Chapter 4

4.1 Rsults and Discussion

4.1.1 Study of fermentation parameters:

4.1.1 .1 Effect of fermentation pH on Bioethanol yeild

Bioethanol yield was investigated at different pH content using rotten rambutan, mango, banana and pineapple (Fig 4.1). It is observed that at lower pH of 4 banana has the highest bioethanol yeild (5.7%) followed by rambutan (5.5%), pineapple (5.2%) and the least in mango (4.3%).

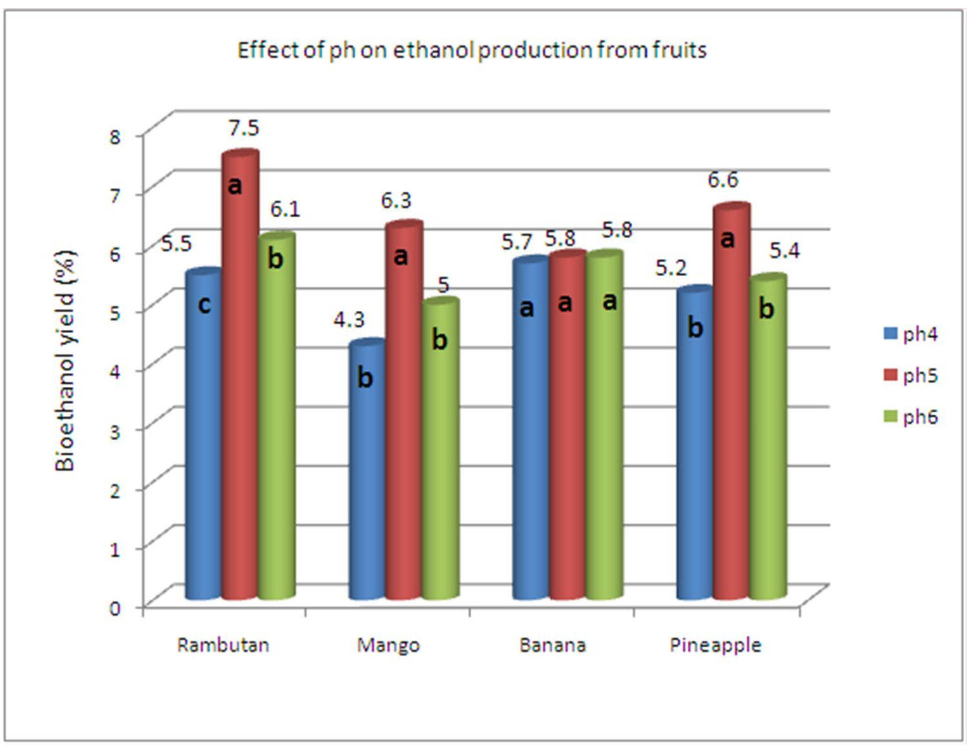


Figure 4.1 Fermentation media pH as a function of bioethanol yeild (max. Standard error ±5%)

Increasing the pH to 5 the bioethanol yeild among these rotten fruits varieties changes, with rambutan having the highest bioethanol yeild of 7.5% followed by pineapple(6.6%), mango (6.3%) and banana having the lowest(5.8%). As the pH increases to 6, the bioethanol yeild decreases among all the fruit tested. Moreover, change in fermentation media pH in rotten banana biomass was observed to have less effect on bioethanol yeild as compared to the other tthree fruits. In general, the optimum pH in both fruits tested was observed to be pH5, with rotten rambutan fruits biomass having the highest overall bioethanol yeild of 7.5%.

This observation was found to be in good agreement with what has been reported in literatures (Hossain *et al*. 2008). In fact, Ogunya *et al.* (2006) reported that the lower pH was found when the experiment was conducted at pH 3.4 and 4.1 which produced much greater of ethanol concentration as well as the rate of ethanol production from pineapples juices.

Furthermore, Muttamara *et al.* (2008) Reported similar observation that pH did not affect the ethanol's yield in the range of 3.5 to 6.0 when used pineapple effluent as substrates. Prior *et al.* (1981) found that bioethanol yield was 4% (v/v) from pineapple cannery effluent when worked with pineapples wastes.

Nonetheless, comparing the consumption of the total soluble solids (TSS), maximum TSS consumption was noticed at pH 5 as compared to pH 4 and 6 respectively (Table 4. 1). Residual glucose was also observed less in samples of pH 5 fermentation as compared to samples in pH 4 and 6 respectively (Table 4.1).

Table 4.1 Fermentation pH as a function of TSS consumption (max. Standard error 5%)

Parameters \ Fruits		Rembutan After fermentation	Mango After fermentation	Banana After fermentation	Pineapple After fermentation
TSS %	**PH 4**	2.17	2.18	2.12	2.15
	PH 5	2.29	2.30	2.20	2.19
	PH 6	2.74	2.62	2.80	2.72
Glucose mg/ml	**PH 4**	4.7	4.5	5.0	4.8
	PH 5	4.44	4.26	4.18	4.23
	PH 6	5.5	5.0	5.2	5.4

From Table 4.1, it is observed that rotten rambutan has the highest consumption of total soluble solid as compared to the rest, this probably may expalin the high ethanol yeild in this fruits biomass. When residual glucose is considered rambutan has the highest residual glucose, though it suppose to have less bioethanol yeild, but due to high TSS consumption this probably resulted in available biomass to both the yeast cells as well as the enzymes that resulted in high biomass conversion which subsequently results in high bioethanol yeild.

4.1.1 .2 Effect of yeast concentration

The effect of different concentrations of yeast (2, 3 and 4 g/L) were investigated to optimize the yeast quantitatively for maximum ethanol production in rotten fruits (Figure 4.2).

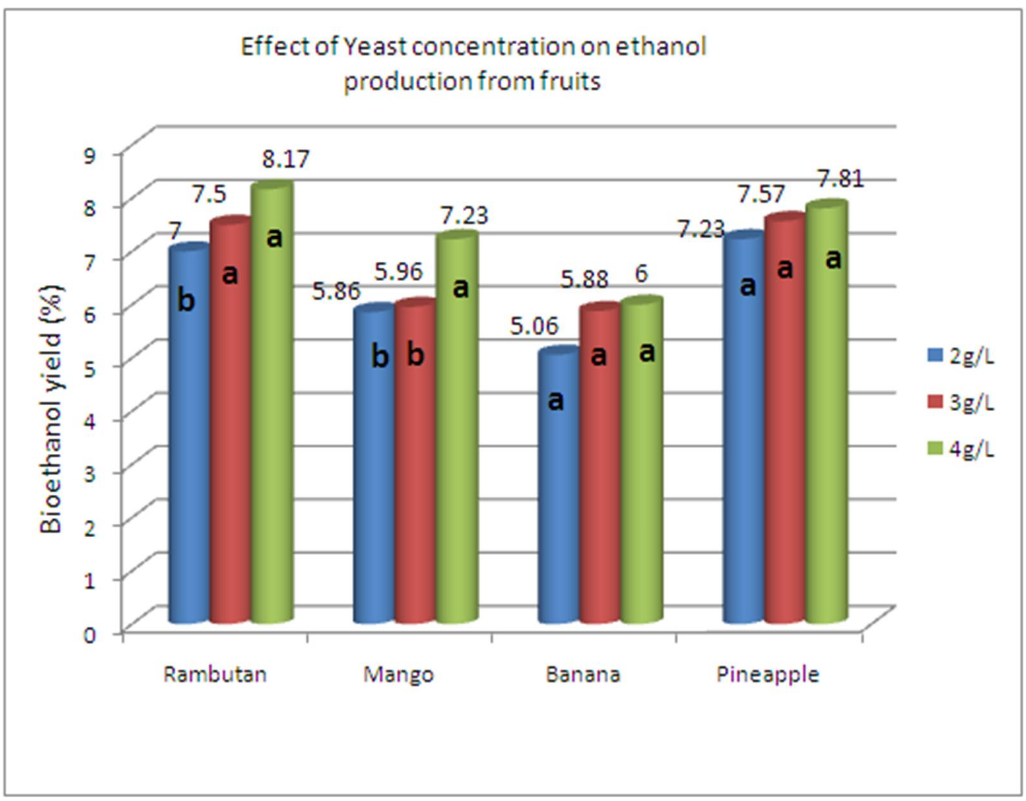


Figure 4.2 Yeast concentrations as a function of Bioethanol production (max. Standard error ±5%)

In Figure 4.2, it is generally observed that *S. Cerevisiae* loading at 4 g/L was found to be more effective in bioethanol production among all the tested fruits. It has also been observed that in all the fruits type the bioethanol production is linear in resepct to the yeast loading. For instance, increase in yeast loading from 2 g/L to 4 g/L in rambutan fruits resulted in net increase in bioethanol production of 1.17% (7% to 8.17%). The net increase in bioethanol production was observed to be more significant in mango fruits, as increase in the yeast loading from 2 to 4 g/L resulted in 1.37% net icrease. Contrary to this observation, the yeast loading was observed to have least effect on bioethaol production in rotten banana (0.94 net increase) with lowest effect in rotten pineapple (0.58 net increase).

