Characterization, Pre-treatment, and Valorization of Pineapple Peels

A Dissertation

Submitted for the partial fulfilment of the Degree

Of

Master of Science (Chemistry)

By

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DECLARATION

I, hereby declare that the dissertation entitled **"Characterization, Pre-treatment, and Valorization of Pineapple Peels"** being submitted in the partial fulfillment of the requirements for the award of degree **of Master of Science in Chemistry** to **School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala** is a record of my own work carried out under the supervision of **Dr. Ranjana Prakash** and **Dr. Anoop Verma** from Jan-July, 2023. Further, any work of this dissertation has not been submitted to any other University for the award of any other degree or diploma.

Eshmit K Dalal

Date: 28/07/2023

Place: Patiala

Full Name of Candidate – Eshmit Dalal

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CERTIFICATE

This is to certify that the dissertation entitled **"Characterization, Pre-treatment, and Valorization of Pineapple Peels"** being submitted by **Eshmit Dalal** to **School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala** in partial fulfillment of the requirements for the award of degree of **Master of Science in Chemistry**, is an authentic record of the work carried out by the candidate under our guidance and supervision. She has fulfilled the requirements for the submission of this dissertation, which to our knowledge has reached the requisite standard. The results embodied in the dissertation have not been submitted in part or full to any other University or Institute for the award of any other degree or diploma.

Date: 28/07/2023

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Date: 28/07/2023
Place: Patiala

Eshmit Dalal

This thesis is dedicated to my family and friends

For their endless love, support, and encouragement.

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ABSTRACT

The pineapple (*Ananas comosus*), a fruit that is in high demand all over the world, produces a lot of waste in the agro-industrial sectors, particularly pineapple peels. The key qualities of pineapple peels as a feedstock for the manufacture of fermentable sugars that might be utilized for ethanol production led to waste valorization. Various nutrient estimations and other characterizations were analyzed for the dried pineapple peel powder, the results of which showed that the dried pineapple peel powder (DPPP) would be a potential feedstock for the enhancement of sugar production. For these, three different pre-treatments were chosen, which were steam explosion, hot water, and ultrasonication pre-treatment. After which, characterizations like XRD, TGA, and SEM were performed for the pre-treated samples in comparison to the dried pineapple peel powder to analyze thermal stability, structural morphology, and functional group changes. Therefore, it was analyzed that sugar concentrations were enhanced along with other nutrients like phenolic content, starch, and amino acids, which can be valorized further for bioethanol production. Lastly, we concluded that the techniques which we used in this study can give rise to the low-cost and environment-friendly ways to produce beneficial fermentable sugars from pineapple fruit peel wastes, which are necessary for the production of ethanol from the dried pineapple fruit peel waste.

CHAPTER 1: INTRODUCTION

The need for food production and processing is growing as a result of the world's growing population, modernization, and urbanization, which are all contributing to a growth in municipal solid waste (Davis et al., 2011; Malikoglu et al., 2013). In municipal waste, biodegradable waste makes up a significant percentage of the total waste generated, often accounting for approximately 50% of the waste stream (Salehi et al., 2020). Fruits processing industries contribute to a large portion of waste. With changing diet habits, the consumption of fruit juices and fruit-derived goods, such as soft drinks, nectars, cordials, and flavored ice cream, have increased recently (Pyar et al., 2014). This has led to an increase in the processing of fruits such as grapes, apples, oranges, pineapples, bananas, watermelons, and mangoes, which has resulted in a considerable increase in the amount of processing waste in the form of peels, seeds, pulp, and stones (Schiebere et al., 2019). The current and rising fruit juice and product consumption has led to the expansion of the fruit processing sector, resulting in the production of large amounts of waste (Kiran et al., 2015). The waste from the fruit processing sector has a high nitrogen and phosphate content, as well as a high carbohydrate and moisture content, which makes it a perfect substrate for bacteria to proliferate and pose a threat to the environment (Ferone et al., 2019).

To reduce the quantity of waste, various alternative technologies are developed that can create new products from waste or reduce the environmental impacts of their operations (Dorta & Sogi, 2016). Wastes derived from food processing are identified as major resources for bio-based process development, and utilizing the food byproducts sustainably to create value-added goods like chemicals, materials, and fuels could help ease environmental concerns and boost economic growth (Cozier, 2014). One of the notable biofuels being used to improve global energy security is bioethanol, which is often produced from renewable resources like plants, fruits, vegetables, and trees (Casabar et al., 2020). It can be used as an alternative fuel for combustion engines.

The pineapple (*Ananas comosus*), a tropical fruit belonging to the *Bromeliaceae* family, is one of the most popular fruits in the world, having a short stem with slender and hard leaves that grow medium to large-sized fruit (Hikal et al., 2021). It is the third most important tropical fruit and is the second most ingested fruit after banana, contributing to about 20% of tropical fruit's total production. It is a medium-tall (1 - 1.5 m) herbaceous perennial plant having 30 or more trough-shaped and pointed leaves 30 - 100 cm long, surrounding a thick stem. It features a regal crown of

spiky, blue-green leaves and a wide cylindrical shape. Its skin is scaly, green, brown, or yellow (Hikal et al., 2021). Various parts of the pineapple, consisting of the core, crown, leaves, and peel, have different applications (shown in **Fig.1**).

The fruits are known to have high moisture, a high sugar content, a high concentration of soluble ascorbic acid, and a low level of crude fiber (Nath et al., 2023). As a result, pineapple can be utilized as a fruit supplement for good health. It is well known around the world for its high nutritional value and medicinal characteristics, which include antioxidant, anticancer, and hypoglycemic capabilities (Sepúlveda et al., 2018). Pineapple waste is an enriched raw material composed mainly of insoluble fibers, pectins, sugars, protein, vitamins, minerals, and phenolic compounds (Diaz-Vela et al., 2013). Agricultural and industrial residues like pineapple wastes (peel, crown, and core) in cannery industries are quality feedstocks for bioethanol production (Ban-Koffi & Han, 1990). Since pineapple is known to have a sweet, sour taste, which indicates the possible occurrence of fermentable sugars and contains a significant amount of lignin (1.5%), cellulose (14%), and hemicellulose (20.2%) in the dry matter, this can be considered as a potential feedstock for bioethanol production.

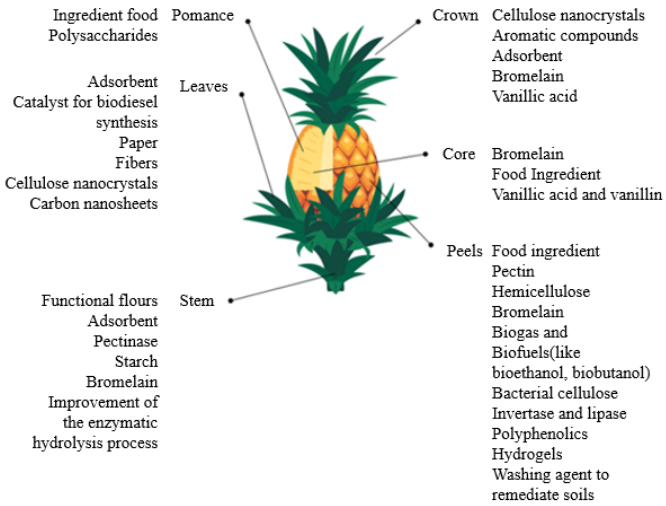

Fig.1. Different parts of Pineapple and most common applications for different fractions of pineapple waste

The objective of this study was to check the potential applications of powder formed from dried pineapple peels in the production of biofuels, sugars, phenolic compounds, and other value-added products after undergoing different pre-treatment methods. The main goal was to find a pre-treatment technique which is cost effective, sustainable, less time consuming, and enhances the

sugar efficacy by utilizing the pineapple waste. The powder obtained from dried peels was tested for basic nutrients like moisture content, ash content, total solids, cellulose hemicellulose, lignin, proteins, phenolic content, carbohydrates, etc., and were tested for the dried pineapple peel powder. In order to utilize the dried pineapple peel powder (DPPP) for the generation of value-added products, various pre-treatment methods were followed, such as steam explosion pre-treatment, hot water pre-treatment, and ultrasonic pre-treatment. DPPP was characterized by Thermo-Gravimetry, X-ray Diffraction, and Scanning Electron Microscope (Hadidi et al., 2020).

CHAPTER 2: LITERATURE REVIEW

The rising demand for fruits and vegetables over the last decade has allowed farmers to produce more of these edible consumables. Fruits are among the most widely used horticultural products. Therefore, they produce a lot of lignocellulosic waste in the form of high-carbohydrate rinds, seeds, pomace, and pulp worldwide (Hassan et al., 2021). Fruits like grapes, apples, oranges, pineapples, bananas, watermelons, and mangoes are increasingly processed for use in soft drinks, nectars, cordials, and flavor-infused ice cream (Schieber et al., 2019). This has resulted in a significant rise in the amount of processing waste in the form of peels, seeds, and pulp.

The pineapple (*Ananas comosus*), a terrestrial tropical plant with coalesced berries, is the most prominent edible member of the *Bromeliaceae* family and is a member of the genus *Ananas* (Hadidi et al., 2020). Pineapple is the third most important tropical fruit produced globally after bananas and mangoes (Hikal et al., 2021). According to the FAO reports, about 28 million tons of pineapple were produced globally in 2021. Whereas, India produced approximately 1.7 million tons of pineapples annually. It contributes to around 20% of tropical fruits total production (Pham et al., 2015). Pineapple is used in various culinary applications, including fresh consumption, juices, canned products, and as an ingredient in different cuisines (Gorinstein et al., 1999). To accommodate rising customer demand, manufacturers have expanded their pineapple plantations and raised output levels, which has resulted in the production of large amount of wastes. Waste from pineapple processing typically consists of peels, crowns, cores, and other byproducts that are thrown (Choonut et al., 2014).

