The Fermentation of Sugar Rich Fruits: jamun *(Syzigium Cumini)*, soursop *(Annona Muricata)*, and papaya *(Carica Papaya)*, with and without additives, in order to produce optimum ethanol yield for commercial purposes

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ABSTRACT

The fermentation of sugar rich fruits: jamun (*syzigium cumini*), soursop (*Annona muricata*), and *Carica papaya* in the absence and presence of additives was achieved under anaerobic condition at a pH of 4-5. Initial and Final brixs were recorded and showed that fermentation was almost completed. The final pH indicates that the filtrate prior to distillation was acidic, due to the presence of carbonic and acetic acid. The reducing sugar content was measured before and after fermentation. Papaya fruit matrix, without additive, yields the highest volume of ethanol, (4.650 ± 0.255, v/v), whilst soursop, the least (4.100 ±0.245, v/v). The effects of the additives were variable at the different percentages of 0.1, 0.5 and 1%. 0.1% promalt, with 0.5% K_3PO_4 produced the highest % yield of ethanol (14.163 ± 0.017, v/v), whereas the lowest % yield of ethanol (4.520 ± 0.08, v/v)) was produced by soursop with $ZnSO_4$ additive. At the 0.5% level, promalt with 0.5% K_3PO_4 produced the highest % yield (9.870 ±0.05, v/v). At the 1% level, K_3PO_4 additive produced the highest yield (7.690 ±0.055, v/v) of ethanol. Compared to the reference compound, glucose, the mean ethanolic content of the fruits, without and with additives,were lower than that of glucose (9.480, v/v). Exception being, the mean ethanolic content with promalt at 0.1 % level with 0.5% K_3PO_4 on soursop (14.163 ± 0.017, v/v) and at the 0.5% level (9.870 ± 0.05, v/v). Gas chromatographic analyses were also done on the distillate, from the fermented matrix, without and

with additives. It was found that the distillate in most cases consists of ethanol and by products of acetaldehyde, methanol, methylacetate, 1-propanol, ethylacetate, 1-butanol, isobutylalcohol, iso-amyl alcohol, 2-methyl-1-butanol, 1-pentanol and furfural in most cases. Our research shows that all of the selected fruits can be used as attractive substrates for the production of ethanol and hence there cultivation should be encouraged as a boost to the Agro Sector of the country and also, a source for the blending with gasoline to produce gas alcohol. However, future work is necessary to intensify the yield of ethanol beyond the recorded in the literature.

Keywords: Fermentation, *Saccharomyces Cerevisae* fruit substrate, jamun, soursop, papaya, ^{0}Brix, pH, temperature, potassium phosphate, zinc sulphate, distillate, ethanol.

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1.0. INTRODUCTION

With, a view to decrease dependence on fossil fuel, as a result of depletion, increasing global fuel prices, increasing population and increasing global warming, there has been increased interest in the use of alternative renewable energy sources of which bioethanol is one [1-33]. Bioethanol (b.p: 78.5°C) can be used for a variety of purposes, of which blending with gasoline to produce gas alcohol to power automobiles is of current utilization in countries such as Brazil and the United

States [1,2,3, 7,10]. In addition, ethanol is a clean burning renewable energy source[1-33]. Ethanol is also an important component of alcoholic beverages such as wine, beer, cider, vodka, gin. whisky, brandy etc. It is also an important starting materials for aldehydes, ketones, carboxylic acid, carboxylic acid derivatives and the hydroxyl group is a component of many pharmaceutical drugs [5]. Ethanol can be used in the perfume, disinfectant, tincture, biological and biofuel industries. Ethanol production via fermentation has been one of the world most significant approaches to aid in the advancement of Commercial Industry[1-33].

Ethanol doesn't have significant environmental impact as fossil fuel combustion [1-33]. It has low air polluting effect and low atmospheric photochemical reactivity, further reducing impact on the ozone layer[1-33]. It contributes little net CO_2 accumulation to the atmosphere and thus should curb global warming[1-33].

Ethanol can be used in three primary ways as biofuel, namely, E10 which is a blend of 10% ethanol and 90% unleaded gasoline, a component of reformulated gasoline, both directly and or as ethyl tertiary butyl ether (ETBE) and as E85 which is 85% ethanol and 15% unleaded gasoline. When mixed with unleaded gasoline, ethanol increases octane levels, decreases exhaust emissions and extends the supply of gasoline. Bio-ethanol is made by fermenting almost any material that contain starch or sugar. Grains such as corn and sorghum are good sources, but fruits that are high in sugar concentration are excellent sources as well, since they contain ready to ferment sugars [10]

To solve the above problem, emanating from fossil fuel, one alternative is to produce bioethanol from fruits, other grown organic matter or waste[3,4,6-29]. Bioethanol can be obtained via the fermentation of glucose, fructose or sucrose under the influence of *Saccharomyces cerevisiae* at room temperature, [4-33]. Also, acid hydrolysis of lignocellulose material followed by subsequent fermentation [3,9-33] . Sugar rich sources include ripe fruits [8-28] etc. Other sources include biodegradable fraction of products, waste and residues from agriculture like vegetables and animal origin [7-28] etc. The percentage yield of ethanol, ranging from 4.0 -10.0 v/v) have been reported [3-33]. Fruits that are high in sugar concentration are favourable to the fermentation process, since they can produce high percentage volume of ethanol [9-33]

The process of fermentation using yeast, *Saccharomyces cerevisiae* occurs under certain factors which is suitable for the production of ethanol. The importance of maintaining specific conditions for fermentation, in which the increase in temperature to 45 °C enabled the system to still show high cell growth and ethanol production rates, while it was inhibited at 50 °C and the

pH. 4.0–5.0 was the optimal range for the ethanol production process [21]. Ethanol fermentation is anaerobic pathway carried out by yeast in which simple sugars are converted to ethanol and carbon dioxide [7-28]. Jamun (*Syzygium cumini*) fruit was utilized for the production of red wine which offers a lot of health benefits by acting as an effective medicine. The alcohol content of the wine varied from 6.62 to 10.25, depending upon the variable concentration of total soluble sugars (7.88 to 10.53%) and varying levels of yeast (5, 10, 15 and 20%). *Saccharomyces cerevisiae* was used in the fermentation process [29].

A red wine from anthocyanin-rich tropical jamun fruit having medicinal (anti-diabetic and curing bleeding piles) properties was prepared via fermentation, using wine yeast, *Saccharomyees cerevisiae*) and the quality attributes compared with commercial grape red wines[30]. A kinetic evaluation of the fermentation of soursop (substrate) by *Saccharomyces cerevisiae* (yeast enzyme) was conducted by determining the effect of various parameters (such as temperature, substrate, pH, and yeast concentration) on the rate of production of CO_2 [31].