Based on the results cited in Table 4.2, the pH values for all the concentrations of yeast were reduced after the fermentation.

Table 4.2 effect of yeast concentration on bioethanol yeild (max. Standard error ±5%)

Fruits / **Parameters**		**Rembutan** **After fermentation**	**Mango** **After fermentation**	**Banana** **After fermentation**	**Pineapple** **After fermentation**
TSS %	**2 g/L**	2.5	3.0	2.9	2.1
	3 g/L	2.7	2.6	2.0	2.3

	4 g/L	2.8	2.3	2.8	2.9
Glucose mg/ml	**2 g/L**	2.6	2.5	2.1	3.0
	3 g/L	2.4	2.9	2.4	2.2
	4 g/L	2.1	2.7	2.5	2.2
pH	**2 g/L**	3.8	3.4	3.6	4.0
	3 g/L	3.8	3.9	3.3	3.8
	4 g/L	3.8	3.7	4.0	3.2

For 2 g/L, the pH values was 5.2 before fermentation and reduced to 3.71, while for 3 g/L, pH was 5.2 befor fermentation but reduced to 3.71 and also for 4 g/L, pH values showed reduction from 5.3 to 3.90 after fermentation. For TSS values, all concentration of yeast showed the reduction of TSS after fermentation. Before fermentation, all concentration of yeast had the same TSS value which was in the range of 11-12.2 and after the fermentation, 2 g/L of yeast was responsible to reduce the TSS from 11 to 2.7 while in case of 3 and 4 g/L of yeast it was reduced from 12.3 and 11.2 to 4.2 and 4.0 respectively. Glucose utilization was noticed 4.2% to 2.17% in case of 2g/L of yeast and 4.4 to 2.46% in case of 3g/L of yeast. Maximum ethanol was produced in the experimental vessel containing 4g/L of yeast but the glucose consumption was not different from the rest of the experimental vessel. It was reduced from 4.0 to 2.6%.

4.1.1.3 Effect of temperature conditions

The effect of fermentation temperature on bioethanol production was observed using different temperature range of 28, 30 and 35°C using rotten rambutan fruits and yeast, *S. Cerevisiae* as depicted in Fig 4.3.

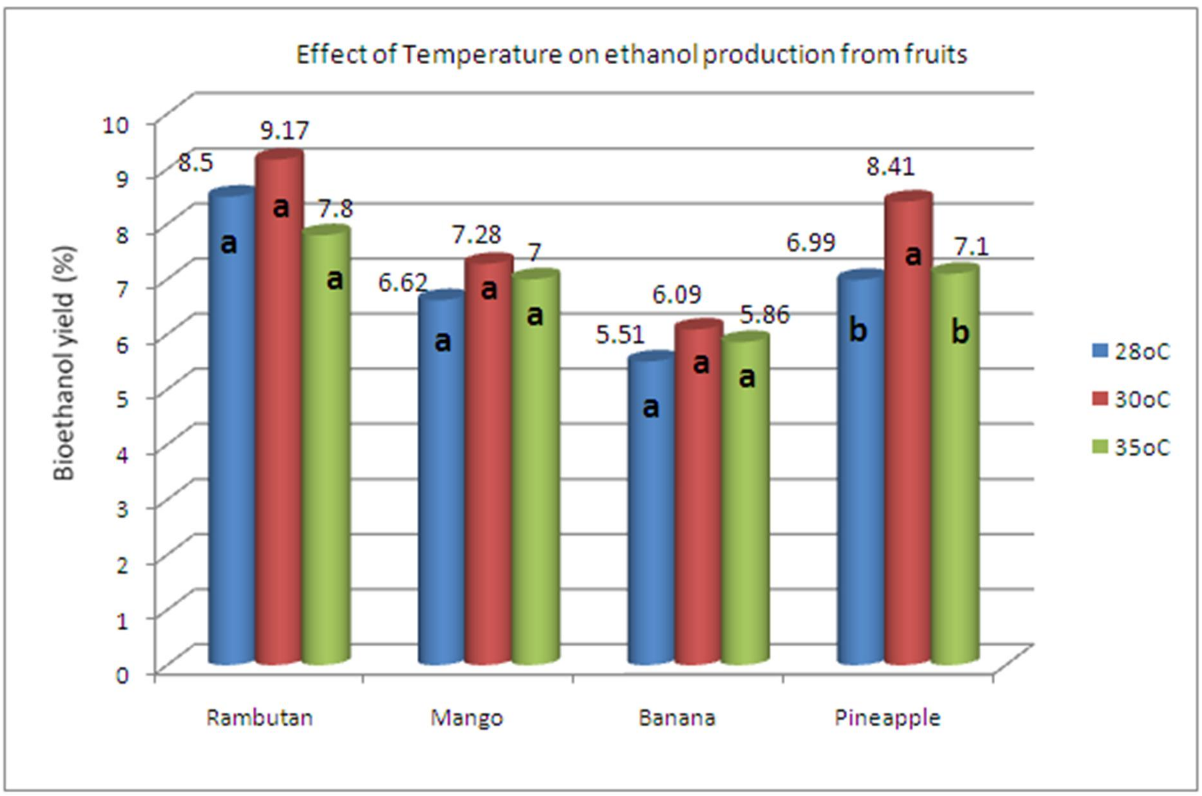


Figure 4.3 Fermentation temperature as a function of bioethanol production (max standard deviation ±5%)

It was observed that the maximum ethanol production was at temperature 30°C with 9.17%, followed by 32°C with 8.5% and at room temperature, 28°C it produced 7.8% of ethanol that was the lowest among of the parameters. Hence, the strain of yeast, *S.cerevisiae* had performed better at 30°C than other temperatures.The experiment at 28°C was observed to produced the lowest yield compared to the others temperature which was 30 and 32°C respectively. This is because, at low temperatures, the reaction rates of all metabolic functions was slowed down and it reduced the substrate and product diffusion rates for higher ethanol yields. In general, temperature of 30 °C was found to be optimum for the bioethanol yeild.

The pH values of the fermented rambutan for all samples of temperatures was observed to decreased gradually, in which the value of the pH for 28, 30 and 32°C was 5.2 but reduced to 3.92, 3.81 and 3.81. respectively (Table 4.3).

Table 4.3 temperature as a function of TSS,glucose consumption and pH (max. Standard error ±5%)

Fruits / Parameters		Rembutan After fermentation	Mango After fermentation	Banana After fermentation	Pineapple After fermentation
TSS %	**28°C**	3.17	3.16	3.11	3.18

	30^{O}C	4.4	4.5	4.4	4.7
	35^{O}C	4.2	4.3	4.8	4.1
Glucose mg/ml	**28^{O}C**	3.7	3.6	3.5	3.6
	30^{O}C	3.3	3.2	3.1	3.5
	35^{O}C	3.6	3.5	3.6	3.3
pH	**28^{O}C**	3.9	3.4	3.4	3.7
	30^{O}C	3.8	3.6	3.2	3.6
	35^{O}C	3.7	3.6	3.1	3.2

The TSS also decreased during the fermentation period in which the initial TSS were 12.3, 12.2 and 12.1 for all temperatures of 28, 30 and 32°C and reduced to 3.17, 4.47 and 4.2, respectively. Kouakou *et al.* (1984) reported similar observation of obtained ethanol concentration ranging from 22.10 to 35.10 g/L at 25°C, 27.17 to 46.60 g/L at 30°C and 27.17 to 40.32 g/L at 32°C.

4.1.1 .4 Comparison of bioethanol yield in fermentation of rotten and fresh fruits

Fermentation was also conducted to evaluate the performance of yeast to produce ethanol using fresh and rotten fruits shows in Fig 4.4. The bioethanol yield was observed to be generally higher in rotten fruits as compared to the fresh fruits, with highest yield in rotten rambutan (7.17%). While the highest bioethanol yield in fresh fruit fermentation was observed in pineapple fermentation (5.99%).

