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# Amberlyst™-15 Catalyzed Hydrolysis of Mango Peel Waste for Bioethanol Production: HPLC Glucose Quantification and GC-FID Analysis of Ethanol Pre- and Post-Distillation

Nur Sahira Adila Che Marzuki[1,2], Mohd Hafiz Abu Hassan[1] and Susi Nurul Khalifah[3]

[1] Faculty of Science and Technology, Universiti Sains Islam Malaysia, 71800 Nilai, Negeri Sembilan, Malaysia.

[2] Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia.

[3] Chemistry Study Program, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University, Malang (65144), Indonesia.

*Correspondence should be addressed to:*
*Mohd Hafiz Abu Hassan; mhafiz.a.h@usim.edu.my*

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***Abstract*— Mango peel waste appears to be an alternative source to lignocellulose materials to produce bioethanol. According to various studies, mango peels are rich in carbohydrates and sugar, which are suitable for generating bioethanol. Today, gasoline, a non-renewable fuel, is massively utilized as a transport fuel. Accordingly, a large amount of bioethanol will have to be generated to replace gasoline. The objectives of this research are to produce bioethanol from mango peel using Amberlyst-15 at various concentrations, characterize the bioethanol, and compare its concentration before and after distillation. For the first time, Amberlyst-15 has been explored as a potential catalyst for hydrolyzing carbohydrates in mango peel to monomeric sugar before the fermentation process. One crucial parameter, which was catalyst concentration (2–4%, w/v), was studied for process optimization. In particular, optimum glucose yield of 23.03 ± 3.64% (High Performance Liquid Chromatography (HPLC)) and 24.64 ± 0% (Blood Glucose Meter (BGM)) was attained based on the following optimum condition: catalyst concentration of 4% (w/v). Meanwhile, the bioethanol was not detected by Gas Chromatography Flame Ionization Detector (GC-FID) after fermentation. However, after distillation, the fermented sample yielded a bioethanol concentration of 4.152 g/L. Overall, the strategy of combining heterogeneous-catalytic hydrolysis and fermentation with Saccharomyces cerevisiae has been a good strategy for producing bioethanol from mango peel biomass.**

***Keywords*—Mango peel; Bioethanol, Amberlyst-15; Glucose, Hydrolysis**

## I. INTRODUCTION

Every year, over 4 billion tons of food are generated globally, yet it is believed that 1.2 to 2 billion tons of food produced are not consumed by humans [1]. Globally, food waste, especially fruit waste, has become a growing concern, with an estimated 492 million tons of fruit, including vegetables, being wasted annually. Notably, food waste contains more than 50% carbohydrate, which can be utilized as a feedstock to produce second-generation bioethanol. Therefore, if a substantial amount of this food waste is utilized in the production of bioethanol, it can reduce waste. This, in turn, improves the country's economy and preserves the environment from pollution [2,3,4].

After bananas, watermelons, apples, oranges, and grapes,

mango is one of the most widely cultivated fruits worldwide [5]. The substantial amount of its production will increase the residual level of mango peels, seeds, leaves, and kernels, which can cause various environmental issues in our society [6]. Following this, a significant proportion of waste ends up in drainage systems or open landfills, posing a threat to water and soil surface quality and increasing the risk of flooding. Over time, it will eventually become a breeding place for disease-carrying pests. Additionally, this excess waste will lead to air pollution or unpleasant odors [7].

Fruit waste, especially mango peel, is commonly obtained as a leftover from restaurants, hotels, juice stalls, and juice processing plants. If these wastes are not utilized, converted into usable products, or disposed of correctly, they can cause significant environmental problems. To address this issue, many researchers have extracted bioethanol from mango peel, which can help mitigate excess waste and increase the use of renewable fuels for transportation, such as bioethanol [8,9].

Ethyl alcohol ($C_2H_5OH$), also known as bioethanol, is produced from the fermentation of monomeric sugar such as fructose, glucose, and sucrose, obtained from food or fruit waste and plant sources. In addition, bioethanol is a colorless liquid that is biodegradable, low in toxicity, and less polluting if spilled [7]. Moreover, according to Bušić et al. [10], bioethanol is a fuel with a high octane number, which significantly differs from gasoline in terms of its physicochemical properties.

In modern industry, bioethanol is widely regarded as a renewable fuel that can contribute to reducing greenhouse gas emissions and lowering reliance on fossil resources. It is compatible with internal combustion engine vehicles and can be blended with gasoline at different concentrations without major modifications. For instance, research using a Worldwide Harmonized Light Vehicles Test Cycle (WLTC) on a hybrid Toyota Prius has demonstrated that higher bioethanol blends improve brake thermal efficiency and reduce $CO_2$ and pollutant emissions. The study further revealed that the engine control unit adapts ignition timing as the bioethanol concentration rises. This indicates that blends of bioethanol with gasoline can enhance energy efficiency and environmental performance in existing vehicle technologies [11]. Generally, no engine adjustments are required to use the gasoline/ethanol blend with such a low ethanol content. Conventional SI engines, on the other hand, require general adjustments to function well at greater ethanol concentrations in a gasoline/ethanol mixture. For instance, 85 vol% ethanol in gasoline (E85), which has been available in Brazil since 2003, cannot be utilized in normal SI engines and must instead be used in Flexible Fuel Vehicles (FFVs). Recently, 90% of new cars sold in Brazil are FFVs, while approximately 8 million vehicles in the United States, including passenger vans, cars, and pickup trucks, are designed with flexible-fuel engines that can run on E85 [12].