The production of ethanol from *Carica papaya* (pawpaw) agricultural waste, using dried active bakers yeast strain (*Sacchromyces cerevisiae*) was investigated. The results of this work show that the rate of alcohol production via fermentation of pawpaw fruit waste by bakers yeast (*Sacchromyces cerevisiae*) increases with fermentation time and peaked at 72 h. It is also increased with yeast concentration at the temperature of 30 °C. The optimum pH for fermentation is 4.5 [32]

The ethanolic content of Papaya (*Carica papaya*) verses Sapodilla (*Manilkara zapota*) via fermentation has been reported[33]. The mean ethanolic content of papaya (1.964, v/v) was lower than that of sapodilla (14.91, v/v). It was found that *Magnifera indica* (mango) in the presence of additives produced the highest mean yield of ethanol of 25.16%, v/v [34]. Other substrates that play a positive role in the production of ethanol are the presence of salts, the pH, and the presence of substances that enhance the activity of the enzymes of the yeast species. As mentioned before, when these conditions are rightly set, the amount of ethanol that will be produced will be significantly greater than the customary amount at room temperature. If these conditions are met it could affect the yeast growth[35] and as a result affect fermentation as depicted in diagram below:

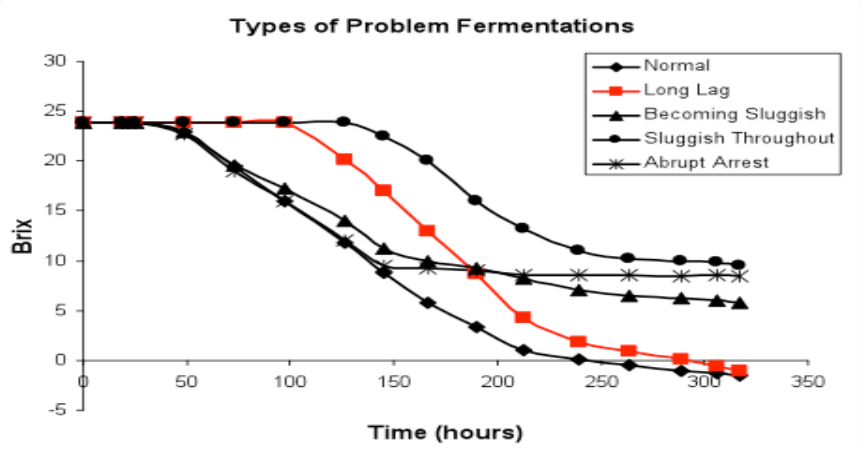


Fig. 1.0 The relationship between fermentation and the growth of yeast

Based on research conducted, nitrogenous compounds, ammonium, urea, magnesium sulphate salts, potassium salts, and also amino acids all enhance the production of ethanol. The addition of nitrogenous compounds, enhances the yield that is obtained, and also ammonium and urea salts play a role in the functioning of the yeast enzymes. The presence of certain salts such as magnesium sulphate and potassium salts is also known to bring about higher yields of ethanol. A high initial sugar concentration brings about a significant yield of ethanol provided that an osmotolerant strain of yeast is being utilized. High substrate concentrations have an inhibitory effect on the yeast strain, because of high osmotic pressure and low water activity, which serves to dehydrate the yeast. Hence, the use of an osmotolerant species of yeast nullifies the dehydration problem and maximizes on ethanol yield. Amino acids are required to act as catalysts, since they convert nitrogen into the ammonium state that is required by yeast.

This paper reports the fermentation of jamun*(Syzigium Cumini)*, soursop*(Annona Muricata)*, and papaya *(Carica Papaya)*, with a view to produce ethanol for commercial use and in the future blending with gasoline to produce gas-alcohol. Guyana has started to use the initiative Brazil has taken over the past forty two years. The first fleet of vehicles belonging to the Ministry of Agriculture was fueled up by bio-friendly ethanol, at the launch of the Bio-ethanol E-10 Fuel brand in Guyana in 2014. The plant is capable of producing fuel blends with 5%, 10%, 15%, 20% and 25% ethanol. The plant is focused primarily on mixing gasoline with ethanol at 10% to produce E-10 blend that is compatible with vehicles in Guyana and which has been tested successfully on

Toyota Corolla[35].

2.0. MATERIALS AND METHODS

2.1. **Methodology**

2.2. **Chemicals**

n-Butanol (BuOH) and ethanol (analytical grade, 99.5%) were purchased from Sigma-Aldrich (Israel) and used thereafter without any pretreatment. Ethyl acetate (analytical grade, 99.5%) was purchased from Frutarom (Israel). Glucose, KH_2PO_4, NH_4SO_4 and $MgSO_4*7H_2O$ were from Sigma (all chemically pure or higher grade).

2.3. Raw materials:

Fruits such as Papaya, soursop were obtained from the local market at Bourda , Georgetown, while Jamun was obtained from a farm in #1 Canal Bagotville, West Bank Demerara. The fruits were transported to Banks DIH Rum Factory Laboratory on the required days, where they were prepared for experimentation.

2.4. Reducing sugar test:

About 2ml of each fruit sample was placed in test tubes, then using a pipette, 10 drops of Benedict's solution was added to the test tube. This was then placed in a hot water bath, until a yellow or orange colour change was observed. This would indicate the presence of reducing sugars. This was done before and after fermentation.

2.5. Preparation of Samples:

Soursops (papaya) were washed thoroughly with distilled water, seeds were removed , then 550g of the fruit pulp and skin were weighed thrice on an electric balance, then proceeded to be blended

using a sterile juice blender (with each 550g portion of fruit being blended in 130ml of distilled water). The fruit pulp & skin matrix was then poured into three (3) plastic jars that were sterilized prior to use (550g of fruit pulp & skin matrix in each jar). The jamun fruit was squeezed manually to separate seed from pulp, instead of blending and all the contents of the fruit was used, then weighed (550g) and distilled water was also added to each matrix. The fruit pulp & skin matrix was then poured into three (3) plastic jars that were sterilized prior to use (550g of fruit pulp & skin matrix in each jar). The initial pH of the fruit pulp & skin matrix, its temperature and its total initial soluble solids content (^{0}Brix) were measured prior to fermentation.

2.6. Yeast Rehydration:

The yeast strain used was wine yeast (*Saccharomyces cerevisiae)*. This was provided by Banks DIH. 6g of the dried yeast each were weighed in three sterilized 100ml beakers, and then 40ml of lukewarm distilled water was added to each beaker. This was mixed properly using a sterilized plastic spatula to ensure all the yeast was completely dissolved. After which each beaker was sealed using plastic paraffin seals and left to stand for 60 minutes.

2.7. Yeast nutrients:

Yeast nutrients, such as a small sample of the fruit pulp & skin matrix and or metal salts solution were added to the beaker with the yeast to aid in rapid yeast replication (growth) by starting a mini culture. The beakers were covered again using plastic paraffin seal and left to stand for 20 minutes.

2.8. Using Promalt:

Promalt was used to aid in the hydrolysis of unevenly modified D- glucose to alpha glucose and also to solubilize amino acids, proteins and polypeptides. Also it has the properties to hydrolyze starch(if present) to glucose, due to the presence of the enzyme alpha amylase. This was achieved by weighing small grams promalt (0.5g, 1.0g, and 1.5g) and adding to 500 ml beaker containing

fruit pulp & skin matrix, then gently heating the mixture to a temperature of 68^0C to allow the enzymes to work optimally.

