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# Production of bioethanol from jackfruit rind-waste using *Saccharomyces cerevisiae* with *crude* enzyme treatment of *Trichoderma reesei* and *Aspergillus niger*

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ABSTRAK

Bioetanol menjadi salah satu energi alternatif yang mulai dibutuhkan, seiring menipisnya cadangan minyak bumi. Bioetanol dapat diperoleh dari berbagai limbah salah satunya kulit nangka dengan bantuan enzim dari *T. reesei* dan *A. niger* dan fermentasi menggunakan *S. cerevisiae*. Tujuan penelitian ini untuk mengetahui kadar gula dan kadar etanol tertinggi dari limbah kulit nangka menggunakan *S. cerevisiae* dengan perlakuan rasio *crude* enzim *T. reesei* dan *A. niger.* Penelitian ini merupakan penelitian eksperimen. Rancangan percobaan menggunakan RAL dengan variabel bebas rasio *crude* enzim *T. reesei* dan *A. niger* yaitu (1:0), (0:1), (1:1), (1:2), (2:1), (1:3), (3:1) dan variabel terikat kadar gula dan etanol hasil fermentasi kulit nangka menggunakan *S. cerevisiae.* Pengukuran kadar gula dengan metode DNS, sedangkan pengukuran kadar etanol menggunakan alkoholmeter. Analisis data menggunakan anova satu jalur (SPSS versi 16). Berdasarkan hasil penelitian, kadar gula tertinggi pada rasio crude enzim *T. reesei* dan *A. niger* setelah perlakuan crude 1:3 (14,21 %) dan setelah fermentasi 1:2 (14,73 %). Kadar etanol tertinggi pada rasio *crude* enzim *T. reesei* dan *A. niger* 1:1 (1,32 %).

Keywords:
*Aspergillus niger*
*Crude enzim*
*Trichoderma reesei*

ABSTRACT

Production of bioethanol from jackfruit peel waste using *Saccharomyces cerevisiae* with crude enzyme ratio treatment of *Trichoderma reesei* and *Aspergillus niger*. Bioethanol is an alternative energy that is starting to be needed, along with the depletion of petroleum reserves. Bioethanol can be obtained from various wastes, one of which is jackfruit skin with the help of enzymes from *T. reesei* and *A. niger* and fermentation using *S. cerevisiae.* The purpose of this study was to determine the sugar content and the highest ethanol content of jackfruit peel waste using *S. cerevisiae* with the ratio treatment of crude enzymes *T. reesei* and *A. niger*. This research is experimental research. The experimental design used RAL with the independent variables being the ratio of crude enzymes *T. reesei* and *A. niger,* namely (1:0), (0:1), (1:1), (1:2), (2:1), (1:3), (3:1) and the dependent variable was the sugar and ethanol content of fermented jackfruit peel using *S. cerevisiae*. The measurement of sugar content using the DNS method, while the measurement of ethanol content using an alcoholmeter. Data analysis used one-way ANOVA (SPSS version 16). Based on the results of the study, the highest sugar content was in the ratio of crude enzymes *T. reesei* and *A. niger* after *crude* treatment 1:3 (14.21%) and after fermentation 1:2 (14.73%). The highest ethanol content was in the ratio of crude enzymes *T. reesei* and *A. niger* 1:1 (1,32%).



## INTRODUCTION

Bioethanol is one of the alternative energy sources as petroleum reserves are depleting. Bioethanol research has been developed using environmentally friendly materials, one of which is jackfruit peel waste. Jackfruit skin is still not used properly by the community and only becomes waste in the trash, even though jackfruit skin has potential as a bioethanol material. Jackfruit skin contains carbohydrates by 15.87%, cellulose by 38.69%, and protein by 1.30% (Hermawani et al., 2019). Conversion of waste into bioethanol can be carried out with the help of cellulase enzymes produced from mushrooms and then continued in the fermentation process. Fungi that produce cellulase enzymes that are easy to apply are *T. reesei* and *A. niger*. The use of *T. reesei* and *A. niger* fungi can be done by producing crude enzymes which are easier and more affordable.