The mango peel sample must undergo four main procedures to obtain a high concentration of bioethanol: pretreatment, hydrolysis, fermentation, and distillation [7]. Accordingly, the pretreatment process enhances the accessibility of lignocellulosic polysaccharides by disrupting lignin structures, thereby making them more susceptible to enzymatic or chemical hydrolysis [13]. Note that the purpose of the hydrolysis or saccharification process is to further break down the polysaccharide observed in the pretreated lignocellulosic of mango peel biomass into some disaccharides and monosaccharide subunits. This includes fructose, glucose, sucrose, and other sugars. Figure 1 illustrates the process of converting lignocellulosic polysaccharides into monomeric sugars, which involves two steps: pretreatment and hydrolysis.

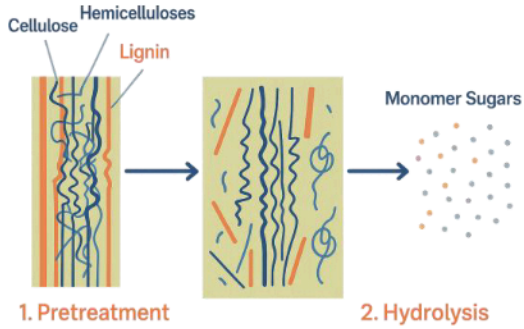


Figure 1. Illustration of pretreatment and hydrolysis processes

Monosaccharides, or monomeric sugars that will be produced after hydrolysis, will improve the fermentation process using Saccharomyces cerevisiae [14]. Reaction 1 below illustrates the chemical reaction that is involved in this process [15]:

$$(C_6H_{10}C_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6 \quad (1)$$

Until now, homogeneous acid catalysts and enzymes have been utilized to convert polysaccharides (hemicellulose and cellulose) from various biomass sources. This includes mango peel, to fermentable sugars in the hydrolysis process. However, the enzyme catalytic technique has several drawbacks, including the difficulty of separating the enzyme from the products and the lengthy hydrolysis process, which increases production costs. Furthermore, a study that employed sulphuric acid as a catalyst to produce bioethanol from Kappaphycus alvarezii (cottonii) discovered that the homogeneous acid hydrolysis technique can also cause various problems. This includes the production of a high number of hazardous chemicals, and the catalyst is not recoverable for future use. Therefore, to reduce production costs and protect the environment from chemical hazards, heterogeneous acid catalysts such as Amberlyst-15 can be utilized in hydrolysis processes [16,17]. Recently, the utilization of several heterogeneous acid catalysts for the hydrolysis of polysaccharides has been described in various studies aimed at determining their activity.

This study focused on the hydrolysis process of pretreated mango peel into fermentable sugars using Amberlyst-15, a novel catalyst applied to raw mango peel. Process variables, such as the concentration of catalyst, as studied by Tan et al., were varied to achieve the maximum sugar yield [17]. Following this, simple sugars were further explored to produce bioethanol.

The usage of a catalyst is vital since the structure of cellulose and hemicellulose is tightly bound to the component of lignin by hydrogen bonding and covalent bonding, respectively. Due to this, the composition is hard and rigid. Cellulose is a

homopolymer consisting of glucose monomers that are connected by β-4 and β-1 glycosidic bonds. It is a polymer with a crystalline and linear structure that is hard to hydrolyze. Additionally, it is surrounded by lignin and hemicellulose, which further restrict its hydrolysis. In the production of bioethanol, first, lignin is eliminated in the process of pretreatment. Subsequently, the catalyst breaks the β-1,4-glycosidic bonds, causing cellulose polymers to be hydrolyzed and producing the sugar glucose. Notably, hemicellulose structure is easier to break than cellulose due to its amorphous properties and branched structure. In summary, a catalyst can efficiently hydrolyze glycosidic bonds in cellulose and break the linkages of hemicellulose-lignin [18,19,20]. The influence of the catalyst has been well-documented in previous studies, which highlight its significant effect on the hydrolysis behavior of lignocellulosic biomass and subsequent ethanol production [21,22].

Tan et al. [17] and Kuznetsov et al. [23] have utilized Amberlyst-15 as a catalyst in the hydrolysis process to break down a polysaccharide to a monosaccharide. According to Tan et al. [17] and Pal [24], Amberlyst-15 is a brown-grey solid with the properties listed in Table I.

Table I. Properties of Amberlyst-15 Catalyst

| **Property** | **Amberlyst-15** |
|---|---|
| Particle size (μm) | 600-800 |
| Surface area ($m^2/g$) | 34.85 |
| Capacity (meq/gm) | 4.20 |
| Average pore diameter (Å) | 260 |
| Supplier | Sigma-Aldrich |

Figure 2 displays the structure of Amberlyst-15. It has a macroreticular polystyrene-based ion exchange resin containing an acidic sulfonic group. As a result, it is a great source of strong acid, which has been applied in a wide range of acid-catalytic processes.