The rising production of pineapple waste can have several negative effects, such as health risks, habitat destruction, and soil degradation. It also causes environmental pollution as pineapple waste decomposes to release methane, a potent greenhouse gas that contributes to climate change, and if the waste gets into water bodies, it can cause water pollution, which harms aquatic ecosystems and upsets the ecosystem's balance (Ferone et al., 2019). To minimize the quantity of waste produced, proper waste management practices are implemented, and various alternative technologies are developed that can create new products from waste and reduce the environmental impacts of their operations (Casabar et al., 2020). The solution to these problems is to find a sustainable pre-treatment method that is cost effective, sustainable, less time consuming, and enhances the sugar

efficacy by utilizing the pineapple waste. We choose these three methods on the basis of these required objectives, which are steam explosion, hot water, and ultrasonication.

Steam explosion: The most typical pretreatment method involves applying heat to the biomass substrates to make them soluble. They have been employed for a long time to improve the breakdown of particulate organic materials at different temperatures (Deepanraj et al., 2017). Due to its low cost and ability to preserve the cellulose in the solid residue for subsequent enzymatic hydrolysis, acid pretreatment is recognized as one of the most promising techniques (Karimi et al., 2006). Sulfuric acid is the most popular acid for the pretreatment of lignocellulosic biomass due to its availability and inexpensive cost. However, sulfuric acid pretreatment results in the breakdown and loss of free sugars as well as the creation of inhibitory chemicals, which has an impact on future fermentation (Chen et al., 2010; Momayez et al., 2017). The use of alkaline pre-treatment primarily deals with the removal of lignin from a plant, which protects and encloses other components of a plant, such as hemicellulose and cellulose (Kim et al., 2019). Kiruthika et al. (2021) studied the steam explosion pre-treatment method for the production of bioethanol and glucose from pineapple peel waste and reported a bioethanol yield of 5.98 g/L and 0.96 g/ml of glucose at the end of pre-treatment. Huynh et al. (2022) also used the steam explosion method for the production of ethanol and total sugars from pineapple peel hydrolysate and concluded that 41.11 g/L glucose and 40.87 g/L fructose were found.

Hot water pre-treatment: According to reports, liquid hot water pretreatment is quick and effective for separating resistant chemicals, which enhances enzyme digestibility. This method does not require the utilization of chemicals, such as acid or alkali, and thus leads to an eco-friendlier process (Imman et al., 2021). Imman et al. (2021) studied the optimization of sugar recovery from pineapple leaves by acid-catalyzed liquid hot water pretreatment for bioethanol production and stated that a low solid loading of 5% resulted in a relatively high percentage of ethanol yield (94.68%).

Ultrasonication pre-treatment: A mechanical pretreatment technique called ultrasonication can be used to prepare substrates for hydrolysis. Ultrasonication is a relatively quick process that, in contrast to heat procedures, solubilizes both extracellular and intracellular molecules, increasing the amount of soluble microbial products (Deepanraj et al., 2017). Various wastes have also been pre-treated with ultrasound to make them more susceptible to enzymatic hydrolysis further on

(Xiaotong et al., 2019). Jennifer et al. (2020) analyzed 5% biomass loading of pineapple peels in the ultrasonic pre-treatment process to produce bioethanol and fermentable sugar with a yield of 197.6 g/L (25.0% v/v) and 571.4 g/L respectively. Similarly, Ratchapol et al. (2016) also used a combination of alkaline and ultrasonic pre-treatment for reducing sugar production from pineapple waste and concluded that the best pre-treatment was using 2% NaOH with ultrasound for 60 min with the maximum total reducing sugar concentration obtained at 218.41 mg/g dried sample. Gabriella et al. (2022) used the ultrasound assisted extraction pre-treatment method for the production of ethanol, glucose, and fructose in pineapple by-product extract and concluded that the highest TPC content i.e., 405.06 mg GAE 100 g^{-1} was from the mixture of ethanol and acid solution and the pineapple by-product extract was found to contain fructose and glucose contents of 6.54 – 7.17 g 100 g^{-1} and 7.56–8.29 g 100 g^{-1}, respectively.

Our aim was to find a suitable pre-treatment method that is cost-effective, efficient, and yields high amounts of fermentable sugars from pineapple fruit peel wastes.

CHAPTER 3: MATERIALS & METHODOLOGY

3.1 MATERIALS REQUIRED:

3.1.1 GLASSWARES/ APPARATUS

Glassware like flasks, test tubes, beakers, glass rods, glass beads, and funnel were used to perform the experiments, along with apparatus like crucibles, desiccators, and Soxhlet assembly.

3.1.2 CHEMICALS OR REAGENTS

Chemicals, namely sulphuric acid (0.255N, 3%, 4%, 67%, 72%, 98%pure), distilled water, sodium hydroxide (pure, 0.313N), potassium sulfate, phosphate buffer (pH-6.9), acetone (100%), ethanol (80%), perchloric acid (52%), hydrochloric acid (2.5N), sodium carbonate (7.5%, 20%), p-bromoaniline, dinitro salicylic acid, petroleum ether, n-propanol and distilled water were used to perform estimation of nutrients, pre-treatments or pH maintenance. Standards like xylose, cellulose, glucose, leucine, gallic acid, etc., were used to perform nutrient estimations. Reagents like anthrone, ninhydrin, iodine, folin-ciocalteu, and di-nitrosalicylic acid, were used to observe changes in the colour of the sample and record the absorbances spectrophotometrically.

3.2 METHODOLOGY

3.2.1 Preparation of raw material:

Pineapple peel waste (PPW) was ripped, and the fruit residue was collected from local vendors in Patiala, Punjab (India). Fresh PPW was cleaned with tap water, cut into small pieces, and oven-dried at 60°C till the removal of moisture and obtaining constant weight. Sample of PPW was ground using a grinder (Havells' Aspro Plus) and sieved through a 1 mm-mesh sieve to get the dried pineapple peel powder (DPPP-1).

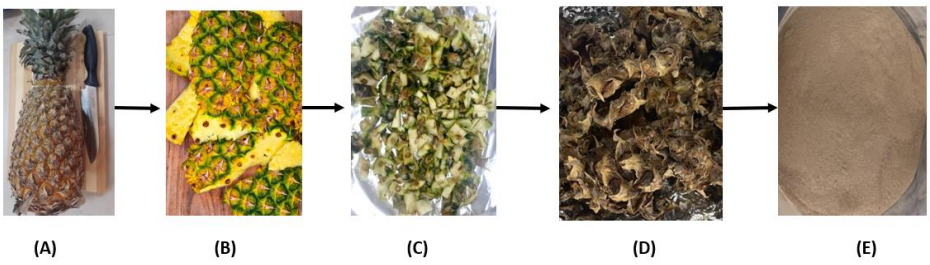

Fig.2. Preparation of raw material: (A) Raw pineapple, (B) Pineapple peels, (C) Chopped pineapple peels, (D) Dried pineapple peels, (E) Pineapple peel powder

3.2.2 Analysis of raw pineapple peel powder (non-pretreated)

3.2.2.1 Particle Size Distribution

The particle size distribution was analyzed by laser diffraction at 200 rpm for DPPP-1 (Dried pineapple peel powder before sieving). It was done by adding 500 mL distilled water in a 600 mL beaker filled with sample up to laser obstruction 2%.

3.2.2.2 Determination of Moisture Content

The moisture content of the samples was analyzed according to the method by APHA 1985 (Sondhi et al., 2020), in which the DPPP-2 (dried pineapple peel powder after sieving) sample was oven-dried at 125°C until a constant weight was achieved. The difference obtained in the weight of the sample before and after drying was its moisture content.

3.2.2.3 Determination of Ash Content

The ash content of the DPPP-2 sample was determined according to the method by APHA 1985 (Sondhi et al., 2020) in which the moisture-free biomass was kept in a muffle furnace at 600°C for two hours and cooled to room temperature in a desiccator and weighed. The difference in the weight of the ignited sample and the initial sample resulted in the ash content of the sample.

3.2.2.4 Total Volatile Solids

Volatile solids in the DPPP-2 sample was determined according to the method by APHA 1985 (Sondhi et al., 2020). The crucible containing the dried residue from the total solids test was kept in the muffle furnace at 550°C ± 50°C for 1 hr. It was then cooled in a desiccator until the weight was constant. The difference in the weight of total solid dried residue and ignited residue upon the mass of total dried residue resulted in the total volatile solids.

3.2.2.5 Total Solids

Total solids in the DPPP-2 sample was determined according to the method by APHA 1985 (Sondhi et al., 2020). Total solids and total dissolved solids were added together to form the total solids. Total dissolved solids were the weight left after moisture loss, and total undissolved solids were the portion of total solids retained on 0.2 µm filter paper during filtration. Both these add up to form the total solids.

