CHAPTER 1

1.0 INTRODUCTION

The single most important driver of the modern world is fuel and this fact has essentially put fuel at the central position to global economy and politics. Unfortunately the major sources of fuel (oil, gas or coal) are non-renewable and they are diminishing fast (Dien *et al.,* 2003). Moreover, these traditional fuels are the major sources of air pollution and green-house effect which is leading to 'Climate Change'- a threat considered to be the greatest ever threat to the human civilization (Ture*et al.,* 1997). On the other hand any alternative source of renewable energy is coming up with extremely high cost and technological intervention. In this backdrop, renewable energy made from organic matter (biomass) is called bio-energy. Plants and animals, or more broadly "biomass" is the sources of bio-energy, that includes agricultural wastes (vegetable, pineapple, apple waste, grape) and forestry residues, municipal solid wastes, industrial wastes, and terrestrial and aquatic crops grown solely for energy purposes (Hossain *et al.*, 2008; Hossain *et al.,* 2010a; Hossain *et al.,* 2010b). Among the biofuel used for transportation, ethanol is the most widely used inform of blended fuel as additive for gasoline and it has become pioneer to the alternative energy revolution (Hansen *et al.,* 2005). Bioethanol, defined as ethyl alcohol originated from biological sources, is derived from fermenting the sugar component of plant material (Atals and Bartha, 1998).

Bioethanol is viewed as an alternative to fossil fuel because it is seen as a renewable resource that may be exploited using more environmental friendly

technologies. Nowadays, biomass resources are used to generate electricity and power by gasification process as well as biofuel production for transportation. Biofuel, mainly bioethanol produced from plants, showed promising marks in the field of renewable energy source. Domestic production of biofuel such as ethanol can decrease the dependency on imported oil, create jobs in the rural areas and help protect the environment by reducing carbon emission (Demirbas, 2006; Demirbas, 2008).

Although methanol and ethanol both reduce emissions in diesel engines, ethanol has the advantage of being a renewable fuel because of having a higher miscibility. Therefore, the use of ethanol in compression ignition (CI) engines has received considerable attention in recent years (Can *et al.,* 2004). Bioethanol can be used in unmodified petrol engines with traditional fueling infrastructure and is easily applicable as additive for gasoline (Hansen *et al.,* 2005). Ethanol may be used as an alternative fuel, for example, in E-85 for flex fuel vehicles, and may also be used as an octane-boosting, pollution-reducing additive to gasoline, such as E-10 that is widely available at gas stations in most parts of the U.S., Brazil and Europe. Although, availability of E-85 is much limited, its' uses is growing and there are currently more than 7 million vehicles on the road today that can use biofuel as the alternative fuel.

However, some difficulties are encountered in case of using alcohols in diesel engines (Qudais *et al.,* 2000). Its limited miscibility with diesel at lower temperatures and the required minor variations in fuel delivery systems restrict the use of ethanol in diesel fuel (Gerdes and Suppes, 2001).

Gasification is an advanced thermochemical and biochemical second generation bio-ethanol technology, which can convert cellulose, hemi-cellulose and lignin efficiently to clean bio-ethanol. It delivers at least 90% greenhouse gas savings compared to petrol.

Two reactions are keys to make understanding how biomass is converted to bioethanol. Firstly, hydrolysis, that converts the complex polysaccharides in the raw feedstock to simple sugars. In the biomass-to-bio-ethanol process, acids and enzymes are used to catalyze this reaction (Demirbas, 2008). Hydrolysis of cellulose is emerging second generation technology. Cellulosic fermentation still has several cost challenges. The cellulose in biomass can be readily converted to C6 sugars for onward conversion to ethanol. It is more challenging to economically convert the C5 sugars from hemi-cellulose to ethanol while the lignin cannot be converted (Demirbas 2005).

Secondly, fermentation is a series of chemical reactions that convert sugars into ethanol. The fermentation reaction is caused by yeast or bacteria, which feed on the sugars. Ethanol and CO_2 are produced as the sugar is consumed. The simplified fermentation reaction equation for the 6-carbon sugar, glucose, is:

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} \longrightarrow \underset{\text{(ethanol)}}{2CH_3CH_2OH} + \underset{\text{(carbon IV oxide)}}{2CO_2} \quad \text{..... Eq. (1)}$$

There are three main approaches to producing bioethanol from biomass materials:

1. Conventional fermentation of sugars obtained from sugar and starch crops.
2. Hydrolysis of cellulose to sugars using acid or enzymes followed by fermentation of the sugars.

3. Gasification of any biomass to syngas followed by catalytic conversion to bio-ethanol.

Ethanol production as bio-fuel for transportation sector has tripled between 2000 and 2007, and share of biofuel in global fuel market is rising significantly every year. Research and practices in the field of biofuel have also increased giving rise to second and third generation biofuels. To utilize this potential resource efficiently, more research is needed specifically on efficient and renewable sources of biofuel to subdue its contradiction with food production, water resource and deforestation. In this connection, this project could examine the potential of using rotten fruits as biomass feedstock for ethanol production.

1.1 Research Objectives

With a view to obtain detailed understanding about the bio-ethanol production from grapes and apple fruit waste as renewable energy, the study aims to observed the following aims:

1. To determine the proper yeast concentration, fermentation initial pH, fermentation time and temperature for bioethanol production using rotten grapes and apples as feedstocks.
2. To determine the yield of bio-ethanol production from different fruit parts (skin and pulp).
3. To characterize the selected physicochemical properties of the produced bioethanol through engine test.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Bioethanol

Bioethanol (CH_3CH_2OH) is an ethyl alcohol that is volatile, colorless liquid having a density of 0.789 g/mL and a boiling point of 78.5 ^{o}C, which is produced from feedstock containing sugar or other material that can be converted into sugar with reasonable effort (Atals and Bartha, 1998). Biomass material which is used for bio-ethanol production divided into sugar, starch, and lignocellulosic materials. Today, over 60 % of the world's ethanol production comes from sugar crops (Rosillo- Calle and Walter, 2006).

The estimated world ethanol fuel production in 2009 has been reported to reach 19.5 billion gallons (about 73.9 billion liters). Brazil and the United States, together both countries were responsible for 89% of the world's ethanol fuel production in 2009 (Hansen *et al.,* 2005). World ethanol production for transport fuel increased three times between 2000 and 2007 from 17 billion to more than 52 billion liters. From 2007 to 2008, the share of bioethanol in global market increased from 3.7 to 5.4 % (United Nations Environment Programme, 2009). Approximately 9% of the ethanol produced synthetically, the rest 91% are produce by fermentation (Wheeler *et al.,* 1991). Ethanol has the advantage of being a renewable fuel because of having a higher miscibility. Therefore, the use of ethanol in compression ignition (CI) engines has received considerable attention in recent years (Can *et al.,* 2004). Bio-ethanol can be used in unmodified petrol engines with traditional fueling infrastructure and is easily applicable as additive for gasoline (Hansen *et al.,* 2005).

Fuel ethanol provides numerous benefits in terms of environmental protection, economic development and national energy security (Yang and Lu, 2007).

The majority of the energy used today is obtained from fossil fuel. Due to the continuing increases in the cost of fossil fuels coupled with environmental effects, demand for clean energy has been increased over the decade. Ethanol being one of the renewable energy sources and obtained from biomass has been tested intensively in the internal combustion engines (Wyman, 1996). Some properties of ethanol with comparison to gasoline are given in Table 2.1. Currently, ethanol for fuel market is produced from sugar or starch at competitive price. However, this raw material were mainly used for human and animal consumption, as such are not sufficient to meet the increasing demand for fuel ethanol (Farrell,2006).

Table 2.1 Properties of gasoline and ethanol

Properties	Gasoline	Ethanol
Chemical formula	C_4-C_{12}	C_2H_5OH
Molecular weight	100-105	46
Oxygen (mass %)	0-4	34.7
Net lower heating value (MJ/Kg)	43.5	27
Latent heat (KJ/L)	223.2	725.4
Stoichiometric air/fuel ratio	14.6	9
Vapor pressure (KPa) at 23.5 ^oC	60-90	17
MON	82-92	92
RON	91-100	111

Source: Das, (1996)

Ethanol has high heat of evaporation, high octane number and high flammability temperature that confer a positive evaporation pressure, which makes it to be safer for storage and transportation.