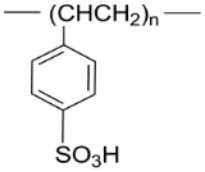


Figure 2. Structure of Amberlyst-15

In addition, Sergi Maicas and Mateo [25] stated that various genera of yeast can synthesize ethanol during the fermentation process, especially Schizosaccharomyces, S. cerevisiae (Baker's yeast), and Pichia. S. cerevisiae is the most widely utilized yeast for commercial bioethanol production. This is mainly due to its rapid growth, high ethanol yield, and resistance to a variety of environmental stresses, including high ethanol concentrations, osmotic pressure, low pH, and temperature variations. Furthermore, its ability to ferment sugars effectively under anaerobic circumstances, along with its 'Generally Recognized as Safe' (GRAS) designation, makes it an excellent biocatalyst for large-scale applications. In industrial applications, S. cerevisiae may reach ethanol yields above 90% of the theoretical maximum, about 0.51 g of ethanol per gram of glucose consumed. Even slight increases in ethanol productivity can lead to substantial economic advantages at the industrial level [26]. In addition, the enzymes invertase and zymase, which are naturally present in this yeast, play a crucial role in converting monosaccharides and some disaccharides generated from hydrolyzed biomass into ethanol [27,28]. According to Sokan-Adeaga et al. [29], Reaction 2 can be used to present the reaction involved in the fermentation process of glucose.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (2)$$

## II. THE MATERIAL AND METHOD

### A. Materials

Chemicals used in this study were Amberlyst-15 and S. cerevisiae. Calcium Oxide (CaO), 2-Pentanone, Potassium Dihydrogen Phosphate (KH2PO4), standard glucose, and ethanol (99%), which were obtained from Sigma Aldrich.

### B. Methods

Figure 3 illustrates the overall process flowchart of the experiment for this study.

This study was conducted through the following six main stages:

*1) Preparation of dried mango peel*: The first step involved rinsing the peels with deionized water to eliminate physically adsorbed impurities, followed by drying them in a hot air oven at 105°C to 110°C to a constant weight [30]. Dried mango peel was selected as the substrate in this experiment due to its reduced moisture content, which allows it to be ground into smaller particles. This, in turn, increases the specific surface area and enhances the efficiency of enzymatic hydrolysis [31].

*2) Physical pre-treatment*: The dry substrate was converted into powder using an electrical grinder and filtered at 40 mesh (0.420 mm). The peels were then placed in individual beakers and wrapped with aluminum foil until further use. Subsequently, the samples were autoclaved for 15 minutes at 121°C using high-pressure steam (15 psi).

*3) Hydrolysis*: At a constant reaction time (1.5 hours) and biomass loading (solid/liquid ratio: 12.5%, w/v), the heterogeneous-catalyzed hydrolysis process was studied by varying one process parameter: the concentration of Amberlyst-15 (2%, 3%, 4%, w/v). This is due to the findings reported by Tan et al. [17], which identified that 4% Amberlyst-15, a reaction time of 1.5 hours, and 12.5% (w/v) of biomass loading as the optimal conditions for hydrolysis of E. cottonii extract. However, this study does not utilize a 5% catalyst, as Tan et al. noted that increasing catalyst loading beyond 4% (w/v) led to a decrease in sugar yield. Additionally, the reaction demonstrates a negative sugar output rate, indicating degradation. This degradation is likely due to the higher acid concentration, which can break down sugar compounds and generate more by-product inhibitors, such as 5-hydroxy-methyl-furfural and organic acids. Their study also revealed that no sugars were produced without a catalyst, and sugar production began when a 2% (w/v) catalyst was used. This implies that Amberlyst-15 facilitates the conversion of the polysaccharide into glucose [17]. Therefore, this study focused on investigating Amberlyst-15 concentrations in the range of 2% to 4% (w/v).

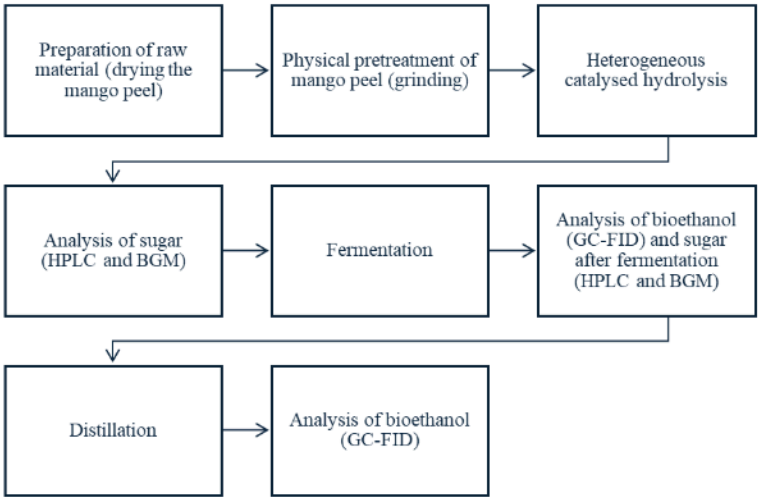


Figure 3. Overall Process Flowchart

The dried mango peel powder (12.5 g) was added to three different 250 ml volumetric flasks. Correspondingly, they were mixed with 100 ml of distilled water to form 12.5% solid/liquid ratios. Various amounts of Amberlyst-15 (2 g, 3 g, and 4 g) were added to each mixture and incubated in the autoclave reactor at a constant temperature (120°C) for 1.5 hours. For all trials, the autoclave's internal pressure and the stirring speed were kept constant at 10 bars and 370 rpm, respectively. Following this, the samples were cooled to room temperature after a specific time of hydrolysis. The residue was separated from the hydrolysate using a Buchner filter. Next, an Agilent High Performance Liquid Chromatography (HPLC) was employed to analyze the hydrolysate containing sugar.

