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The ability of various yeast strains to ferment alcohol from waste rambutan (*Nephelium lappaceum Linn*) fruit

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Abstract

Alcohol is the raw material used in the manufacture of vinegar, it can be distilled to make liquor, and refined alcohol can be used to produce ethanol as an energy source. Raw materials containing sugar, fermented with yeast, are the source of alcohol. Rambutans are fruits containing high content of sugar. After harvesting, the outside appearance of rambutans is no longer acceptable by customers because the fruit deteriorates rapidly within three to five days, resulting in decreasing value. In this research, we aimed to investigate the use of low-quality rambutans for fermentation to produce alcohol. Four single strain, two mix strains, and three combination strains of yeast (*Saccharomyces cerevisiae*) were used in alcohol fermentation of low-quality rambutans. The alcohol content was evaluated every day for 14 days after fermentation. The results showed that for the single strain of *S. cerevisiae*, the TISTR 5020 could produce the highest number of alcohol content at 11.4% after fermented 11 days, followed by the TISTR 5596 strain, which gave 10.8% alcohol on day 8. For the two mix strains of yeast, the combination between TISTR 5596 and TISTR 5194 as well as the combination between TISTR 5094 and TISTR 5596 could provide the best result at 10.5% alcohol on day 9 and day 11 respectively. In the three combinations of *S. cerevisiae*, the combination of TISTR 5596, TISTR 5194 and TISTR 5094 could produce 11.0% alcohol after fermented for 14 days while the mixture of TISTR 5094, TISTR 5020 and could provide 10.8% alcohol on day 4.

Keywords: Rambutan, ethanol product fermentation, waste rambutan, alcohol fermentation, rambutan alcohol
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1. Introduction

Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes [5]. The science of fermentation is known as zymology. In microorganisms, fermentation is the primary means of producing ATP by the degradation of organic nutrients anaerobically [7]. Humans have used fermentation to produce foodstuffs, beverages and industrial production of ethanol by fermentation and distillation [2, 13].

Alcohol fermentation, also known as ethanol fermentation, is the anaerobic pathway carried out by yeasts in which simple sugars are converted to ethanol and carbon dioxide. Yeasts typically function under aerobic conditions, or in the presence of oxygen, but are also capable of functioning under anaerobic conditions, or in the absence of oxygen. When no oxygen is readily available, alcohol fermentation occurs in the cytosol of yeast cells [11]. The basic equation for alcohol fermentation shows that yeast starts with glucose, a type of sugar, and finishes with carbon dioxide and ethanol. One glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules [14, 15].

Since thousands of years ago, yeasts such as *S. cerevisiae* have been used in alcohol production especially in the brewery and wine industries. It keeps the distillation cost low as it gives a high ethanol yield, a high productivity and can withstand high ethanol concentration [8]. Nowadays, yeasts are used to generate fuel ethanol from renewable energy sources [9]. Certain yeast strains such as *Pichia stipitis* (NRRL-Y-7124), *S. cerevisiae* (RL-11) and *Kluyveromyces fagilis* (Kf1) were reported as good ethanol producers from different types of sugars [12]. Yeasts can directly ferment simple sugars into ethanol while other types of feedstocks must be converted to fermentable sugars before it can be fermented to ethanol. The common processes involve in ethanol production are pretreatment, hydrolysis and fermentation. The production of ethanol during fermentation depends on several factors such as temperature, sugar concentration, pH, fermentation

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time, agitation rate, and inoculum size. The efficiency and productivity of ethanol can be enhanced by immobilizing the yeast cells.

S. cerevisiae is the most commonly employed yeast in an industrial ethanol production as it tolerates a wide range of pH [10], thus making the process less susceptible to infection. Baker's yeast was traditionally used as a starter culture in ethanol production due to its low cost and easy availability. However, baker's yeast and other *S. cerevisiae* strains were unable to compete with wild-type yeast which caused contamination during the industrial processes. Stressful conditions like an increase in ethanol concentration, temperature, osmotic stress and bacterial contamination are the reasons why the yeast cannot survive during the fermentation [1]. Flocculent yeasts were also used during biological fermentation for ethanol production as it facilitates downstream processing, allows operation at high cell density and gives higher overall productivity [4, 6]. It reduces the cost of cells recovery as it separates easily from the fermentation medium without centrifugation [3]. There are common challenges to yeasts during sugar fermentation which are rising in temperature (35–45 °C) and ethanol concentration (over 20%) [16]. Yeasts growth rate and metabolism increase as the temperature increases until it reaches the optimum value. An increase in ethanol concentration during fermentation can cause inhibition to microorganism growth and viability. Inability of *S. cerevisiae* to grow in media containing high level of alcohol leads to the inhibition of ethanol production.