2.1.1 Production of bioethanol

Bioethanol is produced from biological materials containing sugar or from other material that can be converted into sugar. This means that different of input can be used including sugar crops, grain crops, cellulosic crops and waste biomass materials such as crop residues (Rosillo-Calle and Walter, 2006). To produce bio-ethanol from sugar crops this process started by extracting the sugar from the plant material by crushing, soaking, or chemical treatment. Afterwards, in the fermentation process, the sugar is converted into alcohol *via* fermentation using microorganisms such as bacteria and yeast. Through this process, sugar containing materials are normally transformed into glucose, which is used as fermentation substrates under anaerobic conditions to give ethanol and carbon dioxide through a process of glycolysis. However, other carbohydrates are converted into bioethanol by the process of phosphorylation, which is carried out through the metabolic pathway. In general, the produced ethanol is then distilled or purified to remove excess water by dehydrating it (Ingram, 1998).

$$(C_6H_{12}O_6)n + nH_2O \rightarrow nC_6H_{12}O_6 \qquad \text{eq. (2.1)}$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{energy (stored as ATP)} \qquad \text{eq. (2.2)}$$

To produce ethanol from grain (such as wheat, barley, or maize), more steps and energy intensive process are needed. The process involves milling of grain, hydrolysis of starch to release fermentable sugar, followed by microbial fermentation (Peterson, 1995). Yeast cannot use starch directly for ethanol production. Therefore, grain starch has to be wholly broken down to glucose by combination of two enzymes, *viz*, amylase and amyloglucosidase, before it is

fermented by yeast to produce ethanol. Alcohol produced from fermented broth and remaining spillages is processed to produce distiller's dried grain and soluble (DDGS), which is an excellent ingredient for animal feed (Sheorain, 2000).

Ethanol production from cellulosic biomass is still under development and has not yet been applied to produce ethanol on a large scale. The process involves the separation of plant materials similar to the pulping process, in a combination of physical and chemical treatment of cellulose to separate it from hemi-cellulose and the lignin. The focus lays in cellulose output which is to be converted into sugar through hydrolysis (IEA, 2004).

2.1.2 Sources of bioethanol

Carbon based feedstock especially agriculture feedstock which is considered renewable, is the main source of bio-energy such as biodiesel or bioethanol. Sugarcane, sugar beet, kenaf, cassava, bagasse, sunflower, fruits, corn, switch grass, grain, wheat, sweet potato, cotton, potatoes other biomass have been used as source of bioethanol in different countries (IEA, 2004; Rosillo-Calle, 2006). For instance, palm oil is used as a main feedstock in Malaysia, whereas coconut in Philippine, cane molasses in Thailand and Vietnam, soybean oil and used oil in Korea. Indonesia also uses palm oil as main source to produce biodiesel. Nippon Oil Corporation and Toyota Motor Corporation are two Japanese companies that have announced to produce oxygen free biofuel from the palm oil. However, it is always recommended to produce bioethanol from non-food stock substrate.

2.1.3 Bioethanol economics

Usually, bioethanol is made from farm-produced raw products (e.g corn, palm oil, sugarcane, lignocellulosic materials etc.) which are normally found in surplus. Bioethanol production is the third largest user of sugarcane, behind domestic livestock feed and export uses (Rosillo-Calle, 2006). As the domestic ethanol industry continues to grow, it is witnessing a surge in the construction of farmer-owned ethanol production facilities. Farmers are realizing the added benefits to the bio-ethanol industry through ownership of manufacturing plants. Ethanol's importance to agriculture is evident like added markets for farmers, stimulating rural economies by increasing agricultural crops prices and rural income (Farrell, 2006).

2.1.4 Bioethanol and environments

Directly related to fossil energy consumptions the question of greenhouse gas emissions, it has the potential to reduce greenhouse gas emissions from automobiles relative to gasoline, therefore, reducing the risk of possible global warming. Because, ethanol contains carbon, combustion of the fuel necessarily results in emissions of carbon dioxide (CO_2), the primary greenhouse gas. Further, greenhouse gases are emitted through the production and use of nitrogen-based fertilizers, as well as the operation of farm equipment and vehicles to transport feedstock and finished products. However, since photosynthesis (the process by which plants convert light into chemical energy) requires absorption of CO_2, the growth cycle of the feedstock crop can serve to some extent as a sink to absorb some fuel-cycle greenhouse emissions. Previous study reported that higher fuel-cycle energy consumption for ethanol production results in higher greenhouse gas emissions (Farrell, 2006).

2.1.5 Production of bioethanol from corn

Corn is the main feedstock used for producing bio-ethanol fuel in the United States, North America is the highest corn yields in the world and it is mainly used as an oxygenate to gasoline in the form of low-level blends, and to a lesser extent, as fuel for E85 flex fuel vehicles (Goettemoeller,*at el.,* 2007). Corn grain contains high amount of starch and long chains of sugar but low in cellulose (Demirbas, 2005). There are two main types of corn ethanol production: dry and wet milling. The products of each type are utilized in different ways. Currently, the majority of ethanol is produced from corn. One bushel of corn produces about 2.7 gallons of bio-ethanol. According to the Renewable Fuels Association, the production of bio-ethanol does not mean less corn available for food, it actually produces much valuable high protein food and feed co-products. For example, an acre of corn produces 313 gallons of ethanol, 1,362 pounds of protein feed for livestock, 325 pounds of 60% gluten meal, and 189 pounds of corn oil in a wet mill process (IEA, 2004). The U.S. ethanol industry consumes 560 million bushels of corn, and boosts the price of corn by 8-104 USD per bushel. When translated to income, this represents additional earnings of $2.2 billion each year to corn producer's nationwide (National Biodiesel, 2008). Kim and Dale (2002) estimated the total energy requirement for producing ethanol from corn grain at 560 KJ/mole of ethanol, indicating that ethanol used as a liquid fuel could reduce domestic consumption of fossil fuels, like petroleum.

2.1.6 Production of bioethanol from sugarcane

Brazil has 851 million hectares from which around 5 million are presently used for sugarcane plantations and only country to use 100% bioethanol from

sugarcane biomass for vehicle. Waste sugar cane could produce bio-ethanol capable of blending 1: 6 of gasoline to bio-ethanol, replacing 1:1 of gasoline to bioethanol as in E85 fuel (Bohorquez and Herara, 2005). Sugar cane bagasse is a co-product in sugar cane food manufacture, and the yield of bagasse is about 0.6 dry kg per 1 dry kg of sugar cane used in food manufacture (Bohorquez and Herara, 2005). Furthermore, lignin-rich fermentation residues from bagasse could generate 103 KWh of electricity and 593 PJ of steam. Waste sugar cane and sugar cane bagasse could produce about 53 gallon of bio-ethanol, replacing 38 gallon of gasoline in an E85 midsize passenger vehicle, or about 3.4% of the global gasoline consumption (Kim and Dale, 2004).

2.1.7 Production of bioethanol from algae

Ethanol from algae is possible by converting the starch (the storage component) and cellulose (the cell wall component). Lipids in algae oil can be made into biodiesel, while the carbohydrates can be converted to ethanol. Algae are the optimal source for second generation bioethanol due to the fact that they are high in carbohydrates/polysaccharides and thin cellulose walls (Shay, 1993). It is reported that algae were one of the best sources of biodiesel and bio-ethanol from residual biomass producing oil content per acre of up to 250 times as soybeans and 7 to 31 times greater than palm oil (Shay, 1993). Among the algae, microalgae have much more oil than macro-algae and it is much faster and easier to grow (Shay, 1993; Hossain, 2008). Moreover, algal biomass can also use to produce bioethanol using cellulase enzymatic fermentation (Hossain *et al.,* 2008).

2.1.8 Production of bioethanol from fruit and fruit waste

Apple fruit and its associated residual biomass are amylaceous and lignocellulosic compounds; therefore, it must be initially hydrolysed to be converted into glucose which can be used as a feedstock to produce ethanol by fermentation and distillation (Kroyer, 1991). It has been reported that all fruits are (10%) sugar, or potentially (5%) ethanol (Dodic*et al,* 2009). In Colombia, banana fruit surplus production amounts to 850.000 t/year and it generates over 1 million tonne per year of associated residual biomass (Bohórquez and Herrera, 2005).

Ethanol content is likely to differ between lipid-rich fruits and sugar-rich fruits, being higher in the latter because simple sugars are more readily fermented than lipids (Levey, 2004). Often more than one species or genus of yeast is present on a given fruit, for example, genus *Candida* is most common on lipid-rich fruits and *Saccharomyces cerevisiae* on sugar-rich fruits (Skinner *et al.,* 1980). Several fruits waste such as pineapple has been reported to be converted to bioethanol (Hossain *et al.*, 2008). The wastes contain valuable components such as: sucrose, glucose, fructose and other nutrients (Sasaki *et al.,* 1991). In addition, the conversion of pineapples waste to useful products such as ethanol production can help to clean the environment from wastes and also, it has economic usefulness, when the wastes are converted to valuable product.

2.2 Ethanol production from grapes and apples

2.2.1 Grapes (*Vitis vinifera*)

Grapes belong to the *Vitaceae* family and native to Eastern Asia, Europe, the Middle East and North America. Native grapes belonging to the *Vitis* genus

proliferated in the wild across North America, and were a part of the diet of many North American first peoples, but were considered by European colonists to be unsuitable for wine. The first grapes *Vitis vinifera* in the old world were cultivated in California where Spain had established a series of monasteries along the coasts to supply their navies with oranges to prevent scurvy and convert natives. In branch grapes grow in cluster of 6 to 300 and color can be crimson, black, dark blue, yellow, green and pink. White grapes are actually green in color, and are evolutionarily derived from the red grape. According to food and agriculture organization (FAO) 75,866 square kilometers of the world are dedicated to grapes. Approximately 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruits (Elzebroek, 2008). A portion of grape production goes to producing grape juice to be reconstituted for fruits canned with no added sugar and 100% natural. The area dedicated to vineyards is increasing by about 2% per year.

