CHAPTER 4

RESULTS AND DISCUSSION

In line with the objectives of the study, bioethanol was produced using rotten dates. The yeast (*Saccharomyces cerevisiae*) concentrations and the optimization on physical parameters such as temperature, pH and production time were observed. Comparison study was also done using fruits and seeds (whole and crashed).

4.1 Bioethanol production in different yeast concentrations

Figure 4.1 shows the amount of the bioethanol produced from dates' biomass using different concentrations of yeast. Of the five readings recorded at different concentrations of yeast, it was evident that the highest level (18.2% v/v) was recorded at 5 g/L. On a descending order 17, 16, 14 and 12% (v/v) yield was achieved from 3, 7, 10 and 2 g/L of yeast concentrations.

Table 4.1 is showing TSS, pH, glucose for bioethanol produced at different yeast concentrations. The TSS recorded before and after the experiment showed that the amount of TSS in the mixture was decreased with the increase of concentration of yeast in the mixture (Table 4.1). The underlying cause would probably due to the consumption of sugar by yeast in mash dates over which the time decayed and reduced the overall content of the soluble solids in the mixture. That was in concurrence with Linde *et al*. (2008) who found decrease in concentrations of water-soluble sugars during fermentation process.

The initial pH of the experiment decreased with increase of yeast concentration. This could be possible due to appearance of acidic ethanol and secondary products (such as acetic acid) during fermentation process (Balat, 2007).

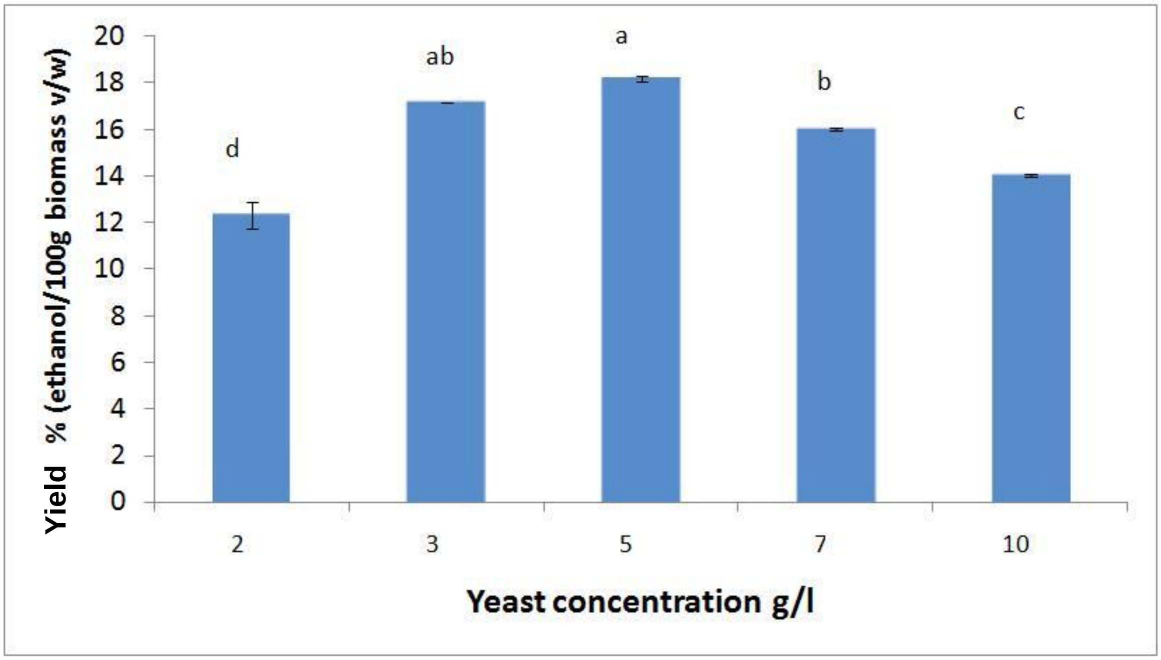

Figure 4.1: Yield of bioethanol at different yeast oncentrations.

Same letters are not significantly different by DMRT ($p \leq 0.05$).

Table 4.1: TSS, pH, glucose for bioethanol produced at different yeast concentration.

Yeast concentration (g/l)	**Total Soluble Solids (TSS) (%)**		**pH**		**Glucose**
	Initial	After	Initial	After	**(%)/100 g**
2	33.0 ± 0.2	24.0 ± 0.2	5.8	4.4 ± 0.1	9.5 ± 0.1
3	32.7 ± 0.2	23.3 ± 0.1	5.8	4.3 ± 0.1	8.9 ± 0.1
5	32.0 ± 0.2	21.2 ± 0.1	5.8	4.3± 0.1	6.2 ± 0.1
7	33.0 ± 0.2	20.0 ± 0.2	5.8	4.4 ± 0.1	7.3 ± 0.1
10	33.0 ± 0.2	17.0 ± 0.2	5.8	4.4 ± 0.1	8.3 ± 0.1

Mean ± SD (n =3).

4.2 Bioethanol production in different fermentation periods

Figure 4.2 presented the amount of the bioethanol production during different periods of fermentation (2, 3, 4, 5 and 6 days). As illustrated in Figure 4.2, the bioethanol production was observed to increase gradually with increasing fermentation time attaining the highest yield of 25.2 % (v/v) at day 5, increasing the fermentation time to day 6 makes no difference in the bioethanol yield. Table 4.2 illustrated the TSS during the fermentation at different periods. It was noted that in general, TSS content has been recorded lower after the fermentation compared to the initial value (Linde *et al.*, 2008). The pH was also observed to decrease with increasing fermentation time, from 4.7 at day 2 to 4.3 at day 3, thereafter the pH stays almost constant. This decrease in pH with fermentation time could probably be due to accumulation of CO_2 with is known to increase medium acidity (Table 4.2).

