The Ethanol Making Out Of Cempedak Seeds (Artocarpus Champedan) With Tofu Dregs Addition As Fermentation Nutrition

Ristu Vini Okawanti, Winni Astuti, Rudi Kartika

Abstract: This researchcombinedtofu dregs powderas the nutrition source with enzymatic for cempedak seeds powder (Artocarpus champedan) which was hydrolyzed inethanol fermentation process by using Saccharomyes cerevisiae has been conducted. This research was conducted in order to know the ethanol content that has been produced by doing nutrient concentration variation andfermentation time variation. Hydrolysis processwas conducted enzymaticallythroughliquefaction phase by using α-amylase and gluco-amylasein saccharification phase. Fermentation process was conducted by usingtofu dregsas the nutrition source. Tofu dregs variations used were 1%, 2%, 3% (b/v) and fermentation time variations were (5, 6 and 7) days. The highest ethanol concentration obtained from the addition of tofu dregs nutrition was 3% (b/v) andfermentation time was6days, withethanol concentration obtained was 22,67%.

Index Terms: Bioethanol, Tofu Dregs, Cempedak Seeds (Artocarpus champedan), Hydrolysis.

1. INTRODUCTION

Energy crisis hashit all over the world, both in developing country or developed country. This is causedby very limited and non-renewable fossil fuels availability. As the result, the renewable alternative energy is necessary, the needs of world energy comes from crude oil, natural gas, and coal. With the fuel consumption level nowadays, it is predicted that fossil fuels source will run out, for oil is in 50 years, natural gas is in 65 yearsandcoal is in 200 years ahead. The researchers focus more on theconsumption of bioethanol fuel and biodiesel ingasoline engine and diesel engine. Wherein, internal combustion engineneedsfuels with relatively high energy.[1] Bioethanol developmentis a step on dealing with the reserve ofworld's crude oil this time. The process of bioethanol making is divided into 4steps; they are preliminary, preparation, hydrolysisand fermentation. The process of the second generation biofuel development is leading to the biomass conversion of non-food organic waste raw material can be an organic waste choice that has lignocellulose which contains cellulose (30-50% by weight), hemicellulose (15-35% by weight) and lignin (13-30% by weight). The important production processes of biofuel from organic waste are hydrolysis and fermentation. Organic waste hydrolysis willproduceglucose reduction. [2] Bioethanol is produced from the material which containsstarch and it is started fromhydrolysis canhydrolyze starchintosugars. α-amylase is divided into two, they are (1)dextrin, fructose, glucose, lactose,amyloseand (2)enzym starch that consists of amylase and gluco-amylase. α-amylase can hydrolyze the starch into maltoseand glucose while gluco-amylase canproducesingleglucose.[3] Tofu dregs is one of the addictive nutritionsas the source of nitrogenwhich contains high protein that ranges (20-23)%. [4]

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2. MATERIALAND METHOD

2.1 Saccharomycescerevisiae Yeast Breeding Making Agar Media

9,75 g Potato Dextrose Agar (PDA) were added in the beaker, dissolved in the 250 mL of aquades, andsterilized with autoclave at the temperature of 121^{o}C. Added 15 mL in test tube, cooled downatroom temperaturefor (15-30) minutes, and stored inrefrigiratoruntil needed.

2.2 Microbe Reneration

Saccharomyces cerevisiaeparent is bred in agar media inside sterilized test tube for ±24 hours at the temperature of 30^{o}C.

2.3 The Making of Cempedak Seeds Flour

Cempedak seeds samples are cleanedfrom the skin, and then they are washed and are cut into small pieces. Then they are dried under the sunfor ± 1 and baked themin the ovenat the temperature of 105^{o}C until dry. Next, the samplesare refined by using food processor, siftedandsterilized by usingautoclave.

2.4 Hydrolysis Process

Liquefaction Process: 900 g of cempedak seeds powder aredissolvedin 4500 mL of aquades until dissolves. Then, the cempedak seeds solution is regulated intomixed pHamong (4-5). The flour pulp is added with 3 mL of α-amylase and stirred foran hour at the temperature of (80-90)^{o}C. Next, the product of liquefactionis cooled downuntil reach the temperature of ± 55^{o}C. Saccharification Process: The sample of liquefaction process product is rearranged the pH among 4-5. Then, add3 mL of gluco-amylase

andheated at the temperature (50-60)^{o}C while stirring it for an houruntilit does not produceblue coloriniodine test. Next,the productof saccharification is cooled downuntil reach the temperature of ± 34^{o}C.