2.2.1.1 Grape pomace

Grape pomace is the waste fibrous material that remains after the juice has been extracted from grape berries and consists of processed skin, seed, and stems (Hang, 1988; Mazza, 1995; Greene, 1998). The carbohydrate fractions of grape pomace (2.4% glucose, 0.26% fructose, 0.006% arabinose, 0.002% glactose and 0.007% mannose) are potential source of fermentable sugars that are of commercial interest to the wine industry (Korkie*et al.,* 2002). Grape pomace consists of the four major poly-saccarides mainly: cellulose, hemicellulose, pectin and starch (Hulme, 1970). Starch serves mainly as an energy reserve in plant, while cellulose, himecellulose and pectin work as an integrated structure to support the cell wall (Glazer and Nikaido, 1995).

2.2.1.2 Nutritional content of grapes

The dietary value per 151 g edible portion contains 27 g carbohydrate, 1 g dietary fiber, 0.72 g proteins and vitamins (Table 2.2). Each serving of grapes contains about 200 mg of potassium and 25% of the daily dietary values for Vitamin C that is needed to take in a day, it can also give 9 mg of phosphorous, 4.6 mg of magnesium, there are also trace amounts of minerals like iron, zinc and selenium.

Table 2.2: Nutritional content of grapes

Nutritional	value per 151 g
Energy	436.80 kJ
Carbohydrate	27 g
Sugar	23 g
Dietary fiber	1 g
Protein and vitamins	0.72 g
Fat	0 g

2.2.2 Apple (*Malus domestica Brokh*)

Apples have been part of the human diet for thousands of year and cultivation practices have existed since at least 1000 BC (Morgan and Richards, 2002). Intense management of apple, *Malus domestica Borkh* (Rosaceae, Maloideae) as a horticultural crop is more recent, with many advancements for production occurring even in the last half century (Westwood, 1978; Childers, 1983; Morgan and Richards, 2002). The apple derives its name from the Latin

pomum, meaning fruit in English, and is classified as a pome, a fruit that has many tiny seeds with in a core at the center, rather than stone (www.vegparadise.com); with more than 7,500 known cultivars of apples (Elzebroek and Wind, 2008).

2.2.2.1 Nutritional content of apple

Apple nutritional contents has been depicted in Table 2.3, in addition to these it is reported to also contained minerals such as magnesium, copper, manganese, copper, calcium, iron, potassium and phosphorus in small quantities as well as rich in Vitamin A and Vitamin C.

Table 2.3: Nutritional content of apple

Nutritional	value per 182 g
Energy	399 kJ
Carbohydrate	25.1 g
Sugar	18.9 g
dietary fiber	4.4 g
Protein and vitamins	0.5 g
Fat	0.3 g

2.2.3 Yeast (*Saccharomyces*)

Saccharomyces is the member of the kingdom of fungi. *Saccharomyces* is an eukaryote and possess a member bound nucleus. *Saccharomyces* is reproduced by budding in which a mother cell will initiate a new replication cycle by formation of an immature bud *Saccharomyces* is not motile and also not chemotaxis which is able to toward or away from specific environmental conditions.

Sacchromyces display the sub-cellular organization of the typical eukaryote. *Saccharomyces* possess the characteristic of sub cellular organization including the plant-like cell wall. Their cell wall comprises of carbohydrate and glycosylated protein consisted of mitochondria, vacuoles, secretory pathway and the nucleus.

2.2.3.1 *Saccharomyces* and ethanol production

Saccharomyces cerevisiae , Baker's yeast , is widely used in ethanol production due to its high ethanol yield and productivity , no oxygen requirement, and high ethanol tolerance (Olsson and Hagn-Hagerdal, 1993). These unusual capabilities are the result of adaptation to efficient ethanol production from hexose sugar during thousands of years (Olsson and Hagn-Hagerdal, 1993). However*, S. cerevisiae* cannot transport and use xylose as substrate, whereas, the isomers of xylose (xylulose and ribulose) can be fermented (Jeffries, 2006). Nevertheless, native *S. cerevisiae* is probably still the best choice for softwood hydrolysed, where glucose and mannose are dominated among other sugars. In addition, the native yeasts are inexpensive and widely available. *S. cerevisiae* is only the yeast that can rapidly grow under aerobic as well as anaerobic condition (Visser, 1990). This unique ability plays a major role in various industrial applications of *S. cerevisiae*, including wine fermentation and large-scale production of fuel ethanol. Bioethanol is most commonly produced by anaerobic fermentation with *Saccharomyces cerevisiae*. Many attempts have been made to increase the overall conversion yield from glucose to ethanol.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Feedstock

The main substrates used are rotten grapes and apple fruits, bought from local market in Bangi, Selangor, Malaysia. After collection, samples were kept for one week to make balance distribution of rottenness in the fruits by visual visions. After that, they were washed and chopped into pieces for fermentation.

3.1.2 Microorganism

Saccharomyces cerevisiae type II (Sigma Aldrich) was activated by heat rehydration, 3 g/L of dry yeast was heated in a warm water bath at 40 ^{o}C for 15 minutes.

3.1.3 Enzymes

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

3.2 Methods

3.2.1 Sample collection and processing

All experiments were conducted using grape and apple fruits (Figure 3.1 (a) and (b)) to produce bio-ethanol and make comparison. The waring blender was used to blend the fruits samples for 20 minutes, while a Juicer (Philips 550w) was used to blend the juice pomaces. Direct separation of juice and pomace were obtained after a short interval (about 2 minutes). The pH and total soluble solid

(TSS) of samples were determined using pH meter and Atago refractometer (Figure 3.1 (c) and (d)) respectively.

(a) Grapes

(b) Apple

(c) pH meter

(d) ATAGO refractometer (Japan)

Figure 3.1 Photomicrograph of the materials used in the study

3.2.2 Fermentation

In order to produce bio-ethanol from grape and apple wastes, two methods were used, which were enzymatic analysis and fermentation. This research focused on bioethanol production *via* fermentation using yeast, specifically

Saccharomyces cerevisiae and enzymes (cellulase and amylase). About 10%, 30% or 50% of water were added to either the juice mixture or the fruits pomace. Out of this mixture, 100 mL of samples were withdrawn into 500 mL Schott bottles and incubated using yeast. The initial pH of culture medium was adjusted to 5.8; all experiments were prepared in triplicates. Samples were labelled properly, capped tightly stored in an incubator at 30 ^{o}C for two days. The initial weight of the content was measured using an analytical balance. The total soluble solids (TSS) of sample was measured before and after fermentation by using digital ATAGO refractometer.

3.2.3 Effect of yeast concentration on bioethanol production from rotten fruits

The optimum yeast concentration for maximum ethanol production was determined by conducting experiments using different yeast concentrations spanning from 2 to 6 g/L, The samples were then placed in the incubator at 30°C for 4 days under orbital shaking to effect homogenous mixing of the fermentation mixture.

3.2.4 Effect of physical parameters on bioethanol production from rotten fruits

3.2.4.1 Effect of temperature on ethanol yield

Studies were also made at different temperatures including 28, 30 and 35 °C by following the similar procedure above.

3.2.4.2 Effect of initial pH on fermentation

Different inintial pH value 4 to 8 were used in the experiment to know the optimum pH. The initial pH was adjusted using 5 M Sodium hydroxide (NaOH) and 1 M acid hydrochloride (HCl). Fermentation was performed at 30 ^{o}C for 4 days using 3 g/L of yeast except otherwise stated.

3.2.4.3 Effect of fermentation time on ethanol yield

Fermentation was conducted at 1 to 5 days following similar procedure mentioned above.

3.2.5 Fermentation of different fruit parts

The skin, pulp and mixture of the fruits (skin, pulp) 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.

3.2.5.1 Effect of different raw materials on bioethanol production

In this study, both rotten and fresh fruits were used as a substrate for the bioethanol production using yeast fermentation following similar procedure mentioned above to compare bio-ethanol yielded.

3.3 Enzymatic hydrolysis in fermentation

Enzymatic hydrolysis using cellulase and amylase were done. 3 g/L of each enzyme were weighed and added together with 3g/L yeast.

3.3.1 Filtration

Filtration was performed after 4 days except otherwise stated, for all experiment fermentation broth was filtered through filter paper (Whatman no. 1) (Figure 3.2 (a)). The apparatus was allowed to settle down for one hour (Figure 3.2 (b)) to ensure all filtrate had drained out from residue. The total volume of the filtrates and weight of residue were taken. Also TSS and pH of the filtrates were measured too.

(a)

(b)

Figure 3.2 Filtration process

3.4 Methods of analyses

3.3.1 Ethanol content

Ethanol was measured using digital refractometer Pal 34-S (Atago, USA) (Figure 3.3) according to manufacturer's guideline. 0.5 mL of filtrate was placed on the digital refractometer sensor and the concentration of ethanol (w/v %) was shown on the digital display.