3.2.2.6 Total Organic Carbon

DPPP-2 was used for total organic carbon content analysis. The dichromate method (Thomas et al., 2018) was used to analyze the dichromate and COD reagent, followed by heating for about 30 minutes at 100°C. Concentrated orthophosphoric acid was added before titration with 0.25M FAS (Ferrous Ammonium Sulfate) in the presence of a ferroin indicator. Total organic content was calculated according to **Eq.1:**

$$Total\ Organic\ Content = \frac{10\times(FAS_{consumed\ in\ blank} - FAS_{consumed\ in\ sample})\times 0.003\times 100}{(blank\ reading \times sample\ weight)} \quad (1)$$

3.2.2.7 Nitrogen as N

Nitrogen content was analyzed using the Kjeldahl method (Barbano et al., 1991), involving a three-step approach to quantify protein following digestion, distillation, and titration. The digestion of organic material was achieved using boiling concentrated sulphuric acid, potassium sulfate (K_2SO_4) (to raise the boiling point), and a catalyst (e.g., selenium) to speed up the reaction. Any nitrogen in the sample is converted to ammonium sulphate during this process. The ammonium sulphate in the digestate was changed to ammonia by adding NaOH, which was then distilled off and collected in a receiving flask of surplus boric acid to create an ammonium borate. The leftover boric acid was titrated using a standard acid and a suitable end-point indicator to determine the sample's total nitrogen concentration. Total nitrogen content was calculated according to **Eq. 2**:

$$Total\ nitrogen\ content(\%) =$$
$$(H_2SO_4 consumed \times 1.4 \times normality\ of\ H_2SO_4 / sample\ weight \quad (2)$$

Fig.3. Kjeldahl Apparatus

3.2.2.8 Probable C/N Ratio

Production of bioethanol depends critically on the C/N ratio of the fermentation medium. A higher ratio will produce a higher yield, whereas a low ratio will produce a decreased yield. The probable C/N ratio is estimated by taking the ratio of carbon content and nitrogen content. as analyzed by the method given by Mariotti et al. (2008).

3.2.2.9 Water-Swelling Capacity (WSC)

Water swelling capacity was analyzed by the method of Dhar et al. (2022) with a minor modification. In this experiment, 1g DPPP-2 was taken in a graduated glass cylinder. 30 mL of phosphate buffer (pH 6.9) was added. The sample was left to swell for 60 minutes, and the final volume of the swollen sample was reported as mL of final volume per g of the initial dry sample.

3.2.2.10 Cellulose

Cellulose content was analyzed by following the method given by Updegraff et al. (1969), where the DPPP-2 sample was treated with the acetic-nitrile reagent in test tubes incubated in a boiling water bath at 100°C for 30 min. After cooling the test tubes, the solution was centrifuged for 15–20 min. at 8000 rpm to separate the residue. The residue was washed with distilled water, and 100 mL of 67% sulfuric acid was added. The test tubes were left undisturbed at room temperature, and 1 mL aliquots of the sample were collected and diluted to a volume of 100 mL using distilled water. Then, 1 mL of that volume was combined with 10 mL of anthrone reagent. For 10 min., the test tubes were incubated in a boiling water bath. After cooling the solution, absorbance at 630 nm was detected. Standard curves were prepared and used to estimate the concentration of cellulose in the DPPP-2 against cellulose as standard.

Cellulose content was calculated according to **Eq. 3**:

$$Total\ cellulose = Absorbance \times Slope \times Dilution\ factor \quad (3)$$

3.2.2.11 Hemicellulose

Hemicellulose content was estimated using the method provided by Deschatelets et al. (1986). In a conical flask filled with 3% H_2SO_4, the DPPP-2 sample was added in a ratio of 1:10. The mixture was autoclaved at 121°C and 15 psi pressure, cooled, and 100mL distilled water was added to it. pH was adjusted to 7.0–7.5. To 1mL aliquots of samples, 5mL of p-bromoaniline was added, and

they were left undisturbed at room temperature for 70 minutes. Absorbance at 540nm was detected. Standard curves were prepared and used to estimate the concentration of hemicellulose in DPPP-2 against xylose as standard.

3.2.2.12 Lignin

Lignin was estimated according to the NREL method (Sluiter et al., 2019). 3 mL (72% H_2SO_4) and 300mg of the dried DPPP-2 sample were combined and agitated at 30°C for one hour. 84 mL of distilled water was then added to the mixture to dilute it to 4% H_2SO_4. The resultant 87 mL mixture was autoclaved at 121°C for one hour. The solution was cooled down, vacuum filtration was done, and the residue was washed with 150mL distilled water 2-3 times. Acid-soluble lignin was present in the filtrate, whereas acid-insoluble lignin was present in the residue. The filtrate's supernatant was collected, and absorbance at 240 nm was measured using 4% H_2SO_4 as the reference. The residue was dried at 105°C for 4 hr in a hot-air oven to achieve a constant weight. The weight of the acid-insoluble lignin was then recorded after being held in the muffle furnace at 575°C 25°C for 18–24 hr.

The acid-soluble lignin was calculated according to **Equation 4**:

$$\%ASL = \frac{UVabs \times Volume_{filterate} \times Dilution}{exODW_{sample} \times Pathlength} \times 100 \quad (4)$$

The acid-insoluble lignin was calculated according to **Equation 5:**

$$\%AIL = \frac{(Weight_{crucible+AIR} - Weight_{Crucible}) - (Weight_{crucible+ash} - Weight_{crucible}) - Weight_{protein}}{ODW_{sample}} \times 100 \quad (5)$$

$$ODW = \frac{Weight_{air\ dry\ sample} \times \%\ Total\ solids}{100} \quad (6)$$

3.2.2.13 Protein

The protein content was estimated using nitrogen factor (NF = 6.25) according to the NREL method (NREL/TP-510-42625) (Karimi et al., 2018).

In order to convert nitrogen content into protein content **Eq. 7** was used.

$$Protein\ content = Nitrogen \times 6.25 \quad (7)$$

3.2.2.14 Starch

Starch content was estimated according to the anthrone method (Kamaraj et al., 2020). 5mL of hot 80% ethanol was used to homogenise 100mg DPPP-2 sample. After centrifugation, the residue was dried, and 5 mL of water and 6.5 mL of 52% perchloric acid were added. The resultant reaction mix were incubated at 0°C for 20 min and centrifuged. The clear supernatant was collected, and the volume was made to 100 mL. The supernatant, 0.1 and 0.2-mL aliquots, were collected and placed in test tubes to make up 1 mL volume with distilled water. After adding 4 mL of anthrone reagent, the test tubes underwent an 8 min. incubation in a boiling water bath. After cooling the solutions, absorbance was measured at 630 nm using the standard plot prepared for total starch estimation with the standard equation y=3.0427x and regression as 0.9977 using standard glucose.

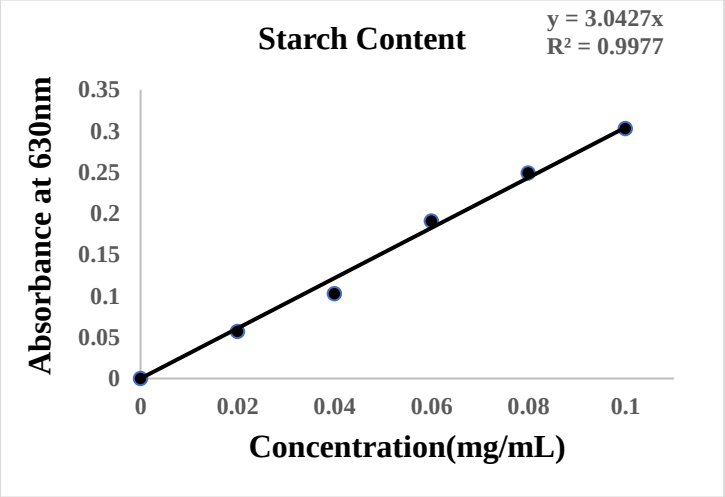

Fig.4. Starch content standard graph (0.01-0.1 mg/mL)

3.2.2.15 Total carbohydrates

Carbohydrates were estimated according to the anthrone method given by (Kamaraj et al., 2020). 5mL of 2.5N HCl was used to homogenize 100mg of DPPP-2 sample in a boiling water bath for 3 hr. The reaction mixture was then neutralized using sodium carbonate until the effervescence stopped. Using distilled water, the reaction mixture's volume was equal to 100 mL. The clear supernatant was collected and placed in test tubes. The test tubes were subjected to an 8 min. incubation in a boiling water bath after adding 4mL of anthrone reagent. After cooling the solutions, absorbance was measured at 630 nm using the standard plot prepared for total

carbohydrates estimation with the standard equation as y=3.0427x and regression as 0.9977, using glucose as standard (same as used in starch estimation).

3.2.2.16 Crude Fiber Content

The AOAC 2005 technique given by Tenkohu et al. (2022) was used to analyze crude fiber. 2 grams of the defatted sample (DPPP-3) was homogenized in 200 mL of 0.255N H_2SO_4, on a hot plate for 30 min. The residue was collected on muslin cloth and rinsed with hot distilled water. After which, the filtered residue was again homogenized in 200 mL of 0.313N NaOH, and digestion was once more carried out for 30 min. on a hot plate. The residue was once more gathered on muslin cloth and washed with hot distilled water, after which it was washed with 15 mL ethanol. The residue was then dried until consistent weight was attained in a hot-air oven at 100 °C. The carbonaceous material was then burned after 5 hr of being held at 550 °C in a muffle furnace. After the sample cooled, its constant weight was recorded. Crude fiber content was calculated as according to **Eq. 8**:

$$Crude\ fiber\ content = (W1 - W2)/W \times 100 \quad (8)$$

where,

W1= weight noted after the sample was dried in a hot-air oven

W2= weight noted after sample kept in a muffle furnace

W= initial weight of sample

3.3 Pre-treatments:

3.3.1 Steam explosion pre-treatment:

DPPP-2 was subjected to different pre-treatments in the presence of alkali (NaOH) or acid (H_2SO_4) and compared against control (only steam explosion).