*T. reesei* is a cellulase-producing fungus that secretes about 80% cellulase. In addition, *T. reesei* is more environmentally friendly, easy to obtain and easy to apply, and does not require high temperatures (Fathimah et al., 2014). *A. niger*, fungi that can degrade crude fiber in waste used in bioethanol (jackfruit skin) and are able to live at high acidity and sugar content. The fermentation process using yeast *S. cerevisiae* is very good in producing ethanol by fermenting hexose sugars such as glucose from hydrolysis of cellulose and converting cellulose into ethanol (Daud et al., 2012).

Based on this background, research on jackfruit skin needs to be carried out so that it can be utilized properly and can reduce waste in the trash into something more useful. Utilization of jackfruit skin as a bioethanol material because jackfruit skin contains lignocellulose, where after pretreatment, cellulose is obtained which is a source of glucose, fructose, sucrose, starch, fiber, and pectin). The use of *T. reesei* and A. niger is important because they can produce cellulase enzymes that can convert cellulose into glucose so that the glucose produced is more optimal. Glucose, with the help of *S. cerevisiae*, will be converted into ethanol. The purpose of this study was to determine the sugar content and the highest ethanol content of jackfruit peel waste using *S. cerevisiae* with the ratio treatment of crude enzymes *T. reesei* and *A. niger.*

Crude enzymes are crude or crude enzymes extracted from agricultural materials. Crude cellulase enzymes have lower performance than enzymes that have been purified but are cheaper to produce and easier to apply in industry. Crude enzyme innovation has started many innovations, both processed and raw materials to produce cheap enzymes (Retnoningtyas et al., 2013).

Jackfruit is a tropical plant that can grow very well and is abundant in Indonesia. Jackfruit plants can live throughout the year as long as there is no long drought. The use of jackfruit plants is still limited to the consumption of the flesh of the fruit by the community. This wastewater from jackfruit skin can produce bioethanol (Rifa'i & Mukti, 2018). Jackfruit rind extract contains ethanol because it contains alkaloids, flavonoids, phenols, and terpenoids. Jackfruit skin has almost the same properties as fruit. Jackfruit skin contains 1.94% crude fiber, while the flesh contains 1.58% fiber. Jackfruit skin also contains carbohydrates such as glucose, fructose, sucrose, starch, fiber, and pectin, which reaches 15.87%, and protein 1.30%, so that jackfruit skin has a high potential in the manufacture of bioethanol (Karim & Sutjahjo, 2013).

*Trichoderma reesei* is a single culture that is often used in food processing because it is able to degrade cellulose and starch into glucose. *Trichoderma reesei* produce cellulolytic enzymes, namely endoglucanase and exoglucanase, which function to hydrolyze cellulose (Rattanapradit et al., 2010). T*richoderma reesei* is a cellulase-producing fungus that secretes about 80% cellulase. The cellulase enzymes produced have different characteristics because they are influenced by several factors, including environmental factors such as temperature, pH and the environment in which the enzyme works, substrate concentration, and incubation time (Fathimah et al., 2014).

*Aspergillus niger* is a type of fungus that produces cellulase enzymes. *Aspergillus niger* metabolism can be fulfilled if the nutrition is appropriate and sufficient, one of which is a source of C (Indriani et al., 2015). Macroscopic characteristics *Aspergillus niger* isolated on PDA medium at the age of 7 days with an incubation temperature of 30$^o$C was black because of the dense conidiophores formed. The surface of the colony is flat with a rough and grainy texture, the margins are uneven and do not produce exudate (fluid that comes out of the colony).

Cellulase is an enzyme that is often used in industry and biomass fermentation. Cellulase was obtained from a mixture of enzymes endoglucanase, exoglucanase, and b-glycosidase. Cellulase can be produced from fungi, bacteria, plants, and ruminants. Fungi that can produce cellulase are filamentous fungi such as *Trichoderma reesei* and *Aspergillus niger*, which also produce crude enzymes commercially (Sulistyarsi et al., 2016).