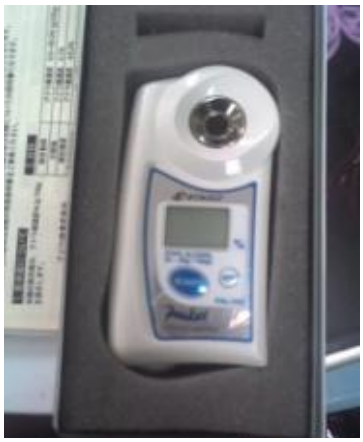

Figure 3.3 Ethanol refractor meter

3.4.2 Glucose content

Glucose content was measured using glucose digital refractometer Pal 15-S (Atago, USA) (Figure 3.4) according to manufacturer's guideline. 0.5 mL of sample was put on the refractometer and the glucose concentration in % (w/v) is seen directly.

Figure 3.4 glucose digital refractor meter

3.4.3 Chemical analysis

Samples of bioethanol fermented at 28, 30 and 35 ^{o}C were sent to Tribology Laboratory at Faculty of Engineering, UM. By using multi element oil analyzer

(MOM II), lube oil analysis was conducted to all samples to determine amount of chemical and metal P, K, Ca, Mg, S, Cu, Pb, and Fe in bio-ethanol.

3.4.4 Engine emission test

Samples of bio-ethanol were sent to Tribology Laboratory at Faculty of Engineering, UM. Samples were run on Gen-2 Multi cylinder Engine and percentage of volume of CO, NO_x, HC, SOx and CO_2 were measured for gasoline. 10% bio-ethanol blended with 90% gasoline to obtain E10 (10% bioethanol + 90% gasoline).

3.4.5 Viscosity

Samples of the bioethanol fermented at 28, 30 and 35°C were sent to Tribology Laboratory at Faculty of Engineering, UM. Using ASTM D445, viscosity of all samples were analyzed by heating up at 40^oC and then measured using viscometer set at 30 rpm and spindle size 63.

3.4.6 Acid value

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

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Effect of yeast concentration

The yeast concentration of 2, 3, 4, 5 and 6 g/L were used in the fermentation of grape and apple biomass as shown in Fig 4.1.

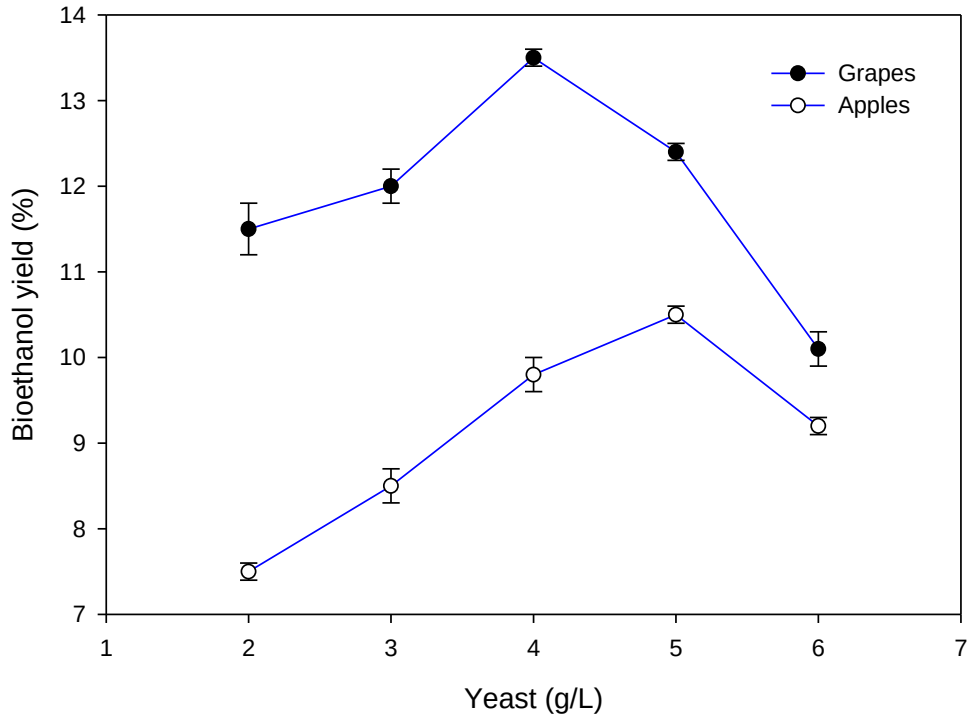

Figure 4.1: Effect of yeast concentration on bioethanol production from grape & apple biomass (max standard error ± 0.1)

The highest percentages of grape bioethanol yeild 13.5% was produced by using 4 g/L of yeast. Meanwhile, 2 and 3 g/L of yeast produced less percentages of bioethanol yield,11.5 and 12% respectively. The amount of bioethanol produced were significantly increased with increasing amount of yeast up to 4 g/L, beyond

this amount, the reduction in bioethanol yeild was observed probally due to microbial cell increase in population with time, which resulted in high CO_2 accumulation. In contrast to grapes, the highest bioethanol yield in apples was observed when using 5 g/L yeast loading, this could be due to high content of cellulosic biomass in apple which may require high microbial loading to effect sucecfful fermentation (Kim and Dale, 2004). Sharma *et al.,* (2007) had reported similar observatiuon that the increase of ethanol poduction was increased in cell concentration of yeast from 2% untill 10 % using 2% (v/v) of *S. cerevisiae.* Based on the results presented in Table 4. 1, the initial pH values for all concentration of yeast were reduced after the fermentation in grape and apple, probably due to increase acidity of the fermentation mixture as a result of microbial CO_2 accumulation.

Table 4.1: Effect of yeast concentration on bioethanol production from grape and apple biomass.

Grapes							
Yeast (g/L)	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
2	5.8	3.3 (±0.1)	10.9	5.1 (±0.1)	14.5	7.2 (±0.3)	11.5 (±0.4)
3	5.8	2.8 (±0.1)	10.9	4.6 (±0.1)	14.5	5.3 (±0.2)	12.0 (±0.5)
4	5.8	2.1 (±0.1)	10.9	4.1 (±0.1)	14.5	4.5 (±0.1)	13.5 (±0.5)
5	5.8	2.0 (±0.1)	10.9	4.5 (±0.2)	14.5	5.2 (±0.2)	12.4 (±0.4)
6	5.8	2.1 (±0.1)	10.9	4.0 (±0.1)	14.5	5.0 (±0.2)	10.1 (±0.3)

Apples							
Yeast (g/L)	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
2	5.8	3.0 (±0.1)	10	6.0 (±0.1)	12	6.0 (±0.1)	7.5 (±0.2)
3	5.8	2.8 (±0.1)	10	4.8 (±0.1)	12	5.5 (±0.2)	8.5 (±0.3)
4	5.8	2.5 (±0.1)	10	4.1 (±0.1)	12	4.8 (±0.1)	9.8 (±0.3)
5	5.8	2.3 (±0.2)	10	3.9 (±0.2)	12	5.0 (±0.1)	10.5 (±0.3)
6	5.8	2.1 (±0.1)	10	4.0 (±0.1)	12	5.1 (±0.1)	9.2 (±0.3)

General increased in ethanol production with increased in yeast concentration has generally been observed in both grapes and apples, attaining maximum ethanol yield of 13.5 (±0.5)% in grapes at yeast loading of 4 g/L and 10.5 (±0.3)% in apples at corresponding yeast concentration of 5 g/L. In both fruits, increasing yeast concentration beyond 4 g/L in case of grapes or beyond 5 g/L in case of apples results in decrease ethanol production, probably due to reduce mass transfer in the media with increasing yeast concentration. In comparison to apple, the grape fruits required lower yeast loading to achieved high ethanol yeild. This could be due to the readily available released sugar of grapes being bramble fruits thus making it easily accessible to the yeast as compared to the apple which is drupe fruits. Intrestingly, glucose consumption was also observed to tally with both increased yeast concentration and ethanol production in both fruits. For example, in grapes glucose consumption was observed to span from 7.3% to 10% whereas in

contrast to apple fermentation, the glucose consumption was observed to range from 6% to 7.2%.

4.2 Effect of temperature on bioethanol production

The percentages of bioethanol production were shown at different temperatures for 28, 30 and 35°C using yeast, *S. cerevisia*e in the grape and apple biomass, Table 4.2 and Fig 4.2.