2.9. **Fermentation procedure:**

The fruit pulp & skin matrix were prepared initially (soursop, then papaya followed by jamun) as described above. Reducing sugar test was carried out as described above. The yeast was then hydrated as described above. The hydrated yeast, *Saccharomyces cerevisiae,* was slowly poured into to each plastic jar containing each fruit mash and each jar was sealed creating an anaerobic environment by only allowing carbon dioxide to be given off, but not for oxygen to be entered. This was done by a rubber bound, with a rubber tube leading out of it at the mouth of the jars. This tube was immersed in a test tube containing paraffin oil. The jars were then left to ferment for three days in a dark room, where the temperature was approximately 28-30ºC. **Yeasts need low pH to replicate but higher acidity than the normal range can inhibit chain elongation which affect DNA replication** After fermentation, the temperature of fruit mash was measured. Filtration was then carried out, using a Whatman filter paper, a glass funnel and another (sterilised) glass jar. This was done for each jar, giving a total of three glass jars with filtrate. After fermentation (for some samples), reducing sugar test was carried out on a small volume (approximately 2ml) of the fermented samples. For each fruit, the same procedure was repeated in triplicate, with varying concentration of metal salts. The procedure was repeated using 5ml of 0.1%, 0.5%, and 1.0% zinc sulphate, respectively which was added to the beaker with yeast prior to mixing with fruit skin & pulp matrix. The procedure was further repeated using 5ml of 0.1%, 0.5%, and 1.0% potassium phosphate respectively which was added to the beaker with yeast prior to mixing with fruit skin & pulp matrix. The above procedure was repeated using 0.5g promalt as described above and 5ml of 0.5% potassium phosphate was added to yeast hydrant. This was then repeated using 1.0g promalt and 0.5% potassium phosphate, then 1.5g promalt and 0.5% potassium phosphate respectively. 220g of glucose was diluted in 1L of water. This was measured equally and placed in three separate jars. The fermentation procedure describe above was repeated. Fig. 2.0. (a) shows the apparatus that was setup to conduct fermentation, whereas Fig. 2.0. (b) is the apparatus used for distillation. Fig. 3.0. shows the density meter used to determine Brix & % yield of ethanol.

Fig. 2.0 (a) above shows how apparatus that was set up to conduct fermentation, whereas Fig. 2.0 (b) is the apparatus used for distillation.

Figure 3.0 shows the Density Meter used to determine Brix and % ethanol.

3.0. Analytical assay

3.1.pH

Measuring pH before and after fermentation was done by placing the probe of a small amount of sample (before fermentation) and filtrate (after fermentation). The brand of the pH meter used was OAKLAND Waterproof Data Meter pH 310 Series.

3.2. Acidity

3.3. Preparation of stock solution:

Sodium hydroxide stock solution was prepared by diluting 12g NaOH pellet in 1L water.

3.4. Standardization:

25 ml aliquot of the stock solution was placed in a conical flask and a few drops of drops of phenolphthalein were placed in aliquot. This was then titrated against a 0.2N hydrochloric acid solution until a pink colour change was observed. The concentration of the stock solution was determined by calculation and was confirmed to be a 0.2N NaOH

3.5. Determining concentration of acetic acid of filtrate:

The 0.2N NaOH was placed in a burette. Using a 25ml volumetric flask, 25ml of filtrate from each jar was measured out and poured into three separate 100ml beaker. The initial pH was checked, and then the initial volume in burette was also written down. 25ml aliquot was titrated against 0.2N NaOH drop-wise and by vigorously swirling the mixture with the probe of the pH meter being kept in the beaker, this was continued until the pH reached 6.2. The final reading on the burette was subtracted from the initial and this value was multiplied by a factor of 8. The answer was recorded as the concentration of acetic acid in each filtrate (the lower the pH the, more acidic the sample).

3.6. ^{0}Brix

The brand of the instrument used was a DMA 5000 Anton Paar Density meter. The initial and final amount of total soluble solid was measured before and after fermentation and was referred to as ^{0}Brix. The value that is obtained for degrees Brix is a good approximation to the true value for total soluble solids. This was achieved by drawing 2ml of the liquid sample into a 2ml syringe (samples were drawn carefully to avoid introducing air bubbles in syringe). With the density meter setting placed as Brix. The sample was carefully injected into the sample collecting tube (samples were carefully injected to avoid introducing air bubbles into the sample tube). Keeping syringe in, the start button was then pressed and the reading was printed on the screen. After that, the syringe was removed and the contents in tube was flushed on into a waste jar using distilled water and air pressure.

3.7. Distillation

100 ml volumetric flask was rinsed with filtrate. The volumetric flask was carefully filled with the filtrate slightly over the mark. This was placed in a water bath with thermometer in flask. The content in flask was attemperated to 20^{0}C(temperature at which volumetric flask was calibrated). If extra was needed to be removed or topped up, a pasteur pipette was used. This content was quickly transferred to a 250ml round bottom flask. The volumetric flask was then rinsed with 50ml distilled water to prevent flask from boiling dry. Boiling chip was added to flask, and then connected to the distillation assembly, ensuring that all ground glass joints were secure. Cold water was constantly being flowed through the condenser. The 100ml volumetric flask was immered in a cold water bath and placed under the distillation apparatus as per figure. The adaptor tubing was placed properly into flask. Heat was applied to the round bottom flask end of the distillation set up which contained filtrate and water, boiling commenced after a few minutes. When the distillation commenced, the condensed distillate was let to accumulate to about 80-100 ml in collecting volumetric flask. The boiling ceased and the flask was removed after this was achieved. The content in flask was then attemperated to 20^{0}C. Then was stoppered and placed in an ultrasonic shaker(Mettler Toledo)

3.8. Determining alcohol percentage:

Using a DMA 5000 Anton Paar Density meter, 2ml of the liquid sample was drawn into a 2ml syringe (sample of the distillate were drawn carefully to avoid introducing air bubbles in syringe). With the density meter setting, placed as OIML V/V. The sample was carefully injected into the sample collecting tube (samples were carefully injected to avoid introducing air bubbles into the sample tube), keeping the syringe in, the start button was then pressed and the reading was printed on the screen. After that, the syringe was removed and the contents in tube was flushed on into a waste jar using distilled water and air pressure.

3.9. Gas chromatogram analyses

Another way to confirm the amount of ethanol in distillate is by the use of a gas chromatogram. However, the method used was not able to quantify the ethanol peaks on the gas chromatogram, but was able to quantify the presence of other forms of alcohol that was in a smaller concentration compared to ethanol and was present because distillate was not 100% pure ethanol. The method used was the External Standard method for determining amount of various forms of alcohol isomers found in distillate. Distillates were analyzed on Gas chromatogram (Agilent Technologies). Distillate samples were placed in glass vials and labeled properly. The corresponding information was inputted on the monitor then the samples were run. The gas chromatogram was later printed on screen, the area under the curve was calculated automatically and the amount of each compound present (except ethanol) was quantified and results printed on screen. Analysis of ethanol and butanol was conducted using SRI GC model 8610C, equipped with a 60 m column (Restec MXT-1, Id 0.53 mm, 5 µM), on-column injector and FID conditions: 250°C; H2, 25 PSI, equivalent to 25 ml/min; air, 2 PSI, equivalent to 100 ml/min; gain set to 'medium'. The GC was also equipped with an internal air compressor and hydrogen generator. N_2 was used as carrier gas with pressure control (24 PSI constant; equivalent to 27 ml/min). The GC was connected to a computer running Peak Simple software version 2.8. Oven temperature (and hence column and injector temperature) was initially set at 50°C and then elevated at the rate of 7°C/min to 100°C, thus giving a total run time of 7 min. Furthermore, 2 µL samples were

injected manually at time 0, using a 5 µl Hamilton syringe and temperature cycle was started. Syringe was thoroughly washed with ethyl acetate between injections to avoid cross-contamination. Each injection was repeated three times, ethanol routinely came out at retention time equivalent to 65°C.

