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Ethanol Production from Fermentation of Arum Manis Mango Seeds *(Mangifera Indica L.)* using *Saccharomyces cerevisiae*

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Abstract

The increase of energy needs coupled with the decline in fossil fuel production, requires other sources of energy to meet those needs. One of the solution is using renewable energy. Bioethanol is one of the alternatives to the fossil fuel. This study was aimed at determining the exact mass of mango seeds in producing high grade bioethanol. Bioethanol was produced by fermentation of arum manis mango fruit seed using baker's yeast, *Sacczharomyces cerevisiae.* The arum manis mango seeds were known to contain carbohydrate contents of 19.53%. The study was conducted by using different mass of mango seeds 25, 35 and 45 g resectively. The study showed that the samples of 25, 35 and 45 g produce ethanol with concentration of 4.78, 6.64 and 1.48%. These results indicated that the mass of 35 g mango seeds produced highest ethanol content.

INTRODUCTION

Indonesia is a country which has abundant natural resources. The abundance of mining material is a wealth of the country that has been used in various sectors. As a consequence, this continuous exploitation leads to the depletion of these resources from time to time. The industrial sector is one of the sectors that relies on fossil fuels whose portion of use in national energy consumption reaches 49.4%. On the other hand, 2025 industrial gas demand is predicted to reach 55% of its total energy needs. In that year, the industry will require 1,553 MMBTU of natural gas. In addition to natural gas, the fossil fuel which becomes the foundation of the industry is coal. 20.3% of energy for industry is produced from coal. By 2025 under the conventional scenario, the coal demand will reach 26.68 million tons. Meanwhile, under the acceleration scenario, the need of coal for the industry can reach up to 53.71 million tons (Indonesian Ministry of Industry, 2015). With increasing energy demand and declining sources of fossil fuels, a renewable energy source is required to meet that need (Padil et al., 2016).

In Indonesia, mango is the third highest productive fruit after banana and orange, which is 1,627,997 tons, equivalent to 10.07% of total national fruit production (Rebin & Karsinah, 2010). In the period of 2003-2005, Indonesia was ranked as the fifth out of the top ten world mango – producing countries. The world's largest mango – producing country is India which reaches 38.58%, China is 12.90%, Thailand reaches 6.20%, Mexico reaches approximately 5.50%, and Indonesia

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Mango Seed Powder | The process of stirring the mango seed powder and distilled water solution using a magnetic stirrer | Fermentation process | Distillation/ Purification | Characterization of the Distillation/ Purification product

Figure 1. Experimental methods

reaches 5.29% of total world mango production (Rebin, 2010). From the mango consumption, this part of the fruit which always becomes waste and is discarded is its seed. In fact, the results of study conducted by Taty and Zulpihri (2012) showed that 10 g of Indramayu mango seeds contained carbohydrate content of 19.53%, 3.80% fat and 3.78% protein.

On the other hand, one of the fossil fuel alternatives is bioethanol. Bioethanol is an ethanol derived from biological sources, especially simple sugar, amylum and cellulose. The polysaccharide – shaped amylum is then hydrolyzed into glucose by heating, using a catalyst and utilizing the enzyme. Furthermore, glucose is fermented and it produces ethanol. In this ethanol fermentation, the activity of breaking down sugars (carbohydrates) into ethanol compound with CO_2 emission is conducted (Megawati & Ciptasari, 2015). This fermentation is carried out under anaerobic condition or in the absence of oxygen. Generally, the microbial production of bioethanol uses *Saccharomyces cerevisiae.* These microbes can be used for conversion of sugars into ethanol with good conversion ability, high grade ethanol resistance, low pH resistance, and high temperature resistance. One of the biological sources which has great potential as bioethanol is the mango seed. The mango seed *(Mangifera indica L.)* is known to contain carbohydrate content of 19.53%.

The production of ethanol using plants containing starch or carbohydrates is conducted through the process of carbohydrate bioconversion into sugar (glucose) that dissolves in water. Glucose can be made from starch by hydrolyzing to break it down into glucose molecules by using an acid chemicals (i.e. sulphuric acid), then fermentation of sugars is conducted to produce ethanol by adding yeast (Fitriana, 2009). The purification of ethanol is achieved in two stages, 1) the purification by distillation to obtain 95.6% concentration of ethanol and dehydration of ethanol to obtain absolute ethanol. Ethanol purification cannot be conducted with a single stage (simple distillation) as ethanol and water form an azeotrope mixture (Errico et al., 2014; Kusuma & Dwiatmoko, 2009). The basic principle of this distillation apparatus is a separation based on the difference of the boiling or melting point of each constituent of a homogeneous mixture. In the distillation process, there are two stages of the process for example the evaporation stage and is continued with the condensation to convert the steam into liquid.

