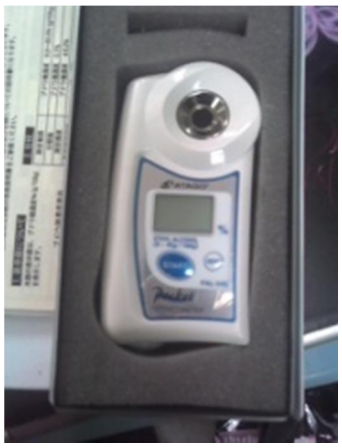
Chapter 3

3.0 MATERIALS AND METHODOLOGY

3.1 Instruments

For this research basic instrumentation (Figure 3.1 (a)-(g)) like refrectrometer, multi-element oil analyzer (MOA II), viscometer, titrator, pH meter, water bath and volumetric flask, conical flasks, incubator, Schott bottles were used.



(a) Ethanol refractometer

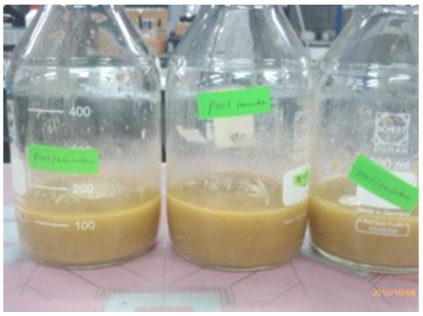
(b) Glucose refractometer

(c) pH meter

(d) Total soluble solid (TSS) meter

(e) Filtration kits

(f) Philips house hold blender



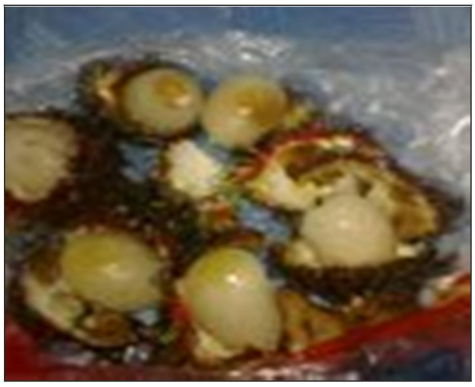
(g) Schott bottles

Figure 3.1 Equipment

3.2. Materials

3.2.1. Raw Material:

The fresh rambutan, mango, banana and pineapple were bought from grocery shop at Petaling Jaya Kuala Lumpur, Malaysia. They were kept at room temperature (27 ^{o}C) for seven days until it fully ripen and became rotten before undergoing the fermentation. To avoid other contamination, the rotten fruits (Figure 3.2 (a)-(d)) were washed before they are cut and chopped for fermentation.



(a) Rambutan

(b) Mango

(c) Banana

(d) Pineapple

Figure 3.2 Rotten fruits

3.2.2. Microorganism:

The microorganisms were obtained from the commercial supermarket (Commercial dry yeast) and ABO lab. S. cerevisiae was used as yeast. It was underwent warming in water bath at 40°C for 15 min that to make it more active and ready to start fermentation (Figure 3.3).

Figure 3.3 Yeast

3.2.3. Enzymes

Enzymes used were cellulase from *Aspergillus niger* and α-amylase from *Bacillus* species. Both were supplied by ABO Laboratory, Kuala lumpur.

3.2.4. Chemicals and reagents:

The chemical reagents were purchased from Chemolab, the chemicals were sodium hydroxide (NaOH), hydrochloric acid (HCl) and Ethano assay reagent (potassium dichromate, sulfuric acid, diphenylcarbazide and 95% ethanol).

3.3. METHODS

3.3.1. Sample Preparation

Rambutan, mango, banana and pineapple waste fruits were used following the same technique of blending. The fruits were washed, cut into small pieces together with their skin and blended in a

Philips household juice blender (Figure3.1 (f)) for 3 minutes. The skin and the juice obtained were mixed together before dispensing them into 1L Schott bottles (Figure 3.1 (g)) and experiments were done in triplicates. Each bottle contained 100 ml of the mixture. The fresh weight was measured. The Total Soluble Solids (TSS) (Figure3.1 (d)), initial ethanol and glucose contents were measured by Figures 3.1 (a) and (b) respectively. The initial pH of the mixture was also recorded using the pH meter (Figure 3.1 (c)).