Cellulose is a glucose polymer with β-1,4 glycosidic bonds which, when hydrolyzed, will produce glucose. Cellulose can be used as a carbon source for microbial growth. Bioethanol can be produced from waste materials containing sugar and cellulose materials. Cellulose is a source of glucose in bioethanol fermentation. In lignocellulose waste, lignin serves to protect cellulose against the attack of cellulose-breaking enzymes. Materials containing cellulose are strong and hard, while the presence of hydrogen bonds causes cellulose to be insoluble in water (Gunam et al., 2011).

*Saccharomyces cerevisiae* is a microorganism that is commonly used in the fermentation process. *Saccharomyces cerevisiae* has several advantages over other types of microorganisms in the bioethanol production process. The advantages are that it is more environmentally friendly, adaptable, easy to obtain, and resistant when interacting with high alcohol content (Azizah et al., 2012). *Saccharomyces cerevisiae* is one of the very good yeasts in the process of making ethanol. Hexose sugar alcohol from fermentation can reach 90%.

*Saccharomyces cerevisiae* can convert glucose into ethanol by fermenting sugar. Sugar will be converted into simple sugars by the invertase enzyme and then converted into ethanol by the zymase enzyme. *Saccharomyces cerevisiae* produces both enzymes so that they are able to convert glucose into simpler sugars. However, *Saccharomyces cerevisiae* cannot convert galactose into ethanol because it is better able to adapt to glucose-containing substrates than galactose-containing substrates (Azizah et al., 2012).

Fermentation can occur due to several influencing factors, including temperature, pH, oxygen, microbes as the perpetrators of fermentation and fermentation time or fermentation time. The growth temperature range is between 20-30$^o$ C, which will grow optimally at a temperature range of 30-35$^o$ C. If the temperature is too high, the enzyme activity produced will decrease due to denaturation. While the temperature is below that temperature, the ethanol fermentation reaction will take place slowly (Azizah et al., 2012). The pH range for the growth of *Saccharomyces cerevisiae* is at pH 3.5-6.5. Under alkaline conditions, *Saccharomyces cerevisiae* cannot grow. Meanwhile, the maximum ethanol production by *Saccharomyces cerevisiae* can reach pH 4.5. Generally use yeast that can convert sugar into alcohol. *Saccharomyces cerevisiae* is a yeast that is often used in alcoholic fermentation. *Saccharomyces cerevisiae* has several advantages over other microbes that can also form alcohol. Within 72 hours, *Saccharomyces cerevisiae* can produce up to 2% alcohol but cannot utilize galactose, so *Saccharomyces cerevisiae* will use glucose as its carbon source instead of galactose.

## METHOD

This research is a type of experimental research. The experimental design used RAL with the independent variable ratio of crude enzymes *T. reesei* and *A. niger*, namely (1:0), (0:1), (1:1), (1:2), (2:1), (1:3), (3:1), and the dependent variable was the sugar and ethanol content of fermented jackfruit skin using *S. cerevisiae*. The measurement of sugar content using the DNS method, while the measurement of ethanol content using an alcoholmeter. Parameters measured in this study were sugar content, pH level, and ethanol content. Data analysis used one-way ANOVA (SPSS version 16). This research was conducted in March 2021 at the Biology Laboratory of Ahmad Dahlan University.

The tools used in this study were blender, mortar, masher, blender, incubator, autoclave, oven, microwave, centrifuge, analytical balance, test tube, tongs, stirrer, funnel, beaker, erlenmeyer, measuring flask, dropper, propipet, measuring pipette, ose wire, bunsen, alcohol meter, and distillator. The materials used in this study were *Trichoderma reesei* and *Aspergillus niger* cultures, *Potato Dextrose Ag*ar (PDA) media, ready-to-use *Saccharomyces cerevisiae* (fermipan yeast), jackfruit peel powder.

