 \times 0{,}00225 \times 2{,}4}{the\ weight\ of\ taro\ flour \times 5} \times 105$$

with:

Mass volume equivalent (1 cm^3 $KMnO^4$ 0.05 M is equivalent to 0.00225 g anhydrous oxalic acid); Dilution factor (2.4 is obtained from the volume of the filtrate 300 ml divided by the volume of filtrate used 125 ml); Molar equivalent of $KMnO^4$ (redox number $KMnO^4$ 5).

Data Analysis

Data analysis was carried out with ANOVA and if there were significant differences in the results of the treatment, then tested by using the DMRT 0.05 further test.

RESULTS AND DISCUSSION

Raw Material Characteristics

Kimpul tubers have a round shape like a cylinder and have a diameter of 5 to 7 cm and a length of 15 cm with a brown color, this tuber has a lot of mucus (17). Taro kimpul if it is cut and left there will be a browning process in the kimpul tubers, the following are the results of research and references used (table 1).

Table 1: Characteristic composition of taro kimpul

Composition	Taro Kimpul	
	Result	Reference
Calsium oxalate (mg/100g)	54,00	95,50[e]
Crude fibre (%)	3,82	0,82[c]
Ash (%)	1,21	1,00[c]
Water content (%bb)	69,26	69,66[c]
Sarch (%)	36,73	21,98[c]
pH	6,92	6,50[d]

Kimpul tubers have a low calcium oxalate content compared to the references presented, Lim 2015 (18) said that the oxalate content of other types of taro is higher than taro types, this indicates that calcium oxalate in kimpul allows for an optimal decrease in the oxalate content.

Kimpul tubers have a calcium level of 54.0 mg / 100 g, this result is different from that obtained which is 95.5 mg/100 (19). The crude fiber content of Kimpul tubers which is 3.82%, the results obtained by Suharti et al (2019)(20) are also different, namely 0.82%. The ash content obtained by Kimpul tubers is 1.21%, this result is almost the same as that obtained by Suharti et al (2019) which is 1.00%. The water content of Kimpul tubers was obtained at 69.26, this value is close to the Kimpul taro namely 69.66%. The total starch obtained was 36.73%, in contrast, the total starch obtained was 2198%. This can occur due to several factors such as climatic factors, where taro is planted, and other environments. The pH of Kimpul tubers was obtained 6.92, and other article it is 6.5 (21). Tubers colocasia spp. showed the lowest pH value when compared to tubers Xanthosoma spp. PH value from 6.56 to 7.59 in the acid to neutral range (22).

Calcium Oxalate

The results of the analysis of variance showed that the fermentation time had a very significant effect ($P < 0.05$) on the decrease in oxalate levels in taro that had been stuck. The results showed that the oxalate value depends on the length of the fermentation process, the longer the fermentation process the oxalate content in kimpul flour will decrease. This is because the microorganisms that play a role during the fermentation process will produce different enzymes depending on the type of carbohydrates, protein, and crude fiber, this process is carried out by hydrolysis of the form of particles that can be broken down more simply when the fermentation process is carried out. The fermentation process has a positive impact and can reduce oxalate levels that are lower or within tolerance(11,14).