*4) Fermentation*: S. cerevisiae (5 g) was added to each hydrolysate prior to being swirled with a shaking incubator [7]. The hydrolysates were then added to a basal medium containing 0.175% (w/v) or 0.175 g KH2PO4 in 100 ml at pH 5. In a 250 ml Erlenmeyer flask, the hydrolysate-to-basal medium volumetric ratio was set at 1:2. Next, the mixture was sterilized in an autoclave reactor at 121°C for 15 minutes before being incubated for six days at 34°C with 135 rpm in a shaking incubator. Before 1.5 ml of the fermented sample was analyzed using Agilent Gas Chromatography Flame Ionization Detector (GC-FID), it was centrifuged for 10 minutes at 10,000 g and 4°C. Lastly, the supernatant was analyzed for bioethanol concentration and glucose residues after centrifugation [17].

*5) Distillation*: This technique was designed to increase the purity of bioethanol. Ethanol was separated from the water using a simple distillation process based on their respective boiling points. To complete this procedure, the fermented solution was placed in a flask and heated for 3 hours at a constant temperature of 78°C to 88°C using a heating mantle until bioethanol stopped dripping. Next, the parameter that resulted in the highest bioethanol concentration was replicated, but with the addition of 5 g of CaO as a drying agent throughout the distillation process. Notably, CaO functions as an adsorbent in the adsorption distillation process, which is used to purify ethanol. In particular, the role of CaO is to enhance ethanol concentration by absorbing water from the mixture through hydration to Calcium Hydroxide (Ca(OH)2) [32]. The bioethanol concentration obtained in this process was analyzed using a GC-FID instrument and then compared to the concentration of the highest bioethanol sample, which had not been added with CaO.

*6) Additional method*: Sugar content in both hydrolysate and fermentation samples was quantified using an HPLC system from Agilent Technologies, equipped with a column and UV detector. A glucose calibration curve was established from the HPLC analysis, and sugar yield was calculated using Equation 1 based on these results. In addition, the Sinocare Blood Glucose Meter (BGM) Monitor was utilized as a supplementary tool to provide quick and cost-effective monitoring of glucose levels (mmol/L) in the same samples, with all measurements performed in triplicate. Ethanol concentration after fermentation was analyzed using an Agilent Gas Chromatograph (GC) equipped with a Flame Ionization Detector (FID).

$$\frac{\text{Concentration (g/l) of sugar at time of t}}{\text{Initial concentration (g/l) of substrate}} \times 100 \quad (1)$$

## III. RESULTS AND DISCUSSION

### *A. Effect of Catalyst Concentration*

The effect of catalyst concentration was studied to identify which parameter produces the highest yield of sugar after hydrolysis. Firstly, Table II summarizes the details of the results from sugar analysis, including the uncertainty or estimated error in the glucose yield measurements when using two distinct instruments (HPLC and BGM).

A Confidence Interval (CI), also known as an uncertainty measurement, is a range with an upper and lower number calculated from a sample where the true value is unknown. In

this work, a 95% CI was considered. Although one might also measure CI of 90% or 99%, in this study, we focused entirely on CI of 95% since it is the most commonly used [33].

Using HPLC, the results in Table II indicate that the highest glucose was released from mango peel with a mean value of 23.03 ± 3.64%, produced at a 4% (w/v) catalyst concentration. Furthermore, the high sugar yield was influenced by the high loading of Amberlyst-15, which accelerated the hydrolysis rate of mango peel. This can be explained by the high number of available active sites as well as surface area for the catalyst [17,24]. Meanwhile, for the hydrolysis that was conducted with less catalyst concentration of 2% (w/v) and 3% (w/v), the mango peel released almost the same and low yield of glucose, which were 6.88 ± 0.68% and 5.94 ± 0.43%, respectively. This suggests that the catalyst was crucial for hydrolyzing the polysaccharide into monomeric sugar [17]. Based on Table II, BGM also presented the highest sugar yield as HPLC at a 4% (w/v) of catalyst concentration. However, with a different yield, which was 24.64 ± 0%.

Table II. The Results of Sugar Analysis at Various Catalyst Concentration When Using HPLC And BGM

| Instrument | Concentration of Catalyst (%, w/v) | Exp. | Sugar Yield (%) | Mean Sugar Yield (%) | Standard Deviation | 95% Confidence Interval |
|---|---|---|---|---|---|---|
| HPLC | 2 | 1 | 6.30 | 6.88 | 0.68 | 0.77 |
| | | 2 | 7.63 | | | |
| | | 3 | 6.70 | | | |
| | 3 | 1 | 6.42 | 5.94 | 0.43 | 0.49 |
| | | 2 | 5.58 | | | |
| | | z | 5.82 | | | |
| | 4 | 1 | 19.02 | 23.03 | 3.64 | 4.12 |
| | | 2 | 23.97 | | | |
| | | 3 | 26.11 | | | |
| BGM | 2 | 1 | 7.35 | 7.40 | 0.08 | 0.09 |
| | | 2 | 7.49 | | | |
| | | 3 | 7.35 | | | |
| | 3 | 1 | 8.50 | 8.70 | 0.17 | 0.19 |
| | | 2 | 8.79 | | | |
| | | 3 | 8.79 | | | |
| | 4 | 1 | 24.64 | 24.64 | 0.00 | 0.00 |
| | | 2 | 24.64 | | | |
| | | 3 | 24.64 | | | |