Table 4.2 Effect of temperature on bioethanol production from grape and apple biomass

Grapes							
Temp (^oC)	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
28	5.8	3.4 (±0.1)	11	5.8 (±0.1)	14.5	6.8 (±0.1)	12.0 (±0.4)
30	5.8	2.8 (±0.1)	11	4.6 (±0.1)	14.5	5.0 (±0.1)	13.0 (±0.5)
35	5.8	3.9 (±0.1)	11	6.0 (±0.1)	14.5	8.0 (±0.1)	11.3 (±0.4)
Apples							
Temp (^oC)	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
28	5.8	3.8 (±0.1)	10	4.9 (±0.1)	12	6.3 (±0.1)	7.5 (±0.3)
30	5.8	3.0 (±0.1)	10	4.0 (±0.1)	12	5.5 (±0.1)	9.3 (±0.3)
35	5.8	4.1 (±0.1)	10	5.3 (±0.1)	12	7.8 (±0.1)	6.8 (±0.2)

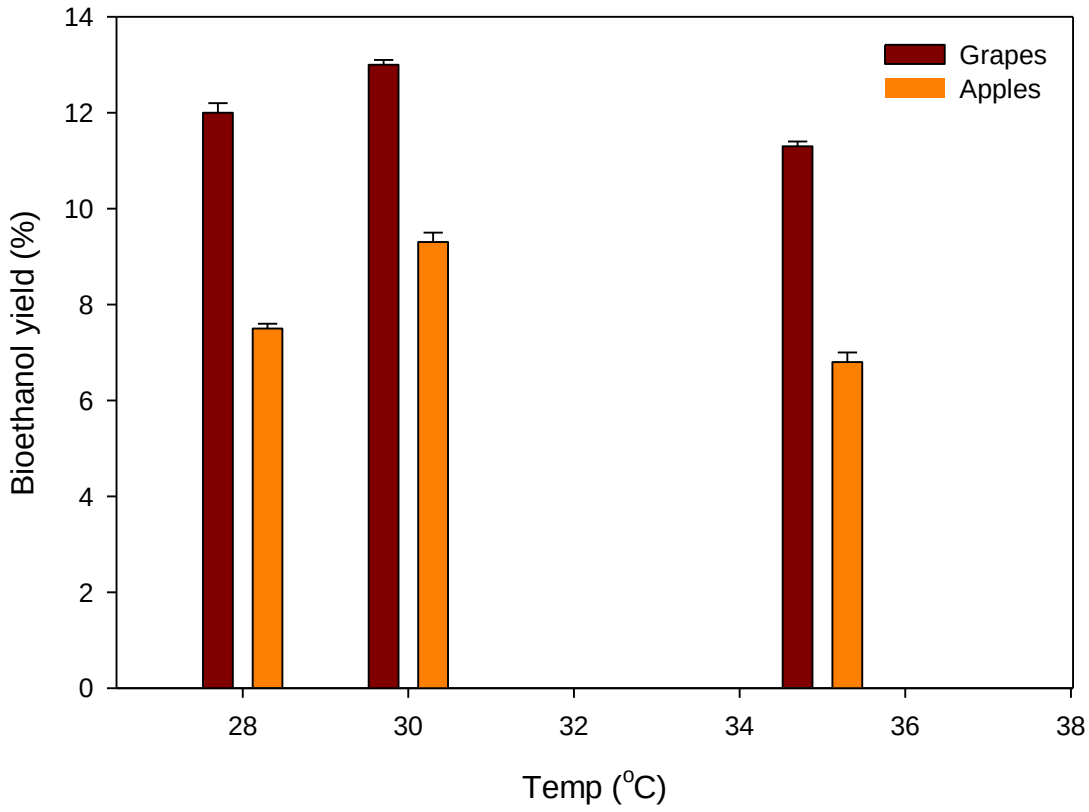

Figure 4.2: Effect of temperature on bioethanol production from grapes and apple biomass (max standard error ± 0.1)

Using both waste grapes or apples, though there is no much significant variation between the temperatures in both fermentations. However, 30 ^{o}C was observed to be the best fermentation temperature. The bioethanol yeild in v/v % has been observed to be high in waste grapes as compared to the waste apples substrate. This could probably be due to high level of readily available released sugar content of grapes as compared to apples. It was observed that the highest bioethanol yield was 13% (v/v) at 30 °C, followed by 12% (v/v) at 28 °C and the least was 11% (v/v) at 35 °C in grape waste whereas, 9.3% was the highest in apple waste and the least was 5.8% at 35 °C. The experiment at 35 °C produced the lowest yield

compared to the others parameters which is 28 and 30 °C. This could probably be due to thermal effect of increased temperature on the yeast which tend to reduce the ethanol yeild by affecting the yeast growth (Sree et al., 2000). This can probably explain the fluctuation in the final fermentation pH among temperatures tested in both fruits. For instance, the final pH at 28 ^{o}C fermentation was observed to be 3.4 and 3.8 for both grapes and apple feremntation respectively. However, running the fermentation at 30 ^{o}C proves to be more favorable for the yeast growth there by increasing the acidic nature of the fermentation broth (Narendranath, 2005) resulting in final pH reduction to 2.8 and 3.0 in grapes and apple respectively. When the fermentation is run at 35 ^{o}C the final pH appears to be higher than the other two tested temperatures. Similar observation on the effect of fermentation temperature on bio-ethanol yeild has been reported by Sree et al., (2000), who reported the optimum temperature to be between 25 to 30 °C. Previously, Liu et al., (2008) published that fermentation at 30 °C for 48 hours yelded the highest bioethanol from sweet sorghum, when used as substrate.

4.3 Effect of initial pH on ethanol fermentation

Effect of initial pH on bioethanol yield has beendepicted in Fig 4.3 and Table 4.3.

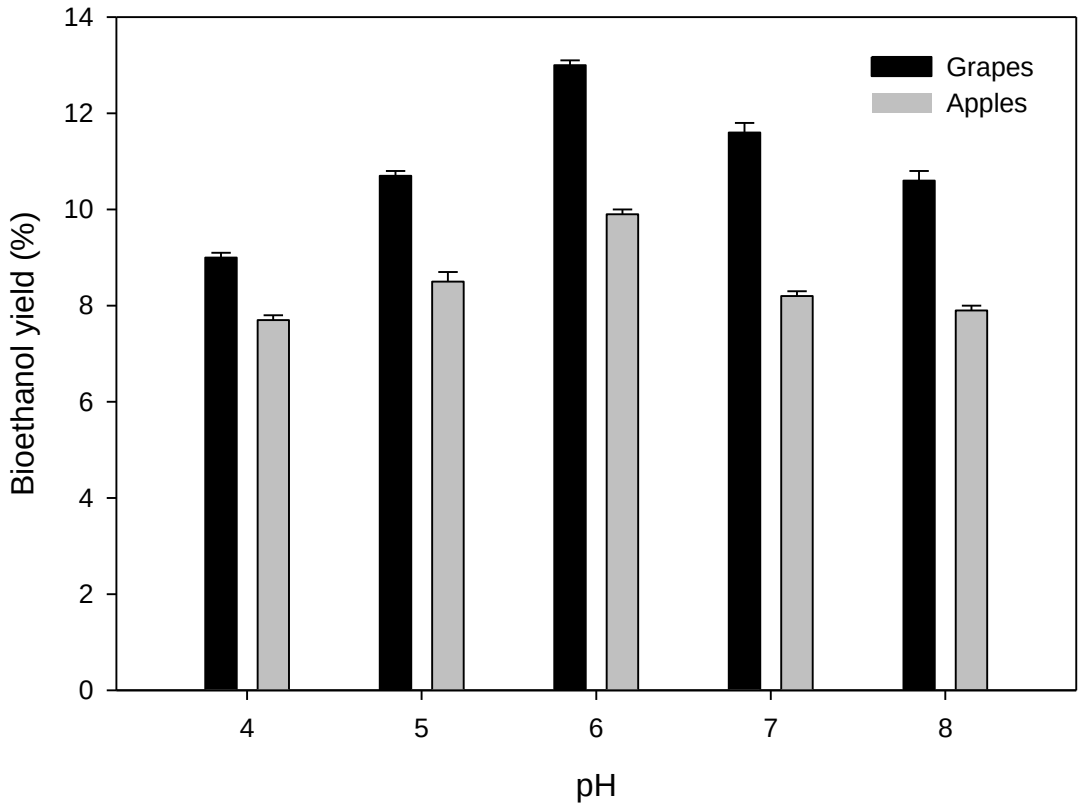

Figure 4.3: Effect of initial pH on bioethanol production (max. standard error ± 0.1)

In both rotten fruits, highest bioethanol production was observed at pH 6 with rotten grapes having the highest yield of 13% (v/v) as compared to rotten apples with 9.9% (v/v). In general, low initial pH is observed to caused reduction in bioethanol yield, for example at pH 4 and 5 the ethanol yield is observed to be 9.2% (v/v) and 10.7% (v/v) in grapes, while 7.7% (v/v) and 8.3% (v/v) in apple respectively. This reduction in ethanol yield at lower pH could be due to acid nature of the media which tends to inhibit the yeast growth (Liu et al., 2008).However, increased in pH beyond 6 to 8 resulted in increased basic condition of the medium which also tends to reduce the ethanol yield by affecting the microbial activity. Similar observation has been reported by Onsoy *et al.,* (2007).

The maximum consumption of the Total Suspended Solids (TSS) was noticed at pH 6 and then a gradual decrease occurred in consumption which was recorded on pH 4 and pH 5 as shown in Table 4.3. Residual glucose was observed being maximum consumed at pH followed by pH 5 and then pH 4. In case of pH 6, the glucose concentration reduced remarkably as shown in Table 4.3.