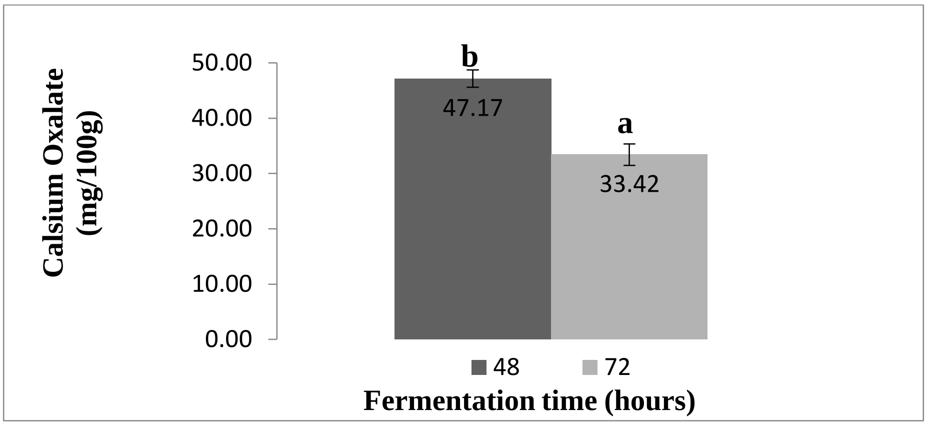

Figure 1: The effect of fermentation time on the calcium oxalate content of flour (different letters indicate a significant difference in the DMRT test at 0.05 level = 2.88, KK = 38.15).

In figure 1. the DMRT test results of 0.05 show that the value of calcium oxalate at the 48 hour fermentation time was higher, namely 47.17 mg / 100 g, and for the 72 hour fermentation time the calcium oxalate value was lower, namely 13.80 mg / 100 g. After fermentation, there was a decrease in calcium oxalate levels in kimpul flour compared to the initial calcium oxalate, namely 54.0 mg / 100 g in kimpul tubers (12).

The role of microorganisms with different characteristics produces different enzymes, namely carbohydrates, proteins, crude fiber, and various complex organic compounds that are broken down or hydrolyzed into simpler forms and used as carbon sources or converted into microbial biomass (11). The decrease in calcium oxalate caused by fermentation may be the leaching effect and enzymes or acid hydrolysis of starch granules during fermentation. Fermentation has a positive impact by reducing calcium oxalate to a more tolerable level (12,14).

Crude Fiber

Based on the analysis of variance, it showed that the interaction between types of microbes and fermentation time had a significant effect (P <0.05) on the decrease in crude fiber content of taro flour. The crude fiber content of taro flour ranged from 1.26-5.92% with an average of 3.28%.

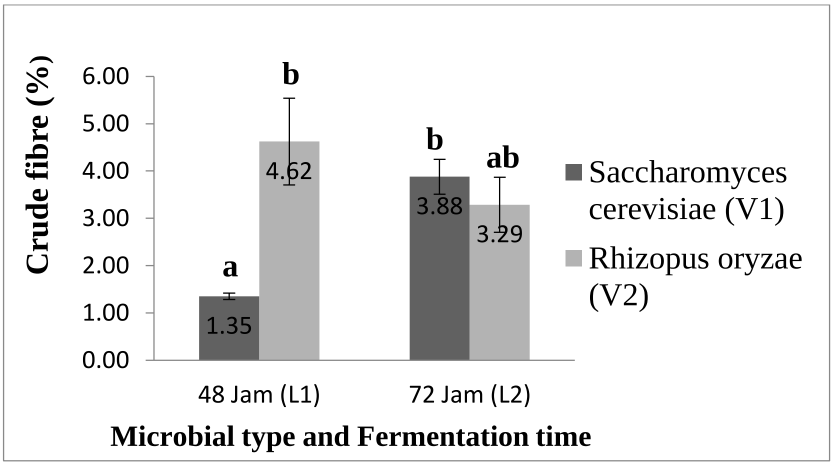

Figure 2: The effect of the treatment of the interaction between types of microbes and fermentation time (VL) on the content of crude fiber flour (Value followed by different letters indicates a significant difference in the DMRT test 0.05, level 1 = 2.02, level 2 = 2.12, and level 3 = 2.18 with KK = 49.76).

In figure 2, the DMRT test result of 0.05 shows the interaction treatment of saccharomyces cerevisiae microbes at the fermentation time of 72 hours and rhizopus oryzae at 48 hours tended to have high crude fiber values, namely 3.88% and 4.62%. The crude fiber content of saccharomyces cerevisiae at the fermentation time of 48 hours had the lowest crude fiber value, namely 1.35%. The results showed a decrease in crude fiber content of kimpul flour which was initially 3.82%. This can be caused by the activity of microorganisms that utilize fiber as their nutrient (23).