Next, HPLC and BGM results presented different trends, as displayed in Figure 4. HPLC demonstrated a decrease in sugar yield (from 6.88 ± 0.68% to 5.94 ± 0.43%) while BGM was increased (from 7.40 ± 0.08% to 8.70 ± 0.17%) when using a catalyst from 2% (w/v) to 3% (w/v). Based on Figure 4, the upward and downward trends of glucose yields are more clearly summarized in Table II.

To clarify, HPLC is commonly utilized for sugar analysis due to its simplicity, specificity, and ability to accurately separate and quantify sugars. Although Refractive Index (RI) detection is the most generally used approach for simple sugars, it has several disadvantages. This includes low sensitivity, limited selectivity, and vulnerability to solvent composition changes. In comparison, the BGM approach, which is a simple method, has even lower accuracy due to poor sensitivity [34,35]. Although HPLC provided the most accurate results, the samples in this study were stored at room temperature for three weeks before analysis. Thus, this storage period likely contributed to a slight deterioration or structural changes in the

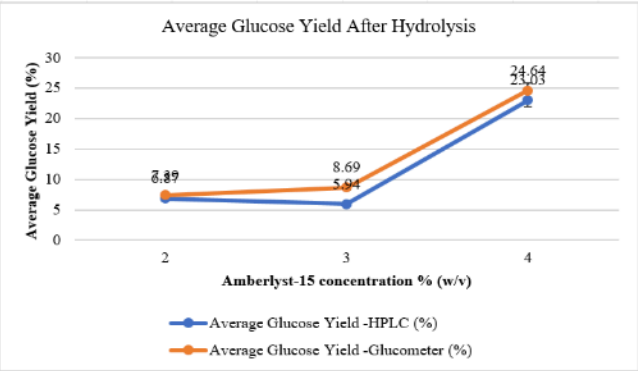


Figure 4. Glucose yield after hydrolysis at various catalyst concentration. Error bar indicated the 95% confidence interval

hydrolyzed sugars, as prolonged exposure to room temperature has been demonstrated to affect sugar stability. Bishnoi et al. [36] reported in their study that the sugar content of fruit pulp decreased after the 12th day of storage at room temperature. However, it was not affected if stored at low temperature (4-15°C). This may explain the downward trend observed in the HPLC graph in Figure 4 for 2% (w/v) to 3% (w/v) catalyst concentration.

### *B. Comparison of sugar concentration post-hydrolysis versus post-fermentation.*

The sugar concentrations in the samples were analyzed using HPLC before and after the fermentation process. Figure 5 presents the HPLC profile of glucose after the hydrolysis process using 4% (w/v) Amberlyst-15, implying a well-resolved glucose peak. Moreover, the results indicate that glucose was present in high concentrations after hydrolysis, confirming the effectiveness of the hydrolysis process in breaking down polysaccharides into simple sugars. In contrast, Figure 6 illustrates the HPLC profile after the fermentation process. The absence of a glucose peak in the chromatogram suggests that all the glucose was consumed during fermentation. This observation is consistent with a significant reduction in sugar content in all samples, indicating that glucose, along with sucrose and fructose, was converted into bioethanol during fermentation [37,38]. Similarly, these findings are consistent with those reported by Pollon et al. [39], who observed that wine fermentation led to nearly complete sugar consumption, leaving no residual fermentable sugars. This is further supported in the present study by the absence of sugar peaks in Figure 6, indicating that glucose was effectively converted into bioethanol.

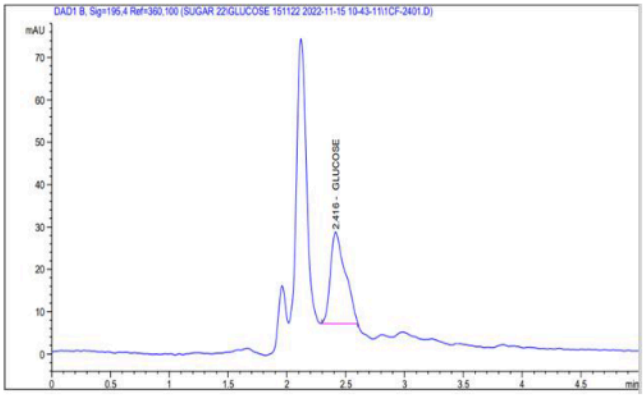

Figure 5. HPLC profile of glucose after hydrolysis process using 4% (w/v) Amberlyst-15

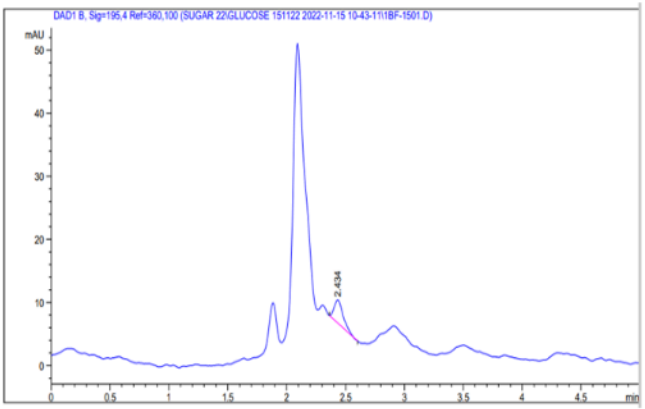

Figure 6. HPLC profile of glucose after fermentation process using 4% (w/v) Amberlyst-15