2.5 The Fermentation of Saccharomyces cerevisiaeSaccharificationProduct

The sample of saccharification process product is strained and added to 12 fermentation containers where each of 4 containers are filled with blank and tofu dregs nutrition with 1%, 2% and 3% concentrations. In each container is filled with blank and the other containers are added with 1%, 2% and 3 % of tofu dregs nutrition with weight ratio from tofu dregs per volume saccharification process product while stirring it. And then, 2 ounces of Saccharomyces cerevisiae are added in each fermentation container and closed the fermentation containers tightly by using plastic wrap and aluminium foil, and then it is fermented with time variations for 5days, 6days, and 7 days at the maximum temperature of 36^{o}C.

2.6 Purification Process

Distillation Process: A set of distillation apparatus is prepared, and then the fermentation product which has been strained is added to distillation flask. During the distillation process the temperature is set to 78^{o}C for 3 hours until all of the ethanol is separated.

2.7 Ethanol Content Analysis

Gas Chromatography Method: The stage of ethanol content analysis by using Gas Chromatography is 1 µL of each distillate is taken and injected to the column through injection site. The ethanol content inside the distillate is determined by readingthe chromatogram result andcalculated the area of ethanol peakfromchromatogram.

3. RESULTANDDISCUSSION

3.1 The Volume of Distillation Result

Distillation for purification of fermentation product that gainedthe resultas shown in Table 1.

Table 1
Distillation Result

Time Fermentation (days)	*Tofu Dregs Concentration (%)*	*Fermentation Volume (before distillation) (mL)*	*Distillation Result Volume (mL)*
5	*0*	*350*	*9,0*
	1	*350*	*10,0*
	2	*350*	*12,0*
	3	*350*	*7,0*
6	*0*	*350*	*9,0*
	1	*350*	*10,0*
	2	*350*	*5,0*
	3	*350*	*7,0*
7	*0*	*350*	*10,0*
	1	*350*	*15,0*
	2	*350*	*15,0*
	3	*350*	*8,0*

Distillation dataaboveproducedifferent distillation volume for each type of time and nutrient concentration. As the result,at the stage ofethanol concentration measurement that is obtained, using the same volume ratio by noticing the highest distillation volume15,0 mL. The aim of this equivalence is fordescribing the percentage ofobtained ethanol contentlooked obviouslydecreasedandincreased in every treatment.

3.2 Ethanol Content Measurement

The determination of ethanol contentwhich isproducedfromthe distillation resulthas the same volume. It has been conducted with gas chromatography analysis method at Biochemistry Laboratory, Faculty of Mathematic and Natural Sciences, MulawarmanUniversity, to obtain the result as shown inTable 2.

Table 2
Ethanol Concentration Determination with Gas Chromatography Method

Fermentation Time (hari)	*Nutrient Concentration (%)*	*Retention Time*	*Area*	*Ethanol Content (%)*
5	*0*	*2,75*	*459244.8*	*2,72*
	1	*2,76*	*1077572.3*	*6,39*
	2	*2,85*	*1897494.3*	*11,25*
	3	*2,77*	*1803492.6*	*10,69*
6	*0*	*2,77*	*1109819.2*	*6,58*
	1	*2,76*	*1137438.5*	*6,74*
	2	*2,79*	*2495828.8*	*14,79*
	3	*2,85*	*3822866.4*	*22,67*
7	*0*	*2,84*	*474379.2*	*2,81*
	1	*2,78*	*627095.4*	*3,72*
	2	*2,78*	*1077122.6*	*6,39*
	3	*2,89*	*2041124.1*	*12,10*
Absolute Ethanol		*2,66*	*16867032.0*	*100*

Based on Table 2, from ethanol content measurement from method, it is found that the optimal addition of tofu dregs for each fermentation time is 3% and the highest ethanol content is obtained on the 6 days of fermentation time. In Table 2, fermentation timewhich producesthe highest ethanol in amount of 22,67% cannot be said as the optimal time, since it is possible when the fermentation is more than 7 days the numberandethanol concentrationproduced is increasing. Graphic data is made with the relation of ethanol concentration with fermentation time at each obtained graphic nutrition addition as shown inPicture 1.