Another method proposed under the title "Analysis of volatile aroma constituents of wine produced from Indian mango (*Mangifera indica L*) by GC-MS is as follows[37]:

The analysis of volatile compounds was carried out by a Hewlett-Packard series 6890, gas chromatograph linked to an HP-5973 mass-selective detector equipped with fused silica capillary column. The flow rate of carrier gas helium was 1 ml/min. The injection volume was 1 µl. The injection temperature was programmed from 60°C for 2 min and then raised to 250°C at 4°C/min, held for 20 min. Injector temperature was maintained at 250°C. Mass spectra (MS) were acquired in the electronic impact (EI) and positive chemical ionization (PCI) modes. The transfer line temperature was 250°C. MS were scanned at 70 ev electron impact mass spectrometry (EIMS) and 230 eV positive chemical ionization mass spectrometry (PCIMS) in the range m/z 29–350 atomic mass unit (amu) 1–s intervals. A sample of 100 ml of wine was adjusted to pH 7, by the addition of NaOH, and 1 ml of 4-methyl-2-pentanol (at a concentration of 10 mg/l) was added as an internal standard. The sample was extracted three times with diethyl ether. The sample was reduced to 1 ml by evaporating the ether solvent in a rotary evaporator at 40°C with low pressure. An aliquot (1 µl) of sample was injected into GC-MS. The identification of the volatile compound was confirmen (MS Chemstation Wiley 7N library) or with their retention times of standards. The analysis was carried out in triplicate

3.10. Standard solutions and calibration curves

Standard solutions of n-butanol were prepared in ethyl acetate (EtOAc) and injected without further treatment. Standard solutions of ethanol (EtOH) were prepared in distilled water containing 1% v/v of n-butanol as an internal standard, extracted and injected. Peak area ratios of the ethanol vs. n-butanol were calculated and plotted against ethanol concentration (% v/v) to afford a calibration curve which served for ethanol quantification in the fermentation samples.

4.0. Results

Table 1.0. shows some physical characteristics of the fruits before and after fermentation

Local (Scientific name)	Initial Degrees Brix (0.05Bx)	Final Degrees Brix (0.05 Bx)	Initial pH of solution	Final pH of Filtrate	Average concentration of acetic acid (± 0.1g/ml)
Jamun (*Syzigium cumini*)	14	2.625	3.20	3.03	170
Papaya (*Carica papaya*)	11.3	1.237	4.81	4.06	65
Soursop (*Annona Muricata*)	16.55	1.433	4.20	3.74	70
Glucose	16.63	0.00	6.67	3.11	55

Table 2.0. Shows the (mean % ethanol ± SD) for the different substrates with the different composition of metal salts

Fruit Type	Mean % v/v Ethanol ± SD , v/v	Mean % Ethanol ± SD with additive ($ZnSO_4$), v/v			Mean % Ethanol ± SD with additive (K_3PO_4), v/v			Mean % v/v Ethanol ± SD with additive (Promalt) and 0.5% K_3PO_4		
		0.1%	0.5%	1%	0.1%	0.5%	1%	0.1%	0.5%	1%
Soursop	4.100 ± 0.245	4.650±0.101	4.520± 0.08	6.740± 0.01	4.780±0.091	8.690± 0.095	7.690± 0.055	14.163±0.017	9.870±0.05	7.430± 0.026
Jamun	4.640 ± 0	4.970± 0.02	5.220±0.1	5.220± 0.355	5.340± 0.16	5.740± 0.065	5.630± 0.081	6.570± 0.098	7.530± 0.026	6.950± 0.05
Papaya	4.650 ± 0.255	5.220±0.173	5.260± 0.036	4.780± 0.043	5.400± 0.021	5.700± 0.1	4.900± 0.173	4.930± 0.435	5.710± 0.494	5.470± 0.13
Glucose	9.480	8.870	9.120	8.940	9.360	9.180	9.120	8.800	8.590	9.030

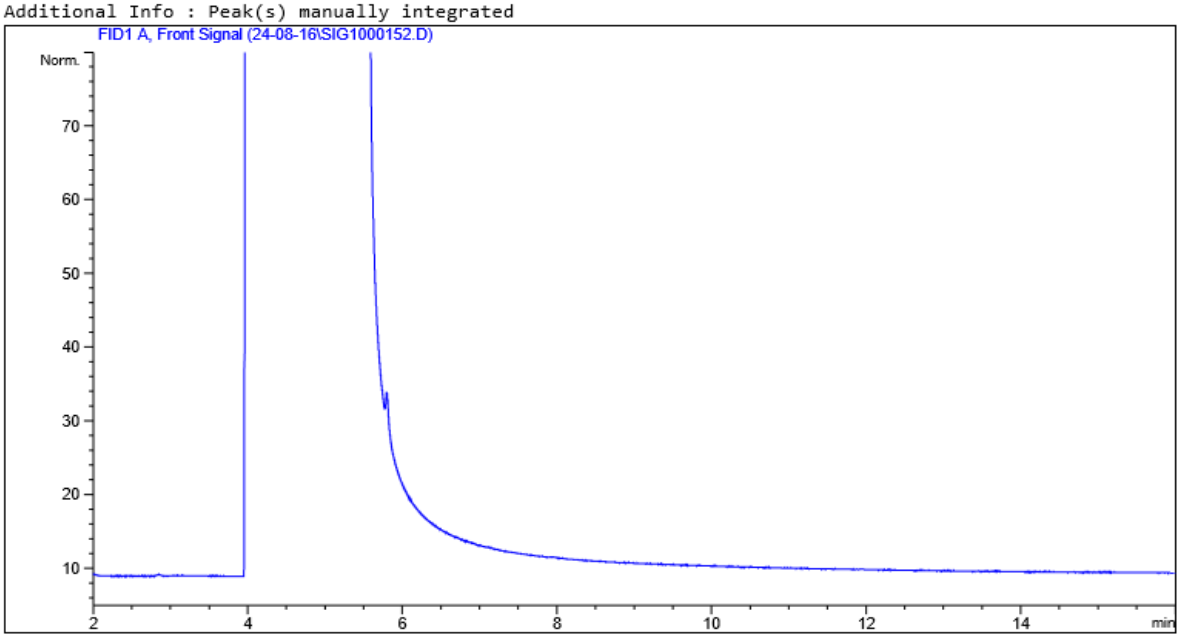
Table 3.0. Below is the List of Standards that were present to analyze the sample injected

Ret timeReRe	Area (pA*s)	Amt/Area	Amount (g/100LAA)	Name of compound
2.851	9.27157	$2.16792e^{-1}$	2.01000	Acetaldehyde
3.074	9.38672	$2.14132e_{-1}$	2.01000	Methanol
-	-	-	-	Ethanol
4.916	11.80068	$1.70329e^{-1}$	2.01000	Methyl Acetate
7.151	15.16423	$1.32549e^{-1}$	2.01000	1-propanol
7.911	13.18315	$1.52467e^{-1}$	2.01000	Ethyl Acetate
8.186	16.82237	$1.19484e^{-1}$	2.01000	2 Butanol
9.081	18.99220	$1.16958e^{-1}$	2.01000	Iso butyl alcohol
10.055	35.05545	$1.16958e^{-1}$	2.01000	1 Butanol
11.834	19.08633	$1.05311e^{-1}$	2.01000	Iso Amyl Alcohol
11.907	21.34000	$9.41893e^{-1}$	2.01000	2 Methyl-1 butanol
12.565	18.55479	$1.08328e^{-1}$	2.01000	1 Pentanol
14.389	12.41335	$1.61922e^{-1}$	2.01000	Furfural
2.851	9.27157	$2.16792e^{-1}$	2.01000	Acetaldehyde

Gas Chromatograph 1.0. shows the profile of absolute ethanol at a 99.99% ethanol (Reference sample)

