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Article

Production of Sugar from Cassava Peel using Different Chemical Pre-treatment

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***Abstract*— Cassava peel has been a notable agricultural waste material to researchers because of its potential to produce sugar, a valuable product in the food, agricultural, and cosmetic industries. The peels constitute lignin, hemicellulose, and cellulose, also known as lignocellulosic biomass. Cassava peels must undergo a pre-treatment method to separate the lignocellulosic material effectively. This study aims to investigate the optimal chemical pre-treatment methods and optimal pre-treatment concentration to produce sugar from cassava peel. Cassava peels were pre-treated with sodium hydroxide, sulphuric acid, and methanol with a catalyst (organosolv). Then, enzymatic hydrolysis was performed using cellulase to hydrolyze cellulose to glucose. The glucose yield is quantified using Dinitrosalicylic Acid Assay and a portable blood glucometer. The results showed that pre-treatment using sodium hydroxide at a concentration of 0.05 M at 121°C for 15 minutes gave the highest glucose yield of 4.53±1.20 mg/ml. Glucose produced from 0.05 M sulphuric acid (H_2SO_4) and 0.2 M organosolv sodium methoxide (MeOH+NaOAc) were 3.55±0.68 mg/ml and 3.29±0.93 mg/ml, respectively. Statistical analysis showed that the effect of different pre-treatment methods and pre-treatment concentrations had a significant glucose yield ($P<0.05$). Similarly, there was a significant difference ($P<0.05$) in the glucose yield under different pre-treatment concentrations. Further study on mechanical-assisted chemical pre-treatment methods is recommended.**

***Keywords*— Cassava peel, acid and alkali pre-treatment, organosolv pre-treatment, sugar production.**

I. INTRODUCTION

Cassava (*Manihot esculenta*) is a starchy root vegetable rich in carbohydrates, resistant starch, and a good source of vitamin C. It can be grown in tropical and subtropical regions [1] and is grown in many African, Latin American, and Asian countries. In 2019, Nigeria, Thailand, and the Democratic Republic of the Congo were the countries with the highest volumes of cassava production, with 61 million, 32 million, and 32 million tonnes, respectively. Cassava may grow under harsh climatic conditions, is durable in a prolonged drought, and may withstand cultural neglect through restricting development by leaf shedding and assuming a dormant state. [1], [2] Since cassava is a cheap alternative calorie, cassava is considered one of the main staple foods in line with maize, rice, and wheat.

The most consumed part of the cassava plant is the root, which can be eaten as a whole, grated, or ground into flour. When producing cassava products, the tuber's roots are peeled to remove a thicker leathery parenchymatous inner covering

and a thin brown outer covering. Cassava peel represents 5 to 15% of the root and has a higher protein content compared to the other parts. The peels constitute lignin, hemicellulose, and cellulose, also known as lignocellulosic biomass [3]. Further process of lignocellulose from cassava peel may produce sugar, a valuable product in the food, agricultural, and cosmetic industries. Cassava peel has been a notable agricultural waste material to researchers because of its potential to produce sugar, a base material for bioethanol production [4]– [6]. In addition, cassava peel may also produce valuable products such as energy sources in animal feed [7] and water treatment [8].

Malaysia generates 168 million tonnes of biomass every year. This positions the country well to promote biomass as a source of wealth. Agro-waste makes up 15% of all garbage produced in Asia, with Malaysia producing 0.122 kg/cap/day of agricultural waste in 2009 and 0.210 kg/cap/day by 2025, respectively [9]. An average of 1.2 million tonnes of agricultural waste are disposed of in landfills every year. By implementing a 22 per cent recycling programme and an 80 per cent intermediate treatment programme that includes material recovery, composting, and waste-to-energy conversion, the Malaysian government hopes to reduce waste discharged in landfills by 40 per cent [10]. Malaysia is a relatively minor cassava producer in The Association of Southeast Asian Nations (ASEAN). However, cassava is a popular traditional food source for Malaysians. Malaysians like to consume cassava as a snack; *kerepek ubi kayu* and as a dessert; *lepat ubi kayu* and *kuih bingka ubi*. Thus, it also produces a lot of cassava waste. Hence, the production of sugar from cassava peel can be one of the alternatives to reduce agricultural waste in Malaysia.

A. Cassava Peel

Cassava is peeled off and cleansed to obtain its peel. As indicated by [11], the peels are 1 mm thick and make up 10 to 13% of the cassava root's dry matter. The brown outer part of the peel is made up of lignified cellulosic material. Therefore, cassava peel is starch-rich, making it useful in many industries. Meanwhile, the white inner part is made up of parenchymatous material and contains toxic cyanogenic glucosides. A study by [11] reported that cassava peel chemical composition consists of 4.5% ash content, 37.9% cellulose, 37.0% hemicellulose, and 7.5% lignin. This finding is in contrast with a study on the nutritional status of cassava peels by [12], where the cellulose is 5.40%, and hemicellulose is 21.65%. This variation might be because of different processing methods that enhance the nutritive value of the cassava peel and its origin [13].

B. Lignocellulosic Material

Lignocellulose is a promising source of biomass because a variety of sugar products can be extracted from it. The three components of lignocellulosic materials are hemicellulose, cellulose, and lignin. The quality and quantity of these three components differ between plants [14].

Cellulose is the most abundant component of lignocellulosic material. Unlike glucose in other glucan polymers, the cellulose chain's repeating unit is the disaccharide cellobiose which is connected by β−1,4 glycosidic linkages. It has extensive intramolecular and intermolecular hydrogen bonding networks that form microfibrils with high glucose binding strength. The cellulose microfibrils relate to hemicellulose fibres, layered by lignin molecules. Therefore, the complex structure formed is difficult to degrade.

Hemicellulose is the second most abundant component of lignocellulosic material. Both cellulose and hemicellulose are made up of sugar monomers. However, unlike cellulose, which comprises exclusively β-glucose monomers, hemicellulose comprises several monomers: galactose, xylose, mannose, arabinose, and rhamnose [15]. Figure 1 shows the structure of lignocellulosic material in biomass.