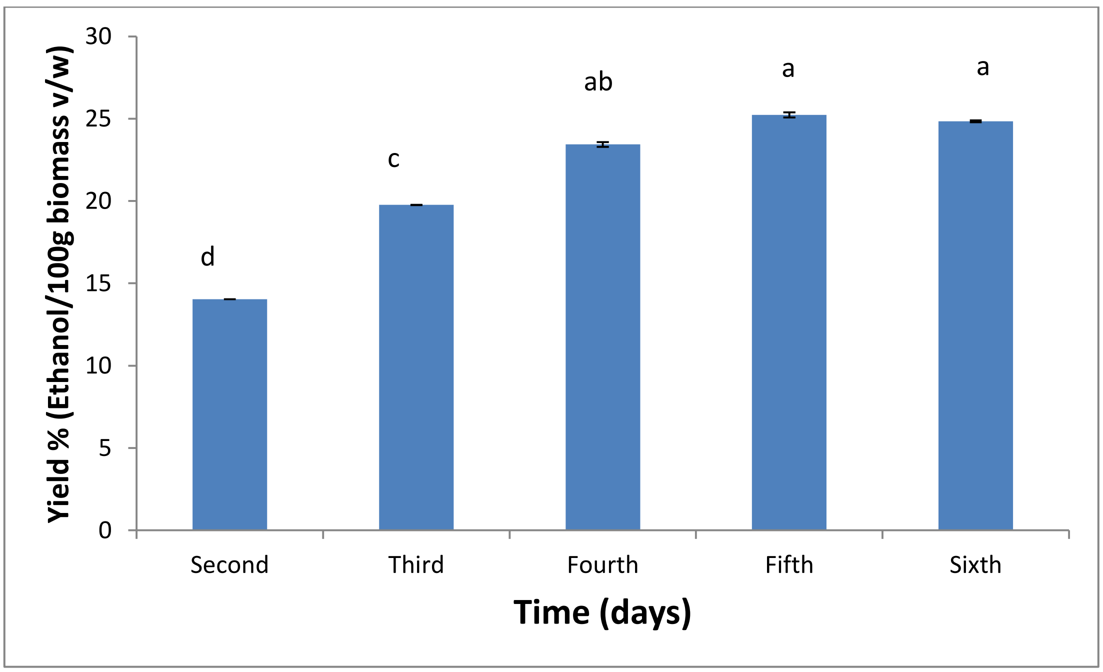

Figure 4.2: Variation in yield of bioethanol at different fermentation periods.

Same letters are not significantly different by DMRT ($p \leq 0.05$).

Table 4.2: TSS, pH, glucose for bioethanol produced at different fermentation periods.

Time	**Total Soluble Solids (TSS) (%)**		**pH**		**Glucose**
(days)	Initial	After	Initial	After	**(%)/100 g**
Second	33.0 ± 0.2	24.0 ± 0.2	5.8	4.7 ± 0.1	13.1 ± 0.2
Third	32.7 ± 0.2	22.3 ± 0.1	5.8	4.3± 0.1	12.2 ± 0.2
Fourth	33.0 ± 0.2	19.2 ± 0.1	5.8	4.3 ± 0.1	10.3 ± 0.1
Fifth	33.0 ± 0.2	19.0 ±0.1	5.8	4.3 ± 0.1	7.3 ± 0.1
Sixth	33.0 ± 0.2	18.8 ±0.1	5.8	4.3 ± 0.1	8.3 ± 0.1

Mean ± SD (*n* = 3).

4.3 Influence of physic-chemical parameters on bioethanol production

4.3.1 Effect of chemical parameters

The effect fermentation pH on bioethanol yield is shown in Figure 4.3, it is observed that bioethanol yield increases with increasing pH from 5 (15.8% v/v) to 5.8 (18.7% v/v). Increasing pH beyond this value results in gradual decrease in the bioethanol yield having lowest yield at pH 7.5 (13.7% v/v). The reduction in yield at higher pH could be due to basic effect on yeast which tend to inhibit its growth (Linde *et al.*, 2008). Table 4.3 shown the TSS, pH, glucose for bioethanol produced at different pH values, the total soluble solid content and glucose concentration of the samples were decreased with the increase of degree of the initial pH. It was also noted that the TSS were higher before fermentation (Linde *et al*., 2008).

4.3.2 Effect of temperature

Figure 4.4 shown the production of bioethanol and Table 4.4 shown the TSS, pH, glucose for bioethanol produced at different temperature. The changes in the pH, TSS before and after the fermentation was observed. The highest yield (18.7%) was found at 28°C while the lowest (6.8%) was found at 40°C. Total soluble solid content and glucose utilization of the samples decreased with increase of degree of the temperature. It was also observed that the TSS was higher in before fermentation than after fermentation (Linde *et al.*, 2008). The production decreased with increase of temperature that because yeast prefer 28°C more than 35 and 40°C which are less suitable temperature for its growth (Linde *et al.*, 2008). Both of pH and TSS decreased after fermentation in case of all temperature.