In previous research, The crude fiber content of purple taro flour decreased from 3.00% to 2.78% by fermentation using bacteria, molds, and yeasts. Reduction in fiber and carbohydrate content can occur because microorganisms take advantage of the fiber and carbohydrate content for their cell growth (13,24,25).

Water Content

The results of the research conducted on variance showed that the water content in the flour during the fermentation process had different values based on the length of the fermentation process. The longer the fermentation process, the less water content will be contained therein. The decrease in water content with increasing fermentation time is probably caused by the soft and porous texture of the tubers after fermentation, resulting in maximum moisture loss. Microorganisms must have utilized moisture for metabolic activity (26)(27)(23).The temperature and drying time factors will affect the quality of the final product.

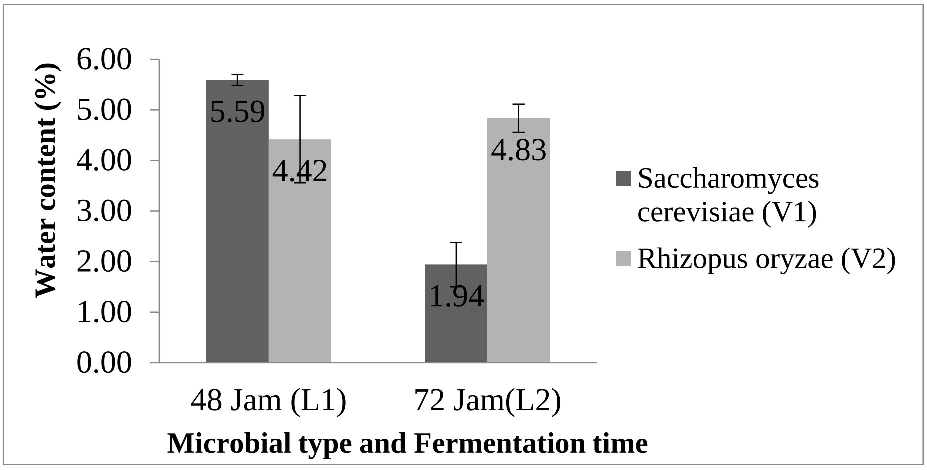

Figure 3: The effect of microbial type treatment and fermentation time (TL) on the moisture content of flour (Different letters indicate a significant difference in the DMRT test 0.05, level 1 = 2.52, level 2 = 2.64, and level 3 = 2 , 72, with KK = 48.53)

In figure 3. shows that the treatment of saccharomyces cerevisiae microbes at the fermentation time of 48 hours had a high water content of the flour, namely 5.59%, and not significantly different from the treatment of rhizopus oryzae at 72 hours of fermentation (4.83%). Saccharomyces cerevisiae at the 72 hour fermentation time had the lowest water content value, namely 1.94%. That there was a decrease in the water content obtained, namely 9.70% to 8.94% (13). The decrease in water content with increasing fermentation time is probably caused by the soft and porous texture of the tubers after fermentation, resulting in maximum moisture loss. Microorganisms must have utilized moisture for metabolic activity (23). The temperature and drying time factors will affect the quality of the final product.

Ash Content

The residual content of inorganic remains after water and other organic materials which are components of food. The results of research conducted based on analysis of variance showed that the interaction between fermentation time treatment and the interaction of microbial types on the ash content of taro flour.

The ash content at 48 hours of fermentation was higher than that of 72 hours during the fermentation process. Kimpul flour increased from the initial ash content, namely 1.21%. The increase in ash content might be caused by microbial degradation of the antinutrient content in the material during fermentation (28). It is concluded that there are more mineral elements in the fermented material. This is the same as the research (13) which obtained ash content (2.40-2.84)% during 96 hours of fermentation. The ash content of colocasia esculenta (2.61%) was lower than that of xanthosoma sagittifolium (4.25%) (21). In other studies, it was found that the ash content was 1.66% in colocasia esculenta tubers and 2.68% in Xanthosoma sagittifolium taro. In general, variations in composition values like this are thought to occur from differences in genetic backgrounds as well as climatic, seasonal, and agronomic factors (21).