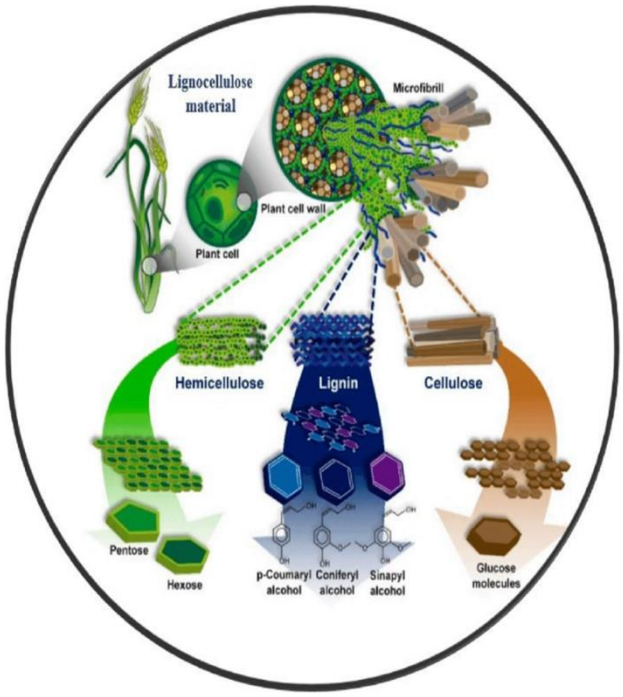

Fig. 1 Structure of lignocellulosic material [16].

Hemicellulose gives the cell wall structural strength by connecting cellulose fibres into microfibrils and cross-linking them with lignin [17]. In comparison to cellulose, hemicellulose can be degraded easily by different types of enzymes, acids, and dilute bases [16], [18].

Lignin is a three-dimensional, complex aromatic polymer. It binds cellulose and hemicellulose together and makes the structure resistant to degradation and more hydrophobic [19]. Lignin has a covalent bonding with hemicellulose, making it a complex structure called the lignin carbohydrate complex [16]. Because of its complex and irregular structure, it is the most rigid component of lignocellulose to break down [20]. Therefore, pre-treatment step is important.

C. Sugar Processing

Sugar from cassava peel can be obtained by the hydrolysis process of the lignocellulosic material. Figure 2 shows several steps involved in biomass processing into sugar. The two main steps in sugar production from lignocellulose in this study are pre-treatment and enzymatic hydrolysis.

1) Pre-treatment: Due to the lignin content that covers up the whole structure of lignocellulose and the strong hydrogen bond between cellulose and hemicellulose, a pre-treatment step is added before the cassava peel is hydrolysed. The basic purpose of any pre-treatment is to change or eliminate

compositional and structural barriers for hydrolysis and subsequent degradation processes to improve digestibility, enzyme hydrolysis rate, and yields of desired products [21].

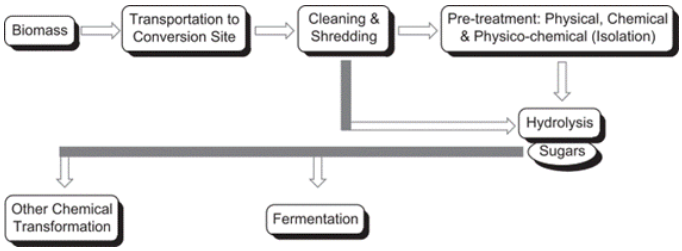

Fig. 2 Several steps involved in biomass processing into sugar [22].

Pre-treatment steps before the enzymatic hydrolysis will decrease cellulose crystallinity, remove lignin, and increase the susceptibility to enzymatic activity, thus increasing the yield of sugar produced.

There are several pre-treatment methods: chemical, physical, mechanical, biological, and physicochemical, and every pre-treatment possesses its pros and cons. [23] stated that the following criteria should be met to achieve an effective pre-treatment process: it should limit the production of inhibitors that resist carbohydrates from being hydrolysed; should preserve and de-crystallize cellulose and depolymerise hemicellulose; should require little energy and may recover value-added products such as lignin, and cost-effective. Based on the findings by [24], every single method has its advantages and drawbacks. More research is needed to find the most efficient method. Chemical pre-treatment methods are more commonly used than physical or biological pre-treatment methods because they are more efficient and enhance the breakdown of complex lignocellulosic compounds. There are three common chemical pre-treatment methods, which are alkaline pre-treatment, acid pre-treatment, and organosolv pre-treatment.

2) Alkaline Pre-treatment: In alkaline pre-treatment, the main process is the solubilisation of lignin and hemicellulose called saponification, which results in the breaking of the intermolecular ester bonds [25]. This alkali solution solubilisation caused the cellulose to interact with enzymes during the enzymatic hydrolysis process. Alkaline pre-treatment is a widely used method that includes various reagents, including sodium hydroxides, potassium hydroxides, and ammonium hydroxides. Advantages of alkaline pre-treatments are: (1) they employ mild reaction conditions, it is possible to recover and reuse alkaline chemicals, (2) they are highly selective for separating lignin., (3) they are efficient at removing uronic substitutions and acetyl groups from hemicellulose, increasing the susceptibility of the carbohydrates to enzymatic hydrolysis and (4) they are less expensive than other procedures, such as acid pre-treatments [26], [27].

3) Acid Pre-treatment: Reactions during acid pre-treatment are based on the hydrolysis of hemicellulose and condensation and precipitation of solubilised lignin [28]. In addition, acid can access the glucosidic bonds that connect cellulose and hemicellulose. Hydronium ions (H_3O^+) due to the acid catalyst cause the precipitation of solubilized lignin as well as the dissolution of long cellulose and hemicellulose chains into sugar monomers [29]. Acid pre-treatment typically uses diluted acids at high temperatures or concentrated acids at low temperatures [30] In addition, according to [29], even though concentrated acid pre-treatment can significantly increase sugar yield, most of the concentrated acids are extremely damaging to equipment, toxic, necessitating high maintenance and operating costs.