The ingredients for making the nutrient solution are distilled water, 70% alcohol, nutrient solution (urea), ammonium sulfate (NH4)2SO4, hydrochloric acid (HCL), glucose, potassium dihydrogen phosphate (KH2PO4), calcium chloride monohydrate (CACL2.H2O), magnesium sulfate heptahydrate (MGSO4.7H2O), and sodium hydroxide (NaOH). For the manufacture of tween solution is a solution of 80% 0.1 tween added with 1 L of distilled water. The material used for the manufacture of DNS solution is a solution of 3.5 dinitrosalicylic acid, NaOH, and sodium sulfate dissolved in aquadest.

The way of working in this research begins with the sterilization of the equipment that will be used in the study. Jackfruit rind is prepared by cutting into small pieces and drying it, then mashed with a blender. Pretreatment of jackfruit skin needs to be done before starting the research because jackfruit skin has a hard texture, so to remove lignin present in jackfruit rind, it is necessary to do pretreatment with NaOH immersion. After soaking the NaOH, the jackfruit peel powder was washed with distilled water to get a pH of 7 (neutral).

The nutrient solution was made by putting all the ingredients into a beaker and adding distilled water, and stirring evenly with a sterile glass stirrer. Preparation of the 80.01% tween solution was carried out by entering the tween solution into a 100 ml volumetric flask and adding aquadest. Mushroom growth was carried out using inclined PDA media, which were cultured for two days to be ready for use. The next stage is the production of crude cellulase enzymes by entering 5 grams of jackfruit peel powder into a 250 mL Erlenmeyer and adding 25 mL of nutrient solution. The mixture was then sterilized at a temperature of 121$^{o}$C for 20 minutes and cooled. The culture of *Aspergillus niger* was suspended in 100 mL of 80% tween solution and incubated for eight days at room temperature. Meanwhile, the *Trichoderma reesei* culture was suspended in 100 mL of 80% tween solution and incubated for eight days at room temperature. After incubation, 100 mL of 0.1% Tween 80 solution was poured into the fermented jackfruit rind sample and stirred at 150 rpm for 120 minutes at room temperature. The solution was then centrifuged at 3000 rpm for 10 min.

The fermentation stage is carried out by inserting 100 grams of jackfruit rind powder into a 1000 mL Erlenmeyer, then adding 900 mL of aquadest, then boiling it until it boils, then the extract is taken. The addition of crude enzymes from T. reesei and A. niger each as much as 10% according to the treatment with variations (1:0), (0,1), (1:1), (1:2), (2:1), (1:3), (3:1) and the control used a measuring pipette aseptically and made three replications, then stirred using a sterile glass stirrer. Erlenmeyer was covered with sterile cotton and coated with aluminum foil then incubated for 24 hours using an incubator at 37$^{o}$C.

Measurement of sugar content was carried out after the production of crude enzymes and fermentation with *S. cerevisiae*. Using the DNS method and then heated in a water bath for 10 minutes at 90$^{o}$C. The next step is adding 40% Rochelle salt and measuring the sugar content using a spectrophotometer at a wavelength of 575 nm. The stage of bioethanol production is to prepare samples with sterilized crude enzymes *T. reesei* and *A. niger*. Then 10% *Saccharomyces cerevisiae* was added and incubated for 72 hours or three days at room temperature. The results of the fermentation with *Saccharomyces cerevisiae* were distilled, the ethanol content of the distillate was measured using an alcoholmeter. Distillation of bioethanol can be done with a simple distillation apparatus. The filtrate from all hydrolysis and fermentation treatments was distilled at 78°C.

## RESULTS AND DISCUSSION

1. Sugar

The following are the results of measuring sugar levels in the study, which include sugar levels before and after pretreatment, which are presented in Table 1.