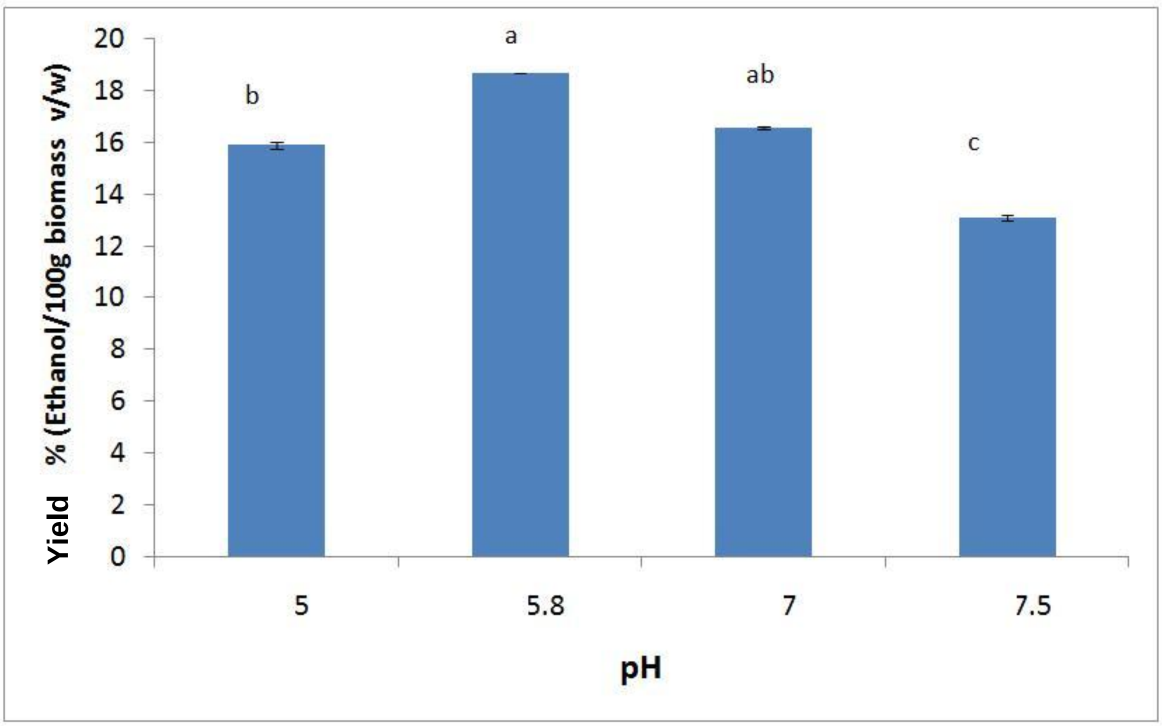

Figure 4.3: Yield of bioethanol at different pH values.

Same letters are not significantly different by DMRT ($p \leq 0.05$).

Table 4.3: TSS, pH, glucose for bioethanol produced at different pH values.

pH	Total Soluble Solids (TSS) (%)		pH		Glucose (%)
	Initial	After	Initial	After	
5.0	33.0 ± 0.2	24.0 ± 0.2	5.8	4.4 ± 0.1	8.3 ± 0.2
5.8	33.0 ± 0.2	23.3 ± 0.1	5.8	4.3 ± 0.1	6.8 ± 0.1
7.0	33.0 ± 0.2	21.2 ± 0.1	5.8	4.3 ± 0.1	8.6 ± 0.1
7.5	33.0 ± 0.2	20.0 ± 0.2	5.8	4.4 ± 0.1	9.7 ± 0.1

Mean ± SD (*n* =3).

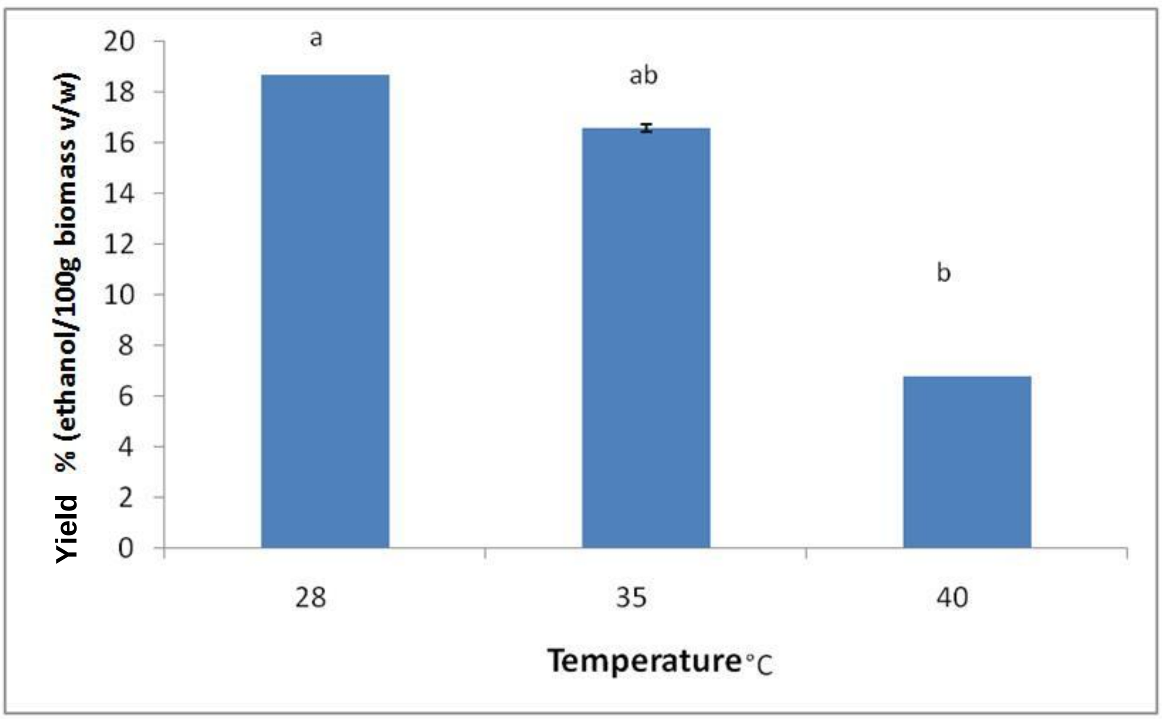

Figure 4.4: Yield of bioethanol at different temperatures (28, 35 and 40 °C).

Same letters are not significantly different by DMRT ($p \leq 0.05$).