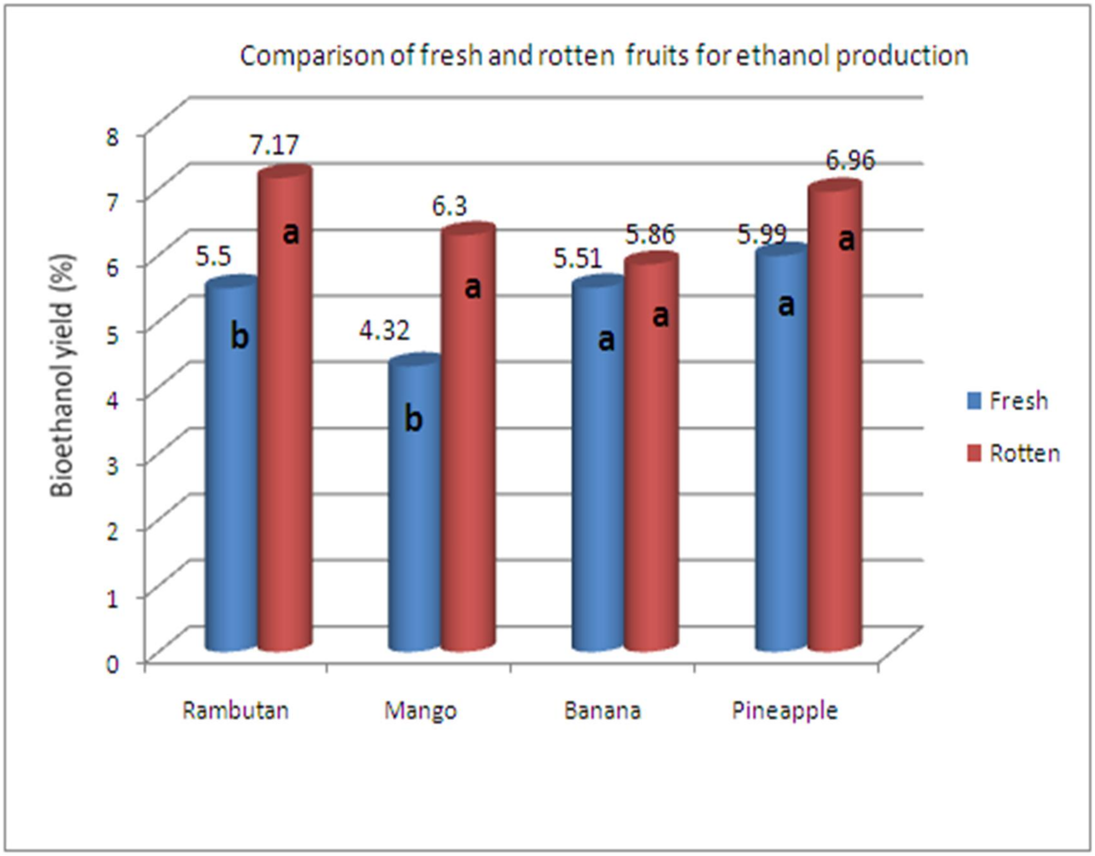


Figure 4.4 Fresh fruits vs rotten fruits as a function of bioethanol yield (max standard error ±5%)

There wasn't much difference observed between fermentation of fresh banana (5.51%) and rotten banana (5.86%). Fresh fruit possessed more TSS as compared to the rotten fruit after fermentation (Table 4.4).

Table 4.4 comparison of fresh fruits vs rotten fruits fermentation (max standard error ±5%)

Fruits / Parameters		Rembutan After fermentation	Mango After fermentation	Banana After fermentation	Pineapple After fermentation
TSS %	**Fresh**	2.8	2.5	2.6	2.3
	Rotten	2.04	2.4	2.7	2.6
Glucose mg/ml	**Fresh**	2.6	2.7	2.9	2.1
	Rotten	2.4	2.03	2.6	2.4
pH	**Fresh**	3.3	3.5	3.7	3.4
	Rotten	3.3	3.6	3.4	3.2

The final TSS values in fresh and rotten were 2.04 and 2.80 which were reduced from 12.00 and 11.30 respectively. The glucose concentration in fresh fruit was observed to be reduced to 2.60 from 4.20.

4.1.1 .5 Effect of fermentation time on bioethanol yield

Fermentation was conducted at different time duration to produce ethanol from rotten rambutan, banana, pineapple and mango fruits (Fig 4.5). Time duration was set from 1 to 4 days and the products were analyzed to determine % bioethanol yield based on different factors including change in pH, TSS and glucose contents.

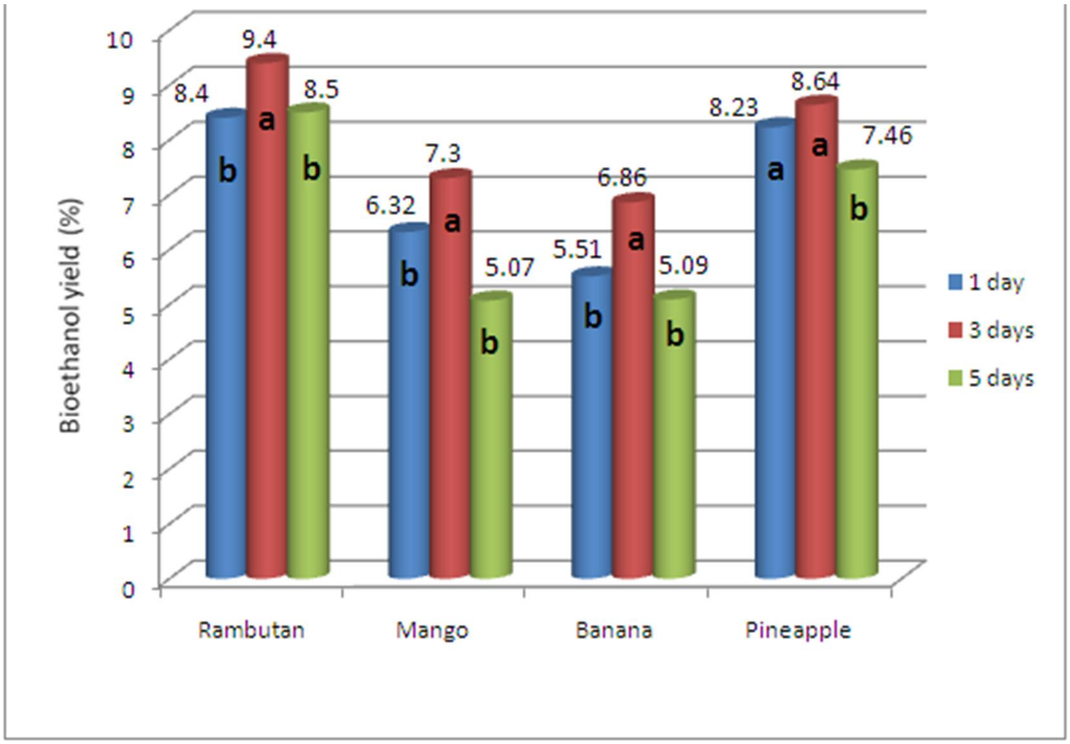


Figure 4.5 Effect of fermentation time on bioethanol yield (max. standard error ± 5%)

There is observed increase in bioethanol yield with increase in time up to 3 days, after that the yield appeared to decrease probably due to yeast cell aging. Rotten rambutan appeared to have highest bioethanol yield of (9.40%) at 3-days fermentation time as compared to pineapple (8.94%), Mango (7.30%) with least in banana(6.86%). Initial pH was recorded as 5.2 in all cases but the pH reduced and was found different as 4.22, 4.19, 3.81 and 3.91 respectively in the cultures grown for 1 to 4 days. Measurement of TSS revealed that the maximum clarity was observed in experimental vessel carrying fermentation for one day, as it reduced the TSS from 12 to 3.0. In case of fermentation with 2 to 4 days, the final TSS values were found to 3.5, 3.17 and 3.17 respectively. The glucose concentration was measured and found that glucose were consumed with the values 3.5, 3.8, 3.6 and 3.6 from one to four days cultures, respectively (Table 4.5) .

Table 4.5 fermentation time as a function of bioethanol yield(max standard error ±5%)

Fruits / **Parameters**		**Rembutan** After fermentation	**Mango** After fermentation	**Banana** After fermentation	**Pineapple** After fermentation
TSS %	**1day**	3.17	3.16	3.11	3.18
	3days	3.4	3.5	3.4	3.7
	5days	3.2	3.3	3.8	3.1
Glucose mg/ml	**1day**	3.5	3.6	3.7	3.9
	3days	3.8	3.4	3.3	3.2
	5days	3.3	3.6	3.8	3.3
pH	**1day**	4.9	4.4	4.4	4.7
	3days	4.8	4.6	4.2	4.6
	5days	3.7	3.6	3.1	3.2

4.1.1 .6 Fermentation with enzymatic hydrolysis

Enzymatic hydrolysis of rambutan fruit was made by using commercial enzymes cellulase and amylase prior to fermentation (Fig 4.6). Saccharification of the cellulosic material was performed by enzymes to release glucose contents for further fermentation by yeast. Furthermore, the enzymes serves as a pretreatment of cellulosic biomass from fruits to facilitate the release of simple sugars from biomass.