(A) **(B)**

Fig.5. Before (A) and after (B) steam explosion pre-treatment

2 grams of DPPP-2 was added in each reaction mixture and volume was made up to 100 mL with distilled water in presence of 1% (v/v) sodium hydroxide (NaOH) for alkali pre-treatment or 1% v/v sulphuric acid (H_2SO_4) for acid pre-treatment. All the samples were autoclaved at 121 °C at 15 psi pressure for 15 min., followed by centrifugation at 4 °C, 9000 rpm, and 30 min. The supernatants were collected for nutrient estimation tests and value-added product formation.

3.3.2 Hot water pre-treatment:

Three variables were picked for the pre-treatment of hot water: temperature, solid loading, and time. Three distinct solid loadings (2 %, 6 %, and 10 %) and three different temperatures (50 °C, 55 °C, and 60 °C) were selected while keeping the time constant (15 min). These settings were used to optimize 9 distinct reactions, each of which was carried out in a different flask. Each flask received a 15-min treatment with hot water, following which they were centrifuged, and the supernatant was gathered for further estimations.

3.3.3 Ultrasonication pre-treatment:

Fig.6. Ultrasonication pre-treatment

To carry out the ultrasonic pre-treatment technique, biomass loadings of 2 %, 6 %, and 10 % were each dissolved in 100 mL of distilled water. The mixture sample was then subjected to sonication using three distinct sonication times (15, 30, and 45 min) under three different temperatures, 50°C, 55°C, and 60°C, at a frequency of 40 kHz and with an ultrasonic power of 100 W. They were then centrifuged, with the supernatant being saved for analysis later.

3.3.4 Analysis of pre-treated pineapple peel liquid hydrolysate:

Nutrient estimations like total sugar, reducing sugar, starch, total phenolic content, and physical

parameters like pH, ºBrix, and specific gravity of all the samples of three different pretreatments were checked.

(i) pH

pH of all the different pre-treated samples was measured using a pH meter.

(ii) ºBrix and specific gravity

ºBrix and specific gravity were measured using a ºBrix refractometer.

(iii) Determination of starch

The estimation of starch was done according to the method provided by Kamaraj et al. (2020). 1mL of the sample was taken in test tubes, and 30µl iodine reagent was added to each. All the test tubes were then mixed well by vortex. The test tubes were then left undisturbed for 1 min. Then, the absorbance was measured at 600 nm, using the standard plot prepared for total starch estimation with the standard equation as y=0.5927x and regression as 0.9974, using glucose standard.

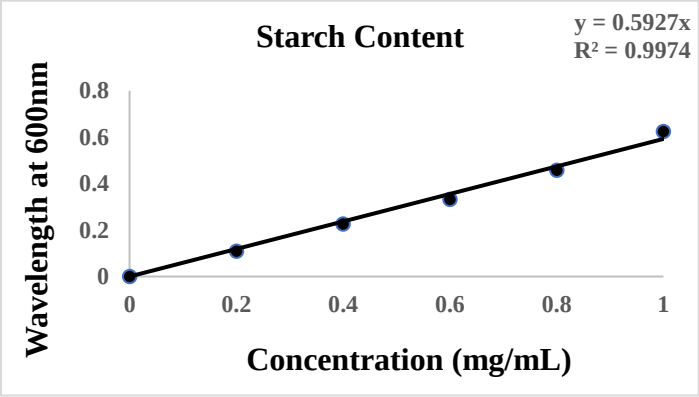

Fig.7. Starch content standard graph (0.1-1.0 mg/mL)

(iv) Determination of reducing Sugars

Reducing sugars were estimated using the method provided by Miller et al. (1959). 20 µL sample volumes were taken in different test tubes, and the total volume of the solutions was made 1mL using distilled water, after which 3 mL of DNS reagent was added to each. The test tubes were then incubated on a water bath for five minutes and then cooled. Then, 7 mL of distilled water was added to each, after which the absorbance at 540 mm was measured, using the standard plot

prepared for total carbohydrates estimation with the standard equation as y=0.9783x and regression as 0.9973, using glucose standard.

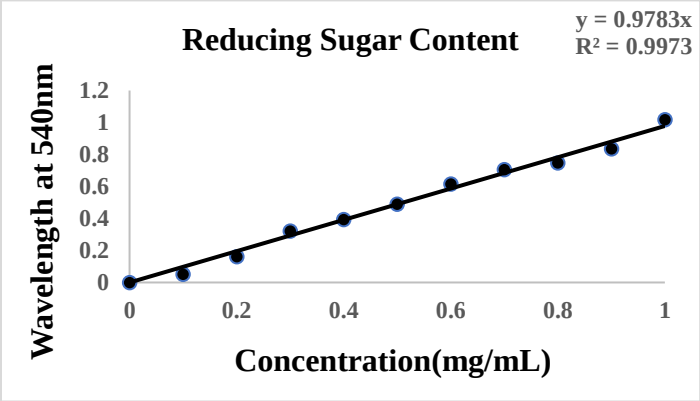

Fig.8. Reducing sugar content standard graph (0.1-1.0 mg/mL)

(v) Determination of total sugars

Total sugars were estimated using the method provided by Rondel et al. (2013). 20 µl of the sample solutions were taken in test tubes, and the total volume of each test tube was made 1 mL using distilled water. After which 5 mL of anthrone reagent was added to each, they were heated in a water bath for 8 min at 100°C and cooled. After which, the absorbance at 630 nm was measured using the standard plot prepared for total carbohydrates estimation with the standard equation as y=3.6413 and regression as 0.995, using glucose standard.

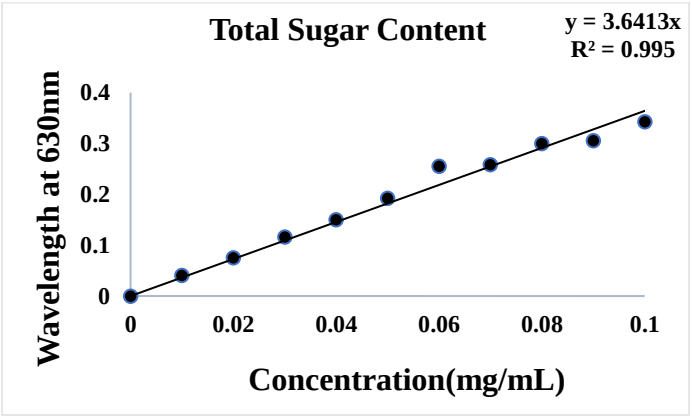

Fig.9. Total sugar content standard graph (0.01-0.1 mg/mL)

(v) Total phenolic content

Total phenolic content was analyzed by the method given by (Conesa et al., 2016). 200µL sample was taken in test tubes, and the total volume was made to 4mL using distilled water. 0.25 mL Folin

& Ciocalteu's phenol reagent was added, followed by the addition of 1mL 20%(w/v) sodium carbonate in all the test tubes. The test tubes were vortexed and left undisturbed at 40°C for 20 minutes in the dark. Absorbance was measured at 685nm using the standard plot prepared for total phenolic content estimation with the standard equation y=10.944x and regression as 0.9996, using gallic acid as standard.

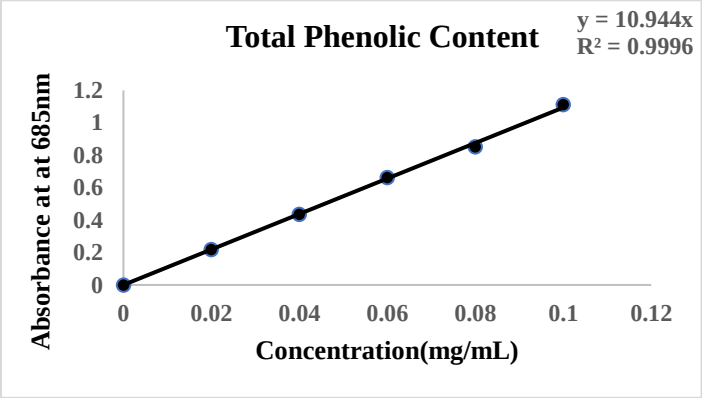

Fig.10. Total phenolic content standard graph (0.01-0.1mg/mL)

(vi) Amino acid content

Amino acid content was measured according to the method given by Mahesha et al. (2012). 20µL sample was taken in test tubes, and the total volume was made 1mL using distilled water, after which 1mL of ninhydrin reagent was added to each, vortexed, and incubated in a boiling water bath for 15 min. The test tubes were cooled, and 5mL diluent was added to each and mixed well. Absorbance was measured at 570 nm, using the standard plot prepared for amino acids estimation with the standard equation as y=16.941x and regression as 0.9976, using the leucine standard.