Rambutan is a tropical fruit native to Indonesia and Malaysia. It grows well in a warm and humid condition. In Thailand, it is planted in the Eastern and Southern parts of the country. There are only three varieties that are popular; Rongrian, Pink and Gold verities. Currently, rambutan production is on the downward trend. Most of them are consumed in the country and neighboring countries along the border accounted for 98.50%. Over the past years, rambutan has been experiencing the problem of price decay nearly every year due to rambutan production of more than 50% come out in the middle of the production season.As a result, price often falls down in May-August. Nonetheless, there is still no solution to solve the problem of rambutan production. Some farmers cut off rambutans tree to grow other crops. This study is interested in bringing rambutan to ferment alcohol in order to use in the future, because rambutan has a natural sugar content up to 18-20%. This sugar can be the good nutrition for fermented yeast.

2. Material and Method

The studies employed unconsumed rambutan quality in Rongrain variety; fermented with 4 yeast strains in 14 days and evaluated alcohol content in percent (w/v)

2.1. Yeast strains for fermentation

Yeast for fermentation design to 3 conditions 10 treatments

1. Single strain as:
   S.cerevisiae TISTR 5194
   S.cerevisiae TISTR 5094
   S.cerevisiae TISTR 5596
   S.cerevisiae TISTR 5020

2. Mixed 2 strains as:
   S.cerevisiae TISTR 5596+TISTR 5194
   S.cerevisiae TISTR 5094+TISTR 5020
   S.cerevisiae TISTR 5094+TISTR 5596

3. Mixed 3 strains as:
   S.cerevisiae TISTR 5094+TISTR 5020+TISTR 5194
   S.cerevisiae TISTR 5020+TISTR 5194+TISTR 5596
   S.cerevisiae TISTR 5596+TISTR 5194+TISTR 5094

2.2. Rambutan preparation

A low quality rambutan was used; peeled and removed seeds. Then, it was crushing and grinding, the natural sweetness of rambutan is around 18%. The pH was also adjust to 4.0 by citric acid, and split the rambutan into 10 treatments.

2.3. Starter preparation

The 4 yeast strains were from National Center for Genetic Engineering and Biotechnology. Those strains were dried yeast, and prepared by growing them in YPD broth 48 hours, then used 5 ml of broth to rambutan juice 100 ml to make a starter. Each starter used for fermentation 5% of juice sample.

2.4. Fermentation method

Each treatment added 5% starter and fermented under anaerobic condition, then evaluated alcohol content by Ebulliometer every day until 14 days.

3. Results

The results of the study unveiled 3 parts of alcohol content from 3 groups of yeast strains. The detail of each result showed as below:

3.1. Alcohol content from single yeast

Table 1 showed that daily alcohol content was likely to increase every day (Fig. 1), which was correlated with the amount of total dissolved solids decreasing from the first day. When brix decreases, more alcohol is produced. The highest alcohol content was TISTR 5020 at 11.4% alcohol (W/V) on day 11, followed by TISTR 5596 at 10.8% alcohol (W/V) on day 8 ($p < 0.05$). Trends of alcohol are slightly increased with time.

Table 1. Alcohol Percentrate (w/v) from Single yeast strain.

Single Srain	Fermentation Day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	3.1^{b}	6.9^{b}	8.5^{b}	9.0^{bc}	9.0^{bc}	9.0^{b}	9.0^{b}	9.3^{a}	9.4^{b}	9.3^{a}	9.4^{a}	9.2^{a}	9.0^{a}	9.0^{a}
2	4.1^{c}	9.3^{c}	8.7^{bc}	8.7^{b}	8.7^{b}	8.8^{b}	9.3^{b}	9.0^{a}	8.7^{a}	9.0^{a}	9.4^{a}	9.3^{a}	8.7^{a}	9.0^{a}
3	4.6^{c}	9.0^{c}	9.4^{c}	9.6^{c}	9.6^{c}	9.3^{b}	10.5^{c}	10.8^{b}	10.5^{c}	10.6^{b}	10.2^{b}	10.2^{b}	10.4^{b}	10.2^{b}
4	2.5^{a}	4.6^{a}	4.6^{a}	4.1^{a}	4.3^{a}	4.3^{a}	4.3^{a}	10.8^{b}	10.8^{c}	10.5^{b}	11.4^{c}	10.6^{b}	10.5^{b}	10.8^{b}