Table 4.4: TSS, pH, glucose for bioethanol produced at different temperature.

Temperature (°C)	Total Soluble Solids (TSS) (%)		pH		Glucose (%)
	Initial	After	Initial	After	
28	33.0 ± 0.2	24.0 ± 0.2	5.8	4.4 ± 0.1	5.5 ± 0.1
35	32.7 ± 0.2	23.2 ± 0.1	5.8	4.7 ± 0.1	8.6 ± 0.1
40	32.0 ± 0.2	21.2 ± 0.1	5.8	4.7 ± 0.1	9.7 ± 0.2

Mean ± SD (n =3).

4.3.3 Effect of different water content

Figure 4.5 and Table 4.5 shows the amount of the bioethanol and the TSS, pH, glucose for bioethanol produced at different water content. Of the six readings recorded at different percentage of water content, it was evident that the highest level of production was (19.3%) was recorded for water content of 60%. As a matter of fact, with increase of water content, yield increased rapidly (Svensson *et al*., 1994). Yield was virtually zero at 20% water content because the amount of free water which important in the enzymatic fermentation was insufficient (Svensson *et al*., 1994).

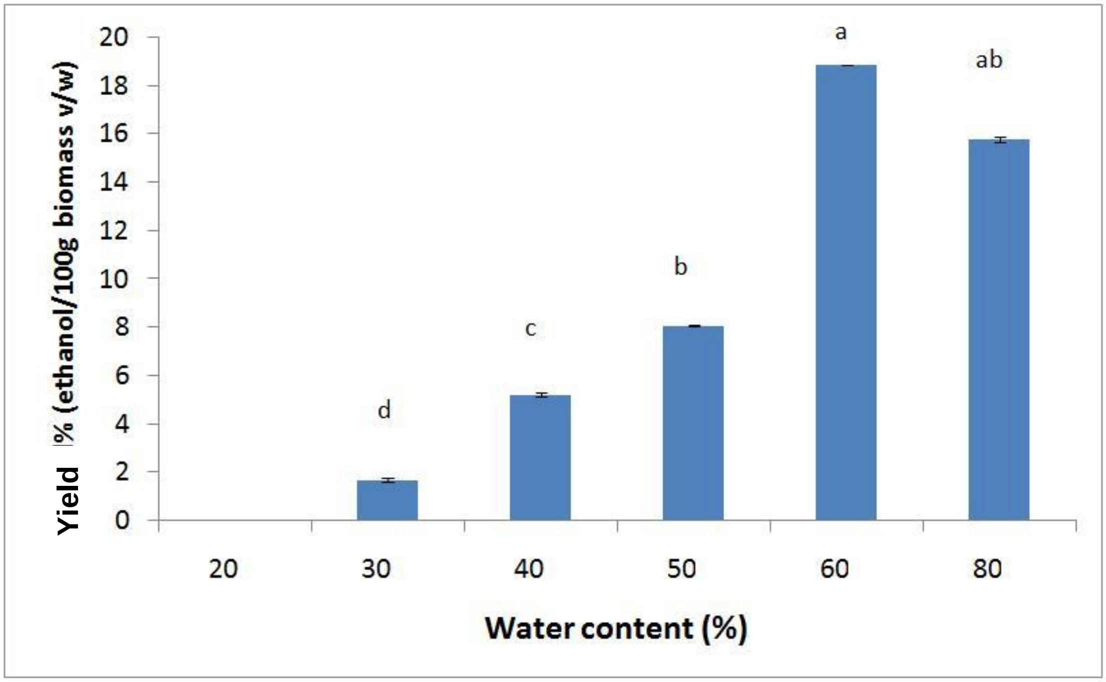

Figure 4.5: Yield of bioethanol at different water contents (%).

Same letters are not significantly different by DMRT ($p \leq 0.05$).

Table 4.5: TSS, pH, glucose for bioethanol produced at different water content.

Water content %	**Total Soluble Solid (TSS) (%)**		**pH**		**Glucose (%)**
	Initial	After	Initial	After	
20	-	-	5.8	5.3 ± 0.1	0 ± 0
30	-	-	5.8	4.7 ± 0.1	10.0 ± 0.4
40	35.0 ± 0.2	32.2 ± 0.2	5.8	4.7 ± 0.1	9.8 ± 0.2
50	34.3 ± 0.2	29.4 ± 0.2	5.8	4.4 ± 0.1	5.9 ± 0.1
60	33.0 ± 0.2	23.2 ± 0.2	5.8	4.2 ± 0.1	4.7 ± 0.1
80	33.0 ± 0.2	17.5 ± 0.2	5.8	4.2 ± 0.1	4.9 ± 0.2

Mean ± SD (n =3).

4.4 Bioethanol production in different dates fruit parts using yeast, amylase and cellulase

As shown in Figure 4.6, it is observed that bioethanol yield was higher in the fleshy part of the date fermented by yeast compared to the ground seed and whole seed fermented by amylase and cellulase. Glucose content also was higher in the fleshy part of the date fermented by the yeast as compared to the ground seed and whole seed fermented by amylase and cellulase (Table 4.6). It was found that highest yield was obtained from fleshy part (18.3%) while lowest from whole seed fermentation (2.6%). The product (bioethanol) from seedless dates, crush seed and the seed (whole) range was 0.6 to 8.30%. The highest yield was from seedless/fleshy dates (8.30%) (v/v) followed by crushed seed (2.6%) (v/v) and the whole seed (0.6%) (v/v). Glucose is usually concentrated in the fleshy part of the fruit. However, lower glucose contents were reported in date seeds (Nancib *et al*., 1997). As a result, compared to the seed components, the seedless fruit flesh containing the highest concentration of glucose (Mustafa *et al*., 1986) gave the highest yield of bioethanol. As shown previously in Table 4.6, the seedless fruit has the highest TSS change, because it contains sugars as soluble solids that present in the mixture. Thus, more sugar leading to more bioethanol yielding, this also affects the pH. The second highest was crushed seed (12%) and the lowest was the whole seed (4%). After the fermentation, TSS of fruit decreased to 24%. For the crushed seed TSS decreased to 9% and for the whole seed decreased to 3.40%. This happened due to the difference in sugar content before and after the fermentation.