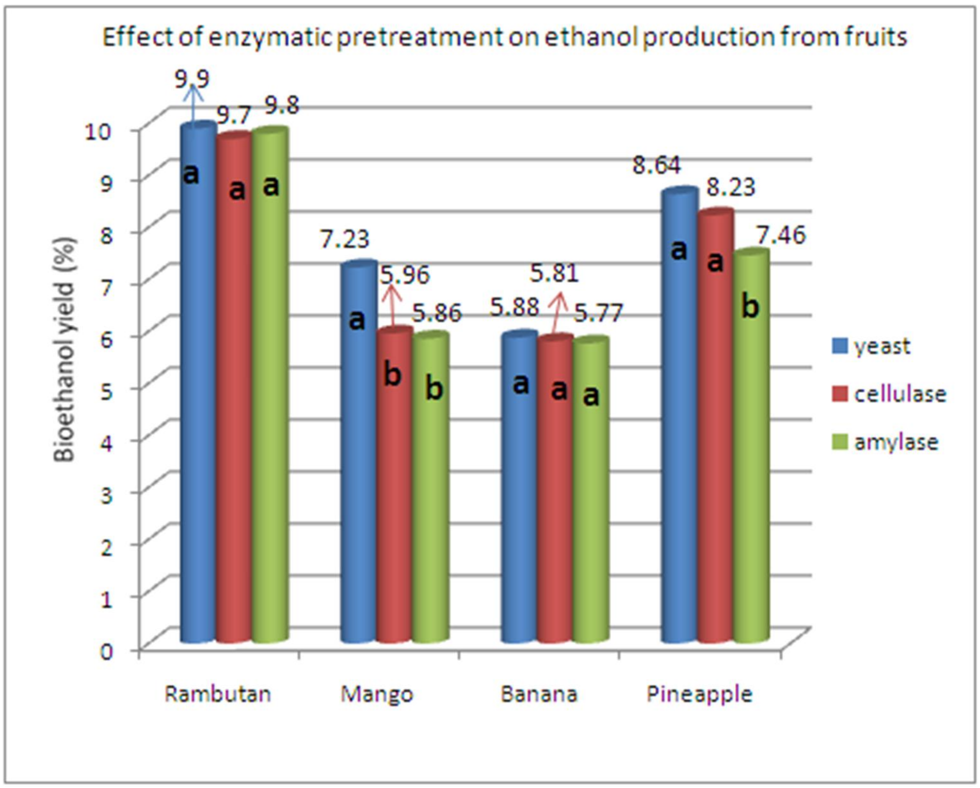


Figure 4.6 Effect of enzymatic hydrolysis on bioethanol yield (max standard error ± 5%).

In rambutan, there is lest effect between yeast fermentation and enzymatic hydrolysis using cellulase or amylase in respect to bioethanol production. As it can be seen, using yeast resulted in 9.90% yield while using cellulase and amylase each resulted in 9.70% and 9.8% respectively. Similar trend was observed in banana fruits with 5.88% yield in yeast and 5.81 and 5.77% in both cellulase and amylase respectively. However, enzymatic hydrolysis under yeast fermentation showed an increasing yield in mango fruits (7.23%) as compared to cellulase alone (5.96%) or amylase(5.86%). Similar trend was observed in pineapple fruits with yeast having highest yield of (8.64%). Generally, enzymatic hydrolysis in combination with yeast fermentation appeared to increase the bioethanol yield, with overall maximum yield in rmbutan (9.90%).

In Table 4.6, general reduction in pH was observed with continuous accumulation of ethanol; probably due to its acidic nature. The initial pH was 5.2 but the final pH was 3.78, 3.57 and 3.23 in case of yeast, cellulase and amylase respectively.

Table 4.6 effect of enzymatic hydrolysis on bioethanol yield (max standard error ±5%)

Fruits / Parameters		**Rembutan** After fermentation	**Mango** After fermentation	**Banana** After fermentation	**Pineapple** After fermentation
TSS %	**Yeast**	3.1	3.6	3.3	3.7
	Cellulose	3.4	3.5	3.4	3.7
	Amylase	3.2	3.3	3.8	3.1
Glucose	**Yeast**	4.5	4.6	4.7	4.9

mg/ml	**Cellulose**	4.8	4.4	4.3	4.2
	Amylase	4.3	4.6	4.8	4.3
pH	**Yeast**	3.9	3.4	3.4	3.7
	Cellulose	3.8	3.6	3.2	3.6
	Amylase	3.7	3.6	3.1	3.2

The TSS values were reduced from initial value 10.0 and reduced by making fermentation with yeast, fermentation with pretreated by cellulase and pretreated by amylase to 3.8, 3.4 and 3.8 respectively. Glucose content was measured and found 4.4 % in case of yeast, 4.6% in case of cellulase treated treated fruit biomass and 4.1% in the vessel with amylase treated fruit biomass. The initial concentration of glucose was measured 6.2.

4.1.1.7 Fermentation of different fruit parts:

Skin, pulp and mixture of the fruits were investigated for ethanol production using yeast fermentation (Fig 4.7). there is general increase in Ethanol production when using pulp as compared to other parts or mixture. Fermentation using rambutan pulp resulted in overall highest ethanol yield of (9.96%) with least yield in banana pulps (5.86%), with no much difference in yield between its pulp and its fruits mixture(5.22%). When using skin parts, there is a clear trend in increasing bioethanol yield between rambutan(7.46%), mango (5.60%) and banana (3.72%)

with slight increase in pineapple (4.31%). Similar trend was also observed using the fruit mixture among the fruits tested, but with much increase in pineapple (8.28%) assuming the highest ethanol yield in this class.

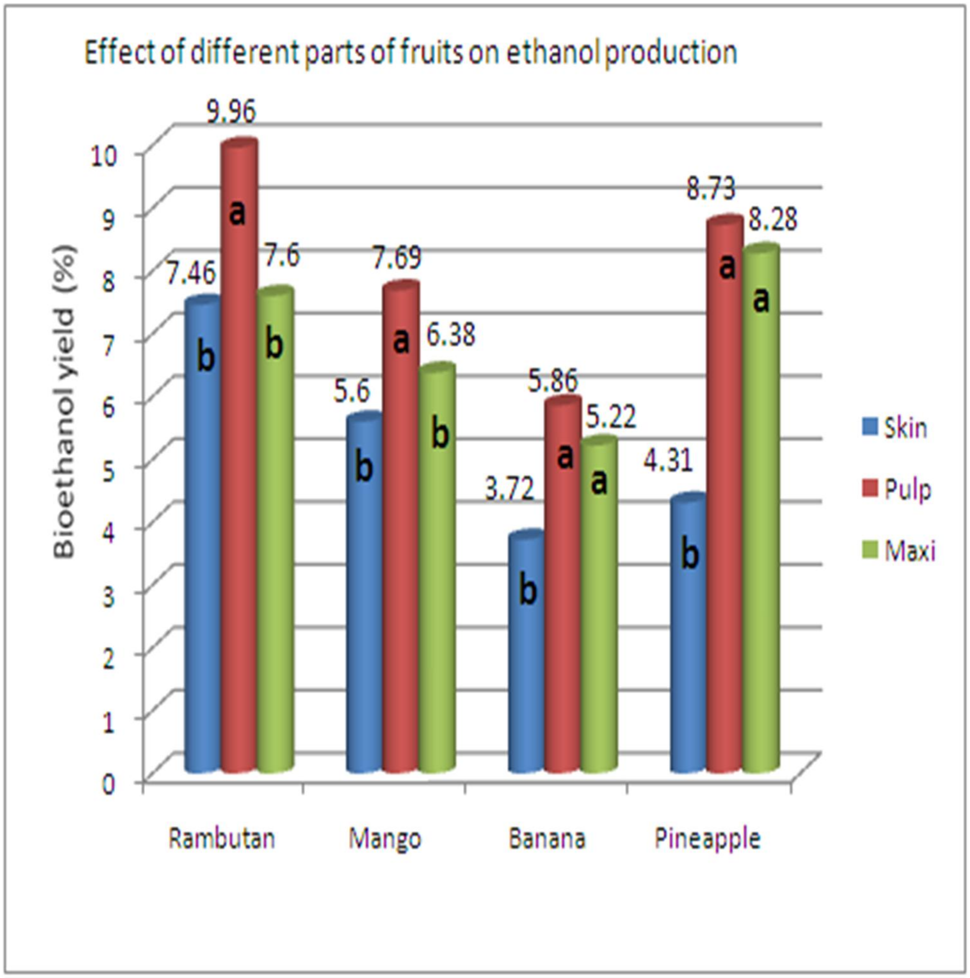


Figure 4.7 Comparison of bioethanol production by different parts of the fruits (max standard error ±5%)

General reduction in pH was also observed here, probably due to the reason discussed earlier. For example, the initial pH was measured at 5.2 but the final pH dropped to 3.04, 3.26 and 3.26 in experimental vessels containing skin, pulp and mixture respectively (Table 4.7).