Sample Info : Actual Strength >>>>99.99 % v/v

Additional Info : Peak(s) manually integrated



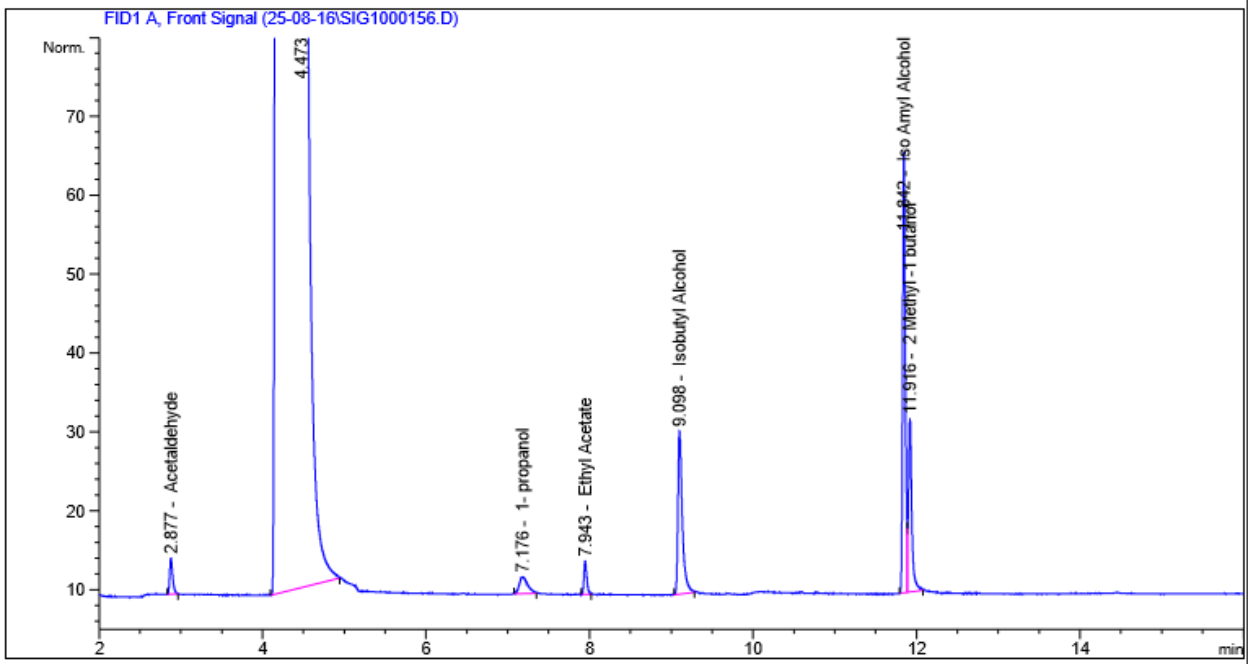
Signal 1: FID1 A, Front Signal

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [g/100LAA]	Grp	Name
2.857		-	-	-		Acetaldehyde
3.068		-	-	-		Methanol
5.492		-	-	-		Methyl Acetate
7.187		-	-	-		1- propanol
7.940		-	-	-		Ethyl Acetate
8.211		-	-	-		2 butanol
9.091		-	-	-		Isobutyl Alcohol
10.061		-	-	-		1 Butanol
11.843		-	-	-		Iso Amyl Alcohol
11.914		-	-	-		2 Methyl -1 butanol
12.574		-	-	-		1 Pentanol
14.396		-	-	-		Furfural

Totals : 0.00000

Gas chromatograph 2.0. below shows the profile of fermented glucose distillate at a 9.030% v/v ethanol

Sample Info : Actual Strength>>>>9.030



Signal 1: FID1 A, Front Signal

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [g/100LAA]	Grp	Name
2.877	BB	11.60114	2.36035e-1	30.32417		Acetaldehyde
3.068		-	-	-		Methanol

trument 1 8/25/2016 2:32:21 PM Rennard

a File C:\CHEM32\1\DATA\25-08-16\SIG1000156.D
ole Name: Glucose, K3PO4 & Promalt (1.5g)

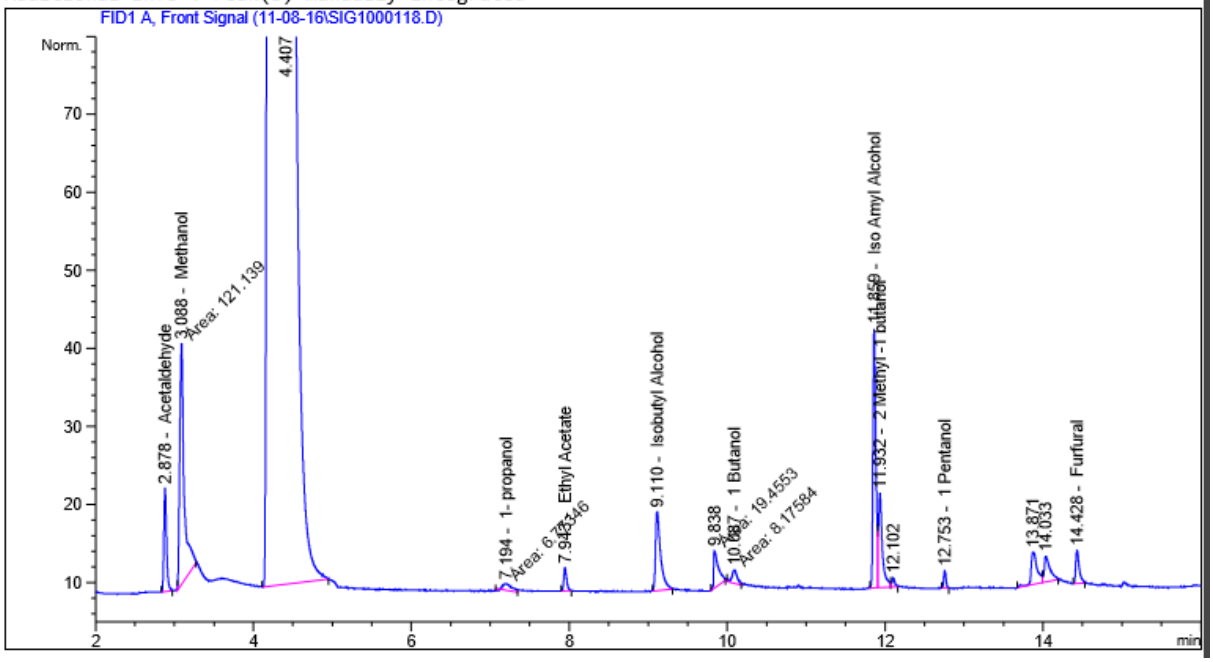
RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [g/100LAA]	Grp	Name
5.492		-	-	-		Methyl Acetate
7.176	BB	14.78742	1.28334e-1	21.01589		1- propanol
7.943	BB	10.18747	2.00610e-1	22.63248		Ethyl Acetate
8.211		-	-	-		2 butanol
9.098	BB	76.84828	1.08521e-1	92.35494		Isobutyl Alcohol
10.061		-	-	-		1 Butanol
11.842	BV	130.68486	1.11727e-1	161.69509		Iso Amyl Alcohol
11.916	VB	62.01458	9.55935e-2	65.65001		2 Methyl -1 butanol
12.574		-	-	-		1 Pentanol
14.396		-	-	-		Furfural