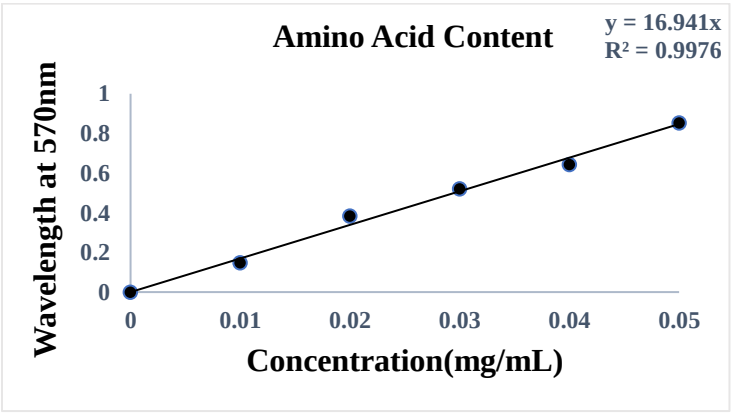

Fig.11. Amino acid content standard graph (0.01-0.05 mg/mL)

3.4 Characterization Techniques

3.4.1 Thermogravimetric analysis (TGA)

The thermal profile of pineapple peel waste was investigated by TGA (Perkin Elmer Diamond TG/DTA). The Thermo-gravimetric analysis was carried out under controlled surroundings of nitrogen and a 300°C–600°C temperature range at a rate of 10°C /min.

3.4.2 XRD analysis

The diffraction pattern of the DPPP-2 was analyzed by X-ray diffraction (Rigaku SmartLab SE Cu K-alpha radiation 1.54 Angstrom) with scanning radiation at 60 kV. All samples dried at 30°C were scanned from a 2θ of 5–80°, with a scan speed of 5°C /min.

3.4.3 SEM analysis

The method given by Lourenço et al. (2021) was used to assess the particle morphology. The microparticles were examined with a scanning electron microscope (Jeol jsm6510lv) at a voltage of 15 kV.

CHAPTER 4: Results and Discussion

4.1 Particle Size Distribution

Physically, increasing the surface area of the powdered pineapple peel powder reduces the particle size, which improves contact between the peel particles and the pre-treatment agents. Better penetration and interaction during the pre-treatment process are made possible by the improved contact (Conesa et al., 2016). So, the particle size distribution of DPPP was checked by laser diffraction at 200rpm, and it was found that more than 80% of particles were of 1000 μm. Therefore, a sieve of mesh size 1 mm was chosen to bring the particles to a uniform size for efficient pre-treatment. Particles of similar size were also used in studies by Momayez et al. (2017) for rice straw to produce ethanol (9.7 g/L).

4.2 Characterization of pineapple peel powder

Essential properties like moisture content, ash content, volatile solids, total solids, total organic carbon, nitrogen, cellulose, hemicellulose, lignin, protein, chlorophyll, water swelling capacity, amino acids, total phenolic content, starch, and carbohydrates were tested for DPPP-2 sample collected after sieving. It was found that the characteristics were in range with the wastes used in studies reported by Damasceno et al. (2016); Kodagoda et al. (2017); Syaliza et al. (2019), Samuel et al. (2020) and others. So, it was inferred that the blended powder produced from dried pineapple peels could be used to produce fermentable sugars that can be converted to bioethanol. The main nutritional characteristics are shown in **Fig. 12**, and results for other important physical parameters are compiled in **Table 1**.

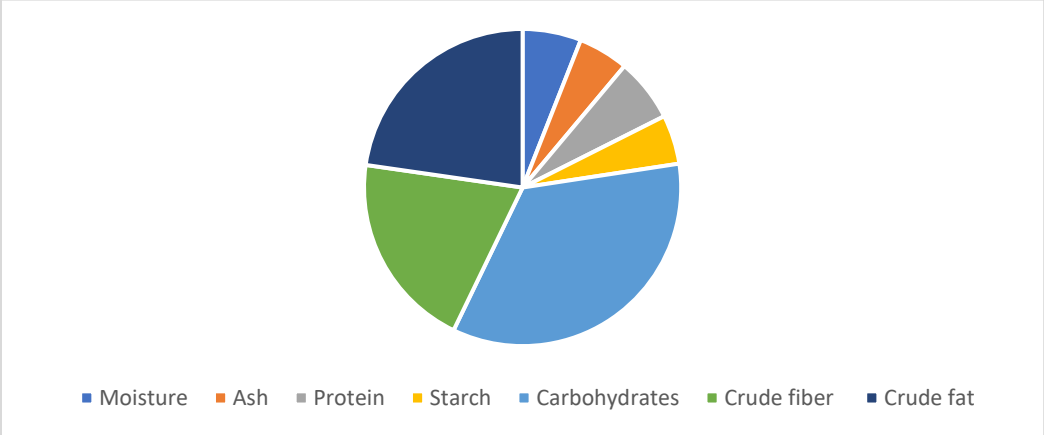

Fig.12. Main nutritional characteristics

Table 1. Test parameters for pineapple peel powder

S.No.	Parameter	Result
1.	Moisture	6.30% ± 0.24
2.	Ash	5.40% ± 0.015
3.	Volatile Solids	94.6% ± 0.123
4.	Total Dissolved Solids	30.42% ± 0.269
5.	Total Undissolved Solids	62.51% ± 0.19
6.	Total Solids	92.93% ± 0.431
7.	Total Organic Carbon	36.6 % ± 0.242
8.	Nitrogen as N	1.09% ± 0.1
9.	C/N Ratio	33.57
10.	Water-Swelling Capacity	0.1ml/g ± 0.001
11.	Cellulose	20 % ± 1.87
12.	Hemicellulose	28.5% ± 1.92
13.	Acid-soluble Lignin	2.71% ± 0.09
14.	Acid-insoluble Lignin	12.73% ± 0.13
15.	Protein	6.81% ± 0.268
16.	Starch	5.28% ± 0.24
17.	Carbohydrates	36.42% ± 0.196
18.	Crude fiber content	21.18% ± 0.372

4.2.1 Moisture

The moisture content of the DPPP-2 sample collected after sieving was checked using the method reported in APHA 1985 (Sondhi et al., 2020). The moisture content is important to understand as it affects the degradation process and the energy content of the waste. A higher moisture level can start microbial activity, which then causes the substance to undergo biological changes. Additionally, a particular amount of moisture is necessary for some chemical reactions, such as hydrolysis, to operate properly. About 6.3% moisture content was found in the sieved powder, almost like the pineapple peel flour, having a moisture content of 6.78% used in studies by Damasceno et al. (2016) for developing cereal bars.

4.2.2 Ash Content

The ash content of DPPP-2 was checked using the method reported in APHA 1985 (Sondhi et al., 2020) to identify the inorganic residue that remains after all the organic material has been completely burned. As it is crucial for determining the purity and energy content of the substrate,

it helps to determine the waste's calorie value. Following the test, an ash content of 5.40% was found, slightly lesser than the value (6.20%) reported by Kodagoda et al. (2017), for pineapple wastes used in developing non-alcoholic wines.

4.2.3 Volatile Solids

The test for volatile solids was performed according to the APHA 1985 method (Sondhi et al., 2020) to measure the amount of organic material that is quickly biodegradable or decomposable. It assists in quantifying the quantity of organic matter that is capable of being fermented, which is present in the substrate, that is directly related to the probable production of ethanol yield or other value-added products. So, the number of volatile solids present in the sample was found to be 94.6%, similar to observations by Syaliza et al. (2019) for biogas production from pineapple peel waste having 94.5% volatile solids.

4.2.4 Total Solids

The test for total solids was performed according to the method reported in APHA 1985 (Sondhi et al., 2020). Total solids refer to the portion of the waste that remains after all the moisture has been removed. It indicates the overall concentration of all solid components in the sample, as well as the organic content of the waste, which helps in determining the concentration of the sugars that can be fermented and other components that are available for valorization. A low total solids concentration could result in ineffective use of the reaction media, whereas a high total solids content could cause excessive viscosity, which would impact mixing and mass transfer. In our study, 92.93% total solids were estimated by adding 30.42% total dissolved solids and 62.51% total undissolved solids. Samuel et al. (2020) also performed this test and reported total solids content as 92.3% in their studies of the adsorption and biodegradation of phenols by pineapple peels.

4.2.5 Total Organic Carbon

The Dichromate Method (Thomas et al., 2018) was used to calculate the total organic carbon (TOC) present in DPPP-2. Proteins, lipids, carbohydrates, and organic acids are organic substances that form any sample's total organic content. Understanding the possible availability of organic carbon that can be changed into fermentable sugars during the pre-treatment and fermentation processes is important. About 36.6% of TOC was determined using this method, and the value was

found to be comparable with the one reported by Abdullah et al. (2008), i.e., 38.90% in their study of characterization of solid and liquid pineapple waste.

4.2.6 Nitrogen as N

The test for nitrogen was performed using the Kjeldhal method (Barbano et al., 1991). The carbon-to-nitrogen (C:N) ratio, which is essential for effective microbial growth and the production of ethanol, is directly impacted by the nitrogen content, which plays a vital role in the fermentation process. Its value was found to be 1.09%, greater than the 0.97% reported by Abdullah et al. (2008).

4.2.7 Probable C/N Ratio

The carbon-to-nitrogen (C:N) ratio is a key measure for determining how much carbon is present in an organic substance or substrate in relation to nitrogen. It is quantified as the proportion of total organic carbon to total nitrogen. The C:N ratio is important in the synthesis of bioethanol, especially during pre-treatment, because it affects many biological processes, such as microbial development, breakdown, and bioconversion. Bioethanol production depends critically on the C/N (Carbon: Nitrogen) ratio of the fermentation medium. It significantly affects microbial development, metabolism, and the effectiveness of fermentation as a whole. So, a suitable ratio will produce a high yield, whereas an unsignificant ratio will produce a decreased yield. It was analyzed by the method given by Mariotti et al. (2008) and estimated to be 33.57, similar to the 33.47 reported by Sondhi et al. (2020), for the kitchen waste used to produce bioethanol.