**Table 1.** Sugar content of jackfruit rind substrate

| Repetition | Reducing sugar level (%) | |
|---|---|---|
| | **Before pretreatment (%)** | **After pretreatment (%)** |
| 1 | 8.33 | 9.18 |
| 2 | 9.35 | 10.29 |
| 3 | 11.06 | 9.72 |
| Average | 9.58 | 9.73 |

Calculation of sugar content before pretreatment and after pretreatment was carried out to determine the ability of delignification/breakdown of cellulose so that higher sugar content was obtained after pretreatment. Based on Table 1, the results of reducing sugar content before pretreatment are 9.58, and reducing sugar levels after pretreatment is 9.73. These results indicate that the sugar content increased after pretreatment. The pretreatment stage is the stage of removing lignin in lignocellulose and hydrolyzing cellulose and hemicellulose into simple sugars so as to increase the sugar content, which will later be converted into bioethanol (Hendrasarie & Mahendra, 2020). The sugar content of jackfruit rind substrate after crude enzyme treatment is in Table 2.

**Table 2.** Sugar content of jackfruit skin substrate after crude enzyme treatment

| *Crude* enzyme ratio | Reducing sugar level (%) | | | Average reducing sugar level (%) |
|---|---|---|---|---|
| | **Repetition 1** | **Repetition 2** | **Repetition 3** | |
| K | 9.37 | 10.80 | 9.92 | 10.03 |
| P1 | 10.05 | 9.53 | 11.62 | 10.40 |
| P2 | 11.54 | 11.08 | 11.78 | 11.47 |
| P3 | 10.99 | 10.48 | 10.35 | 10.61 |
| P4 | 12.66 | 14.76 | 12.94 | 13.46 |
| P5 | 12.60 | 9.90 | 9.90 | 10.80 |
| P6 | 14.58 | 13.03 | 15.02 | 14.21 |
| P7 | 14.62 | 8.55 | 8.31 | 10.49 |

Description: K(Control); P1(*Crude* enzyme ratio *T. reesei* dan *A. niger* 1:0); P2*(Crude* enzyme ratio *T. reesei* dan *A. niger* 0:1); P3*(Crude* enzyme ratio *T. reesei* dan *A. niger* 1:1); P4*(Crude* enzyme ratio *T. reesei* dan *A. niger* 1:2); P5*(Crude* enzyme ratio *T. reesei* dan *A. niger* 2:1); P6*(Crude* enzyme ratio enzim *T. reesei* dan *A. niger* 1:3); dan P7(*Crude* enzyme ratio *T. reesei* dan *A. niger* 3:1)

Calculation of sugar content of jackfruit rind substrate after treatment was analyzed with SPSS. The prerequisite tests, namely normality test and homogeneity test. The test results show that the data is normally distributed and homogeneous, so it is continued to the ANOVA test with the results in Table 3.

**Table 3.** ANOVA test results sugar content after crude enzyme treatment

| | Total square | Df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between-group | 50.366 | 7 | 7.195 | 2.940 | .035 |
| In group | 39.155 | 16 | 2.447 | | |
| Total | 89.521 | 23 | | | |

ANOVA test results show Sig. $0.035 < 0.05$, then H0 is rejected, meaning that the average crude sugar content of the jackfruit rind substrate after treatment is significantly different, then it can be continued with the significant difference test (BNT) with a 5% confidence level, which is presented in Table 4.

**Table 4.** BNT Test Results 5% Sugar Content After Crude Enzyme Treatment

| ***Crude* enzyme ratio** | **Average sugar content (%)** | **Notation** |
|---|---|---|
| Control | 10.034 | a |
| P1 | 10.397 | a |
| P7 | 10.492 | ab |
| P3 | 10.607 | ab |
| P5 | 10.801 | ab |
| P2 | 11.469 | abc |
| P4 | 13.455 | bc |
| P6 | 14.208 | c |

Description: The same letter in the column indicates not significantly different (p < 0,05) K(Control); P1(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:0); P2(Crude Enzyme Ratio *T. reesei* dan *A. niger* 0:1); P3(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:1); P4(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:2); P5(Crude Enzyme Ratio *T. reesei* dan *A. niger* 2:1); P6(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:3); dan P7(Crude Enzyme Ratio *T. reesei* dan *A. niger* 3:1)