Acid pre-treatment can be organic or inorganic acids like phosphoric acid, nitric acid, hydrochloric acid, maleic acid, and sulphuric acid [16]. Among the acids, sulfuric acid is the most favourable for treating lignocellulosic feedstocks [31]. The study by [32] found that fermentable sugar yield was higher when 1.5 per cent (w/v) cassava peel was hydrolyzed by 0.1 M sulfuric acid at 135°C under the pressure of 15 lb/inch2 for 90 minutes as compared to 0.025 M hydrochloric acid (63.29 per cent) and 0.25 M acetic acid (30.36 per cent) under the same temperature, pressure and time.

4) Organosolv Pre-treatment: The primary principle of organosolv pre-treatment is pre-treatment technology of lignocellulosic materials by solubilising hemicellulose and extraction of lignin using an organic solvent such as formic acid, acetic acid, phenol, glycerol, ethanol, and methanol. In addition, the organic solvent can be used with or without a catalyst, such as sodium acetate, magnesium chloride, sulphuric acid, and sodium hydroxide. In this pre-treatment, almost pure cellulose is obtained in the solid phase with only a little amount of degradation, while hemicellulose and lignin are obtained in the liquid phase [33]. Moreover, organosolv pre-treatment can extract relatively clean and almost pure lignin, which can become a valuable co-product instead of an undesired byproduct [33], [34]. The advantages of the organosolv pre-treatment are easy solvent recovery, mild conditions, high efficiency, and by-product that can be converted into a valuable product [33], [35]. In this study, different chemical pre-treatment: acid, alkali, and organosolv, were conducted to evaluate which pre-treatment will result in the highest yield in sugar production.

5) Enzymatic Hydrolysis: Enzymatic hydrolysis is the process of converting pre-treated lignocellulosic biomass into fermentable sugars using enzymes. According to [36], enzymatic hydrolysis consists of several crucial steps: (1) enzymes are transferred from the bulk liquid phase to the cellulose surface, (2) enzyme adsorption on the cellulose surface, which forms enzyme-substrate complexes, (3) cellulose hydrolysis process, (4) products of hydrolysis are transferred from the surface of the cellulose particle to the bulk liquid phase., and (5) cellodextrins and cellobiose are hydrolysed to glucose in the liquid phase. Factors influencing the rate of enzymatic hydrolysis yield include cellulose crystallinity, the particle size of lignocellulosic material, and the accessible surface area and pore volume of lignocellulosic material [37].

In this study, cellulase was used in the hydrolysis stage. Cellulase is made up of three different kinds of complex enzymes, which are endoglucanases or CMCases (EC 3.2.1.4),

β-glucosidases (EC 3.2.1.21), and cellobiohydrolases (EC 3.2.1.91), they are collaborating to convert complex lignocellulosic materials into glucose [38]. Cellulase can be produced by several microorganisms, bacteria, fungi, and actinomycetes. However, fungi are known for the secretion of cellulase in bulk amounts [39]. Among fungi, *Aspergillus* sp. and *Trichoderma* sp. have received a lot of attention and are being used for the commercial production of cellulase [40]

Cellulases are widely used to produce valuable products from lignocellulosic biomass. Besides, commercial cellulase is an essential tool for academicians to research lignocellulosic biomass. Advanced research on cellulase enzyme demonstrates its biotechnological potential in textile, food processing, biomass refining, detergent, agriculture, and animal feed industries [41]– [43].

A few parameters, such as pH and temperature, must be closely monitored to carry out enzymatic hydrolysis optimally. [44] reported that the optimal temperature and pH range is 50°C and 4.0 to 5.0, respectively.

D. The Uses of Cassava Peel

1) Sugar: Sugar is used to make thousands of different foods, ranging from frozen fruits to confections and cured meats to preserves [45]. Cassava peel can be used to extract a variety of sugars, including D-glucose, which is produced by the hydrolysis of cellulose. Glucose syrup is in demand and widely used in the food industry. Glucose syrup is utilised as a food additive in the industry to prevent the crystallisation of sugar, add volume, enhance flavour, and soften texture [46]. A study by [46] found that the cassava peel generated a significant amount of glucose. A sensory evaluation revealed that it was sweet and smelled pleasant, indicating that it could be a suitable raw material for manufacturing glucose syrup. Therefore, cassava peel can be used to replace feedstock in producing sugar.

Nowadays, boba milk tea, also referred to as pearl or bubble tea, is a popular beverage. The boba or pearl balls are the main ingredient in this drink, and tapioca starch is used to create these boba balls. Tapioca starch is made from cassava tubers. Hence, a bulk amount of tapioca starch production might produce a copious amount of cassava peel. Therefore, the cassava peel waste may be processed into sugar syrup to be used in the boba milk tea. This will save the industrial cost of making sugar from primary raw materials.

2) Animal Feed: Cassava produces abundant cassava peel after post-harvest and processing. Cassava peel is a good source of energy for the ruminant eating system as it contains high nitrogen-free extract (NFE), crude fibre, and carbohydrates [47]. However, cassava peel has a high content of hydrogen cyanide (HCN), which is toxic. HCN gives a bitter taste and reduces cassava peel's palatability [48]. Many studies on methods to reduce HCN content have been conducted. A study by [48] reported that HCN content can be reduced by sun-drying, soaking, or ensiling the cassava peels.

Cassava peels can be served either as a supplement or as the main basal diet. Research by [49] found that sheep fed with cassava peel gained more weight than sheep fed with only grass. The researchers agree that grazing animals should be given supplements such as cassava peel during the dry season for optimum production, as cassava peels have a high rumen degradability.

3) Bioethanol: Bioethanol is commonly utilised in the industrial sector as an industrial raw material for alcohol derivatives, pharmaceutical raw materials, a blend of liquor, and cosmetics [50]. Cassava peel is widely recognized as one of the greatest options for replacing edible sources in the production of gasoline-ethanol without jeopardising food security. Bioethanol production from lignocellulosic biomass is also known as second-generation bioethanol [51]. This generation of bioethanol involves several steps similar to sugar production except for the fermentation process after the saccharification of cellulosic.