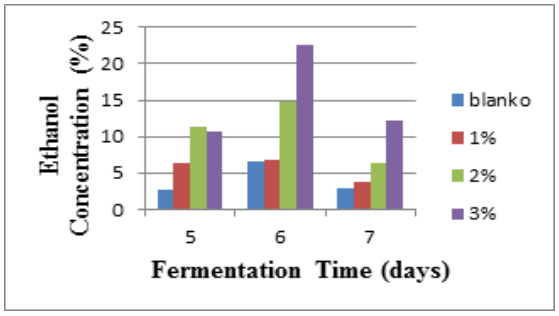

Fig 1: *The Graphic of Ethanol ContentwithFermentation Time Relation*

Based onPicture 1, obtained ethanol contentkeeps increasing from 5 days to 7 days of fermentation time, so the optimal time cannot be obtained forthe fermentation of cempedak seeds flourwith tofu dregs nutrition mixture. Meanwhile, the aimof tofu dregs addition in each fermentation time affects the obtained result a, where the result increases slowly. It shows thatSaccharomyces cerevisiae needs additional nutritionto stay alive, as empty nutrition is not enough for growth. Thus, it is obvious when 1% of tofu dregs were addedit was increasing. Until 2% and 3% addition it is increasing pretty high. On the 5th day offermentation time, ethanol content produced was still a little, it is because on that time was still entering the growth phase of lag phase, where on that phase the microorganism still adaptedwith thenew growth media, so thata little amylase was producedandethanol contentproduced was a little as well. On the 6 days of fermentation time, ethanol content was increasing. It is because on that time the growth of microorganism was entering the exponential phase, so thatthe amylase activitywhich was produced was increasing and ethanol content produced was increasing as well. The ethanol content wasdecreasing on the 7 days of fermentation time. It is because the number of glucosefrom the samplehas been completely decomposed into ethanoland alsosome ethanol producedhave beenoxidizedinto acetic acid which can cause death to Saccharomyces cerevisiae.[5]

3.3 Gas ChromatographyChromatogram

The following picture is the chromatogram result of ethanol measurement andthe result of tofu dregs fermentation with 3% concentration.

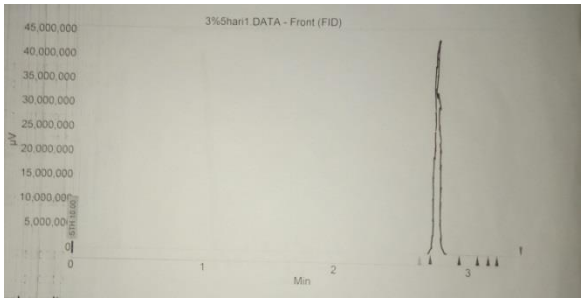

Fig 2: *Chromatogramon the 5 days of fermentation timewith 3% of nutrition addition.*

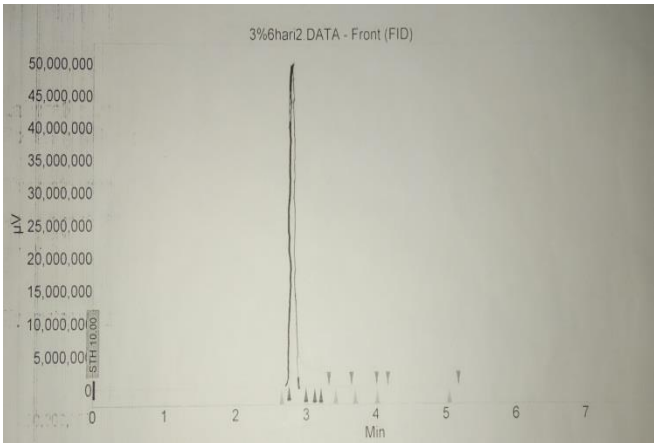

Fig 3: *Chromatogram on the 6 days of fermentation timewith 3% of nutrition addition.*

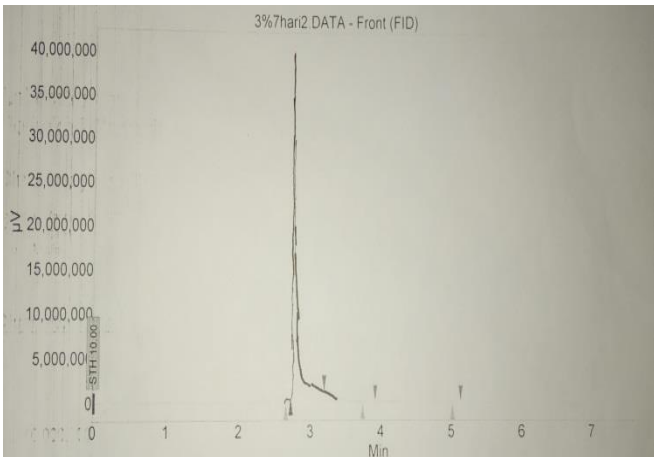

Fig 4:*Chromatogram on the 7 days of fermentation time with 3% of nutrition addition.*

4. CONCLUSION

It is obtained the optimal ethanol content in amount of 22,67% with tofu dregs additionin amount of 3% with 6 days duration of fermentation time.

5. DECISION

The researchers statedthatthere is no conflict of interest regarding the publicationof this atricle.

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