Based on the research results of the sugar content of jackfruit skin substrate after crude enzyme presented in Table 2 shows that the average sugar content ranges between 10.03%-14.21%. Control sugar levels were lower than all the treatments given. Sugar levels in all crude enzyme treatments proved to be effective because all calculation results were higher than control sugar levels. The highest sugar content was found in treatment 6 with a crude ratio of *T. reesei* : *A. niger* enzyme 1: 3 with a sugar content yield of 14.21%. In comparison, the lowest sugar content of all treatments was found in treatment 1 with the ratio of *T. reesei* enzyme crude: *A. niger* 1: 0, which is 10.40%.

The ratio of crude *T. reesei*: *A. niger* 1:3 got the highest sugar content because it was caused by an increase in the activity of the crude enzyme *Aspergillus niger* capable of producing high glucosidase (Oktavia et al., 2014).In the ratio of crude enzyme *Trichoderma reesei* : *Aspergillus niger* 1:0 to get the lowest sugar content, the causal factor is a decrease in enzyme activity where the longer the enzyme is used, the activity decreases, sugar levels also decrease due to glucose that has been hydrolyzed a lot so that sugar levels tend to fall or remain constant (Oktavia et al., 2014). The sugar content of the jackfruit rind substrate after crude enzyme treatment proved that there was a significant difference in the process of adding crude enzymes *Trichoderma reesei* and *Aspergillus niger because* based on the results of the study, there was an effect of increasing sugar content seen from the high sugar content of the treatment than the control sugar content.

**Table 5.** Sugar content of jackfruit peel substrate after fermentation with *S. cerevisiae*

| ***Crude* enzyme ratio** | **Reducing sugar level (%)** | | | **Average reducing sugar level (%)** |
|---|---|---|---|---|
| | **Repitition 1** | **Repitition 2** | **Repetition 3** | |
| K | 10.79 | 9.64 | 9.64 | 10.02 |
| P1 | 9.59 | 10.32 | 10.89 | 10.27 |
| P2 | 10.89 | 11.17 | 11.89 | 11.32 |
| P3 | 10.57 | 10.68 | 10.35 | 10.54 |
| P4 | 14.91 | 15.23 | 14.06 | 14.73 |
| P5 | 13.64 | 9.17 | 9.07 | 10.63 |
| P6 | 14.52 | 11.46 | 14.51 | 13.50 |
| P7 | 12.06 | 9.55 | 9.22 | 10.28 |

Description: K(Control); P1(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:0); P2(Crude Enzyme Ratio *T. reesei* dan *A. niger* 0:1); P3(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:1); P4(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:2); P5(Crude Enzyme Ratio *T. reesei* dan *A. niger* 2:1); P6(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:3); dan P7(Crude Enzyme Ratio *T. reesei* dan *A. niger* 3:1)

Calculation of sugar content of jackfruit rind substrate after fermentation was analyzed with the help of SPSS, with prerequisite tests, namely normality test and homogeneity test. The test results show that the data is normally distributed and homogeneous, so it is continued to the ANOVA test with the following results:

**Table 6.** Results of ANOVA analysis of sugar levels in jackfruit peel substrate after fermentation with *S. cerevisiae*

| | Total square | Df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between-group | 63.832 | 7 | 9.119 | 5.260 | .003 |
| In group | 27.739 | 16 | 1.734 | | |
| Total | 91.571 | 23 | | | |

ANOVA test results show Sig. 0.003 < 0.05, then H0 is rejected, meaning that the average sugar content of jackfruit rind substrate after fermentation with *S. cerevisiae* from several treatments is significantly different so that it can be continued with a significant difference test (BNT) with a confidence level of 5%.