Totals : 393.67259

Gas chromatograph 3.0 below shows the profile of Soursop*(Annona Muricata)* distillate at a (14.163% v/v ethanol)

Sample Info : Fermented using Promalt & K3PO4
Actual Strength @ 14.163% v/v

Additional Info : Peak(s) manually integrated

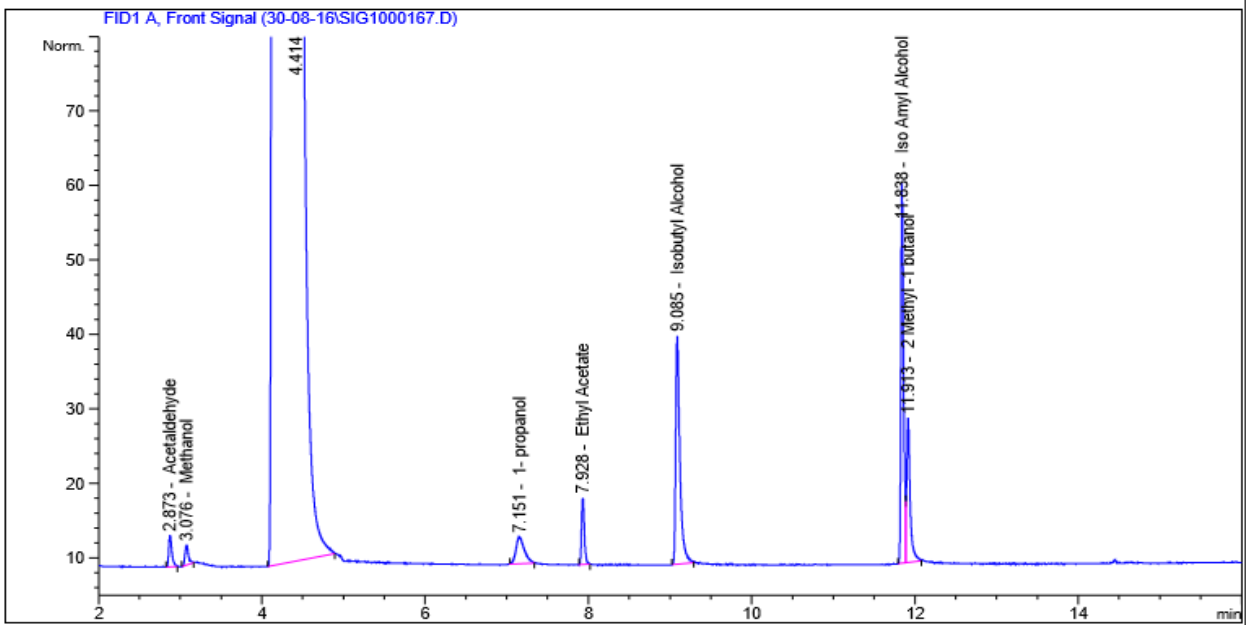


Signal 1: FID1 A, Front Signal

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [g/100LAA]	Grp	Name
2.878	BB	30.80594	1.83376e-1	39.88617		Acetaldehyde
3.088	MM	121.13864	4.07782e-1	348.78311		Methanol
5.505		-	-	-		Methyl Acetate
7.194	MM	6.77346	1.90227e-1	9.09763		1- propanol
7.943	BB	7.58150	3.08540e-1	16.51624		Ethyl Acetate
8.222		-	-	-		2 butanol
9.110	BB	44.92321	1.72741e-1	54.79113		Isobutyl Alcohol
10.087	MM	8.17584	1.98188e-1	11.44073		1 Butanol
11.859	BV	85.52952	1.65365e-1	99.86300		Iso Amyl Alcohol
11.932	VV	41.22959	1.52718e-1	44.45743		2 Methyl -1 butanol
12.753	BB	4.32826	2.18705e-1	6.68370		1 Pentanol
14.428	BB	13.01132	1.30563e-1	11.99464		Furfural
Totals :				643.51377		

Gas chromatograph 4.0 below shows the profile of jamun *(Syzigium Cumini)* distillate at a 7.53%v/v ethanol

Sample Info : Actual Strength>>>7.533%



Signal 1: FID1 A, Front Signal

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [g/100LAA]	Grp	Name
2.873	BB	11.07269	2.36035e-1	34.69452		Acetaldehyde
3.076	BB	7.68654	3.20765e-1	32.73031		Methanol

:rument 1 8/30/2016 2:40:23 PM Rennard

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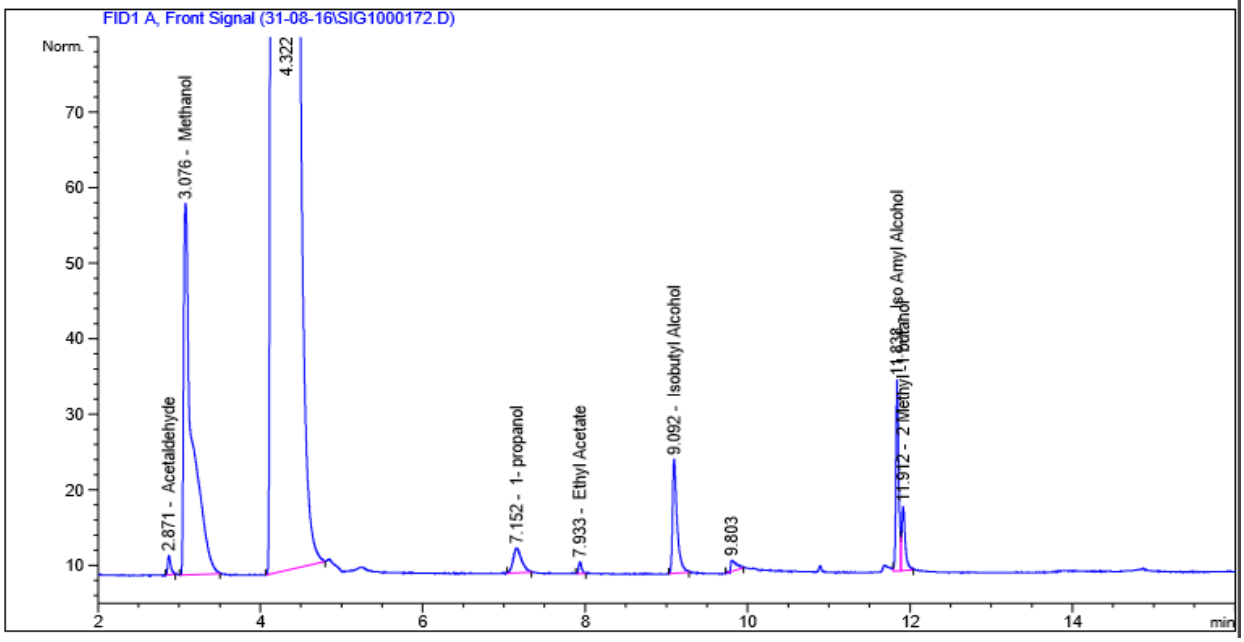
le Name: Jamun,Promalt & K3PO4

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [g/100LAA]	Grp	Name
5.492		-	-	-		Methyl Acetate
7.151	BB	25.69700	1.28334e-1	43.77814		1- propanol
7.928	BB	21.45957	2.00610e-1	57.14871		Ethyl Acetate
8.211		-	-	-		2 butanol
9.085	BB	110.01751	1.08521e-1	158.49199		Isobutyl Alcohol
10.061		-	-	-		1 Butanol
11.838	BV	118.15273	1.11727e-1	175.24066		Iso Amyl Alcohol
11.913	VB	57.46461	9.55935e-2	72.92241		2 Methyl -1 butanol
12.574		-	-	-		1 Pentanol
14.396		-	-	-		Furfural
Totals :				575.00674		