Bioethanol made from cassava peel has better properties than petrochemical-based gasoline fuel, such as a higher-octane rating and lower pollution levels [52], [53]. However, there are several problems with using bioethanol for biofuel. [54] reported that due to its hygroscopic nature, bioethanol can corrode fuel injectors and electric fuel pumps, cause issues with engine startup in cold weather due to its difficulty in vaporising and affect the characteristics of lubricants and engine performance. According to them, the use of synthetic oil is one option to solve the above-mentioned problems.

4) Value-added biochemical: Lignocellulosic materials content in cassava peels can be converted into other chemical derivatives through biorefineries processes [44]. Sugar produced from cellulose and hemicellulose through the hydrolysis process can be further derivatized to different acids, alcohols, and Hydroxymethyl Furfural [14], [55]. One of the acids is levulinic acid. Levulinic acid has drawn a lot of interest. For its use in the preparation of pharmaceuticals, polymers, plastics, and resins, it can be used as a fuel alongside gasoline without requiring any modifications to engine architecture [55].

As lignin molecules' predominant C-O type linkages are broken, a variety of chemicals known as phenols, aldehydes, and acids are produced as a result of thermochemical degradation [14]. One of the phenolic compounds is ferulic acid. Ferulic acids have low toxicity and are extensively utilised in the pharmaceutical, food, and cosmetics industries [56]. Additionally, it has been discovered that ferulic acid is a good precursor in the creation of vanillin, the primary component of vanilla flavour, which is widely used in the food, beverage, pharmaceutical, and cosmetics industries [57]. This is because ferulic acid can be naturally released through a combination of physical and enzymatic processing, making it a substance that is almost universally present and simple to access [58].

II. MATERIAL AND METHOD

A. Material

Figure 3 shows 5 kg cassava tubers bought from a local market, Pasar Borong Sri Kembangan, Serdang, Selangor. Chemicals and reagents used in this study were Sodium hydroxide (NaOH), sulphuric acid (H2SO4), methanol (MeOH), sodium acetate (NaOAc), enzyme blend cellulase (Sigma Aldrich), citrate buffer solution, glucose anhydrous, Dinitrosalicylic Acid (DNS reagent), and Benedict reagent.

Fig. 3 Cassava tubers bought at a local market.

B. Method

1) Substrate Preparation: Cassava tubers were peeled to get their inner and outer layer of peels. Then, the peels were washed under running water to eliminate any non-structural sugar, soil contaminants, fertilisers, and other soluble components linked to biomass waste. The peels are then sliced into smaller pieces to facilitate the drying process, which takes around 24 hours at 60 degrees Celsius. The moisture content of the peel is determined by weighing them before and after drying. Following that, the moisture content is determined to make sure it is not higher than 10%. The small pieces of peel were then milled in a blender until the peels became powder [59].

2) Pre-treatment: 5g of cassava peel powder was suspended in 50 mL of 0.05 M to 0.2 M Sodium Hydroxide (NaOH) and placed in an autoclave at 121°C for 15 minutes in a 100 mL conical flask. Then, the mixture was filtered to collect the solid residues and thoroughly rinsed with tap water until a neutral pH was reached before the enzymatic hydrolysis process [60].

5g of cassava peel were suspended in 50 mL of 0.05 M to 0.2 M Sulphuric Acid (H_2SO_4) and autoclaved at 121°C for 15 minutes in a 100 mL conical flask. Then, the mixture was filtered to collect the solid residues neutralised with 2 M NaOH, and rinsed with tap water until neutral pH was reached before the enzymatic hydrolysis [60].

5g of cassava peel were suspended in 50 mL of methanol with different concentrations of sodium acetate (0.05 M to 0.2 M) as a catalyst. The mixture was then autoclaved in a 100 mL conical flask for 15 minutes at 121°C. The ratio for the mixture is 60:40 (methanol: sodium acetate). After that, the solid residues were collected and washed under running tap water for 10 minutes before the enzymatic hydrolysis process [3].

3) Enzymatic Hydrolysis: This hydrolysis process aims to convert cellulose into glucose. 1 L of 0.1 M sodium citrate buffer solution (pH 4.8) was prepared in 1 L Schott bottle by mixing 8.7785 g citric acid with 17.1240 g sodium citrate by using a magnetic stirrer. Then, about 0.1 g of pre-treated samples were mixed with 120 µl cellulase enzyme blend and transferred into 10 ml falcon containing 5 ml of 0.1 M sodium citrate buffer with pH 4.8. After that, 40 µl of tetracycline solution was added to the solution to prevent any possible contamination during hydrolysis. Then, distilled water is added to the solution until it reaches 10 mL in the falcon tube. Enzymatic hydrolysis continued in a shaking incubator at 150 rpm at 50℃ for 72 hours. Figure 4 shows the hydrolysates of pre-treated cassava peels in a falcon tube.

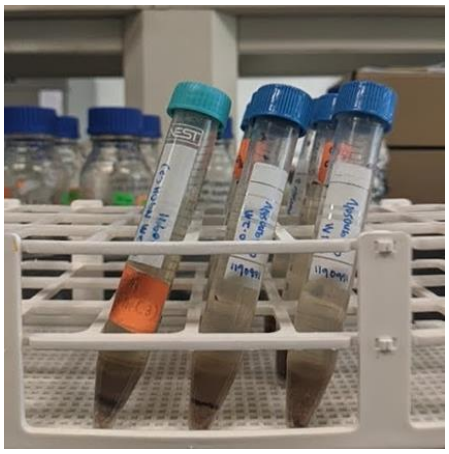

Fig. 4: Hydrolysates in a falcon tube.

4) Benedict's Test: Benedict's tests were conducted to test the presence of reducing sugar. Approximately 2 ml of the hydrolysed sample was placed into a clean test tube, and 5 ml of Benedict's reagent ($CuSO_4$) was placed in a test tube. The solution was heated in a boiling water bath for 3-5 minutes. The colour change and the precipitate formation were observed.