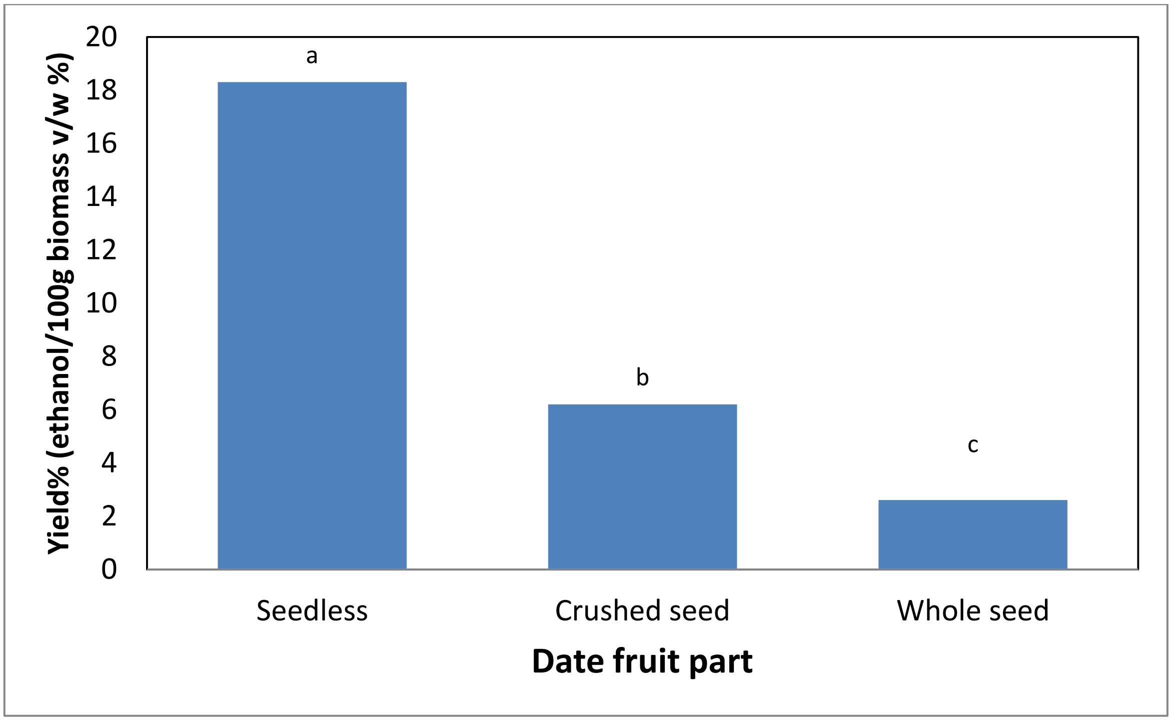

Figure 4.6: Bioethanol production from Different part of date fruit. Same letters are not significantly different at 5% level of Significant by DMRT. Seedless: fleshy date, crushed: ground seed, whole seed.

Same letters are not significantly different by DMRT ($p \leq 0.05$).

Table 4.6: Content of glucose, TSS and pH of different parts of fruits.

Fruit part	**Total Soluble Solid (TSS) (%)**		**pH**		**Glucose (%)**
	Initial	After	Initial	After	
Fleshy/seedless Dates	33.00 ± 0.20	24.00 ± 2.0	5.8	3.5 ± 0.5	2.35± 0.10
Crushed/ground Seed	12.00 ± 0.40	9.00 ± 0.00	5.8	3.1 ± 0.5	3.22± 0.12
Seed (whole)	4.00 ± 0.30	3.40 ± 0.00	5.8	3.3 ± 0.5	6.52± 0.3

Mean ± SD (*n* =3).

4.5 Chemical properties of bioethanol produced from dates fermentation

Table 4.7 showed the viscosity and acid value at different days and the results are in the range of ASTM standard. Both the viscocity and acid values were observed to decrease with increasing fermentation time reaching a viscosity value of 1.9 (0.1) cst at fifth day of the fermentation. This observation was found to be in agreement with previously reported literatures (Hadeel and Hossain, 2011; Hossain *et al.*, 2011). The result of the element analysis from fermented date fruits showed that, the value of bioethanol metal element content range from 0.2 to 158 ppm (Figure 4.7). 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. Among the elements presents, magnesium was observed to be in highest abundance (158 ppm) in waste date fruits at the first day. This was found to be in contrary to was has been reported 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 waste date fruits was observed to have 110 ppm as the highest argentum content. In similarity to previous literature (Hossain *et al.*,2011), 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.

Table 4.7: Viscosity and acid value measurements at different days of fermentation.

	Fermentation periods (Days)			
Properties	**1**	**3**	**5**	**ASTM standard**
Viscosity (cst)	2.1 ± 0.2	2.1 ± 0.4	1.9 ± 0.1	1.9 – 6.0
Acid Value (mgKOH/g sample)	0.5±0.01	0.4±0.005	0.3±0.005	0.0 - 0.5

Mean ± SD (*n* =3).