Table 4.7: Comparison of Bioethanol yield from different fruit parts (max standard error ±5%)

Fruits / Parameters		Rembutan After fermentation	Mango After fermentation	Banana After fermentation	Pineapple After fermentation
TSS %	**Skin**	2.1	2.6	3.3	3.7
	Pulp	2.4	3.5	2.4	3.7
	Maxi	2.2	2.3	2.8	3.1
Glucose mg/ml	**Skin**	4.4	4.8	4.3	4.9
	Pulp	4.8	4.2	4.7	4.2
	Maxi	4.2	4.7	4.2	4.9
pH	**Skin**	3.1	3.9	3.8	3.3
	Pulp	3.2	3.9	3.7	3.5
	Maxi	3.4	3.8	3.3	3.1

The TSS values were reduced from initial value 11.0 to 2.81, 12.3 to 3.6 and 12.2 to 2.96, for skin, pulp and mixture respectively. Residual glucose content was measured and found reduced from 5.2 to 3.06, 6.4 to 4.04 and 6.0 to 3.4 in case of skin, pulp and mixture respectively (Table 4.7).

4.1.2 Determination of media viscosity

Different viscosity 1.23, 1.58, 1.62 and 1.75cst were recorded from fermentation using different fruit biomass (Table 4.8).

Table 4.8: Viscosity and acid value (max standard error ±5%)

Feedstock	Viscosity (cst)	Acid value (mg KOH/g)
Rambutan	**1.23**	**0.36**
Banana	**1.58**	**0.38**
Pineapple	**1.62**	**0.48**
Mango	**1.75**	**0.49**

The viscosities results were compared with research done by (Ghobadian *et al.* 2008) which measured the viscosity value of bioethanol with 1.1 cst. The viscosity of ethanol was 1.52 cst at 20°C (Sinor *et al.*, 1993). From the results, all the samples had acceptance value of viscosity since it had a standard value same as the pure petrol and did meet the requirement of petrol standards. The anhydrous ethanol was blended with the petrol to get the results of lower viscosity and was used for fuel engines. The lower fuel viscosity led to the greater pump and injector leakage which can reduce maximum fuel delivery and power output. Lower viscosity also, can overcome hot restart problems as insufficient fuel is injected at cracking speed, when fuel leakage in the high pressure pump is amplified due to the reduced viscosity of fuel.

4.2 Discussion

4.2.1 Bioethanol production from rotten fruit biomass

1- Optimization of different pH

Bioethanol yield was investigated at different pH using rambutan fruits (Fig 4.1). The bioethanol production at pH 5 showed the highest with 7.5%, followed by pH 6 and pH 4 in which bioethanol was decreased to 4. 16% and 3.17% respectively. The range percentage of bioethanol production betweenpH 4 to 6 showed a significant difference. The maximum consumption of the Total Suspended Solids (TSS) was noticed at pH 5 and then a gradual decrease in consumption was recorded on pH 4 and pH 6 as shown in Table 4.1.Glucose contant was observedat pH 5minimun. In case of pH 5, the glucose content was reduced remarkably as shown in Table 4.1.

All biological processes are affected by pH because all biological processes are catalyzed by enzymes which are by definition proteins, and tertiary structure can be broken by extremes in Hydrogen and Hydroxyl ion concentration which is what pH measures. The suitable pH found for fermentation of fruit was pH 5 to facilitate the enzymatic catalysis of the available sugars into ethanol.(Gao *et al.* 1988) has reported the survival and growth of *Saccharomyces cerevisiae*, according to the authors this yeast can tolerate the ethanol concentration up to 15% but the sensitivity of yeast cells to ethanol was marginally increased on decrenasing the pH from 6-0 to 3–0. It showed the pH had an important impact on ethanol production and yeast cell concentration. (Ogunya *et al.* 2006) reported that when the experiment was conducted at 3.4pH and 4.1 ethanol production was enhanced from pineapples juices. It was also reported that the pH did not affect the ethanol's yield in the range of 3.5 to 6.0 when pineapple effluent was need as substrates (Stuckey and Hamza, 1982*)*.

2- Optimization of yeast concentration

According to the parameter stated in (Fig 4.2), the results of bioethanol production from different concentrations of yeast (2, 3 and 4 g/L), shows that *S. Cerevisiae* with 4 g/L concentration is highly effective to produce bioethanol from fruit's cellulosic stuff. The bioethanol productions were linear to the concentration of yeast. As the concentration of yeast increases, higher percentages of bioethanol yield were produced. The 2 g/L concentration of yeast produced 7.0% of ethanol yield, while 3 g/L produced 7.5% and 5 g/L produced the highest of ethanol yield with 8.17 (v/v)%.(Ghose and Tyagi 2004) has reported a study on the strain of *Saccharomyces cerevisiae* in batch and continuous cultures at pH 4.0 and 30°C temperature by using a 23.6 g/Liter cell concentration, a concentation of 9.7%

(w/v) ethanol was developed in a period of 6 hs. They found that the rate of fermentation was found to increase with supplementation of yeast vitamins in the reaction mixture. It was also reported that the ethanol productivity was found to decrease linearly with ethanol concentration. This results are found to br in good agreement with the reported literature (Ghose and Tyagi 2004).

3- Optimization of temperature conditions

The percentages of bioethanol production were shown at different temperatures for 28, 30 and 35°C using rotten rambutan wastes and yeast, *S. Cerevisia*e (Fig 4.3). It was observed that the maximum ethanol production was at temperature 30°C with 9.17%, followed by 32°C with 8.5% and at room temperature, 28°C produced 7.8% of ethanol that was the lowest among the parameters. Hence, the strain of yeast, *S.cerevisiae* has performed better at 30°C than other temperatures. The experiment at 28°C produced the lowest yield compared to the others parameters which is 30 and 32°C. This is because, at low temperatures, the reaction rates of all metabolic functions was slowed down and it reduced the substrate and product diffusion rates for higher ethanol yields.(William and Munnecke 1979) reported the immobilized cells of *Saccharomyces cerevisiae*could resist the adverse conditions of temperature and pH but the free cells had usually a range of moderate temperature, in this case the optimum temperature was found 30°C for maximum ethanol yield, reduction of the TSS and glucose contents.

4- comparison of bioethanol yield between the rotten and fresh fruits

Fermentation was also conducted to evaluate the performance of yeast to produce ethanol using fresh and rotten fruits (Fig 4.4). The ethanol produced in case of rotten fruit was 7.17% as compared to the fresh fruit yielding only 5.5% of ethanol. Fresh fruit possessed the more TSS as compared to the rotten fruit after fermentation (Table 4.4). The fruits are usually rotten because of the microbial/enzymatic activity. The availability of the reducing sugars was greater in the rotten fruits as compared to the fresh fruits. It is like an enzymatic pretreatment of the fresh fruit pulp or other parts before fermentation. (Ugwuanyi and Obeta, 1999) have reported pectinolytic and cellulolytic activities of heat resistant fungi and their macerating effects on mango and African mango. The overall results of the microbes on fruit results into conversion of cellulosic biomass into simple sugars followed by ethanol production. In case of our study, it was also noticed that the rotten fruit is more promising to ethanol production by yeast as compared to the fresh fruits. The authors also reported that enzymatic activities were significantly higher in fruit tissue (mango and African mango) media than in pectin medium showing efficient production of the simple sugars and other products.

5- Fermentation at different days

Fermentation was conducted at different time duration to produce ethanol from rambutan fruits (Fig 4.5). Time duration was set from 1 to 4 days and the products were analyzed for different factors including change in pH, TSS, glucose contents and ethanol. Ethanol production from one to four days was 8.4, 9.4, 8.5 and 7.4 respectively. Two days or 48 hours fermentation retention time was chosen best for ethanol production which is 9.4%. Initial pH was recorded as 5.2 in all cases but the lowering in pH was found different as 4.22, 4.19, 3.81 and 3.91 respectively in the cultures grown for 1 to 4 days. (Sharma *et al.* 2007) has reported that maximum ethanol can be produced within 48 hours of incubation time. Measurement of TSS revealed that the maximum clarity was observed in experimental vessel carrying fermentation for one day, as it was reducing the TSS from 12 to 3.0. In case of fermentation with 2 to 4 days, the final TSS values were found to 3.5, 3.17 and 3.17 respectively. The residual glucose concentration was measured and found that glucose content was as 3.5, 3.8, 3.6 and 3.6 in 1, 2, 3, and 4 days cultures respectively.