5) Quantification of Sugar Content: Reducing sugar was analysed using the dinitrosalicylic acid (DNS) method developed by [61]. The preparation of the DNS reagent is shown in Appendix A. About 1 mL of hydrolysed samples was put into a test tube containing 2 mL of DNS reagent, and it was heated in boiling water for 5-7 minutes and cooled at room temperature. After that, the spectrophotometer's absorbance of the reaction mixture was read at 540 nm to quantify glucose yield from cassava peel. The absorbance reading was translated into glucose equivalent using a glucose standard graph. In this research, the standard glucose graph was prepared to determine sugar concentration, as shown in Appendix B. Total glucose content was calculated based on the glucose standard curve plotted.

Glucometer is a device that measures approximate glucose concentration directly. In this research, an electrode-type portable glucose meter, which is a current-mode enzyme sensor, was used. Glucose oxidase, an enzyme in strips, reacts with glucose in the samples and produces gluconic acid and hydrogen peroxide. At a platinum catalytic anode, hydrogen peroxide is oxidized. The amount of electron transfers can be quickly determined by the electrode, and this electron flow is proportional to the number of glucose molecules in the sample [62]. This portable blood glucose meter has already been widely applied in analysis, especially biomedical, food, and environmental [63]. A small amount of hydrolysed sample was dropped onto the test strips, and the glucometer displayed the sugar concentration in seconds.

6) Statistical Analysis: Quantitative experimental tests were carried out in triplicate, and the results were reported as the mean ± standard deviation. The data obtained were subjected to Analysis of Variance ANOVA by employing MINITAB 19 software. Analysis (ANOVA) was performed at

a confidence level of 95% to investigate the glucose yield from different chemical pre-treatment and pre-treatment concentrations. The overall process of sugar production is shown in Figure 5.

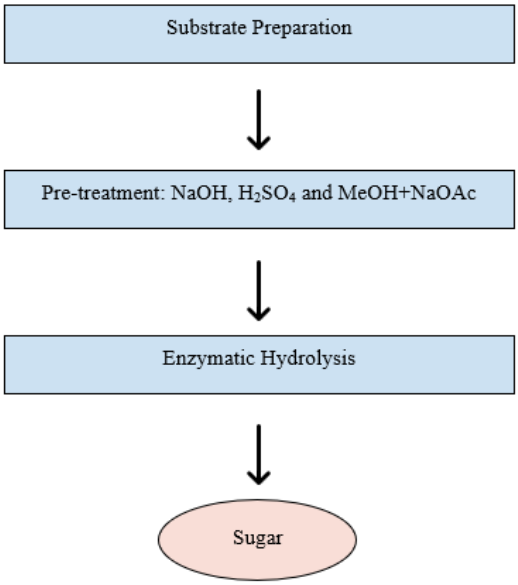

Fig. 5 Overall process of sugar production

III. RESULTS AND DISCUSSIONS

A. Analysis of Reducing Sugar in Cassava Peel

Benedict's test was done to investigate the presence of reducing sugar in the hydrolysed cassava peels. This test was done in triplicate for all pre-treatment types (acid, alkaline, and organosolv pre-treatment) and concentrations (0.05 M, 0.1 M, 0.2 M). Figure 6 shows Beenedict's Test conducted before boiling in a water bath for 5 minutes. Figures 7, 8, and 9 showed Benedict's Test conducted before and after boiling in a water bath for 5 minutes for acid, alkaline, and organosolv pre-treatment of cassava peel. The solution's colour changed from blue to green, and there was an orange precipitate at the bottom of the test tube. These indicated that a reducing sugar is present in hydrolysed cassava peel. Cellulose and hemicellulose in the cassava peel composition was the possible reason for the presence of reduced sugar. This finding was similar to [64] found cellulose, a reducing sugar, was obtained in the cassava peels. A study by [11] reported that cassava peel chemical composition consists of 37.9% cellulose, 37.0% hemicellulose, and 7.5% lignin. In addition, the presence of reduced sugar is confirmed by a study by [65], who found that in the dry matter of the cassava peel, glucose accounted for about 83 %. Meanwhile, xylose and arabinose only accounted for 2.31% and 2.35% each.

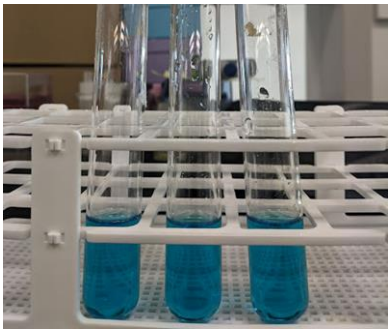

Fig. 6 Benedict's Test for Reducing Sugar before boiling in the water bath.

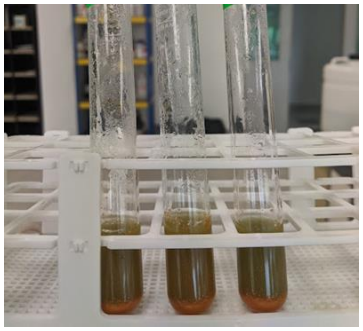

Fig. 7 Benedict's Test for acid pre-treated cassava peel after boiling in the water bath.

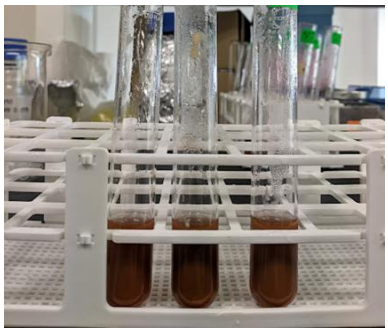

Fig. 8 Benedict's Test for alkali pre-treated cassava peel after boiling in the water bath.

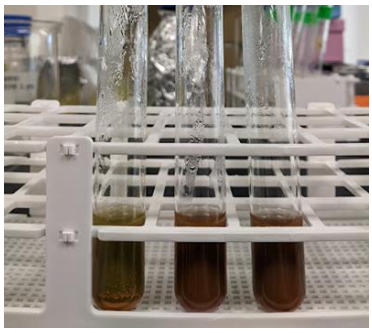

Fig. 9 Benedict's Test for organosolv pre-treated cassava peel after boiling in the water bath.