### *C. Comparison of bioethanol concentration pre- and post-distillation.*

Firstly, the bioethanol concentration before distillation is indicated by the concentration of bioethanol after fermentation. Chromatograms in Figure 7 and Table III suggested that bioethanol in all samples was not detected by GC-FID after six days of fermentation. Still, the glucose concentration decreased (Figure 6), which indicated that the glucose was converted to ethanol. Based on the calibration curve, the bioethanol produced after fermentation might be very low. Rusli [40] noted in his study that low ethanol concentration was not detected by GC-FID. Instead, UV-Vis detected the small concentration of ethanol in the same sample, which was 11.8 mg/L. Due to the high volatility of ethanol, it was challenging to determine its concentration in a small volume (0.2 µL) in the GC-FID column. Another factor that might affect the results was air interference during the injection of samples into the injection port. Fortunately, after distillation, the chromatograms obtained from GC-FID confirmed the presence of bioethanol in all samples, as portrayed in Figure 8. Essentially, the concentration of bioethanol in the three samples increased after distillation due to the effective separation of ethanol from water and other by-products in the fermentation mixture, and the results are recorded in Table III [41]. In particular, the maximum bioethanol concentration of 4.152 g/L achieved with the highest catalyst concentration (4% (w/v)) was comparable to the 4.2 g/L reported by Somda et al., who also used the strain S. cerevisiae, though they employed a specialized strain, B1 [42].

Table III. Bioethanol Concentration Before And After Distillation

| **Amberlyst-15 Concentration % (w/v)** | **Bioethanol Concentration Before Distillation (g/L)** | **Bioethanol Concentration After Distillation (g/L)** |
|---|---|---|
| **2** | Not detected | 2.163 |
| **3** | Not detected | 3.697 |
| **4** | Not detected | 4.152 |

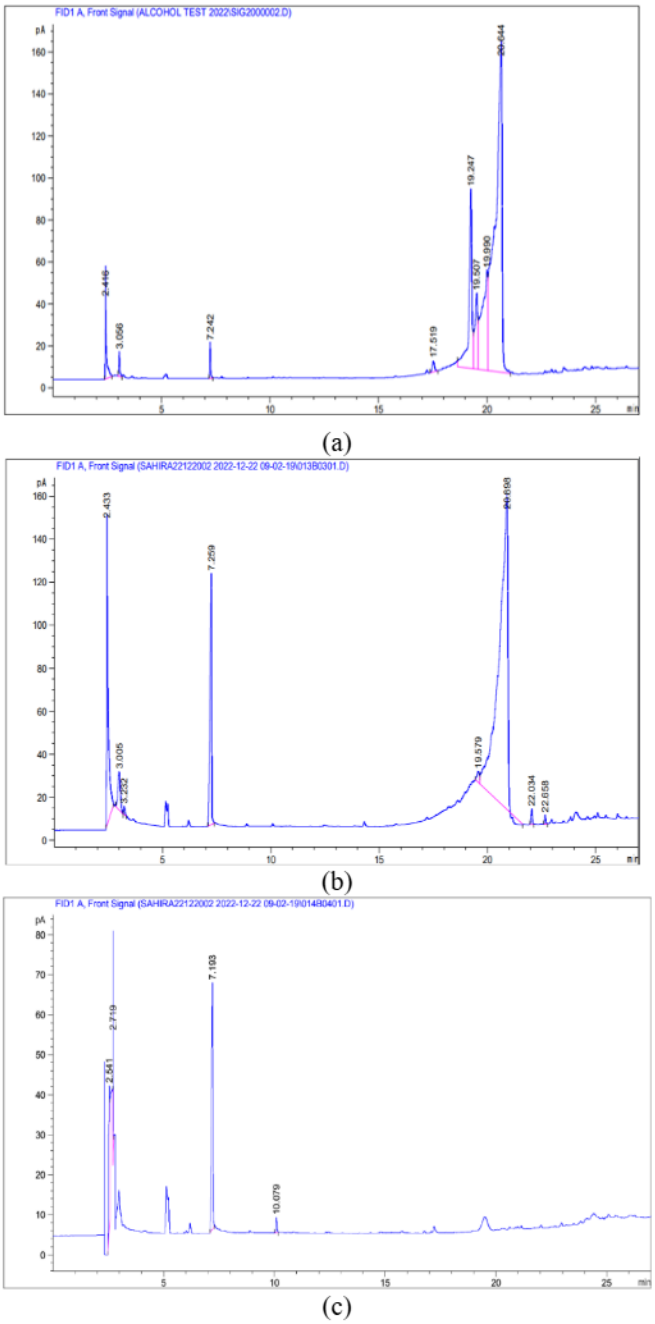


Figure 7. GC-FID chromatogram of ethanol obtained after fermentation from various catalyst concentration: a) 2% (w/v) Amberlyst-15 b) 3% (w/v) Amberlyst- 15 c) 4% (w/v) Amberlyst-15.