**Table 7.** Results of 5% BNT test sugar content of jackfruit peel substrate after fermentation with *S. cerevisiae*

| ***Crude* enzyme ratio** | **Average** | **Notation** |
|---|---|---|
| K | 10,023 | a |
| P1 | 10,269 | a |
| P7 | 10,276 | a |
| P3 | 10,537 | a |
| P5 | 10,625 | a |
| P2 | 11,318 | ab |
| P6 | 13,499 | bc |
| P4 | 14,731 | c |

Description: The same letter in the column indicates not significantly different (p < 0,05) K(Control); P1(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:0); P2(Crude Enzyme Ratio *T. reesei* dan *A. niger* 0:1); P3 (Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:1); P4(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:2); P5(Crude Enzyme Ratio *T. reesei* dan *A. niger* 2:1); P6(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:3); dan P7(Crude Enzyme Ratio *T. reesei* dan *A. niger* 3:1)

Based on the results of the study, the sugar content of the fermented jackfruit peel substrate by *Saccharomyces cerevisiae* which is presented in Table 5 shows that the average sugar content of the fermented jackfruit peel substrate ranged from 10.02% - 14.73%. The highest fermented sugar content was treated with 4 ratio crude *Trichoderma reesei* : *Aspergillus niger* 1:2, which was 14.73%. Meanwhile, the lowest fermented sugar content was treatment 1, the ratio of crude enzyme *Trichoderma reesei : Aspergillus niger* 1:0 was 10.27%. During the fermentation process, the pH level was measured and obtained a pH of 5. This indicates that the pH conditions are good because the microbial growth range of *Saccharomyces cerevisiae* is between 3.5-6.5. Under these conditions, *Saccharomyces cerevisiae* can produce up to 2% alcohol in 72 hours. In this study, the temperature range is 25-30$^o$C. The fermentation temperature is low because it is already in the room because the fermentation process has stopped so that the resulting alcohol production is low because the optimal growth temperature of *Saccharomyces cerevisiae* is in the range of 30-35$^o$C. Fermentation in the slow condition and at highly temperatures will cause *Saccharomyces cerevisiae* to die so that the fermentation process cannot take place (Widyastuti, 2019).

2. pH Level

The pH level of the jackfruit rind substrate before the crude enzyme and after fermentation with *S. cerevisiae* was 7, because the pretreatment process was carried out before the crude, which was then washed with distilled water until the resulting pH was neutral as a sign that the lignin present in the jackfruit skin had been removed to proceed to the process. Furthermore, the jackfruit skin sample was sterilized before the fermentation process so that the resulting pH was 7 or neutral. The pH level after the measured crude enzyme is 8, which means it is in an alkaline state. The pH level after fermentation was measured on average 5. The measured pH was in accordance with the theory where pH with acidic properties for fungi has an optimum pH of 5 to 7, but can also be found from a pH range of 2 to 8.5. This is because each microorganism has a different level of acid tolerance, and enzymes do not work if they are too acidic or too alkaline (Larasati et al., 2015).

3. Etanol Level

Based on the results of the study, it can be obtained that the measured ethanol content is as follows:

**Table 8.** Ethanol content of jackfruit peel substrate after fermentation with *S. cerevisiae*

| ***Crude* enzyme ratio** | **Etanol level** | | | **Average (%)** |
|---|---|---|---|---|
| | **Repetition 1 (%)** | **Repetition 2 (%)** | **Repetition 3 (%)** | |
| K | 0.76 | 1.08 | 1.28 | 1.04 |
| P1 | 1.18 | 1.16 | 1.44 | 1.26 |
| P2 | 0.66 | 2.28 | 0.98 | 1.31 |
| P3 | 1.20 | 1.16 | 1.60 | 1.32 |
| P4 | 1.26 | 1.44 | 0.86 | 1.19 |
| P5 | 1.54 | 1.24 | 0.78 | 1.19 |
| P6 | 1.10 | 0.88 | 1.36 | 1.11 |
| P7 | 1.68 | 0.74 | 1.28 | 1.23 |

Description: K(Control); P1(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:0); P2(Crude Enzyme Ratio *T. reesei* dan *A. niger* 0:1); P3(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:1); P4(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:2); P5(Crude Enzyme Ratio *T. reesei* dan *A. niger* 2:1); P6(Crude Enzyme Ratio *T. reesei* dan *A. niger* 1:3); dan P7(Crude Enzyme Ratio *T. reesei* dan *A. niger* 3:1)

Calculation of ethanol content of jackfruit rind substrate was analyzed with the help of SPSS, with prerequisite tests, namely normality test and homogeneity test. The test results show that the data is normally distributed and homogeneous, so it is continued to the ANOVA test. The results shown in Table 9.