4.2.8 Water-Swelling Capacity (WSC)

The swelling capacity of the sample was analyzed by the method suggested by Dhar et al. (2022). The term "water swelling capacity" describes a substance's capacity to absorb and hold water, which causes them to swell or gain volume. It influences factors such as pre-treatment considerations, viscosity, nutrient diffusion, enzyme accessibility, moisture content, and fermentation efficiency. In our case, the DPPP-2 sample collected after sieving possessed 0.1mL/g WSC.

4.2.9 Cellulose

As cellulose comprises long chains of connected glucose molecules, it is a crucial source of dietary fiber and a key structural element of plant cell walls. Understanding these elements is helpful in

determining the substrate's potential for bioconversion and in optimizing pre-treatment techniques to reduce the complexity of compounds and improve sugar accessibility. Its content in DPPP-2 was found to be 20%. The result was in range with one reported by Aophat et al. (2019) to produce ethanol from pineapple peel waste having 21.98% cellulose content.

4.2.10 Hemicellulose

Hemicellulose was analyzed by the method of Deschatelets et al. (1986). and about 28.5% of hemicellulose was determined after the tests, which is a value comparable to 29.39%, reported by Paulo et al. (2022) in his study of the effect of chemical treatment sequence on pineapple peel powder. Efficient pre-treatment leads to the breakdown of hemicellulose content into enhanced quantities of fermentable sugars.

4.2.11 Lignin

Lignin was analyzed by the NREL method (Sluiter et al., 2019), which is an amorphous and highly cross-linked polymer composed of phenolic compounds. It is of two types: acid-soluble lignin and acid-insoluble lignin. It is essential for maintaining the structural integrity of plant cell walls and serves as a barrier, making it challenging for enzymes to access and break down the substrate's cellulose and hemicellulose. Estimation of lignin assist in selecting the best pre-treatment technique for the particular lignocellulosic material. In the present study, acid-soluble lignin was found to be 2.71% only, but acid-insoluble lignin was found to be 12.73%. Samuel et al. (2020) reported a nearby value of 11.52% of acid-insoluble lignin in the studies of the adsorption and biodegradation of phenols by pineapple peels.

4.2.12 Protein

The protein content was estimated using nitrogen factor (NF = 6.25) according to the NREL/TP-510-42625 for the nitrogen content obtained from the Kjeldahl method (Karimi et al., 2018). The content was checked to analyze its importance in maintaining the nitrogen content of the sample, thereby maintaining the C/N ratio. The protein content in DPPP-2 was found to be 6.81%, similar to the studies by Damasceno et al. (2016) for the development of cereal bars containing pineapple peel flour.

4.2.13 Starch

As an easily fermentable carbohydrate, starch can greatly increase the substrate's overall fermentable sugar concentration. The amount of starch, which was estimated by the anthrone method reported by (Kamaraj et al., 2020), was calculated to be 5.28% in DPPP-2.

4.2.14 Carbohydrates

Estimating carbohydrates can reveal how much sugar is there altogether in the pineapple peel powder. This comprises both sugars that can be easily fermented (such as glucose and xylose) and sugars that can be potentially fermented but are encased in complex carbohydrates (such as cellulose and hemicellulose). Knowing the substrate's original sugar concentration is essential for determining its potential for producing bioethanol since it informs the selection of the best pre-treatment and fermentation techniques. The carbohydrate content, was estimated using the anthrone method and was observed to be 36.42%, a value slightly lesser than 43.95%, a value reported by Kodagoda et al. (2017).

4.2.15 Crude Fiber Content

The AOAC 2005 technique was used to analyze crude fiber content in the DPPP-2 sample collected after sieving. The majority of lignocellulosic biomass is made up of fibers. Although they cannot be directly fermented by yeast, pre-treatment techniques can hydrolyze them into sugars that can be. In our research. it was estimated that DPPP-2 contains 21.18% fiber content, slightly lesser than the value reported by Khedkar et al. (2017) in the studies for biobutanol production from pineapple waste.

4.3 Characterization of pre-treated pineapple peel liquid hydrolysate

Nutrient estimations like total sugar, reducing sugar, starch, total phenolic content, and physical parameters like pH, ºBrix, and specific gravity of all the samples of three different pre-treatments were analyzed as shown in **Table 2, Table 3, and Table 4**.

4.3.1 Steam Explosion Pre-treatment:

The steam explosion pre-treatment uses water and high-pressure steam to break down the biomass but can cause the formation of fermentation inhibitors as part of its process (Kokta et al.,

1992). An autoclave reaction was performed on three samples at 121ºC and 15 psi pressure for 15 minutes. The different results analyzed for this pre-treatment are shown in **Table 2.**

Table 2. Results for steam explosion pre-treatment

Sample Name	pH	ºBrix	Specific Gravity	Total Sugar (mg/mL)	Reducing Sugar (mg/mL)	Starch Content (mg/mL)	Total Phenolic Content (mg GAE/mL)	Amino Acid Content (mg/mL)
SEP (C)	4.33	0.6	2.4	2.45 ±0.09	0.3 ±0.2	13.81 ±0.03	0.68 ±0.003	0.22 ±0.01
SEP (AL)	13.5	2.4	9.6	5.71 ±0.14	0.83 ±0.19	37.3 ±0.08	1.46 ±0.01	0.37 ±0.01
SEP (AC)	0.82	3.0	12.0	7.63 ±0.41	5.97 ±0.1	47.78 ±0.15	2.07 ±0.05	0.39 ±0.02

The most effective results were shown in steam explosion pre-treatment in the presence of acid [SEP(AC)], as it showed the highest total sugar content of 7.632 mg/mL, reducing content of 5.979 mg/mL, and starch content of 47.7813 mg/mL, with a ºBrix value of 3.0 and specific gravity of 12.0. Steam explosion pre-treatment in the presence of alkali [SEP(AL)] showed nutrient concentrations less than [SEP(AC)], steam explosion pre-treatment control [SEP(C)], showing the least amount of each nutrient. So, it can be concluded that steam explosion pre-treatment in the presence of acid SEP(AC) positively affected all the nutrients, such as total sugar, reducing sugar and starch contents, and playing an essential role in increasing them. Alkali pre-treatment removes the lignin, and hemicelluloses and increases accessible surface area (Kim et al., 2016). Whereas acid pre-treatment Hydrolyses both hemicelluloses and cellulose and hence gives a high sugar yield (Sharma et al., 2019).

4.3.2 Hot Water Pre-treatment:

The cell wall rigidity of lignocellulosic biomass can be significantly reduced by hydrothermal pre-treatment using liquid hot water. With comparatively little investment, it improves the conversion of polysaccharides into monosaccharides, especially cellulose, into glucose (Shilin et al., 2017). In this study, hot-water pre-treatment was performed on different combinations of solid loading and temperature, keeping the time constant.

The most effective results were shown by HWP-3 treatment, which represented 50 ºC, 10% solid loading, and 15 minutes of extraction time. HWP-3 positively affected all the nutrients (total sugar,

reducing sugar, starch content, total phenolic content, and amino acid content) by giving the highest value for each.

Table 3 Results for hot water pre-treatment

Sample Name:	Temp (ºC)	Solid loading (g)	Extraction Time (min)	pH	ºBrix	Specific Gravity	Total sugar (mg/mL)	Reducing sugar (mg/mL)	Starch Content (mg/mL)	Total Phenolic Content (mg GAE/mL)	Amino acid content (mg/mL)
HWP-1	50	2	15	5.05	0.6	2.4	5.17± 0.08	1.46± 0.01	6.23± 0.02	0.59± 0.01	0.29± 0.07
HWP-2	50	6	15	4.50	2.0	8	10.48± 0.09	9.81± 0.01	11.44± 0.03	1.44± 0.01	0.74± 0.02
HWP-3	50	10	15	4.45	3.8	15.2	23.61± 1.93	21.76± 0.4	21.02± 0.05	2.12± 0.03	1.32± 0.03
HWP-4	55	2	15	4.71	0.8	3.2	6.21± 0.42	1.63± 0.03	6.43± 0.01	0.55± 0.06	0.2± 0.009
HWP-5	55	6	15	4.69	2.0	8	12.8±0. 18	9.46± 0.04	10.89± 0.01	1.44± 0.01	0.68± 0.02
HWP-6	55	10	15	4.51	3.2	12.8	20.51± 0.36	17.14± 0.15	18.74± 0.04	1.89± 0.05	1.26± 0.04
HWP-7	60	2	15	4.86	0.6	2.4	4.94± 0.18	2.04± 0.01	4.71± 0.03	0.56± 0.06	0.22± 0.09
HWP-8	60	6	15	4.61	2.2	8.8	14.37± 0.32	10.86± 0.66	11.85± 0.05	1.55± 0.01	0.81± 0.03
HWP-9	60	10	15	4.46	3.2	12.8	17.35± 0.02	15.59± 0.12	18.33± 0.03	2.01± 0.05	1.23± 0.05

It showed highest total sugar content of 23.618mg/mL, reducing sugar content of 21.765mg/mL, starch content of 21.023mg/mL, total phenolic content of 2.126mg GAE/mL, and amino acid content of 1.329mg/mL, supported by the highest ºBrix value of 3.8 and specific gravity of 15.2. It is also noted that as the solid loading was increased, keeping the temperature and time constant, the concentrations of each nutrient increased, proving that the one with the highest solid loading turned out to be the best pre-treatment run, yielding the best results. Pineapple peel polysaccharides yield extraction is highly impacted by the solid loading. This phenomenon could be explained by the fact that when the solid loading rises, the surface area that the solvent has to interact with the solid material likewise gets better, causing the polysaccharide to dissolve out of the material entirely (Prakash et al., 2014). The main benefit of hot water treatment is that it uses lower temperatures and reduces the formation of degradation products. The necessity for a final washing step or neutralization was removed by this procedure. Another benefit for widespread application is the solvent's low cost (Nakashimad et al., 2005).