6- Fermentation with enzymatic hydrolysis

(Patle and Lal. 2007) has reported the enzymatic digestion of agricultural wastes using mixed culture of Zymomonas mobilis and Candida tropicalis, they found that the maximum yield of reducing sugars from apple and carrot pomace was in the hydrolysate treated with pectinase (5 U enzyme g_1 substrate) however the pineapple, mango and sapota hydrolysate yielded maximum reducing with xylanase (5 U enzyme g_1 substrate) treatment . There was not much significant increase in reducing sugars yield with increasing concentrations of enzymes. Enzymatic hydrolysis of rambutan fruit was made by using commercial enzymes cellulase and amylase prior to fermentation (Fig 4.6). *Saccharification* of the cellulosic material was performed by enzymes to release glucose contents for further fermentation by yeast. The ethanol production was 9.9% in case of only yeast while it was negligibly low in experimental vessels which were pretreated with enzymes like cellulase and amylase, as it was shown 9.7% and 9.9% respectively. Usually an enhancement of releasing sugars and ethanol concentration is reported in the pretreated stuff by enzymes leading to fermentation. But in this study, it was not creating any remarkable difference to enhance ethanol production. It might be due to the rotten nature of fruits in which simple sugars have already been released by the environmental microbes/enzymes activity. (Retamal *et al.* 1987) reported that the enzymatic pretreatment using prickly pear cladodes and fruits and different yeast strains of the genus Saccharomyces, enzymatic digestion of fruits was noticed as best to release the sugars as compared with the acidic treatment.

7- Fermentation of different fruit parts:

Skin, pulp and maxi of the fruits were investigated for ethanol production using yeast fermentation (Fig 4.7). Ethanol produced in case of fermentation of rambutan skin was 7.46%, from maxi, it was 7.6% and maximum ethanol was produced from pulp which was recorded as 9.96%. (Ugwuanyi and Obeta 1999) have reported maximum hydrolase activities in fruit tissues instead of the skin and maxi. It is always in practice to make treatment of the hard cellulosic tissues to soften enough for the penetration of enzymes. In case of pulp, it looks enough soft and pretreated to be acted upon by enzymes properly as compared to the other parts of the fruits. According to (Reddy and Reddy, 2007), there were three types of sugars that had been identified in mangoes, namely glucose, fructose and sucrose.

4.2.2 Comparison of Bioethanol Production From Different Fruits Biomass

The ethanol production from rotten rambutan fruit was compared to the ethanol production from mango, banana and pineapple. The parameters involved were included the pH, temperature, retention time, status of the fruit (rotten/fresh), yeast concentration, enzymatic pretreatment and different parts of fruits. As shown in (Fig 4.1), at pH 5, the ethanol (v/v) was produced as 7.5, 6.3, 5.88 and 6.64 % from rambutan, mango, banana and pineapple respectively. The efficiency of the rambutan to produce ethanol was also higher at pH 4 and pH 6. The other fruit biomass (Gao and Fleetn, 1988) reported that the pH has vital impact on the efficiency of survival of the *Saccharomyces cerevisiae*, The comparative study (Fig 4.2), regarding yeast concentration among fermentation of four different fruits also revealed that the maximum ethanol was produced by rambutan as compared to the other fruits e.g. the yeast concentration, 4g/L was responsible to produce ethanol (v/v) as 8.17, 7.23, 6.0 and 7.8% from rambutan, mango, banana and pineapple respectively. (Ghose, and Tyagi 2004) has reported a linear relationship in yeast cells and ethanol production. In case of studying the temperature as a factor to influence the fermentation and ethanol production from different fruits, at 30 ^{O}C, the ethanol (v/v) was produced 9.17, 7.28, 6.09 and 8.41% from rambutan, mango, banana and pineapple respectively (Fig 4. 3). In case of different condition (rotten and fresh) ethanol production from rotten fruit is 7.17 % (Fig 4.4). As the incubation time of the fermentation is concerned, the 48 hours were chosen as the optimum incubation time (Fig 4.5). (Sharma *et al.* 2007) has reported that maximum ethanol could be produced within 48 hours of incubation time. It was found that, after two days the ethanol (v/v) was produced as 9.4, 7.3, 6.86 and 8.64% from rambutan, mango, banana and pineapple respectively. All of these details were lead to the fact that rambutan had more efficient fruit biomass to be acted upon by the enzymes for its conversion into bioethanol. However, ethanol production was different from the same material under different conditions, but the priority reflection was evident in all cases to nominate the rambutan as a best feedstock of ethanol production among the said fruits. It was also revealed that the enzymatic treatment was also made a difference to elaborate the soft nature of rambutan as compared to the other fruits under investigation (Fig 4.6). The fermentation of mixture was able to produce ethanol (v/v) as 7.6, 6.38, 5.22 and 8.28% from rambutan, mango, banana and pineapple respectively showing higher ethanol production from pineapple (Fig 4.7). The pulp was able to produce bioethanol (v/v) as 9.96, 7.69, 5.86, and 8.73% from rambutan, mango, banana and pineapple respectively. In case of rambutan or pineapple bioethanol was higher than maxi than of other fruits. It might be due to the fermentation in which yeast activated well having more sugars in the samples.

4.2.3 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. From the result obtained in table 8, it could be seen that the bioethanol produced from fermentation of mango pulp at temperature of 30°C with different amount of yeast were in the range of ASTM standard considered, which were within l to 5 centi stroke. This would give an indication that ethanol produced from fruits was suitable as a possible biofuel substitute. Additionally, low viscosity value was good for engine and reduced the problems of corrosion to the engine. The samples from fermentation using rambutan and banana showed a lower viscosity than mango and pineapple biomasses. The increased of viscosity value in mango and pineapple was mainly due to the presence of higher glycerol in the solution. Glycerol was a major byproduct of ethanol fermentation by Saccharomyces cerevisiae. Thus, the yeast cells produced glycerol under anaerobic and glucose-repressing growth conditions in order to function to help maintain a cytosolic redox state conducive to sustain glycolytic catabolism (Alberol *et al.* 1996). So, the higher glycerol content could cause higher viscosity to the solution. However, the viscosity obtained was still maintained under ASTM standards, presenting it as a qualified alternative fuel. (Table 4.8) shows the results of acid value test from samples fermented at different fruit biomasses. From the result, the acid values measured were almost the same for all fermentation reactions of fruits. The results obtained were in the best range and under ASTM standard specification. Through there was not highest different between all fruit samples (Table 4.8).

4.2.4 Chemical analysis

The metal contents in bioethanol produced were analyzed and tabulated in Table 4.9.

Table 4.9 Chemical Analysis (max standard error ±5%)

Chemical	Value (ppm)			
	Time (hour)			
	Rambutan	Banana	Pineapple	Mango
Fe	1.2	2.5	5.5	3
Pb	0	0	0	0
Cu	0	0.5	0	0
Al	0	0	0	0

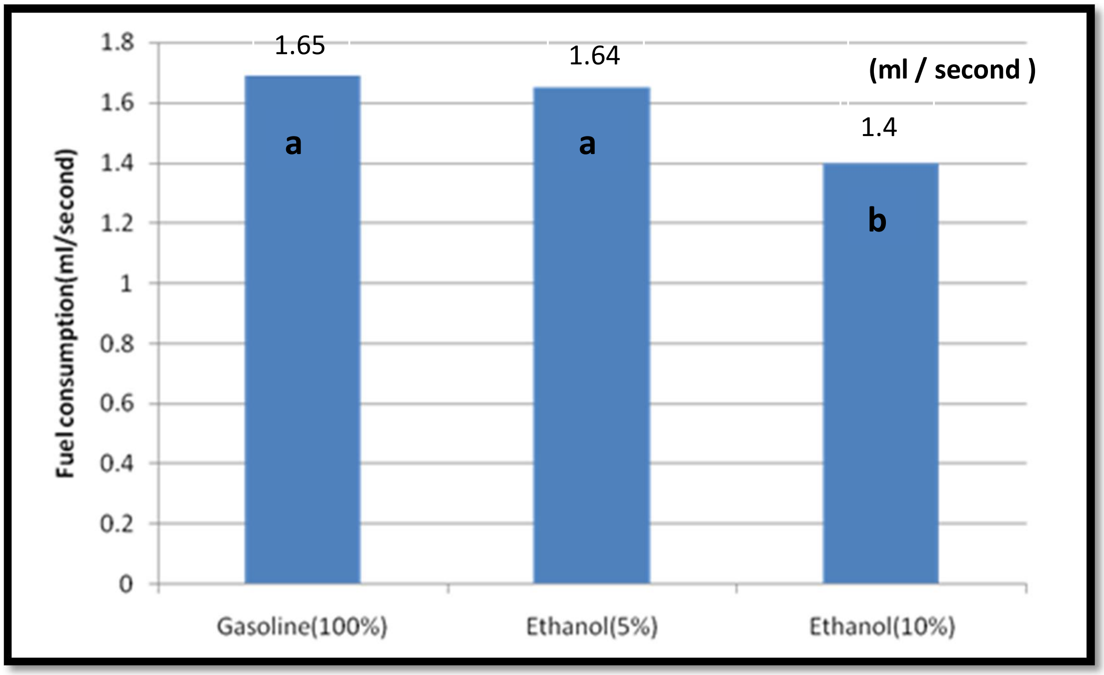
Sn	4.2	4.2	4.3	4.15
Mn	6	4	2.5	4.5
Ag	18.5	00.5	20.0	19.65
Zn	9.5	6	7	5
P	91	75.5	70	65
Ca	3.16	5.8	4.25	5.3
Mg	1.67	185	1.72	8.1
Si	1.65	1.65	1.65	18
Na	13.8	14.05	8.75	11.35