The result obtained by 2% (w/v) Amberlyst-15 produced the least bioethanol concentration (2.163 g/L) after distillation with a retention time of 3.656 minute, followed by 3% (w/v) Amberlyst-15 (3.697 g/L) with a retention time of 3.626 minute. Lastly, 4% (w/v) of Amberlyst-15, which produced the highest glucose concentration during hydrolysis, with values of 23.03 ± 3.64% (HPLC) and 24.64 ± 0% (BGM). It also yielded a bioethanol concentration of 4.152 g/L after distillation with a retention time of 3.624 minute. In other words, the highest Amberlyst-15 concentration produced the highest sugar yield after hydrolysis of mango peel and the highest bioethanol concentration after distillation. These findings indicate that the rate of ethanol production increased with higher glucose yields, as greater availability of glucose molecules enhances the rate of bioethanol conversion within the sample [43]. Flores et al. [44] revealed this in their study when the average rate of ethanol production increased from 0.00135 ppm/min to 0.00420 ppm/min as 0.0 M and 0.15 M glucose concentrations were added to the solution, respectively. Although their study focused on glucose concentration, the trend supports the idea that higher glucose levels, whether measured as concentration or yield, promote greater ethanol conversion efficiency.

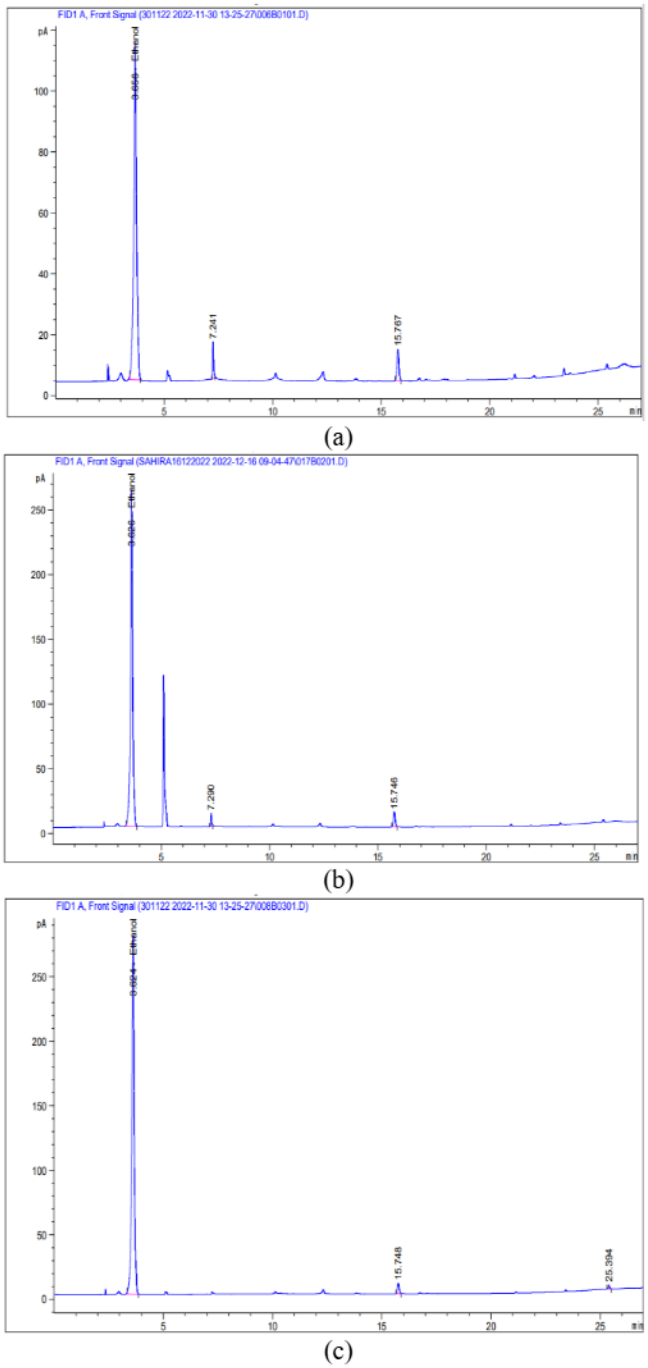


Figure 8. GC-FID chromatogram of ethanol obtained after distillation from various catalyst concentration: a) 2% (w/v) Amberlyst-15 b) 3% (w/v) Amberlyst-15 c) 4% (w/v) Amberlyst-15.

The increase in glucose yield is also consistent with the findings of Andary et al., who indicated that a higher catalyst concentration enhances sugar yield, with an observed increase of up to 0.06% [45]. The increase in bioethanol concentration with higher Amberlyst-15 catalyst concentrations can be attributed to more effective hydrolysis of carbohydrates into sugar. Accordingly, higher enzyme loads provide more active sites to catalyze the breakdown of cellulose, enhancing sugar yield and, subsequently, ethanol production [46].

### *D. Effect of Calcium Oxide Addition during Distillation*

Based on the results above, the sample that used 4% (w/v) Amberlyst-15 as the catalyst in the hydrolysis process produced the highest sugar yield (23.03 ± 3.64% by HPLC and 24.64 ± 0% by BGM) and the highest bioethanol concentration (4.152 g/L). Therefore, it was replicated with the addition of CaO during distillation to enhance the ethanol purity. Unfortunately, the ethanol was not detected by GC-FID after fermentation and distillation, although the sugar yield after hydrolysis was high. Their chromatogram, generated by GC-FID, is displayed in Figure 9.