Table 4.3: Effect of pH on bioethanol production from grape and apple biomass

Grapes						
pH		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
4	3.1 (±0.1)	11	6.3 (±0.1)	14.5	7.0 (±0.3)	9.0
5	2.8 (±0.1)	11	5.0 (±0.2)	14.5	6.0 (±0.2)	10.7
6	2.1 (±0.1)	11	4.2 (±0.1)	14.5	4.5 (±0.1)	13.0
7	2.0 (±0.1)	11	4.5 (±0.1)	14.5	5.2 (±0.1)	11.6
8	2.1 (±0.1)	11	4.0 (±0.2)	14.5	5.0 (±0.2)	10.6

Apples						
pH		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
4	3.4 (±0.1)	10	7.5 (±0.1)	12	7.0 (±0.2)	7.7
5	3.0 (±0.1)	10	5.5 (±0.1)	12	5.7 (±0.1)	8.5
6	2.2 (±0.1)	10	4.5 (±0.1)	12	4.9 (±0.1)	9.9
7	2.3 (±0.2)	10	3.9 (±0.1)	12	5.0 (±0.2)	8.2
8	2.1 (±0.1)	10	4.0 (±0.1)	12	5.1 (±0.1)	7.9

As reported in literature, *S. cerevisiae* perform well in pH 4 to 6 but they can survive in pH 2.5- 8.5 (Narendranath, 2005). Based on the data depicted in Fig

4.3, it was clearly shown that pH 6 had the most yeild of bioethanol as compared to other pH tested. This result was found to be in good agreement with Jovana *et al.,* (2009). Fermentation efficiency remained more or less same over the pH range of 5.0- 6.0, and decreased marginally 6.5 (Mohanty *et al.,*, 2009).

4.4 Effect of fermentation time

Effect of fermentation time during bioethanol production was observed over a period of five (5) days as shown in Table 4.4 and Fig 4.4 respectively. At the end of first day ethanol yield of 11.9%, from grape and 6.2% from apple were observed, this increased significantly after 48 hours to reach a maximum yield of 13.5% and 9.2% in both grapes and apples respectively. Prolonging the fermentation time beyond two days results in less ethanol yield probably due to decrease in yeast cells growth as they approach death phase. Initial pH was recorded as 5.8 in all cases but with increasing fermentation time pH was observed to decreased, probably due to CO_2 accumulation, which results in reduced media final pH. The TSS values span from 11 to 4.2. In case of fermentation after 2 to 3 days, decrease in TSS values was observed in both grapes and apples respectively. The residual glucose was found to decrease from 14.5% in grape to as low as 4.9% corresponding to bioethanol yield of 13.5% (v/v), whereas in apple fruits it was observed to decrease from 10% to 4.0% after 48 hours fermentation yielding ethanol of 9.2% (v/v). This could probably be due to high content of released available sugar in grapes as compared to apples.

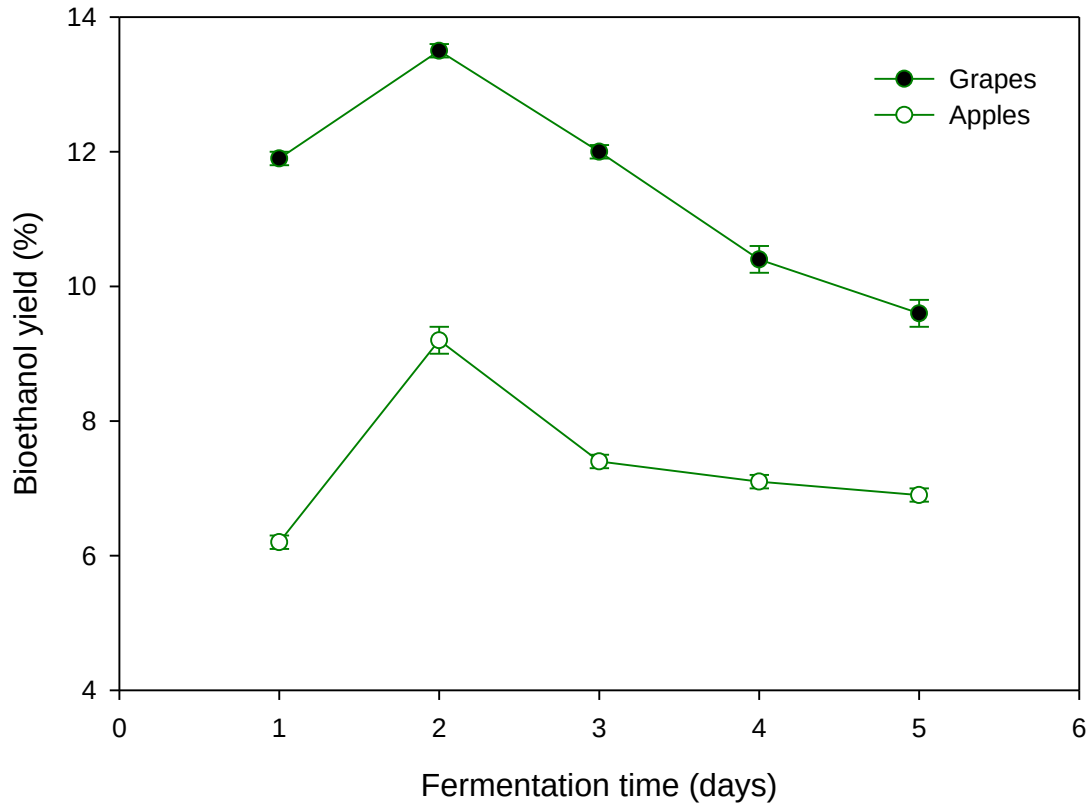

Fig 4.4: Effect of fermentation time on bioethanol production from grape and apple biomass (max standard error ± 0.1)

Table 4.4 Effect of fermentation time on grape and apple biomass

Grapes							
Time (days)	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
1	5.8	3.5 (±0.1)	11	6.0 (±0.2)	14.5	6.0 (±0.3)	11.9
2	5.8	2.0 (±0.1)	11	4.2 (±0.1)	14.5	4.9 (±0.2)	13.5
3	5.8	2.8 (±0.1)	11	5.0 (±0.1)	14.5	5.3 (±0.1)	12.0
4	5.8	2.8 (±0.1)	11	5.2 (±0.1)	14.5	5.2 (±0.1)	10.4
5	5.8	2.9 (±0.1)	11	5.7 (±0.1)	14.5	5.0 (±0.2)	9.6

Apples							
Time (days)	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
1	5.8	3.8 (±0.1)	10	6.3 (±0.1)	10	7.0 (±0.1)	6.2
2	5.8	2.1 (±0.1)	10	4.9 (±0.1)	10	4.0 (±0.1)	9.2
3	5.8	3.0 (±0.1)	10	5.5 (±0.1)	10	5.9 (±0.1)	7.4
4	5.8	3.2 (±0.1)	10	5.7 (±0.1)	10	6.2 (±0.1)	7.1
5	5.8	3.1 (±0.1)	10	5.8 (±0.1)	10	6.4 (±0.1)	6.9

4.5 Fermentation of rotten and fresh fruits

Ethanol yield from rotten and fresh fruits feedstock was compared in Table 4.5. High ethanol yield was observed in rotten grape with 13% (v/v) than rotten apple with 9.2% (v/v), while produced lower yield in amount of 11.5% (v/v) bioethanol in grape fresh fruit and 7.8% (v/v) in fresh apple probably due to high released sugar content in the rotten fruits. High TSS consumption was also observed in rotten fruit compared to the fresh after fermentation (Table 4.5)

Table 4.5 Effect of fruit condition on ethanol yield

Grapes							
Fruits status	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (%)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
Fresh	5.8	3.5 (±0.1)	10	5.9 (±0.2)	14.5	8 (±0.3)	11.5
Rotten	5.8	2.8 (±0.1)	11	4.8 (±0.1)	14.5	5 (±0.2)	13.0

Apples							
Fruits status	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (%)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
Fresh	5.8	3.8 (±0.1)	9	6.3 (±0.2)	10	8.3 (±0.1)	7.8
Rotten	5.8	3.0 (±0.1)	10	4.9 (±0.1)	10	5.5 (±0.1)	9.2

4.6 Fermentation of different fruit parts

The yield of ethanol using different fruits parts (skin, pulp and their mixture) as feedstock in yeast fermentation was studied Fig 4.5 and Table 4.6.