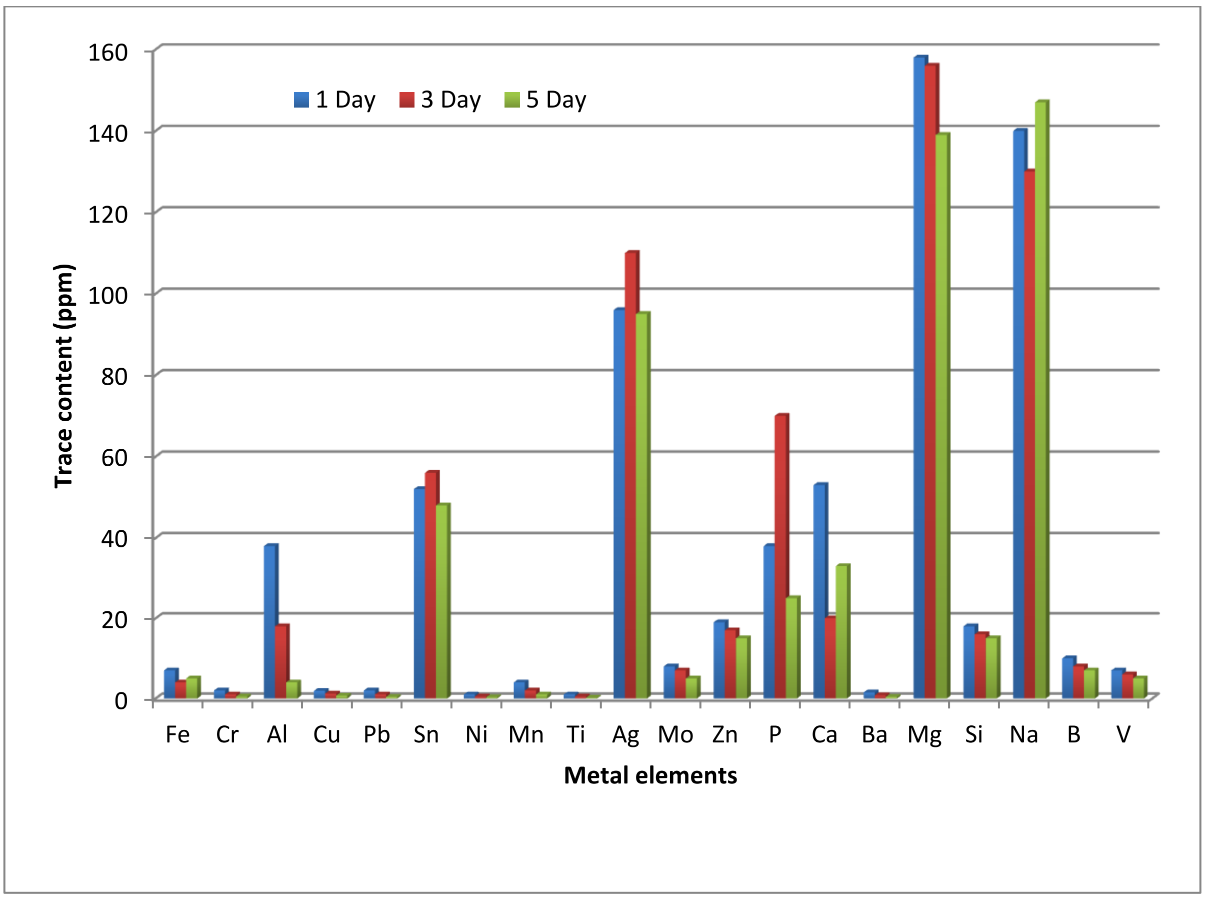

Figure 4.7: Metal elements of bioethanol from date biomass.

4.6 Engine performance and gas emission

Figure 4.8 shows the lower fuel consumption in E5 and E10 than 100% pure gasoline. The fuel consumption (ml/sec) was observed to reduced significantly with increasing ethanol content in the blended fuel. In comparison to 100% gasoline, the fuel consumption was observed to reduce by 5.1% and 18.3% in E5 and E10 respectively. This observation is in accordance with previously reported literature (Hadeel *et al.*, 2011).

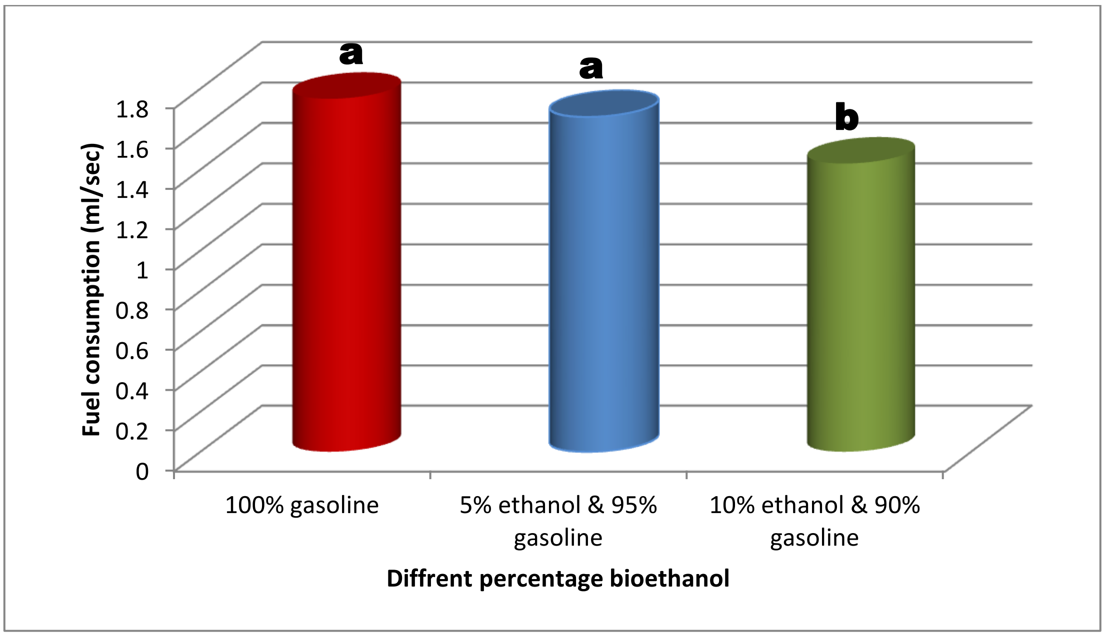

Figure 4.8: Gasoline and ethanol percentage in fuel consumption. Same letters are not significantly different by DMRT (p>0.05).