The four hazardous chemicals were Lead (Pb), Iron (Fe), Copper (Cu) and aluminum (Al).Are ASTM

From table 4.9, it has been observed that there is a clear reduction of hazardous metal content especially Pb, Al, Fe and Cu in the bioethanol produced from waste banana, rambutan, mango and pineapple. However there were some metal contents in bioethanol which were found to be high in bioethanol i.e Sn, Ag and Na. The metal content in bioethanol should be less or reduced, so that it is more suitable for used as biofuel. This observed high metal contents may probably be due to high nutrient content of the fruits biomass, as it is well known that the nutrients comprises of metallic elements. However, most of the metal such as Pe, Mn, Zn, P, Ca, Mg, Si, Na, B and V were observed to be lowered, thus decreasing the risk of corrosion to the engine. The other metals seem to be zero as compared to the values of including Cr, Al, Cu, Pb, Ni, Ti, Mo and Ba indicate of that bioethanol produced have the quality to be used as biofuel on generating the engine car

4.2.5 Engine Test

Burning of fossil fuel and emission of hazardous gases are considered as a main source of global warming and environmental pollutions. The utilization of bioethanol is quite environment friendly and has remarkable effects to reduce the emission of hazardous gases. The ethanol produced from this experiment was tested by using the Proton Gen 2 multi-cylinder engine for 1 hour at 2000rpm (60km/hour). A drastic decrease in exhaust emission and fuel consumption was observed and analyzed when the 5 and 10% of bioethanol rnixcd with gasoline on generating the engine Multicylinder of Proton Gen-2 car. Based on the graph (Figure 4.8), there was a reduction in the emission of Nitrogen oxide (NOx) (Fig 4.9) using 5 and l0 % of bioethanol in the mixture of gasoline.



Engine performance

Figure4.8: Comparison of fuel consumption of Gasoline (100%), Ethanol (5%) and Ethanol (10%) (max standard error ±5%).

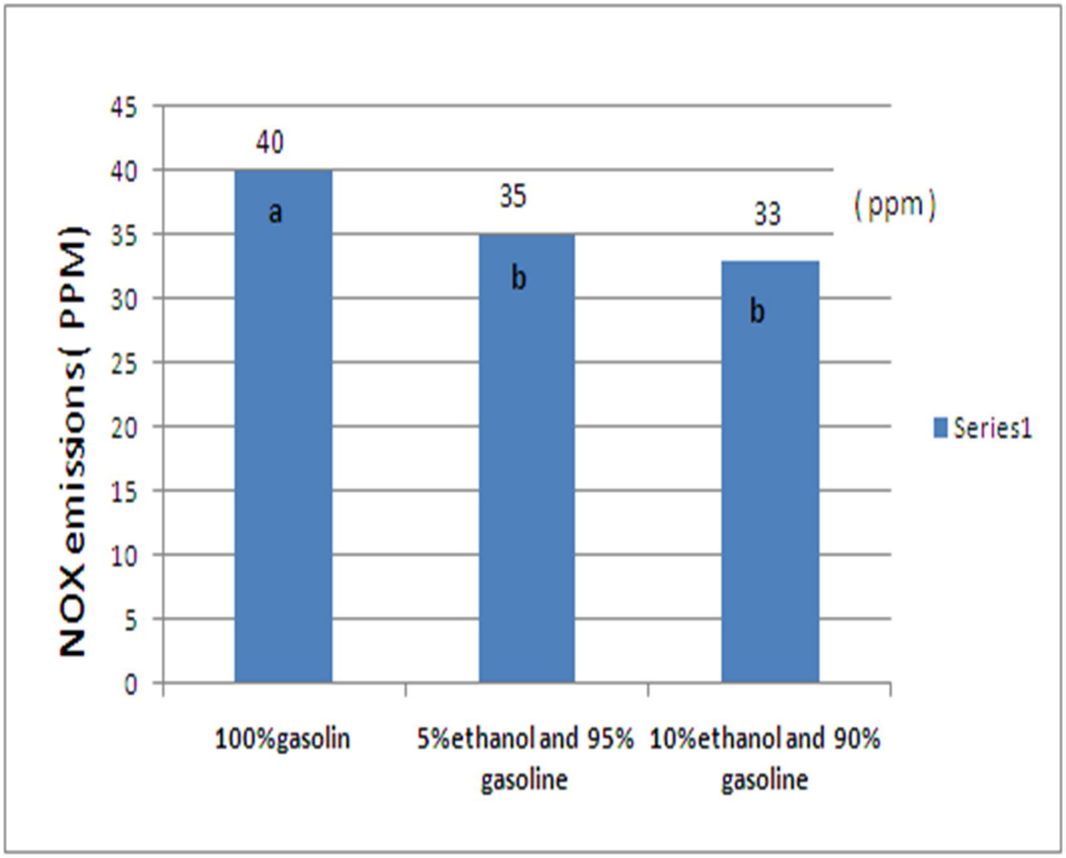
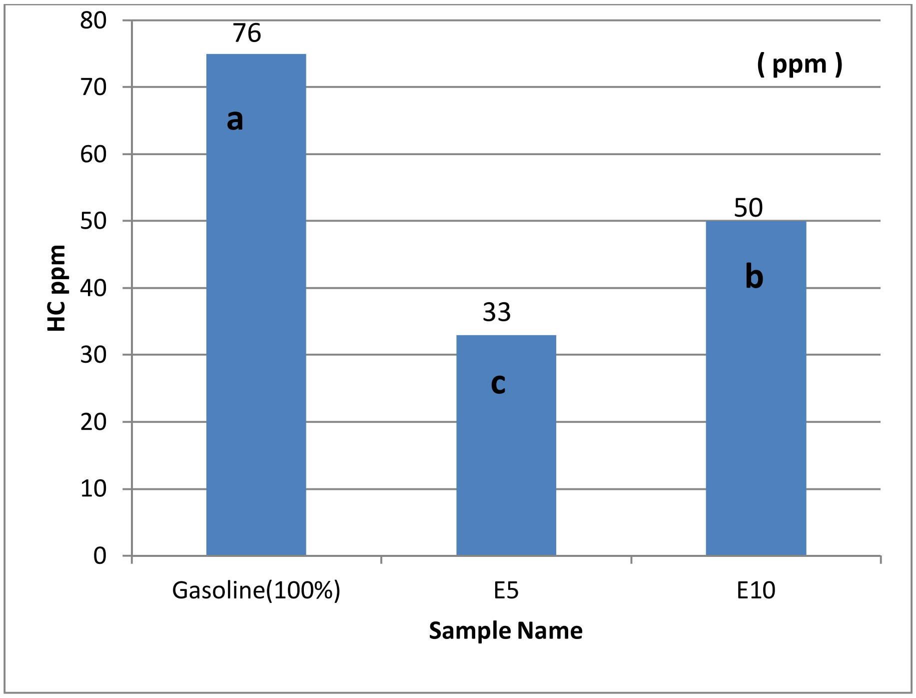


Figure 4.9 Amount of hydrocarbon (NO_X) from engine test of 100% gasoline, a blend of 5% bioethanol 95% gasoline and a blend of 10% bioethanol 90% gasoline (max standard error)