**Table 9.** Results of ANOVA analysis of ethanol levels on jackfruit peel substrate after fermentation with *S. cerevisiae*

| | **Total square** | **Df** | **Mean square** | **F** | **Sig.** |
|---|---|---|---|---|---|
| Between-group | .191 | 7 | .027 | .156 | .991 |
| In group | 2.807 | 16 | .175 | | |
| Total | 2.998 | 23 | | | |

ANOVA test results show Sig. 0.991 > 0.05 then Ho is accepted, meaning that the average ethanol content of several treatments is not significantly different, so there is no need for a significant difference test. The ethanol content based on the research in table 8 was found in the range of 1.04% - 1.32%. The highest ethanol content was found in the third treatment ratios of *T. reesei* and *A. niger* (1:1) which was 1.32%. Meanwhile, the lowest ethanol content was found in the treatment of 6 *T. reesei* and *A. niger* (1:3) which was 1.11%. The results of the study are also in line with the existing theory where the ethanol content of jackfruit peel is in the range between 1.04% - 1.32%. Meanwhile, according to the theory, the ethanol content produced by jackfruit peel is around 2% (Rifa'i & Mukti, 2018).

The ethanol content produced in the study was low due to several factors, including the ratio of crude enzymes given in various variations, not always causing the sugar content to increase or decrease (Seftian et al., 2012), so that at high sugar content high levels of ethanol are not produced, because the possibility of variations in the volume of enzymes in the crude ratio is too little. In addition, if the number of carboxyl groups of the enzyme decreases, the formation of the enzyme glycosyl complex will be slightly inhibited so that the activity of the enzyme decreases

(Safaria et al., 2013). The highest sugar content in treatment with sixth ratios of *T. reesei* and *A. niger* (1:3) was 14.21%, because at that ratio, the increase in crude enzyme activity was able to produce high glucosidase. In contrast, the lowest sugar content in the treatment of 1 *T. reesei* and *A. niger* (1:0) was 10.40%, due to the decrease in enzyme activity due to glucose which had been hydrolyzed a lot so that the sugar level tended to fall. The highest ethanol content was found in the third treatment ratios of *T. reesei* and *A. niger* (1:1) which was 1.32%. Meanwhile, the lowest ethanol content was found in treatment with sixth ratios of *T. reesei* and *A. niger* (1:3), namely 1.11%.

The high sugar content during the fermentation process in the research conducted could not produce high ethanol levels. Even though based on theory, the higher the sugar content, the higher the ethanol content produced. However, high sugar content cannot be used as a reference for the high levels of ethanol produced because of the evaporation of ethanol during fermentation which cannot be controlled, so that the evaporated ethanol comes out with carbon dioxide. Therefore, the ethanol content decreases when the fermentation process is carried out (Hendrasarie & Mahendra, 2020).

In addition, the research has not produced high levels of ethanol because it is caused by the amount of cellulase enzyme that is not sufficient to hydrolyze starch substrates. Because of that, when entering the saccharification process for a longer time, it cannot affect the ethanol content to be greater because the stability of the enzyme decreases.

## CONCLUSION

Based on the results and discussions, it can be concluded that the highest sugar content in the ratio of crude enzymes *T. reesei* and *A. niger* after crude treatment 1:3 (14.21%) and after fermentation with *S. cerevisiae* 1:2 (14.73 %). The highest ethanol content was in the ratio of crude enzymes *T. reesei* and *A. niger* 1:1 (1.32 %).

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