B. Analysis of Sugar Content

1) Glucose Quantification by DNS Assay: The yield of sugar production from cassava peels was investigated by quantifying glucose from the absorbance reading translated into glucose equivalent using a glucose standard graph. Figure 10 shows the variation in glucose production from cassava peels with different pre-treatment. The results showed that the highest glucose produced was from alkaline pre-treatment (NaOH), with a yield of 4.53±1.20 mg/ml. Glucose produced from acid (H_2SO_4) and organosolv (MeOH+NaOAc) were 3.55±0.68 mg/ml and 3.29±0.93 mg/ml, respectively. Thus, the optimum pre-treatment in this study is alkaline.

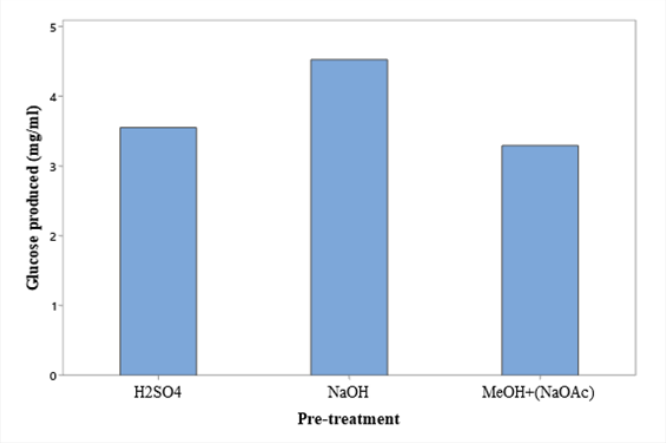

Fig. 10 Glucose produced (mg/ml) from cassava peels under different pre-treatment methods by DNS Assay.

This result is different from research by [60], who found that hydrolysing cassava peels with sulphuric acid pre-treatment gave a higher glucose yield than alkali and organosolv. This difference might be due to the different enzymatic hydrolysis methods after the pre-treatment. In their research, *Aspergillus terreus* SC1 and *Pseudomonas fluorescens* B9 were used for saccharification. Both microbes yielded different percentages of reducing sugar, where 88% and 98% for *Pseudomonas fluorescens* B9 and *Aspergillus terreus* SC1, respectively. This study uses a commercial cellulase enzyme blend to break down the cellulose. Hence, different microbes producing enzymes led to a different sugar yield after the pre-treatment. This reason agrees with a study by [66], who reported that multienzyme produces the highest reducing sugar (11.0267 g/L) while Effective Microorganism 4 (EM4) and cellulase produce 8.7900 g/L and 8.0667 g/L, respectively.

A two-way ANOVA was performed to analyze the effect of pre-treatment and pre-treatment concentration on glucose produced. A two-way ANOVA output performed on the glucose produced from cassava peels under different pre-treatments was provided in Table I. The result revealed that there was a statistically significant interaction between the effects of pre-treatment and pre-treatment concentration ($P<0.05$).

In this study, the optimum concentration for each pre-treatment was investigated. Figure 11 shows glucose variation produced under different pre-treatment methods and concentrations. The result shows that 0.05 M alkali pre-treated peels produce an optimal glucose of 44.66%. The percentage of glucose produced shows a decreasing trend from 0.05 M to 0.1 M and 0.2 M NaOH. This finding is agreeable as in [60], [67] when they found that pre-treatment with a low concentration of NaOH yielded significantly higher total reducing sugars than that with a high concentration of NaOH.

TABLE I. ANALYSIS OF VARIANCE OF DATA FOR GLUCOSE YIELD AFTER CASSAVA PEELS WERE TREATED WITH DIFFERENT PRE-TREATMENT AND PRE-TREATMENT CONCENTRATIONS BY DNS ASSAY.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Pre-treatment and Concentration	4	11.642	2.9104	6.40	0.002
Pre-treatment	2	7.611	3.8057	8.37	0.003
Concentration	2	2.224	1.1120	2.45	0.115
Error	18	8.185	0.4547		
Total	26	29.662			

According to [67], although higher NaOH concentrations had higher lignin removal, they resulted in lower solid recovery, leading to less reduced sugar production. Table II shows there were significant differences ($P<0.05$) in the glucose produced under alkaline with concentrations of 0.2 M, 0.1 M, and 0.05 M.

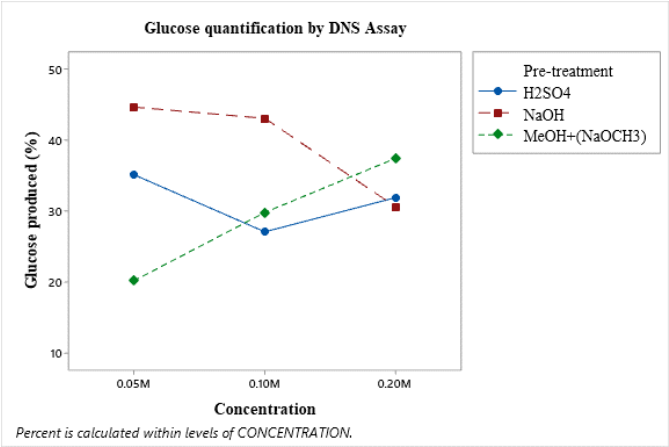

Fig. 11 Glucose produced (%) from cassava peels under different pre-treatment methods and different concentrations by DNS Assay.