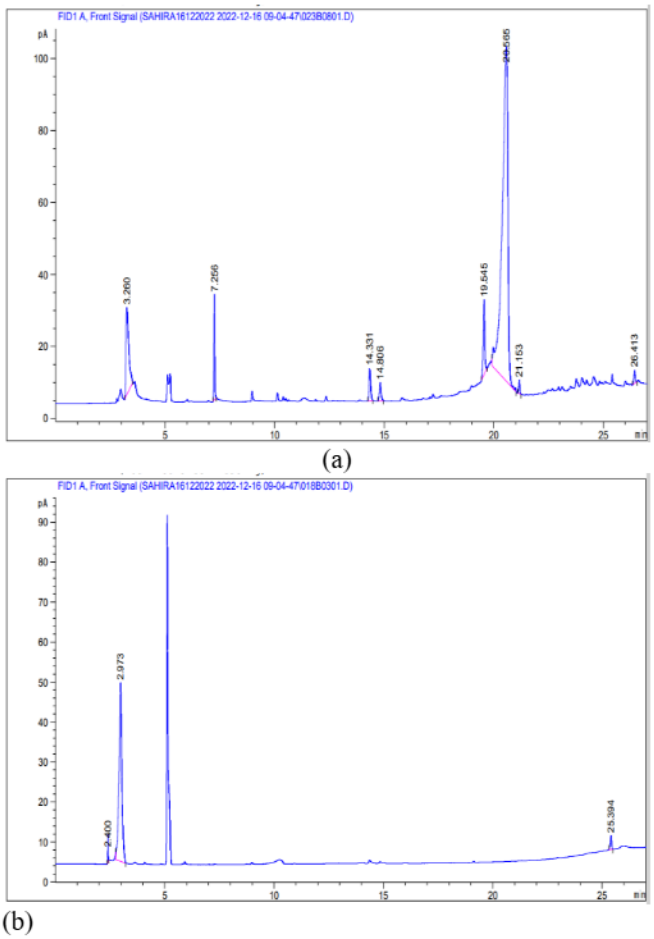


Figure 9. GC-FID profile of ethanol absence; (a) after fermentation; (b) after distillation

These might result from the formation of toxic chemicals or secondary metabolites such as furans, organic acids, and phenolic compounds, generated during the fermentation. These compounds might have triggered the death phase of S. cerevisiae at an early stage of the microbial's growth [26]. Knutsen et al. [47] stated in their study that thermally treated fruit juices can produce furan when stored at 35°C, whereas fermentation in this experiment was taking place at 34°C. At the same time, Andary et al. [48] discovered that furans remained stable in the presence of sugar at the pH values of 0.2, 4, 6, and 10. Since pH and surrounding temperature were not controlled in this experiment, it was expected that furan was generated from sugar and might have been the factor preventing fermentation in the replicated sample. To clarify further, the glucose yield after hydrolysis was high (24.6 ± 0.25%). However, after fermentation, it only decreased to 23.97 ± 0.25% which proved that glucose was not converted into ethanol. In addition, the glucose yield in this step was only determined using BGM as an alternative to HPLC analysis, and the results are provided in Table IV.

Table IV. Sugar Yield And Bioethanol Concentration Of Replicated Sample In Optimum Condition

| **Sugar Yield (%)** | | **Bioethanol Concentration (g/L)** | |
|---|---|---|---|
| **After Hydrolysis** | **After Fermentation** | **After Fermentation** | **After Distillation** |
| 24.60 ± 0.25 | 23.97 ± 0.25 | Not detected | Not detected |

## IV. CONCLUSIONS

This study successfully converted mango peel waste into bioethanol, helping to reduce waste disposal and proving that mango peel is a potential feedstock for bioethanol production. Amberlyst-15, which was a heterogeneous catalyst, can work efficiently as a solid acid catalyst during the sugar hydrolysis process.

This study indicated that the concentrations of Amberlyst-15 can significantly affect the release of fermentable sugars during hydrolysis and ethanol concentration after fermentation. It was proven when the yield of glucose and ethanol concentration increased with catalyst concentration for all samples. To conclude, 4% (w/v) of Amberlyst-15 was an efficient concentration to produce the highest fermentable sugars during hydrolysis, which then produced the highest bioethanol concentration after distillation. Notably, although the GC-FID did not detect ethanol after fermentation, the chromatogram clearly resolved after distillation, indicating the presence of ethanol. Correspondingly, ethanol concentration continuously increased after distillation since the water and by-product have been separated from the mixture [39]. Furthermore, the addition of CaO during distillation as a drying agent for replicated samples was able to improve the concentration of ethanol. However, due to furan formation, the ethanol was not produced whether after fermentation or distillation [46]. Lastly, this experiment did not require a large amount of ingredients, as bioethanol can be produced through the fermentation process. This simple yet effective method could also facilitate a cost-effective and energy-efficient conversion of mango peel biomass into bioethanol. Lastly, produced bioethanol can

reduce the utilization of non-renewable fuel since it can be mixed with gasoline and used as a transportation fuel.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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