By looking at the content of the arum manis mango seed and its potential to be processed into bioethanol, it is necessary to study the processing of the mango seed. This study was aimed at determining the exact mass of the arum manis mango seed that to produce high content of bioethanol.

METHODS

The materials used in this study were the Arum Manis mango seeds *(Mangifera indica L.)* which were then crushed, a solution of distilled water and *Saccharomyces cerevisiae* yeast with "Fermipan" brand. The equipment used in this study were knife, arum manis mango seed crusher, 3 pieces of an Erlenmeyer flask and its lid, a magnetic stirrer, a digital scale, a microwave, and a beaker glass.

The stages of this study were illustrated on Figure 1. The first step was the preparation of raw materials. The mango seeds were washed. Furthermore, the seeds were dried under the sun for 2 weeks. Then after they were dry, the mango seed shells were separated from the flesh of mango seeds

Table 1. Distillation product of fermented solution

No.	Mass of Mango Seed (g)	Mass of Yeast (g)	Fermentation Time (hr)	Distillation Time (minutes)	Distillation Temperature (°C)	Ethanol Volume (mL)
1.	25	6	240	60	78 - 80	11
2.	35	6	240	60	78 - 80	8
3.	45	6	240	60	78 - 80	1

by peeling it. The results were then heated by using a microwave, then they were pounded until they were turned into powder. The second stage was the fermentation process. Each mango seed powder with the mass of 25, 35 and 45 g which was put into the Erlenmeyer. Then 100 ml of distilled water was added and it was stirred evenly by using a magnetic stirrer. After that, 6 g of baker's yeast *Saccharomyces cerevisiae* was added into each of the solution. Erlenmeyer was tightly closed using plastic to keep fermentation anaerobically, then it was kept for 10 days.

The next stage is the process of distillation and purification. Distillation equipment was arranged appropriately. Then, the temperature was kept at a temperature of 78 ° C which is the boiling point of ethanol. Sample testing phase involved testing glucose content Arum Manis mango seed using Luff Schoorl method and the testing of the bioethanol content was conducted using gas chromatographic analysis or Gas Chromatography (GC) which compared the mass of the spectrometry of bioethanol as measured by the standard bioethanol.

RESULTS AND DISCUSSION

Luff Schoorl testing method resulted in the arum manis mango has glucose content of 15.29%. The result showed that the maximum volume of ethanol by distillation process from 25 g of mango seed was 11 mL. However, the volume of ethanol decreased as the mass of mango seed increased. The data of distillation results of fermentation solution is shown in Table 1.

Significant decrease of ethanol volume occurred in mango seed with the mass of 45 g with 1 mL ethanol volume. This showed that there was still a lot of glucose that was not fermented by microorganisms to ethanol. This result contradicted with the study of Suri et al. (2013) which explained that more microorganisms lead to more ethanol production. The decrease of glucose content at 45 g of mass was assumed to be caused by the lack of yeast mass during the fermentation process which lead to the decrease of the ethanol concentration, meaning that the existing bacteria were exhausted to ferment the mass of 35 g and the addition of mango seed mass above 35 g resulted in the increase of water content of ethanol.

The reaction of the ethanol production from the fermentation of sugar by yeast can be formulated as follows:

$$C_6H_{12}O_6 \xrightarrow[\text{cerevisiae}]{\text{Saccharomyces}} 2\ C_2H_5OH + 2\ CO_2 \qquad (1)$$

The ethanol from the result of the study was then tested for their ethanol content. The mango seed with the mass of 25 g showed the sample area of 25765.79940 au. It showed that the ethanol content was 4.78%. The graph showing the sample area is shown in Figure 2.

Furthermore, the sample area of the mango seed with the mass of 35 g is 35755.90805 au with ethanol content obtained by 6.64%. The graph of the sample area is shown in Figure 3.

The sample area of the mango seed with the mass of 45 g is 7991.14789 au. Therefore, the ethanol content was 1.48%. The graph of the sample area of the material mass can be seen in Figure 4.

Based on the analysis, 25 g of mango sample produced ethanol content of 4.78% which was lower than 35 g of mango seed sample because the amount of available nutrients was not proportional to the amount of yeast. Therefore, the ethanol content was low, meaning that the amount of bacteria was very excessive. However, they could not produce optimal bioethanol because of the small amount of mango. This result is in accordance with the testing glucose content that reached 15.29 % and the study of Khoiriyati (2013) stating that the concentration of glucose which is good for the growth of yeast *(Saccharomyces cerevisiae)* is between 10% -18% because high glucose concentration (over 18%) inhibits the yeast growth resulting in a small amount of ethanol content.