*Different letters in vertical means different significant ($p < 0.05$)

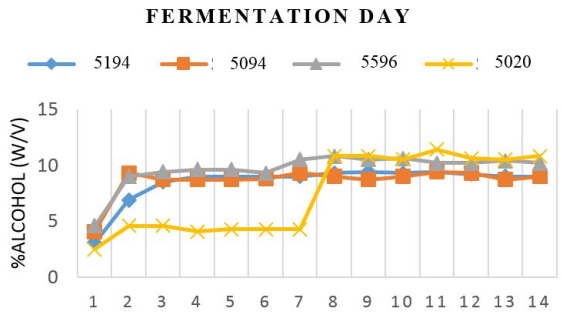

Figure 1: The percentage of alcohol (%W/V) from single yeast strain.

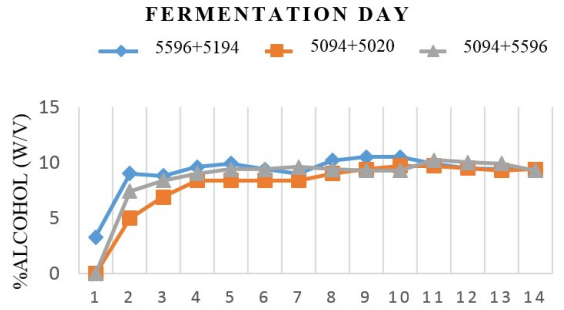

Figure 2: The percentage of alcohol (%W/V) from mixed 2 yeast strains.

3.2. Alcohol content from mixed 2 yeast strains

Daily alcohol intake was likely to increase every day (Fig. 2), which was correlated with the amount of total dissolved solids decreasing from the first day. When brix decreases, more alcohol is produced. For the use of two yeasts, the highest alcohol contents were yeast TISTR5596 + TISTR 5194 at 10.5% alcohol (W/V) on day 9, followed by TISTR 5094 + TISTR 5596 at 10.2% alcohol (W/V) on day 11 ($p < 0.05$). Trends of alcohol are slightly increased with time.

3.3. Alcohol content from mixed 3 yeast strains

Mixed 3 yeast strains, daily alcohol intake was likely to increase day by day (Fig. 3), which was correlated with the amount of total dissolved solids decreasing. When brix decreases, more alcohol is produced. The highest alcohol contents were TISTR 5596 + TISTR 5194 + TISTR 5094 at 11% alcohol (W/V) on day 14 followed by TISTR 5094 + TISTR 5020 + TISTR 5194 at alcohol content of 10.8. %(W/V) on day 4 ($p < 0.05$). Trends of alcohol are slightly increased with time as other yeast.

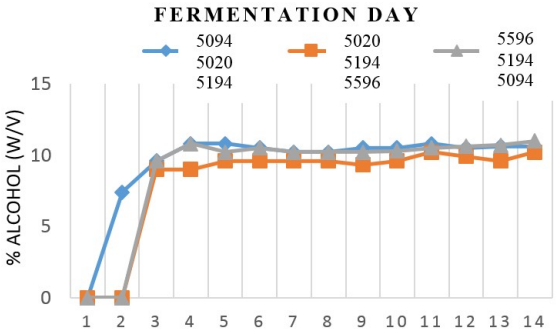

Figure 3: The percentage of alcohol (%W/V) from mixed 3 yeast strains.

4. Conclusion

All strains of *Saccharomyces cerevisiae* were significantly different in alcohol fermentation. Hence, in the future for simplicity of yeast preparation, TISTR 5020 can be used as a starter for fermentation because alcohol percentage is higher than other strains in a same fermentation day.

Low quality rambutans with 18-20% content of sugar could provide the best result of alcohol production at 11.4% on day 11 after fermentation from *S. cerevisiae* TISTR 5020. Our finding suggested that when the price of the rambutan is decreased from the market owing to its low quality after harvesting, it can be used as a source of alcohol production to produce vinegar, liquor or ethanol in the future.

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