4.3.3 Ultrasonication pre-treatment:

The ultrasound-assisted procedure is among the most efficient pre-treatment methods. In this procedure, the ultrasonic radiation’s frequency, power, and duration damage the plant’s cell wall,

enabling particular feedstocks' cellulose and hemicellulose enzymes access (Casabar et al., 2020). This pre-treatment was performed on different combinations of temperature, solid loading, and time. The different results analyzed for this pre-treatment are shown in **Table 4.**

Table 4. Results for ultrasonication pre-treatment

Sample Name:	Temp (°C)	Solid (g)	Time (min)	pH	°Brix	Specific Gravity	Total Sugar (mg/mL)	Reducing Sugar (mg/mL)	Starch Content (mg/mL)	Total Phenolic Content (mg GAE/mL)	Amino Acid Content (mg/mL)
USP-1	50	2	30	4.72	0.8	3.2	5.28 ± 0.09	1.38 ± 0.03	8.05 ± 0.05	0.59 ± 0.03	0.31± 0.04
USP-2	50	6	15	4.47	2.2	8.8	18.7 ± 0.07	11.97 ± 0.33	15.14 ± 0.06	1.51 ± 0.02	0.66± 0.01
USP-3	50	6	45	4.51	2.2	8.8	14.11 ± 0.32	12.38 ± 0.18	15.85 ± 0.001	1.54 ± 0.06	1.16± 0.01
USP-4	50	10	30	4.42	3.8	15.2	24.56 ± 0.17	21.19 ± 0.19	26.69 ± 0.09	2.34 ± 0.04	1.61± 0.03
USP-5	55	2	15	4.73	0.6	2.4	4.05 ± 0.14	1.15 ± 0.01	6.88 ± 0.06	0.6 ± 0.09	0.24± 0.05
USP-6	55	2	45	4.83	0.6	2.4	3.29 ± 0.07	1.16 ± 0.04	13.57 ± 0.04	0.68 ± 0.02	0.3± 0.04
USP-7	55	6	30	4.54	2.2	8.8	14.6 ± 0.05	12.6 ± 0.13	15.0 ± 0.03	1.67 ± 0.01	0.96± 0.01
USP-8	55	10	15	4.42	3.6	14.4	21.6 ± 0.64	19.67 ± 0.17	23.86 ± 0.05	2.32 ± 0.02	1.39± 0.03
USP-9	55	10	45	4.46	3.6	14.4	18.88 ± 0.49	21.19 ± 0.18	23.86 ± 0.08	2.29 ± 0.01	1.57± 0.08
USP-10	60	2	30	4.78	0.6	2.4	5.13 ± 0.07	1.11 ± 0.1	12.81 ± 0.032	0.64 ± 0.01	0.29± 0.04
USP-11	60	6	15	4.48	2.2	8.8	17.39 ± 0.57	9.78 ± 0.05	14.69 ± 0.09	1.54 ± 0.01	0.94± 0.03
USP-12	60	6	30	4.57	2.2	8.8	14.71 ± 0.72	9.38 ± 0.05	17.32 ± 0.04	1.48 ± 0.01	0.92± 0.01
USP-13	60	6	45	4.54	2.2	8.8	14.3 ± 0.08	11.14 ± 0.06	18.49 ± 0.04	1.26 ± 0.01	0.9± 0.03
USP-14	60	10	30	4.51	3.8	15.2	23.87 ± 0.24	20.52 ± 0.13	25.83 ± 0.06	2.12 ± 0.03	1.28± 0.03

USP-4, which signified 50°C temperature, 10% solid loading, and 30 minutes extraction time, was the best pre-treatment, showing the highest results for each nutrient estimation. It showed the

highest total sugar content of 24.561mg/mL, reducing sugar content of 21.19mg/mL, the starch content of 26.69mg/mL, phenolic content of 2.34 mg GAE/mL, and amino acid content of 1.61mg/mL, supported by the ºBrix value of 3.8 and specific gravity of 15.2. Among USP-8 and USP-4, when the temperature was decreased, it increased the content of all nutrients, whereas when the time was decreased, the content of all the nutrients decreased. The other runs that showed the best values were USP-4, USP-14, USP-11, and USP-9, which also showed significant amounts of sugars, phenols, and amino acids. As the solid loading was increased from 2% to 6% to 10%, we observed a significant increase in the nutrient values of each pre-treatment, yielding the highest amount of nutrients at a 10% solid loading. It was also proved that the best results were from 10% solid loading among all the pre-treatments. One of the most crucial elements in the optimization of ultrasound assisted extraction is the water/solid material ratio (solid loading). Pineapple peel polysaccharides yield extraction is highly impacted by the water/solid material ratio. This phenomenon could be explained by the fact that when the water-to-solids ratio rises, the surface area that the solvent has to interact with the solid material likewise gets better, causing the polysaccharide to dissolve out of the material entirely (Prakash et al., 2014). The total sugar and reducing sugar concentrations increased as the temperature increased. Hence, ultrasonication became an efficient pre-treatment method for many essential nutrients and enhanced fermentable sugars, which can be converted to high-value products like bioethanol.

4.4 Characterisation of DPPP

The best ultrasonic pre-treated sample (USP-4) was compared with DPPP-2 in the characterizations. USP-4 showed the highest amount of the nutrient estimations, which was compared with our initial sample DPPP-2, which was used for nutrient estimations of dried pineapple peels. Both of these were compared to analyze the changes, like structure and stability, after pre-treatment of the sample.

4.4.1 TGA analysis:

One of the crucial factors during the thermal processing of food unit operations is the thermal stability of a polysaccharide. TGA was performed to differentiate the changes in the thermal behaviours and thermal stability of DPPP-2 and ultrasound-assisted dried pineapple peel waste, as shown in **Fig. 13.**

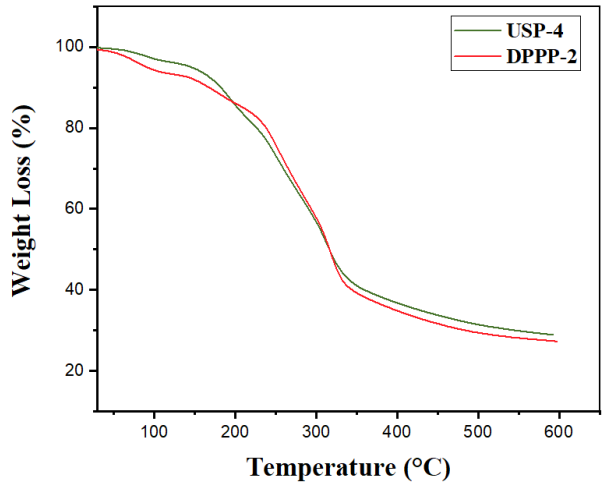

Fig. 13. Thermo-gravimetric analysis of Pineapple peels powder (With/without ultrasonication)

A temperature range of 0 ºC to 700 ºC was used for TGA. Results showed variations in every sample at the various temperature ranges (40 ºC -200 ºC, 200 ºC -325 ºC, and 325 ºC -450 ºC up to 630 ºC), and it was noticeable that the degradation kinetics of the samples varied at these temperatures. Temperature degradation was seen in pineapple waste DPPP at 35 ºC to 100 ºC, 100 ºC to 200 ºC, 200 ºC to 295 ºC, and even up to 625 ºC (Dhar et al., 2022). The findings showed that the first temperature range of 30 ºC to 140 ºC was where the absorbed water evaporated and low molecular mass components degraded (Ma et al., 2016). According to studies, hemicellulose degrades relatively quickly between 210 and 35 ºC (Moriana et al., 2011; Yang et al., 2007), whereas cellulose (cleavage of cellulose glycosidic bonds) degrades at 315 ºC and continues to degrade up to 400 ºC (Liu et al., 2021). Variable thermal breakdown temperatures have been noted in DPPP-2 due to the presence of various functional groups, including lignin, cellulose, and hemicellulose. This outcome revealed that dried pineapple peel waste, aided by ultrasound, absorbed more water. The temperature ranges show that cellulose degradation and hemicellulose depolymerization occurred (Begum & Deka, 2019). The thermal breakdown of char was prolonged when the pyrolytic temperature rose to the second temperature range (200-315 ºC). The weight loss of ultrasound-assisted dried pineapple peel was more than DPPP according to the temperature range investigation (40 ºC -200 ºC, 200 ºC -325 ºC, and 325 ºC -450 ºC up to 630 ºC), showing that ultrasound-assisted dried pineapple peel waste samples had more vital thermal stability than pineapple waste DPPP (Paulo et al., 2022) (Dhar et al.,2022).