In this study E5 and E10 were used in the ordinary petrol engine without engine modification. It is necessary to modify the engine if bioethanol is used more blends (E15, E20 to E85) than E10. Bioethanol can be used in different blends to fuel vehicles. It was reported that E85 was used in ethanol based vesicle fuel with having engine modification low blends in petrol (E5, E10 and E15) were used without having engine modification. Modified vehicles are required for high bioethanol blends while not required in low bioethanol blends. High blends contain a high proportion of bioethanol and effectively substitute fossil fuels. It was stated that E85 was a mixture of 85% ethanol and 15% gasoline, and was generally the highest ethanol fuel mixture found in the United States and several European countries, particularly in Sweden as this blend was the standard fuel for flexible-fuel vehicles (Whyatt *et al*., 2004). Thailand introduced E20 in 2008 and E20 demand increased rapidly due to the most vehicle models launched. E20 is compatible and sales of E20 are expected to grow faster once more local automakers start producing small E20-compatible fuel-efficient cars (Praiwan, 2008).

E95 (96.5 % hydrous bioethanol, 3.5 % additives) was used in bioethanol buses, converted diesel vehicles and dedicated heavy diesel vehicles, such as waste collection trucks. E100 **(**100 % hydrous bioethanol) was used in modified petrol engine in Nanyang and petrol cars in Brazil (Janssen *et al*., 2007). Blending bioethanol into vehicle fuels had been enacted at the national level, USA with most mandates requiring a blend of 10% ethanol with gasoline without engine modification (UNEP, 2009). It was reported that E95 designated a blend of 95% ethanol and 5% ignition improver and was used in modified diesel engines where high compression was used to ignite the fuel as opposed to the operation of gasoline engines where spark plugs were used (Green Car Congress, 2008). The diesel engine run on ethanol had also a higher compression ratio and an adapted fuel system. It was reported that E100 was pure ethanol fuel and more recently used for flexible-fuel vehicles (Chakravorty *et al*., 2009, Szulczyk *et al*., 2010).

The higher oxygen content in the blending fuel favours conversion of the CO produced during combustion into CO_2. In Figure 4.9, both the SOx and HC emissions were observed to deccrease with increasing ethanol content in the blended fuel. In 100% gasoline, SOx emission of about 1030 ppm with corresponding HC emission of 80 ppm were observed. Blending the fuel with 5% ethanol the SOx emission reduces by about 3.3 fold. On further blending with 10% ethanol the SOx emission is observed to reduce to 180 (about 5.7 fold!), while the hydrocarbon reduces to 35 ppm, a 2.3 fold emission decrease as compared to 100% gasoline. 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 (Wu *et al.*, 2004, Najafi *et al.*, 2009b).

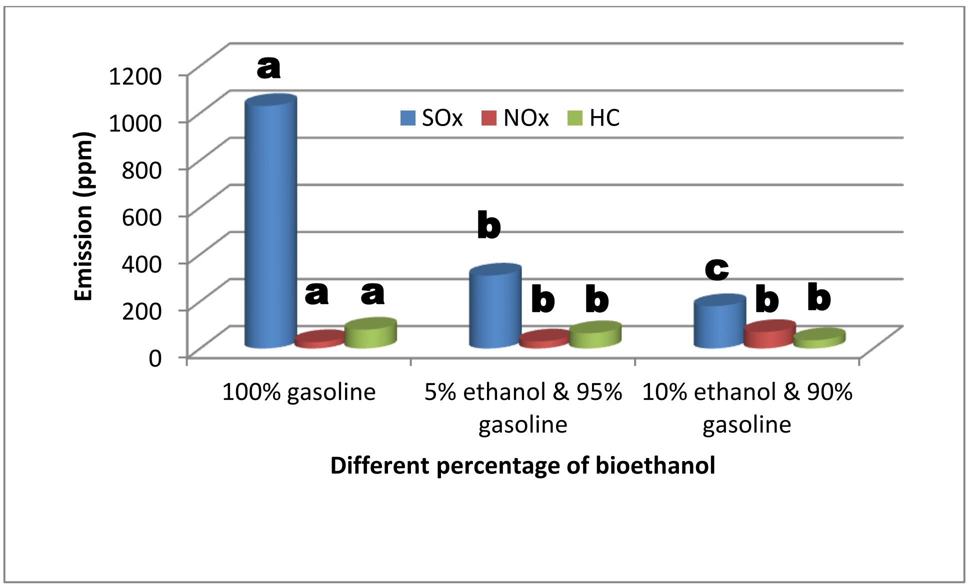

Figure 4.9: Percentage of gasoline and ethanol in engine emission. Same letters are not significantly different by DMRT ($p>0.05$).

In contrast, NOx emission was observed to increase with increasing ethanol content (Figure 4.9). pure gasoline was observed to emit 27 ppm of NOx, in comparison to 5% and

10% blended fuels, NOx emission was observed to increase by 11.1% (30 ppm) and 62.8% (70 ppm) respectively. This observation was found to be in good agreement with previously reported literatures (Wu *et al.*, 2004, Najafi *et al.*, 2009b). Najafi et al., (2009b) reported an increase in NOx emission of 12.6% and 33.9% in E5 and 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 in-creased, particularly by the increase of thermal NOx (Hsieh *et al.*, 2002; Najafi *et al.*, 2009).