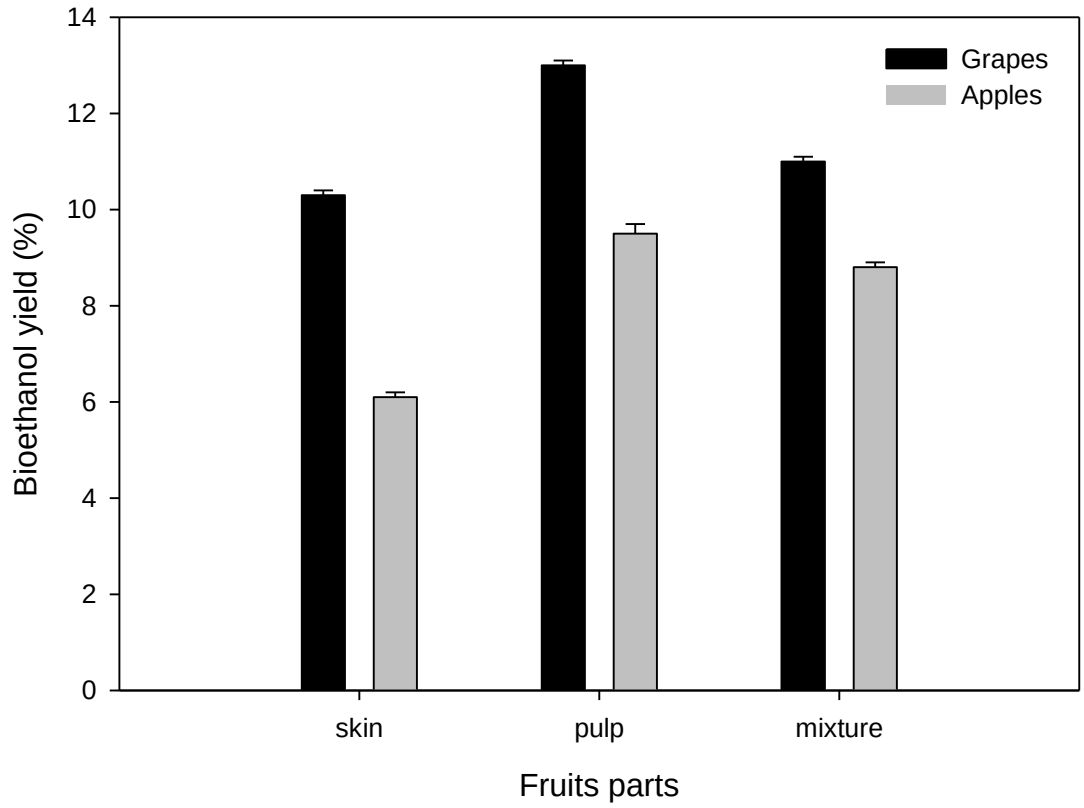

Fig 4.5: Effect of different parts of fruits on bioethanol production (max. stand. error ± 0.1)

Generally, there is high ethanol yield in grapes (13.0%) as compared to that of apple (9.5%). In all the fruits parts tested pulp was observed to have high ethanol yield of 13% (v/v) in grapes and 9.5% (v/v) in apple as compared to the skin or mixture, this could be due to low lignin and high cellulosic contents of the pulp which are readily been converted to the sugar for the ethanol fermentation as compared to the other parts tested (Demirbas, 2005).

Table 4.6 Effect different fruit part on fermentation of grape and apple biomass

Grapes							
Fruit part	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
Skin	5.8	3.8 (±0.1)	10.5	9.3 (±0.2)	11.2	9.8 (±0.3)	10.3
Pulp	5.8	2.5 (±0.1)	12.5	6.5 (±0.1)	15.3	5.5 (±0.2)	13.0
mixture	5.8	3.1 (±0.1)	11.5	7.5 (±0.1)	13.0	6.2 (±0.1)	11.0
Apples							
Fruits status	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (% v/v)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
Skin	5.8	4.0 (±0.1)	10.2	9.2 (±0.2)	11.2	8.0 (±0.1)	6.1
Pulp	5.8	2.8 (±0.1)	11.5	6.8 (±0.1)	15.0	6.0 (±0.1)	9.5
mixture	5.8	3.8 (±0.1)	11.0	7.8 (±0.1)	14.2	7.0 (±0.2)	8.8

4.7 Fermentation with enzymatic hydrolysis

Commercial enzymes amylase and cellulase were used in enzymatic hydrolysis prior to fermentation in order to aid the sccharification of the cellulosic material to release glucose contents that is fermented to ethanol by the yeast (Fig.

4.6). The bioethanol yield (% v/v) after hydrolysis of the cellulosic material using each enzyme is shown in Table 4.7.

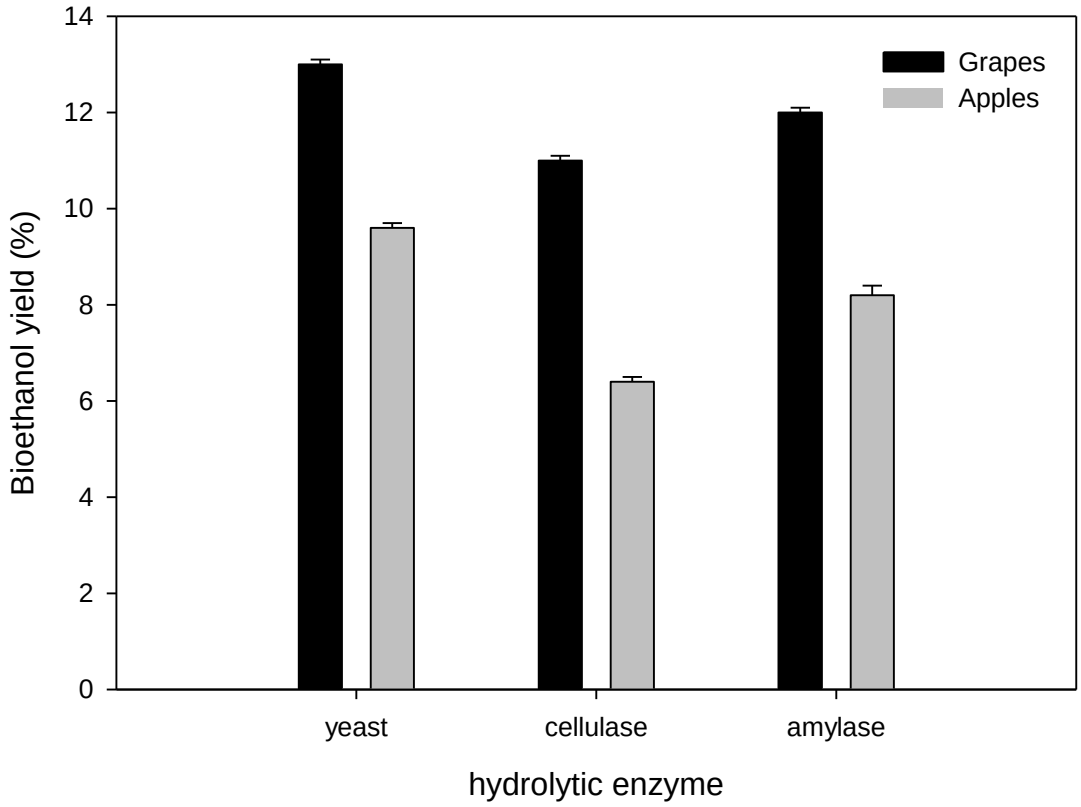

Fig 4.6 Effect of enzymatic treatment on bioethanol production (max. stad. error ± 0.1)

The ethanol production was observed to be in yeast hydrolyzed samples, with overall highest yield in grapes as compared to apples. When grapes were used, the highest ethanol yield was observed to be 13% in yeast hydrolysis followed by 12% in amylase hydrolyzed samples with cellulase having the least ethanol yield (11%). In comparison to apple fruits samples, the same trend was observed. Yeast hydrolyzed apple samples produced the highest ethanol yield (9.6%) followed by amylase sample (8.2%) and lowest in cellulase sample (6.5%).

This difference in ethanol yield based on enzyme hydrolysis could probably be due to the media pH effect on the enzyme. Since the increase acidity of the fermentation media tends to affect the activity of the free the enzyme. As it is shown in Table 4.7, the pH was observed to reduce due to increase the accumulation of CO_2 and its acidic nature. The initial pH was 5.8 but the final pH was 2.5, 4.0 and 3.2 in case of yeast, cellulase and amylase respectively. The TSS values were reduced from initial value 12% and 11% in grapes and apple and reduced to 5.2%, 7.5%, 6.11% in grape and 6.8%, 9.0% and 7.8% in apple by using yeast, cellulase and amylase respectively. Glucose contents were measured and found decreased from 15% in grape to 6% in the yeast hydrolyzed sample, 8% and 7% were observed when cellulase and amylase were used in the fermentation vessel respectively. Similar reducing trend is found in glucose contents after fermentation in the case of apple (Table 4.7).