Gas chromatograph 5.0 below shows the profile obtained for of papaya *(Carica Papaya)* distillate at a 5.360%v/v ethanol

Sample Info : Actual Strength>>>5.360 %



Signal 1: FID1 A, Front Signal

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [g/100LAA]	Grp	Name
2.871	BB	6.70889	2.36035e-1	29.54351		Acetaldehyde
3.076	BB	355.81830	3.20765e-1	2129.36989		Methanol

rument 1 8/31/2016 11:54:59 AM Rennard

File C:\CHEM32\1\DATA\31-08-16\SIG1000172.D
le Name: PawPaw, Promalt & K3PO4 (2)

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [g/100LAA]	Grp	Name
5.492		-	-	-		Methyl Acetate
7.152	BB	23.82680	1.28334e-1	57.04848		1- propanol
7.933	BB	4.06040	2.00610e-1	15.19698		Ethyl Acetate
8.211		-	-	-		2 butanol
9.092	BB	58.43101	1.08521e-1	118.30206		Isobutyl Alcohol
10.061		-	-	-		1 Butanol
11.838	BV	62.37073	1.11727e-1	130.00957		Iso Amyl Alcohol
11.912	VB	25.41054	9.55935e-2	45.31874		2 Methyl -1 butanol
12.574		-	-	-		1 Pentanol
14.396		-	-	-		Furfural

Totals : 2524.78922

Gas chromatograph 6.0. below shows the profile of Soursop (*Annona muricata*) and Potassium phosphate distillate at 8.494% ethanol.