It was referred that bioethanol can be blended with petrol or used as neat alcohol in dedicated engines due to the higher octane number, low octane number and higher heat of vaporization and vehicle can diminish greenhouse gas emissions by 41–61% /km driven, compared to gasoline-fuelled vehicles (Green Car Congress, 2008; Szulczyk *et al*., 2010). Present data were analysed on the percentage basis of different gasoline and gasoline-bioethanol blend fuel. The automotive engine speed was considered 2000 rpm. The effects of different volumetric percentages of bioethanol–gasoline blends, ranging 0%, 5%, and 10%, on engine emissions were tested on local engine (Gen-2 proton engine). The present study showed that the variations of the NO_X, CO_2, CO and HC emissions depending on the blending ratio at 2000 rpm engine speeds. Figure 4.10 showed that carbon II oxide (CO) emission decreases with increasing ethanol blend. For instance, 100% gasoline produces 8.7 ppm CO emission while E5 and E10 found to produce 8.1 and 6.9 ppm CO emission 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, 2009b). 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, 2009b; 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).

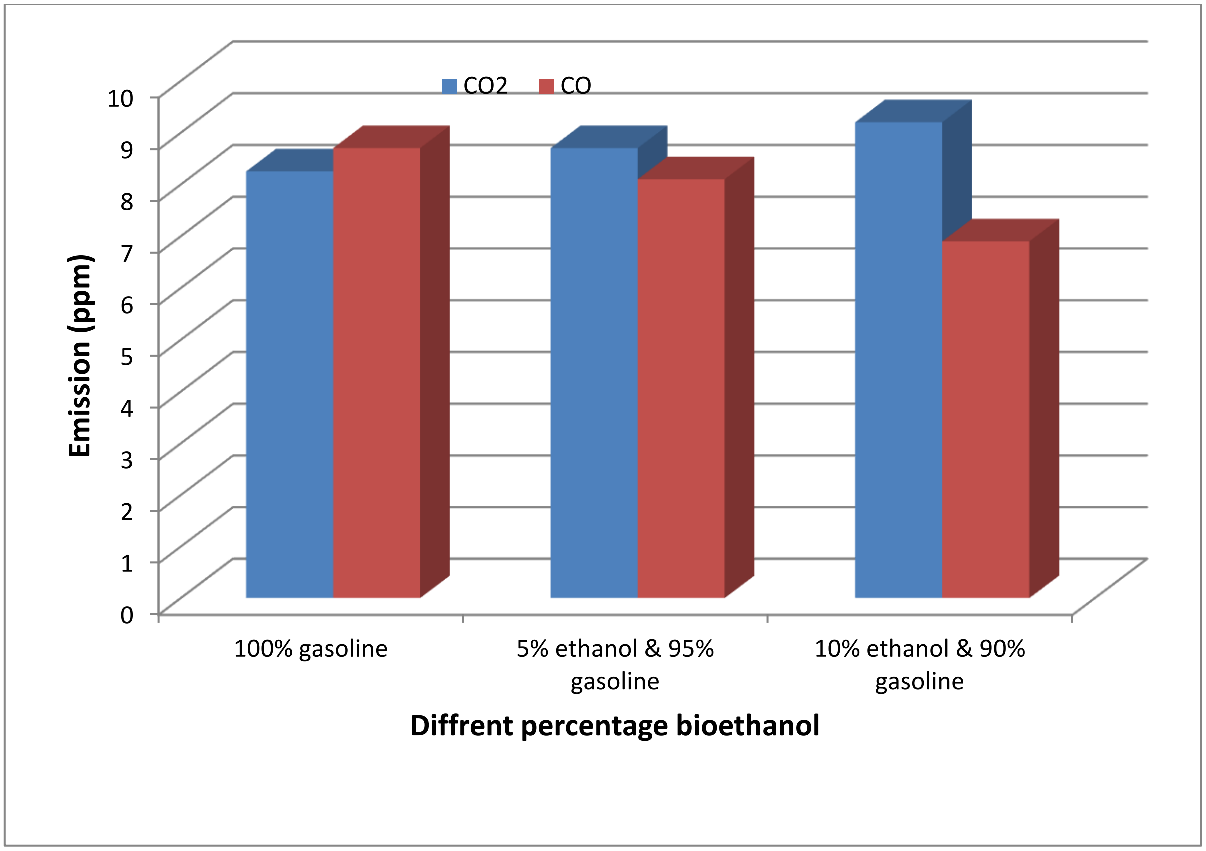

Figure 4.10: Percentage of gasoline (100%) and ethanol (5% and 10%) in engine emission.

The CO_2 emission is observed to increase with increasing ethanol blend from 8.25 ppm in 100% gasoline to 8.7 and 9.2 ppm in E5 and E10 blended fuels respectively. This is not suprising as it has been reported that CO_2 emission depends on relative air–fuel ratio and CO emission concentration (Najafi and Ghobadian, 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 and Ghobadian, 2009; Wu et al., 2004).

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

The study revealed that the fleshy parts of the date have shown more ethanol yield compared to only seed used as fermentation substrate. Optimum yeast concentration for fermentation was found to be 5 g/L and optimum pH was found to be 5.8. Bioethanol production was higher at 28^oC which tended to decline at higher temperatures.

The reducing sugar content (glucose), total soluble solids (TSS) and pH values were reduced after fermentation due to conversion of glucose into ethanol and carbon dioxide by yeast. The chemicals content, viscosity and acid values of the bioethanol produced were within ASTM (American Society for Testing and Materials) standard specifications. Viscosity and acid value and metal content indicated that bioethanol was safer to be used for engine purpose and reduced corrosion problem to the engine. Furthermore, the engine test result showed that the hydrocarbon (HC) NO_x, SO_x, CO and CO_2 content in E5 and E10 respectively, were significantly lower than in 100% gasoline having less fuel consumption and able to generate automotive, (Proton Gen 2 multi cylinder) car