Table 4.7 Effect of fermentation with enzymatic hydrolysis on grape and apple biomass

Grapes							
Enzyme	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (%)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
yeast	5.8	2.5 (±0.1)	12	5.2(±0.2)	15.5	6.0 (±0.3)	13.0
cellulase	5.8	4.0 (±0.1)	12	7.5 (±0.1)	15.5	8.0 (±0.2)	11.0
amylase	5.8	3.2 (±0.1)	12	6.1 (±0.1)	15.5	7.0 (±0.1)	12.0

Apples							
Enzyme	**pH**		**TSS (%)**		**Glucose (%)**		**Ethanol yield (%)**
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	
yeast	5.8	2.8 (±0.1)	11	6.8 (±0.2)	12.5	8.6 (±0.1)	9.6
cellulase	5.8	3.9 (±0.1)	11	9.0 (±0.1)	12.5	9.9 (±0.1)	6.4
amylase	5.8	3.2 (±0.1)	11	7.8 (±0.1)	12.5	9.2 (±0.2)	8.2

4.8 Measurement of Viscosity and Acid Value

The viscosity of the bioethanol produced was important when considering the spray characteristics of the fuel within the engine, since the change in spray could greatly alter the combustion properties of the mixture. The produced bioethanol was observed to have a respective viscosity and acid values of 2.0(±0.1) cSt, 0.4(±0.1) mg KOH/g in grapes and 1.9(±0.2) cSt, 0.5 (±0.1) mg KOH/g in apples (Table 4.8). These values were observed to be within the range of ASTM standard. Indeed, the viscosity values were found to correlate with previously reported values of 1.5 cSt in bioethanol obtained from banana fruits (Hossain *et al.,* 2011) and 1.1 cSt in bioethanol obtained from potato's waste (Najafi *et al.,* 2009). Hadeel *et al.* (2011) reported a viscosity of 1.6 to 2.0 cSt and acid value of 0.4 mg KOH/g in a bioethanol produced from rotten Rambutan fruits.

Table 4.8. Measurement of viscosity and Acid Value

	Viscosity (Cst)	Acidvalue (mgKOH/g)	ASTM standard
Grapes bio-fuel	2.0 ±0.1	0.4±0.1	1.9-6 cst
Apple bio-fuel	1.9±0.2	0.5±0.1	0.5 cst

Mean ± SE (n=3) .SE=Standard Error

ASTM: American Society for Testing and Materials

4.9 Chemical analysis

The result of the element analysis from fermented date fruits showed that, the value of bioethanol metal element content range from 0 to ≈76 ppm (Table 4.9). The data demonstrates that the samples did not contain the toxic elements based on American Society for Testing and Materials (ASTM) D4806 and ASTM D5709 standards. In both fruit samples, sodium (Na) was observed to be the highest abundant element (75.5±0.3 ppm) in grapes and (52.5±0.2 ppm) in apple. This was found to be in contrary to previously reported data in bioethanol produced from rotten rambutan where the highest metal element is argentum (Hadeel and Hossain, 2011). Furthermore, Hossain *et al.*, (2011) reported argentum to be the highest metal element content (407 ppm) in bioethanol obtained from rotten banana. In this regard, the fruits samples were found to have ≈30 pmm in grape and ≈50 ppm in apple as the highest argentum content. Furthermore, in contrast to previous literatures (Hossain *et al.*,2011; Hadeel et al., 2011) where such elements like Chromium (Cr), aluminium (Al), cuprum (Cu), plumbum (Pb), nickel (Ni), titanium (Ti), molybdenum (Mo) and barium (Ba)

were found to be lower content (>10 ppm) throughout the fermentation time. In this studies these elements were absent in both samples.

Table 4.9: Chemical analysis of bioethanol produced from grape and apple

Metals	Apple	Grape
Fe	0.5±0.01	0.3±0.01
Cr	0	0
Al	0	0
Cu	0	0
Pb	0	0
Sn	42.5±0.2	22.5±0.3
Ni	0	0
Mn	0	0
Ti	0	0
Ag	50.0±0.3	30.0±0.2
Mo	0	0
Zn	1±0.1	0.5±0.1
Mg	29±0.2	14.5±0.2
Si	0	0
Na	52.5±0.2	75.5±0.3
B	1± 0.1	0.4±0.1
V	4.5±0.1	2.25±0.1
P	0	0
Ca	15.5±0.5	7.7±0.6
Ba	0	0

Mean ± SE (n=3) .SE=Standard Error

4.10 Engine test

The ethanol produced from this experiment was tested by using the Proton Gen 2 multi-cylinder engine for 1 hour at 2000rpm (60km/hour). The higher oxygen content in the blending fuel favors conversion of the CO produced during combustion into CO_2. In Table 4.10, both the SOx and HC emissions were observed to decrease with increasing ethanol content in the blended fuel. In 100% petrol, SOx emission of about 90 ppm with corresponding HC emission of 75 ppm were observed. Blending the fuel with 10% ethanol produced from grapes, the SOx emission is observed to reduce to 8 ppm (about 10 fold reductions!), while the HC emission reduces to 40 ppm, a 2.3 fold emission decrease as compared to 100% petrol. Similarly, using 10% ethanol obtained from apple samples, SOx emission of 10 ppm and HC emission of 50 ppm were observed. This result indicates that ethanol can significantly reduce HC emissions. The concentration of HC emission decreases with the increase of the relative air–fuel ratio, the reason for the decrease of HC concentration is similar to that of CO concentration described above (Najafi *et al.,* 2009).

In contrast, NOx emission was observed to increase with increasing ethanol content (Figure 4.9). Pure gasoline was observed to emit 5 ppm of NOx, in comparison 10% blended fuels, NOx emission was observed to increase by 91.7% (60 ppm) in grapes sample and 90% (65 ppm) in apple sample. This observation was found to be in accord with previously reported literatures (Najafi *et al.,* 2009). Najafi et al., (2009) reported an increase in NOx emission of 33.9% in E10 blended fuels. This increase in NOx concentration could be due to the known reason that NO_X formation is a strong function of peak chamber temperature. Hence When the combustion process is closer to stoichiometric, flame temperature increases,

therefore, the NOx emission is increased, particularly by the increase of thermal NOx (Najafi *et al.*, 2009).

The carbon II oxide (CO) emission decreases with increasing ethanol blend. For example, 100% gasoline produces 8.24 ppm CO emission while the blended E10 found to produce 6.0 in grapes and 6.6 ppm in apple respectively. Several reasons were attributed to this the observed reduction in CO emission with increasing ethanol blend. Some researchers hypothesized that the reduction in CO concentration using blended fuels is due to the fact that ethanol (C_2H_5OH) has less carbon atoms than gasoline (Najafi *et al,* 2009). Others attributed the decrease to be due to the reason that the oxygen content in the blended fuels increases the oxygen-to-fuel ratio in the fuel-rich regions. The most significant parameter affecting CO concentration is the relative air–fuel ratio (Najafi *et al,* 2009; Wu *et al.,* 2004). Hence, as the ethanol content of the blended fuel increases, the relative air–fuel ratio approaches 1 and consequently combustion becomes complete (Hsieh *et al.,* 2002; Wu *et al.,* 2004).

The CO_2 emission is observed to increase with increasing ethanol blend from about 7.0 ppm in 100% gasoline to 9.1 ppm in both grapes and apple samples respectively. This is not surprising as it has been reported that CO_2 emission depends on relative air–fuel ratio and CO emission concentration (Najafi *et al.,* 2009; Wu *et al.,* 2004). This increase in CO_2 concentration in exhust gas emission at 2000 rpm with increasing ethanol blend has been reported to be due to the lean burning associated with increasing ethanol percentages, the CO_2 emission increased because of the improved combustion (Najafi *et al,* 2009; Wu et al., 2004).

Table 4.10: Measurement of engine emission

	Grapes		Apple	
Fuel emission (ppm)	Petrol%	E10%	Petrol%	E10%
CO_2	7.0 ±1	9.1 ±1	7.1 ±1	9.1 ±1
CO	8.2 ±1	6.0 ±1	8.2 ±1	6.6 ±1
HC	70 ±2	40 ±1	75 ±1	50 ±2
SO_x	80 ±2	8.0 ±1	90 ±2	10 ±1
NO_x	5.0 ±0.1	60 ±1	6.5 ±1	65 ±1

Mean ± SE (n=3) .SE=Standard Error

CHAPTER 5

5.0 CONCLUSIONS

This study was investigated to utilize the waste fruits for ethanol production and reduce the possible pollution because of the waste fruit material. The results of this study have revealed that the fruit wastes of grape and apple can efficiently be utilized for ethanol production with the help of *Saccharomyces cerevisiae* in a process of fermentation. A comparison of the yield of ethanol from different fruits has made it evident that the grape is the most efficient fruit waste to produce maximum ethanol (13.5% v/v) in 2 days as compared to the apple fruits (9.2% v/v). The efficiency of fermentation or the yield of ethanol production was dependent on the optimum: time (2 days), concentration of yeast 4 g/L in grapes and 5 g/L in apples, temperature (30^{o}C) and initial fermentation pH (6.0). In addition the nature of the fruits feedstock was also found to influence bioethanol yield. In all fruits, rotten fruits and the pulp part of the fruits revealed high ethanol yield, hence fruits with high pulp content could produce higher ethanol. Furthermore, based on the data obtained from the chemical and viscometric analyses, the produced ethanol is found to be within ASTM standard specifications. The engine test showed low amount of hazardous chemicals content, thus this bio-ethanol could potentially be used as good bio-fuel.