Fuel consumption of the mixture of bioethanol and gasoline was less and the emission of NO, was reduced. This was because of the highly oxygenated component of ethanol fuel (Yoon *et al.* 2009). There was a little difference in the amount of emissions of nitrogen oxides from ethanol-blended fuels in relation to conventional fuels. Reports were cited regarding this difference in the range of 5% decrease to 5% increase for low-level ethanol blends. For ethanol blends in the range of 5-95%, the reduction in emissions of nitrogen oxides may be of the magnitude of 20% (Environment Canada, 1989). However, in comparison to using 100% gasoline as fuel, in this study, the emission of nitrogen oxide was reduced approximately 80%, when a mixture of 5% of I bioethanol with 95% of gasoline (E5) and 10% bioethanol with 90% gasoline were used. The hydrocarbon content for fuel consumption (ml/sec) was measured at 100% gasoline, E5 (A blend of 5% bioethanol/95% gasoline) and E10 (A blend of 10% bioethanol/90% gasoline). From Figure10, the hydrocarbon content in E5 and E10 was found 33 and 50 ppm respectively, were significantly lower biomasses than in 100% gasoline with hydrocarbon of 75 ppm. This showed that the fuel was burned more completely in E5 and E10 as compared to the 100% gasoline permitting emission with fewer unburned hydrocarbons (HC). This phenomenon reduced air toxics, which ware associated with the unburned or partially burned HC (Fig 4.10) emissions. So_2 (Fig 4.11), with more completely burning of fuel, the amount of hazardous gaseous emissions such as carbon dioxide (Figure 4.12), carbon monoxide and nitrogen oxide were reduced to generate cleaner environment. The emissions produced by burning ethanol are less reactive with sunlight than those produced by burning gasoline.



Engine emission of (HC)

Figure 4.10: Amount of hydrocarbon (HC) from engine test of 100% gasoline, E5: A blend of 5% bioethanol 95% gasoline and E10: A blend of 10% bioethanol 90% gasoline (max standard error ±5%)

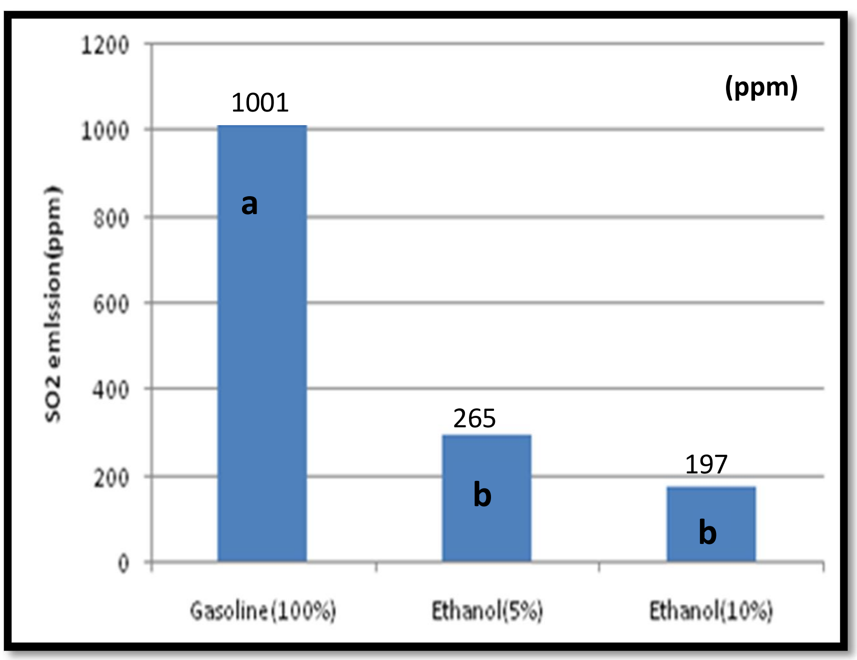


Figure 4.11: So_2 emissions of Gasoline (100%), Ethanol (5%) and Ethanol (10%) from rambutan fruit waste (max standard error ±5%)

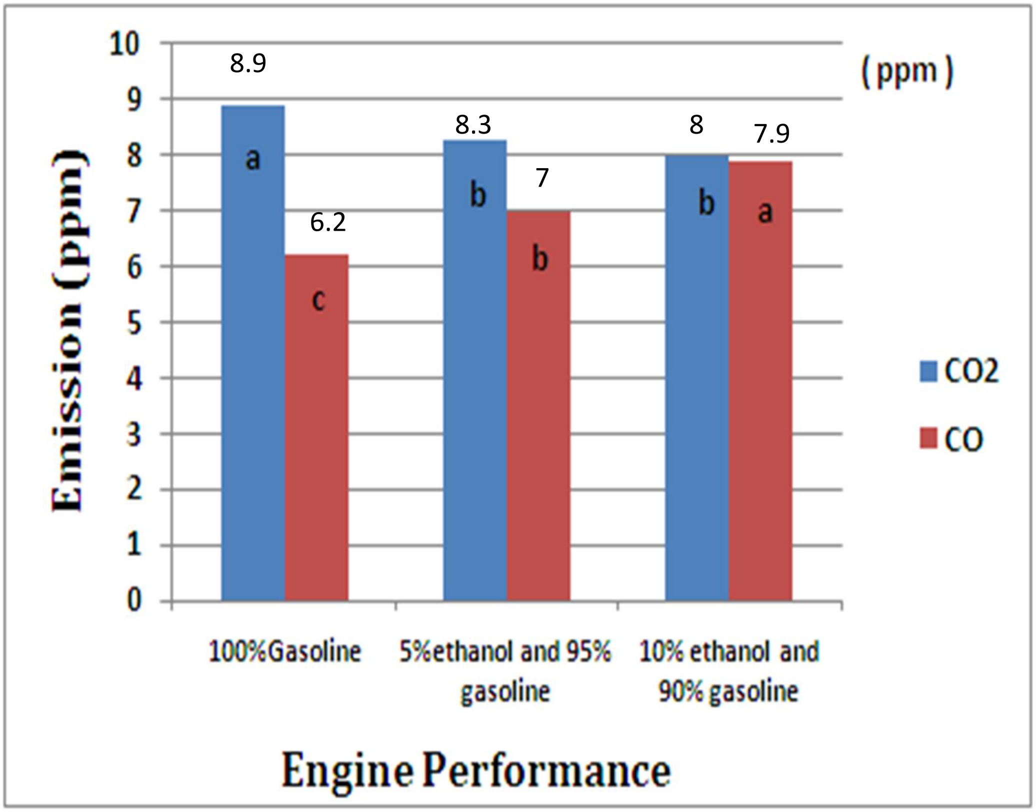


Figure 4.12: Comparison of carbon dioxide (CO_2) and carbon monoxide (CO) from engine test of 100% gasoline, a blend of 5% bioethanol 95% gasoline and a blend of 10% bioethanol 90% gasoline. (max standard error ±5%).

This results in lower potential for damaging the ozone layer (Vincent et al, 2003). The performance of the engine and emission of gases were strongly supporting the quality of the ethanol produced by the fermentation of fruits. The engine test was not made by using 100% ethanol but it was reported that Brazil operated almost 50% of their vehicles on pure ethanol while a 10% blend requiring no engine modifications at all (Vincent et al, 2003). Furthermore, E5 could be used in any unleaded car on the road in the UK and E10 was well applied in all motor vehicles manufacture since the 1970s and did not require engine modifications.

Chapter 5

5.0 CONCLUSION

This study was designed to utilize the waste fruits for ethanol production and reduce the possible pollution because of the waste fruit material. The results of this study has revealed that the fruit wastes including rambutan, banana, mango and pineapple 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 rambutan was the most efficient fruit waste to produce maximum ethanol as compared to the other fruits, The efficiency of fermentation or the yield of ethanol production is dependent on the time, concentration of yeast and optimum conditions as described in results and discussion section. The chemicals content, viscosity and acid values of the bioethanol produced were within ASTM (American Society for testing and Materials) specifications. The reducing sugar content, total soluble solid (TSS) and pH values were reduced as a result of fermentation due to conversion of glucose into ethanol and carbon dioxide by yeast. The engine test showed low amount of hazardous chemicals content, thus this bioethanol could potentially be used as good biofuel. Viscosity and acid values measured indicated that this bioethanol was safer to be used for engine purposes and reduced corrosion problem to the engine. Finally it can be concluded that the bioethanol produced from different fruit biomasses is of good quality and can be used for petrol engine as well as it can be a good waste management production and recycling reprocess .

5.1 Recommendations

We humbly recommend that further study concerning process optimization should be undertaken especially using waste rambutan biomass. As it has been known that this fruit is among Malaysian indigenous fruits and is found abundantly in Malaysia, hence using the waste biomass of this fruit will not only add to the reduction in the production cost of the bioethanol but also will help in environmental cleaning. Therefore, we strongly believe that further research on process optimization could lead to scale-up of the bioethanol production process using the cheap and available biomass from laboratory scale to mass production scale.