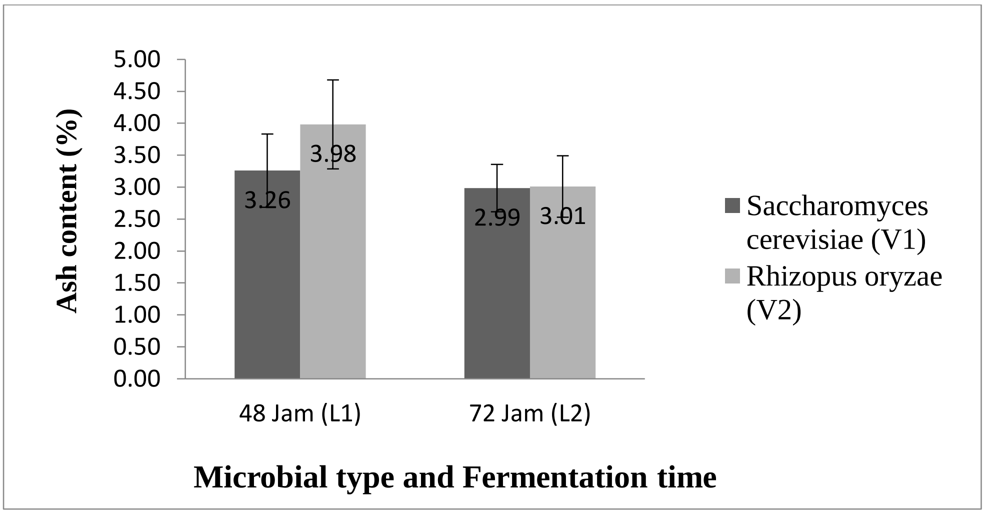

Figure 4: The effect of the type of microbial treatment and fermentation time (VL) on the ash content of flour (Different letters indicate a significant difference in the DMRT test 0.05, level 1 = 0.33, level 2 = 0.35, and level 3 = 0 , 36, with KK = 8,12).

In figure 4. Shows from the DMRT test, 0.05 kimpul flour fermented with rhizopus oryzae at 48 hours of fermentation has the highest value, namely 3.98%. In saccharomyces cerevisiae, the fermentation time of 72 hours has the lowest value of ash content, namely 2.99%. The high level of ash in food shows the total mineral content (21). However, this treatment interaction also decreased the ash content of the flour from the long fermentation time. The changes in chemical properties that occur during fermentation can be caused by process conditions such as tuber size, water: tuber ratio during immersion, the addition of microbial types, and methods of separating water and tubers after fermentation (14).

pH

The results of the research with pH analysis showed that from the DMTR test, 0.05 at 48 hours of fermentation time had the highest pH value, namely 6.62 for saccharomyces cerevisiae and 6.46 for rhizopus oryzae. The fermentation time of 72 hours has a low pH value, namely 5.58 with saccharomyces cerevisiae and 6.04 with rhizopus oryzae. Judging from the initial pH, namely 6.73 for purple taro and 6.5 for Kimpul taro, there was a decrease in pH after fermentation for 48 hours and 72 hours. The results of previous research showed that the pH decreased from 5.68 to 3.75 from the fermentation time of 0 to 96 hours (13). The decrease in pH could be caused by the production of organic acids in materials fermented by microorganisms. During the fermentation process, there is the production of hydrolysis enzymes which break down organic compounds into simpler ones(29).

Total Microbes

Total microbial analysis was carried out using the TPC method on purple taro and kimpul taro with the types of microbes Saccharomyces cerevisiae and Rhizopus oryzae. Based on the results of the analysis of variance, it shows that the factors of taro varieties, types of microbes, and fermentation time as well as the interaction between treatments did not significantly affect the total microbes. Total taro microbes ranged from 2.81 to 4.54 log cfu / g with an average of 3.65 log cfu /g.