3.3.2. Fermentation:

The general process of fermentation is described as a reaction in a Schott bottle containing fruit contents and yeast. The optimum ethanol production as a result of fermentation was investigated by using different parameters as following: The optimum yeast concentration for maximum ethanol production was determined with the addition of 4g/L of yeast in the mixture containing fruit contents, and the bottles were shaken so that the yeast was mixed together with the samples. The samples were then placed in the incubator at 30°C and left there for 3 days. Parameters that were studied were fermentation incubation time, fermentation temperature, fermentation by using different components of fruits and fermentation by using rotten and fresh fruits. Fermentation incubation time was conducted at 1 day, 2 days and 5 days. The skin, pulp and mixture of the fruits were separated to be used for the fermentation involving different components of fruits. For fermentation with skin, water was added to the skin that has been blended to activate the yeast. Fermentation was followed after the enzymatic digestion of the pH was adjusted using 5 M natrium hydroxide (NaOH) to increase the pH and 1 M acid hydrochloride (HCl) to decrease the pH whenever needed.

3.3.3 Fermentation for different fruits parts:

For fermentation of different parts used pulp, skin and seed were separated to be used for the fermentation; 200ml of water was added to 1 kg of fruit skin that has been blended to activate the yeast.

3.3.4. Fermentation under different pH

Using 5 M Sodium hydroxide (NaOH) was used to adjust the pH. In the experiment 4, 5, 6 different pH values were used to know optimum pH. For fermentation kept it for 4 days at 30^{o}C using 4 g/L of yeast.

3.3.5. Fermentation at different Yeast Concentration

The optimum yeast concentration for maximum ethanol production was determined by experiment with the addition of 2, 3 and 4g/L of yeast in the mixture containing 1 kg fruit contents, the Schott bottles were shaked in order to mixed the yeast together with the samples properly. After which, the bottles were incubated at 30°C for 4 days.

3.3.6. Fermentation at different Temperature

The effect of different fermentation temperature 28, 30, and 35 ^{o}C on the product yield was also studied following the same procedure of fermentation.

3.3.7. Fermentation at different Days

The effect of fermentation time was studied over a period of 24, 48 and 72 hours.

3.3.8. Comparison of bioethanol yield between rotten and fresh fruits wastes:

Comparison of bioethanol yield and quality between the rotten fruits fermentation and fresh fruits was also observed under the same fermentation process, except changing the biomass composition.

3.3.9. Enzymatic hydrolysis in rambutan, banana, pineapple and mango fruits:

Enzymatic hydrolysis was done using cellulase and amylase. 3mg/L of each enzyme were weighed and add with 3g/L yeast.

3.4. Filtration

After a specific reaction time, the mixtures in the bottles were then filtrated using a beaker covered with a piece of folded cheese cloth (Figure 3.1(e)). The liquid obtained inside the glass was the raw bioethanol. The volume of the raw bioethanol was measured using the measuring cylinder and it was then transferred into a labeled plastic bottle. The pH and TSS of the raw bioethanol were measured and weight of the residues was also checked.

3.5. Bio-Ethanol measurement:

Sample bioethanol yeild was measured using ethanol kit (ATAGOrefrectrometer) (Japan) (Figure 3.1 (a)) as follows: 0.6 ml of bio-ethanol sample was put on the digital refractometer and ethanol (%) was shown directly .

3.6. Glucose measurement:

The sample's glucose content was measured by using glucose meter (Glucose refrectrometer) (Japan)(figure 3.1 (b)) as follows: 0.6 ml of sample was put on the refractometer using pipette and percentage glucose was recorded directly

3.7. Chemical analysis:

Samples from fermentation pH parameter were tested for chemical compo nents and viscosity test. Chemical analysis by using Multi Element Oil Analyzer (MOA) II was conducted to measure various chemical components that can be found in the bioethanol.

3.8. Viscosity:

Samples for rambutan, banana, pineapple and mango were also analyzed for the viscosity of the bioethanol produced (Table 8). Viscosity was analysed using a viscollite 700 (hydramotion, New York) viscometer at 40^{o}C temperature.

3.8.1 Acid Value

Total acid number was measured for all samples by using ASTM D445 standard

3.9. Engine Test

Samples that have been tested for chemical and viscosity analysis were then being tested to run the multicylinder engine of Proton Gen2 by using 5% and 10% bioethanol and compared with 100% pure petrol. (Synersy 97, shell)