Dilute sulphuric acid was used in this study instead of concentrated acid because, as mentioned in the literature, although concentrated acid may produce a high sugar yield, it is very corrosive. Besides, [68] reported that a low concentration of acid at a high temperature is preferable to a high concentration of acid because it prevents the degradation of fermentable sugars to other unfavourable compounds, which reduces hydrolysis yield. The optimal sugar produced for acid was 35.15% by 0.05 M H_2SO_4. However, ANOVA outputs in Table III show that glucose yield produced from cassava peels pre-treated with acid under concentrations 0.2 M, 0.1 M, and 0.05 M were found to be no significant difference ($P>0.05$). The acid concentration range chosen might not significantly affect the glucose yield. Thus, a comparative study on the effect of using various concentrations of sulphuric acid pre-treatment may be conducted in further research. Methanol with sodium acetate as a catalyst was used in this study for organosolv pre-

treatment. A catalyst was used to lower the pre-treatment temperature.

If the methanol is used alone, a high temperature is needed to ensure the pre-treatment efficiency. The result shows that methanol with 0.2 M sodium acetate pre-treated peels produces optimal glucose of 37.46%. The graph in Figure 11 shows that higher organosolv concentration produces higher glucose yield. This finding was similar to [69], who found that delignification increased with the increase of ethanol concentration as an organic solvent in organosolv pre-treatment. This is similar to previous research that reported increases in delignification cause higher cellulose exposure and cellulose conversion to glucose [70]– [72]. On the contrary, [3], [60] found no trend in the glucose yield under different organosolv concentrations. Different pre-treatment conditions, such as time and temperature, are possibly the reason for the variation in findings [73].

Similar to alkaline pre-treatment, glucose produced under organosolv with concentrations 0.2 M, 0.1 M, and 0.05 M were significantly different (P<0.05). The ANOVA outputs are shown in Table IV.

TABLE II. ANALYSIS OF VARIANCE DATA FOR GLUCOSE YIELD FROM CASSAVA PEELS PRE-TREATED WITH ALKALI UNDER DIFFERENT CONCENTRATIONS BY DNS ASSAY.

Source	DF	Adj S.S.	Adj MS	F-Value	P-Value
Alkaline pre-treatment concentration	2	8.390	4.1950	8.14	0.020
Error	6	3.094	0.5157		
Total	8	11.484			

TABLE III. ANALYSIS OF VARIANCE DATA FOR GLUCOSE YIELD FROM CASSAVA PEELS PRE-TREATED WITH ACID UNDER DIFFERENT CONCENTRATIONS BY DNS ASSAY

Source	DF	Adj S.S.	Adj MS	F-Value	P-Value
Acid pre-treatment concentration	2	0.7573	0.3787	0.77	0.506
Error	6	2.9661	0.4944		
Total	8	3.7234			

TABLE IV. ANALYSIS OF VARIANCE DATA FOR GLUCOSE YIELD FROM CASSAVA PEELS PRE-TREATED WITH ORGANOSOLV UNDER DIFFERENT CONCENTRATIONS BY DNS ASSAY

Source	DF	Adj S.S.	Adj MS	F-Value	P-Value
Organosolv pre-treatment concentration	2	4.718	2.3592	6.66	0.030
Error	6	2.125	0.3541		
Total	8	6.843			

2) Glucose Quantification by Glucometer: The yield of sugar production from cassava peels was then evaluated by direct readings from portable blood glucose meters called a glucometer. Glucometer is a biosensor in which the principle combines a biological component with a physicochemical detector to detect chemical analytes and electrical components. In addition to glucose testing, glucometers have been used in food analysis for testing foodborne pathogenic bacteria, mycotoxins, prohibited additives, pesticides, and veterinary drug residues [63]. The advantages of using a glucometer are that it provides a glucose reading in a matter of seconds, is simple to use, is inexpensive, produces results that are precise and easily understood and does not involve any other chemicals.

Figure 12 shows the variation in glucose production from cassava peels with different pre-treatment. Results showed that the highest glucose produced is from acid pre-treatment (H2SO4), with a yield of 2.63±0.17 mg/ml. Glucose produced from alkali (NaOH) and organosolv (MeOH+NaOAc) were 2.37±0.24 mg/ml and 2.55±0.17 mg/ml, respectively. This finding was comparable with [60] [74], who found that acid pre-treatment gave the highest glucose yield from cassava peels. The two-way ANOVA outputs performed on the glucose produced from cassava peels under different pre-treatment and pre-treatment concentrations are provided in Table V. The result showed there was a statistically significant interaction between the effects of pre-treatment and pre-treatment concentration (P<0.05).

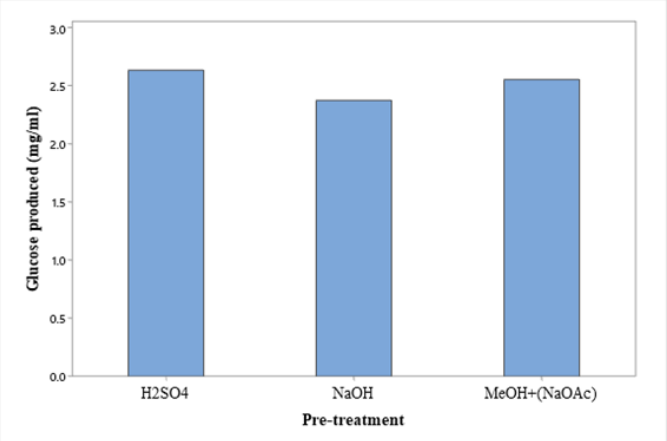

Fig. 12 Glucose produced (mg/ml) from cassava peels under different pre-treatment methods by glucometer.

TABLE V. ANALYSIS OF VARIANCE OF DATA FOR GLUCOSE YIELD AFTER CASSAVA PEELS WERE TREATED WITH DIFFERENT PRE-TREATMENT AND PRE-TREATMENT CONCENTRATIONS BY GLUCOMETER.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Pre-treatment and Concentration	4	0.5069	0.12673	8.71	0.000
Pre-treatment	2	0.3192	0.15960	10.97	0.001
Concentration	2	0.1607	0.08036	5.53	0.013
Error	18	0.2618	0.01454		
Total	26	1.2486			

Figure 13 shows glucose variation produced under different pre-treatment methods and concentrations. The results show that the optimal glucose produced for 0.05 M alkali is 32.90%. The optimal glucose produced for 0.1 M organosolv is 36.42%, while 0.1 M acid-pre-treated peels produce an optimal % glucose of 36.49%.