Table 2: Total microbe in taro kimpul

Type of microbes	Taro Kimpul	
	48 hours	72 hours
Saccharomyces cerevisiae	4,67 log cfu/g	4,54 log cfu/g
Rhizopus oryzae	3,13 log cfu/g	2,81 log cfu/g

Table 2 in kimpul tubers, the number of microbes obtained from saccharomyces cerevisiae is 4.67 log cfu/g at 48 hours fermentation time to 4.54 log cfu/g at 72 hours fermentation time and rhizopus oryzae is 3.13 log cfu/g at the fermentation time of 48 hours to 2.81 log cfu/g at the fermentation time of 72 hours. The temperature of fermented taro tubers increased from 27 oC to 35 oC as a result of the heat generated during sample metabolism by fermented organisms (13). The pH can also affect the number of microbes obtained in taro. The type of taro colocasia spp. shows the lowest pH value when compared to the type of taro xanthosoma spp (22). During fermentation there were differences in the number and characteristics of microbes in the growth of the number of microbes, this seems to be largely determined by the number, type, and composition of ingredients (tubers or cereals with their respective chemical composition) such as the number and types of microbes added, the characteristics of soaking water, and the fermentation method applied (22). In other studies said that each dilution factor distributed affects the colony number of each sample, showing the diversity of data according to the dilution factor carried out (30). This shows that the higher the dilution is carried out, the lower the number of colonies obtained or vice versa.

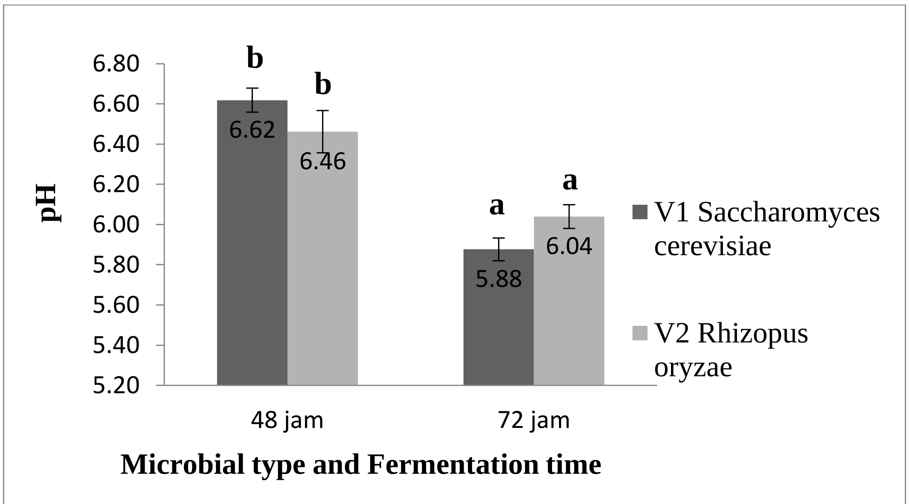

Figure 5: The effect of treatment of microbial types and fermentation time (VL) on pH (Different letters indicate a significant difference in the DMRT test, 0.05, level 1 = 0.22, level 2 = 0.23, and level 3 = 0. 24, with KK = 2.83).

Total Starch

The total starch analysis was carried out on samples with the lowest levels of calcium oxalate taro flour, namely at the 72 hour fermentation time.

The total initial taro starch ranged from 35.65% for Kimpul taro. taro Kimpul, Saccharomyces cerevisiae at 72 hours fermentation time was 16.94%, and taro Kimpul, rhizopus oryzae at 72 hours fermentation time was 17.07%. A very significant decrease can be expected to occur when taro drainage, a lot of starch is wasted along with the fermented water. The decrease in total starch from 29% to 19.37% in the 72 hour fermentation time (22). After experiencing the fermentation process, modified taro flour decreased starch content, because the starch of organic matter has been used to meet the energy needs of microorganism growth.

CONCLUSION

From the results of this study, the very good fermentation time in reducing oxalate levels was found in the 72-hour fermentation process using Rhizopus oryzae microbes with a mean of 24.81 mg / 100 g, from these results obtained analysis of crude fiber content of 4.11%, water content 4, 44%, ash content 3.69%, pH 6.07, protein content 1.09%, water absorption 1.94 g / g, total microbes 2.81 log cfu / g, total starch 17.07%,

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