4.4.2 XRD analysis

An XRD study was carried out to determine the changes in the crystallinity and amorphous region of DPPP-2 and to identify the cluster region of the ultrasound-aided dried pineapple peel powder USP-4. Compared with the pineapple waste DPPP-2, XRD patterns of USP-4 were obtained. The X-ray diffractogram was used to access the variations in crystallinity of the dried pineapple peel powder produced with ultrasonic assistance and pineapple waste DPPP-2, as shown in **Figure 14.** Analysis of the DPPP-2 and USP-4 patterns showed prominent peaks at 21.49 and 35.17, whereas non-crystalline minor peaks were found at 16.0 (Dhar et al., 2022). The sharp peak in USP-4 signifies that it was the best effective pre-treatment method, as the amorphous portion was removed and the crystalline nature was increased (Dhar et al., 2022).

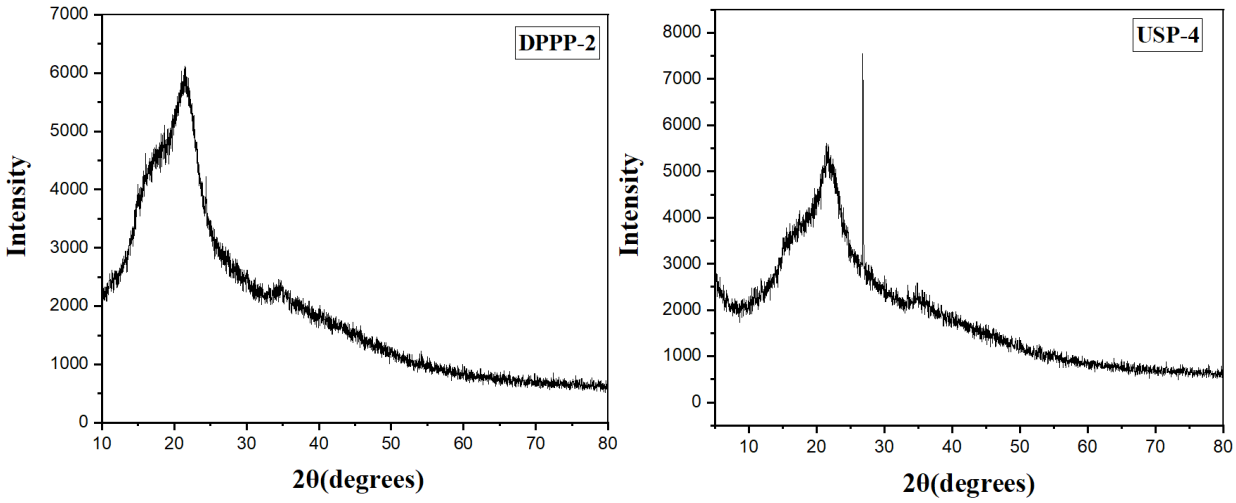

Fig.14. XRD analysis of Pineapple peels powder (With/without ultrasonication)

(a) DPPP-2 (b) USP-4

According to the graph, it can be seen that ultrasonication treatment substantially disrupted the cell structure of ultrasound-assisted dried pineapple peel powder, which makes hydrolysis of cellulose and hemicellulose part of the sample into strong ordered crystalline structure from less ordered crystalline or even amorphous structure. Thus, the characteristic crystalline structure was found in USP-4, and the interaction between pineapple peel molecules weakened due to the removal of the amorphous structure (Kaur et al., 2023).

4.4.3 SEM analysis

SEM was employed to analyze the morphological properties of the ultrasound assisted sample USP-4 and the DPPP-2 sample and further compare the change in morphologies of the DPPP-2 sample on pre-treatment. As shown in **Fig. 15.**, DPPP-2 exhibited uneven morphologies with varying particle sizes, massive wrinkled and depressed blocks (i.e., a form of flakiness), as seen with greater magnification, and a dense structure because non-cellulosic components, including pectin, lignin, and hemicellulose were unaltered (Paulo et al., 2022). USP-4 exhibited the best results as it can be seen that its surface was the smoothest, it had loosened and is more exposed, creating huge pockets/sites for enzyme attack. The microstructure variation implied that the ultrasonication treatment disrupts the solute with the solvent, resulting in a honeycomb structure of ultrasound-assisted pineapple peel powder (Begum et al., 2019). Thus, the removal of lignin and hemicellulose and alteration of external and internal structure may improve cellulose and hemicelluloses accessibility to enzymes, which contributes to an increase in the efficiency of enzymatic hydrolysis (Waesarat et al., 2016).

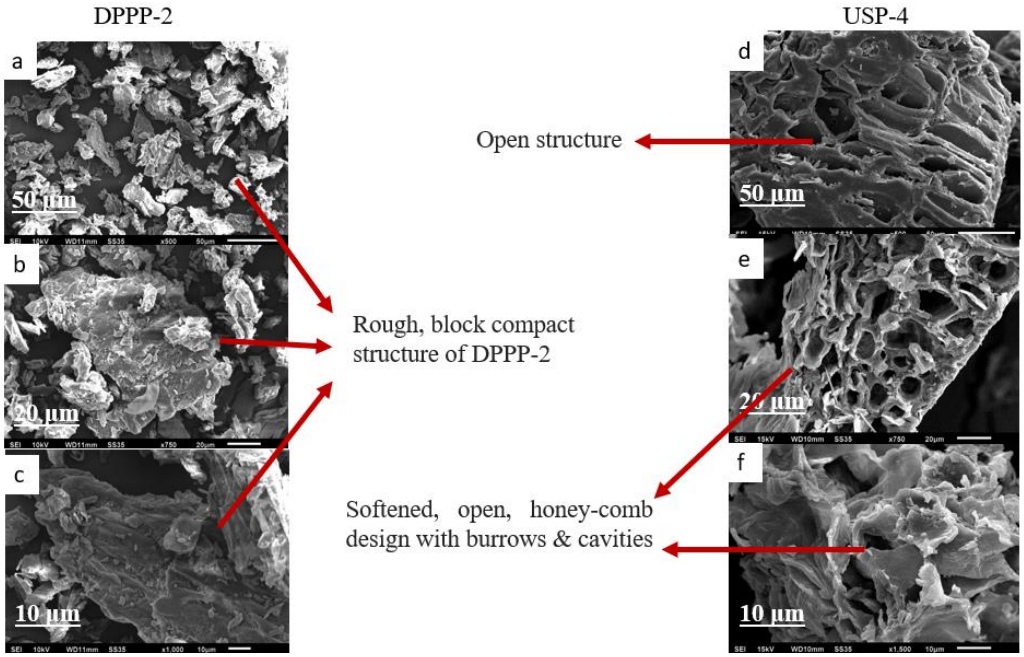

Fig.15. SEM images of pineapple peel powder without ultrasonication pre-treatment having rough, block compact structure at a) 50 µm b) 20 µm c) 10 µm, and with ultrasonication pre-treatment having open, softened, honeycomb design with burrows & and cavities at d) 50 µm e) 20 µm f) 10 µm

CHAPTER 5: CONCLUSION

In the agro-industrial sectors, the pineapple (*Ananas comosus*), a fruit that is in high demand worldwide, generates a lot of waste, specifically pineapple peels. Waste valorization was made possible by the essential properties of pineapple peels as a feedstock for the creation of fermentable sugars that could be used for ethanol production. Various nutrient estimations and other characterizations were analyzed for the dried pineapple peel powder (DPPP), the results of which showed that the dried pineapple peel powder would be a potential feedstock for the enhancement of sugar production. For these, three different pre-treatments were chosen, which were steam explosion, hot water, and ultrasonication pre-treatment. After which, characterizations like XRD, TGA, and SEM were performed for the pre-treated samples in comparison to the dried pineapple peel powder to analyze thermal stability, structural morphology, and functional group changes. Based on the results, we have concluded that pineapple peels are a potential feedstock for sugar production. The study results have shown that USP-4, which symbolized 50°C temperature, and 10% solid loading in 30 min of extraction time, exhibited the highest nutrient estimates and emerged as the best pre-treatment condition. Comparing an ultrasonic extracted pre-treated sample to dried pineapple peel powder also showed that the former had greater functional qualities. In comparison to the powder made from dried pineapple peel, the ultrasound-extracted sample's FTIR, XRD, SEM, and TGA analyses revealed that it had all the functional groups, a semicrystalline pattern, a honeycomb structure with a smooth surface, and good thermal stability. Therefore, it was analyzed that sugar concentrations were enhanced along with other nutrients like phenolic content, starch, and amino acids, which can be valorized further for bioethanol production. Lastly, we concluded that the techniques which we used in this study can give rise to the low-cost and environment-friendly ways to produce beneficial fermentable sugars from pineapple fruit peel wastes, which are necessary for the production of ethanol from the dried pineapple fruit peel waste.

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ABSTRACT The pineapple (Ananas comosus), a fruit that is in high demand all over the world, produces a lot of waste in the agro-industrial sectors, particularly pineapple peels. The key qualities of pineapple peels as a feedstock for the manufacture of fermentable sugars that might be utilised for ethanol production led to waste valorization. Various nutrient estimations were analysed for the dried pineapple peel powder, the results of which showed that the dried pineapple peel powder would be a potential feedstock for the enhancement of sugar production. For these three different pre-treatments were chosen, which were steam explosion, hot water, and ultrasonication pre-treatment. After which characterisations like XRD, TGA and SEM were performed for the pre-treated samples in comparison to the dried pineapple peel powder to analyze thermal stability, structural morphology and functional group changes. Therefore, the fermentable sugar concentration increased in the end. Lastly, we concluded that the techniques which we used in this study can give rise to the low-cost and environment-friendly ways to produce beneficial fermentable sugars from pineapple fruit peel wastes which are necessary for the production of ethanol from the dried pineapple fruit peel waste.