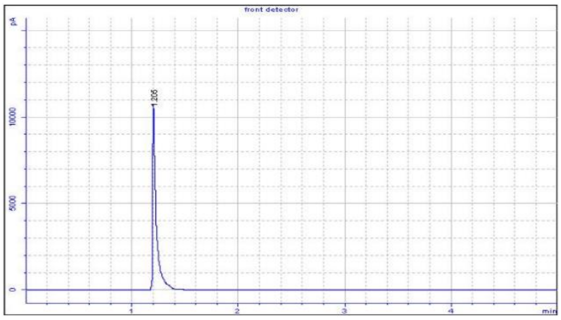

Figure 3. Chromatogram of 25 g mango seed sample

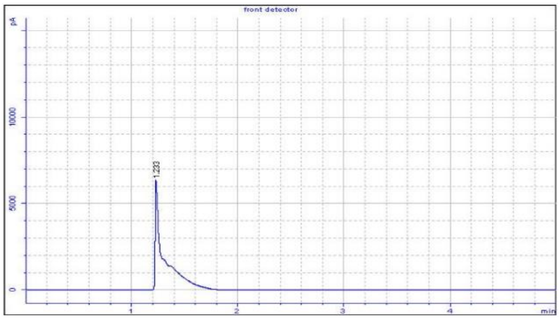

Figure 3. Chromatogram of 35 g mango seed sample

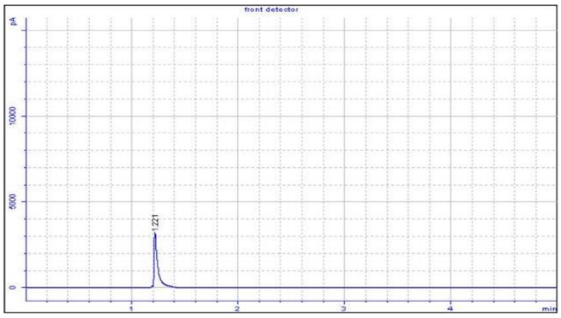

Figure 3. Chromatogram of 45 g mango seed sample

The highest ethanol content was 6.64%, it was produced by using 35 g of mango seed. It explained that higher ethanol volume is not always has highest ethanol content. Ethanol content can not be represented by the ethanol volume. During distillation process, it was assumed that there was only small amount of water vapor which vaporized with the ethanol vapor. The low water content, might be caused by high concentration of bioethanol contained in the sample. With the same number of bacteria, sample with mass lower than 35 g of mango seed only able to produce lower bioethanol content. It is due to the lack of fermented material and during distillation process mostly it only vaporized the water. From the experiment result, it can be seen that the microorganism able to produce maximum ethanol content using mango seed with mass of 35 g. From the previous study on durian seed, highest ethanol content was obtained when the ratio of *Saccharomyces cerevisiae* to durian seed was 1:25 (Nurfiana et al., 2009). Meanwhile, in this study, maximum ethanol content was obtained when the ration of yeast to mango seed was 1:6. Therefore, it can be concluded that ratio of seed mass to yeast is different for each fruit seed to obtain maximum ethanol content.

It is a different case with a sample of 45 g mango seed. The ethanol content of 1.48% was considered as the lowest one. It was caused by the inappropriate mass of *Saccharomyces cerevisiae* which reacted with all mango seed. Therefore, the ethanol production was less optimal. It meant that the abundant amount of nutrients that was not balanced with the amount of bacteria resulted in the obstructed fermentation reaction.

The production of bioethanol can also be produced from several types of fruits. Snake fruit is able to produce bioethanol by 49.92% using 5% (w/w) of *Saccharomyces cerevisiae* yeast (Thamrin et al., 2011). In addition, Woldu et al. (2015) showed that bioethanol produced by avocado seeds was 6.365%, while jackfruit seed produced bioethanol of 11.5% (Chongkhong, 2012).

CONCLUSION

Different seed mass of Arum Manis mango *(Mangifera indica L.)* has different effect on the ethanol content. Mango seed with the mass of 25 g produced ethanol content of 4.78%. Furthermore, mango seed with the mass of 35 g produced a maximum ethanol content of 6.64%, while the minimum ethanol content was produced by the mass of 45 g mango seed which was 1.48%.

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