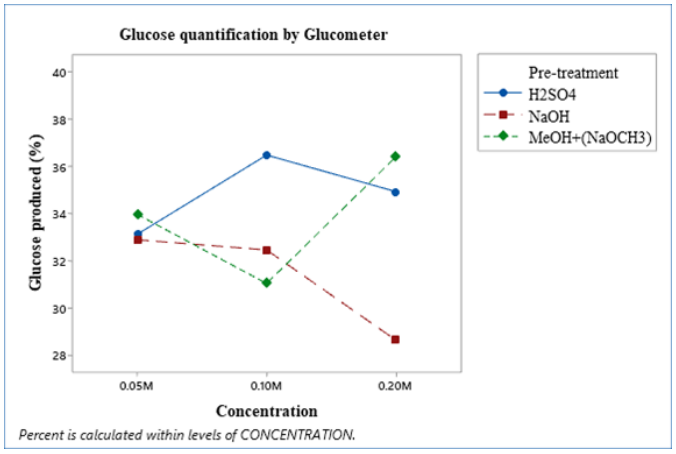

Fig. 13 Glucose produced (%) from cassava peels under different pre-treatment methods and different concentrations by glucometer.

Table VI shows the ANOVA output of glucose produced from cassava peels pre-treated with alkali under different concentrations. The glucose produced under alkaline concentrations of 0.2 M, 0.1 M, and 0.05 M were significantly different ($P<0.05$).

Similar to alkaline pre-treatment, glucose produced under organosolv with concentrations 0.2 M, 0.1 M, and 0.05 M were significantly different ($P<0.05$). The ANOVA outputs are shown in Table VII.

In contrast, Table VIII shows no significant difference between glucose produced under different concentrations of acid pre-treatment. The acid concentration range chosen might not significantly affect the glucose yield. Thus, a comparative study on the effect of using various concentrations of sulphuric acid pre-treatment may be conducted in further research.

TABLE VI. ANALYSIS OF VARIANCE OF DATA FOR GLUCOSE YIELD AFTER CASSAVA PEELS WERE TREATED WITH DIFFERENT PRE-TREATMENT AND PRE-TREATMENT CONCENTRATIONS BY GLUCOMETER.

Source	DF	Adj S.S.	Adj MS	F-Value	P-Value
Alkaline pre-treatment concentration	2	0.40810	0.204048	26.24	0.001
Error	6	0.04666	0.007776		
Total	8	0.45475			

TABLE VII. ANALYSIS OF VARIANCE DATA FOR GLUCOSE YIELD FROM CASSAVA PEELS PRE-TREATED WITH ORGANOSOLV UNDER DIFFERENT CONCENTRATIONS BY GLUCOMETER.

Source	DF	Adj S.S.	Adj MS	F-Value	P-Value
Organosolv pre-treatment concentration	2	0.15926	0.07963	5.67	0.041
Error	6	0.08424	0.01404		
Total	8	0.24350			

The quantity of enzyme able to produce 1 mol of glucose in 1 minute (U/ml) under experimental conditions is referred to as one unit of cellulase enzyme activity.

TABLE VIII. ANALYSIS OF VARIANCE DATA FOR GLUCOSE YIELD FROM CASSAVA PEELS PRE-TREATED WITH ACID UNDER DIFFERENT CONCENTRATIONS BY GLUCOMETER.

Source	DF	Adj S.S.	Adj MS	F-Value	P-Value
Acid pre-treatment concentration	2	0.1003	0.05015	2.30	0.181
Error	6	0.1309	0.02182		
Total	8	0.2312			

Cellulase activity is defined as the ability of cellulase to digest crystalline cellulose [43]. It is essential because it shows cellulase's effectiveness in hydrolyzing cellulose into glucose. Cellulase may be produced by several microorganisms: actinomycetes, bacteria, and fungi. Each cellulolytic microorganism has a difference in the cellulase activity produced [75]

A study by [42] found that Aspergillus niger produced the highest glucose concentration with the highest enzymatic activity (0.13 U/ml), followed by a monoculture of *Trichoderma reesei* (0.11 U/ml). Besides, [42] reported that *Penicillium citrinum* and Bacillus subtilis have the activity of 1.72 IU/ml and 2.8 IU/gds, respectively. A different strain of microorganisms producing cellulase also has different cellulase activity, as reported by Bhati et al. (2021). Aspergillus niger A 20 and Aspergillus niger NRRL3 have 27.5 U/ml and 215 U/ml, respectively.

IV. CONCLUSION AND RECOMMENDATION

In this study, different chemical pre-treatment methods were used in the pre-treatment of cassava peels before enzymatic hydrolysis. NaOH, H2SO4, and MeOH+NaOAc were evaluated for their ability to produce the highest sugar yield from cassava peels. In this experimental study, hydrolysing cassava peels with alkaline and acid produced more sugar than organosolv pre-treatment. Besides, the optimum pre-treatment concentration for NaOH and H2SO4 is 0.05 M, while MeOH+NaOAc is 0.2 M. It can be concluded that the types of pre-treatments and concentration used can significantly affect the sugar production from cassava peel. The sugar was quantified by using a DNS Assay and Glucometer. It is found that a glucometer is more feasible than a DNS Assay and saves time.

Sugar production from cassava peels will always be of interest for industrial use. as it can provide a renewable energy source, and its supplies are not limited and will not affect the food supply and security. Besides that, cassava peels can be a source to produce animal feedstocks, bioethanol production, biofilm batteries, and other value-added products such as vanillin, glycolic acid, formic acid, and levulinic acid [44]. Thus, further research on other pre-treatment methods of cassava peels is needed as pre-treatment is the fundamental process for hydrolysing lignocellulosic biomass. In the future, a combination of mechanical and chemical pre-treatment may be used to increase sugar yield. High-performance liquid chromatography (HPLC) with refractive index (RI) may be used in further study because it can separate, quantify, and identify each component in the mixture of the pre-treated sample.

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