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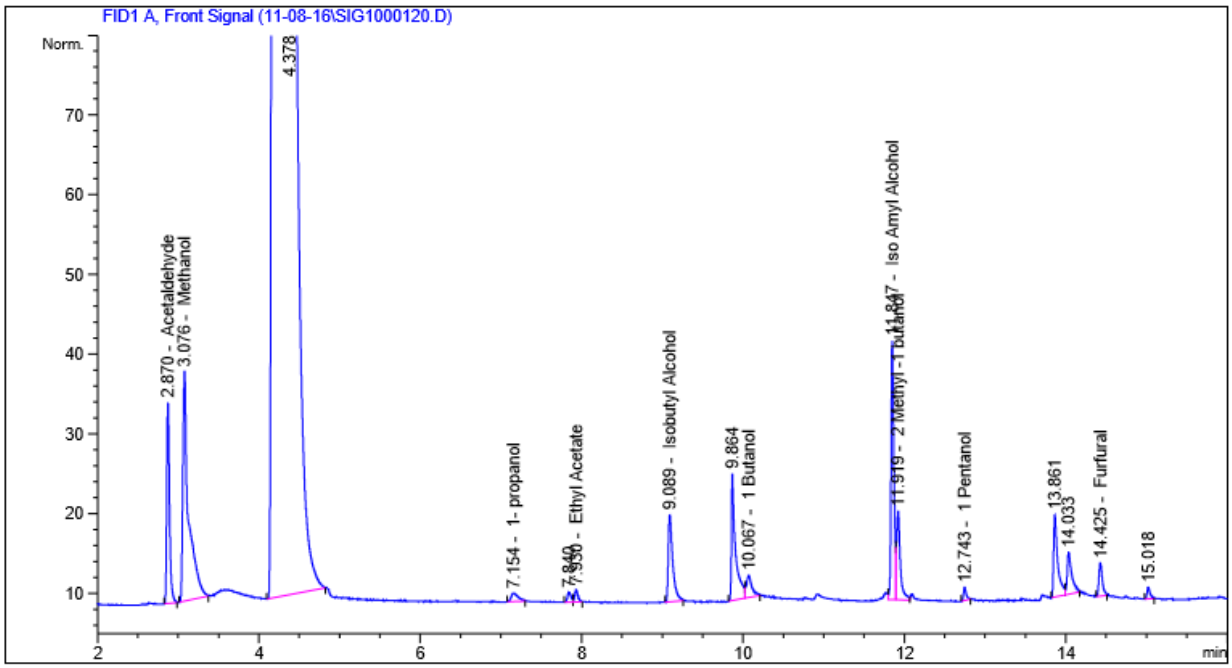
Sample Info     : Fermented using K3PO4
                  Actual Strength @ 8.494% v/v
```



Signal 1: FID1 A, Front Signal

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [g/100LAA]	Grp	Name
2.870	BB	60.89134	1.83376e-1	131.45785		Acetaldehyde
3.076	BB	142.98489	4.07782e-1	686.44547		Methanol
5.505		-	-	-		Methyl Acetate
7.154	BB	6.16340	1.90227e-1	13.80323		1- propanol
7.930	VB	4.10394	3.08540e-1	14.90732		Ethyl Acetate
8.222		-	-	-		2 butanol
9.089	BB	41.38349	1.72741e-1	84.16078		Isobutyl Alcohol
10.067	VB	13.32025	1.98188e-1	31.07969		1 Butanol
11.847	VV	81.71901	1.65365e-1	159.09437		Iso Amyl Alcohol
11.919	VB	35.01500	1.52718e-1	62.95535		2 Methyl -1 butanol
12.743	BB	4.11880	2.18705e-1	10.60516		1 Pentanol
14.425	BB	12.39461	1.30563e-1	19.05206		Furfural
Totals :				1213.56128		

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6.0. Statistical analysis:

Each experiment was repeated thrice and results are expressed as means ± standard deviations. The data obtained were analyzed by Anova test[1] in Microsoft excel 2010 by using single factor and two factors with replication of variance. It was carried out to test for any significant differences between the means values at a 95% confidence level. Null Hypothesis for the test: there is no significant difference in the % volume of ethanol produced. If p-value is < 0.05 there is significance difference between the values. Also, if F value calculated is < than F critical, there is no significance difference between the values.

Table 4.0. Shows a summary of the results obtained from Anova: Single factor test to compare % ethanol obtained for all fruit substrates without additive

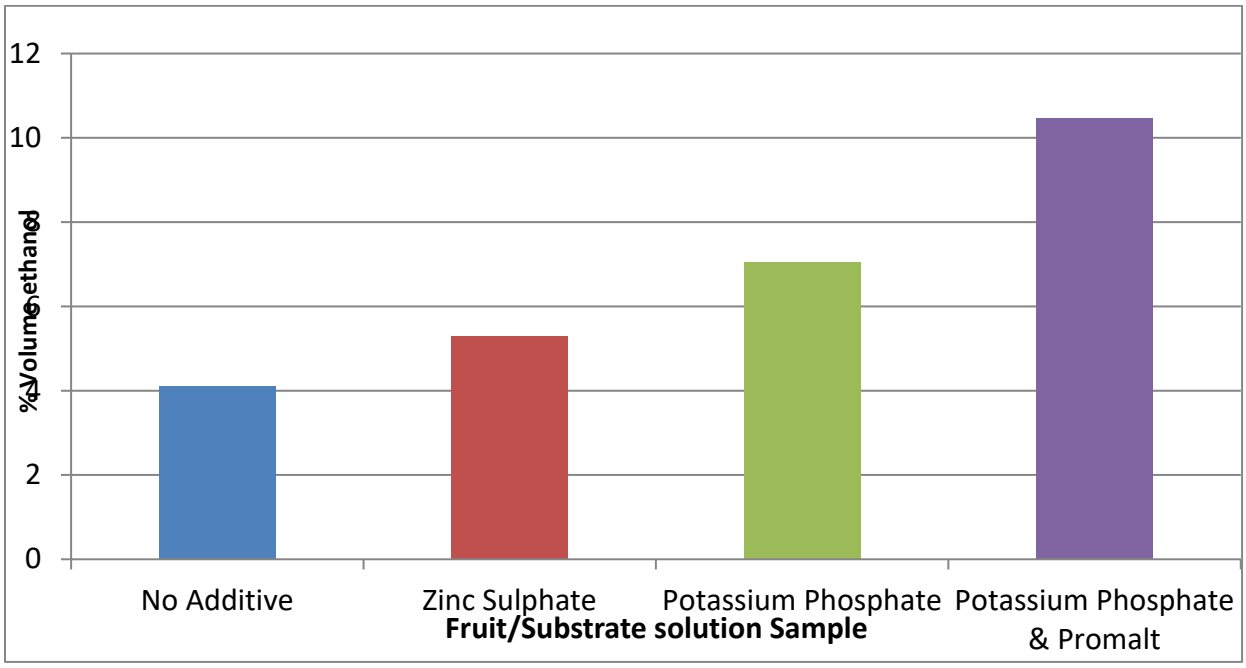
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F critical*
Between Groups	0.590689	2	0.295344	7.073177	0.026416	5.143253
Within Groups	0.250533	6	0.041756			
Total	0.841222	8				

Table 5.0. Shows a summary of the results obtained from Anova: Two factors with replication test to compare % ethanol for all fruit substrates with selected additive

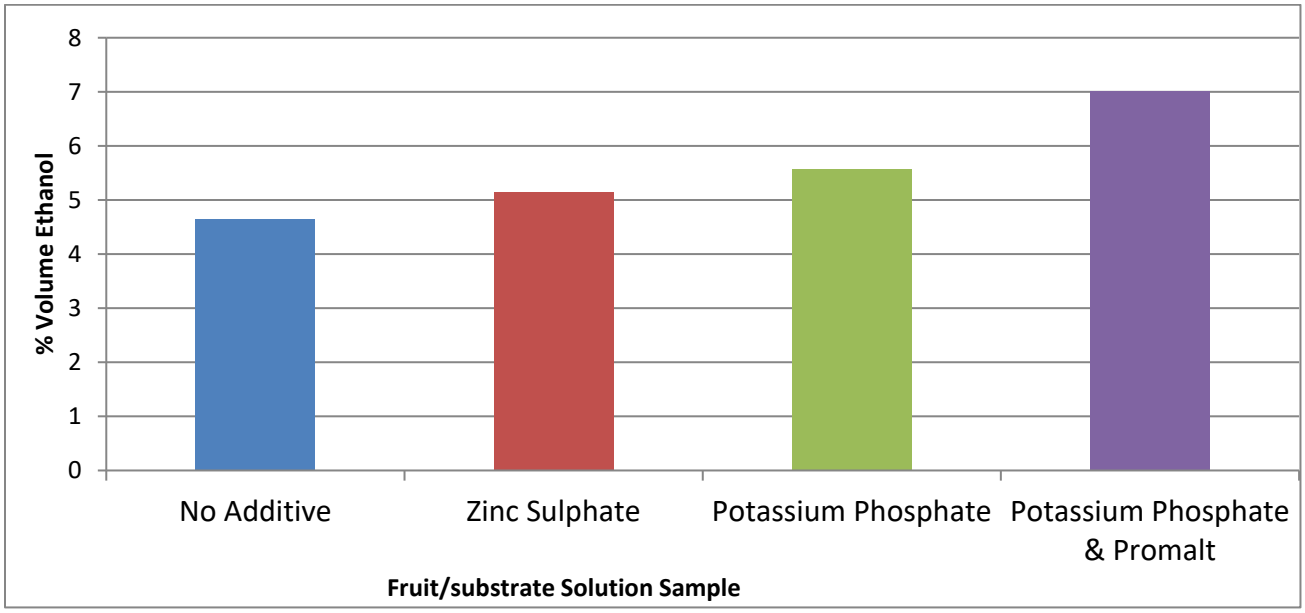
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	79.95382963	2	39.97691481	1374.535232	4.88105×10^{-47}	3.168245967
Columns	112.3597111	8	14.04496389	482.9111448	2.26522×10^{-47}	2.115223279
Interaction	138.9890815	16	8.686817593	298.6807985	9.97375×10^{-47}	1.834629446
Within	1.570533333	54	0.029083951			
Total	332.8731556	80				

7.0. Graphical Analysis

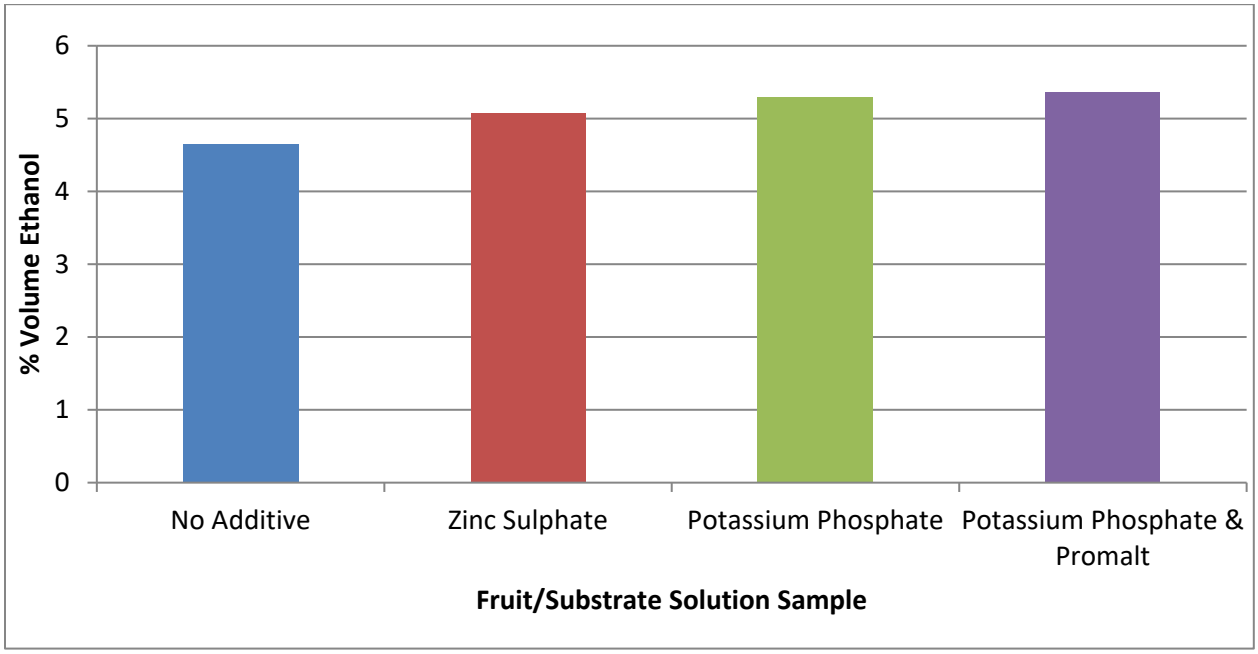
Bar graph 1.0 below showing average % v/v of ethanol that was obtained for Soursop*(Annona Muricata)* using selected additives



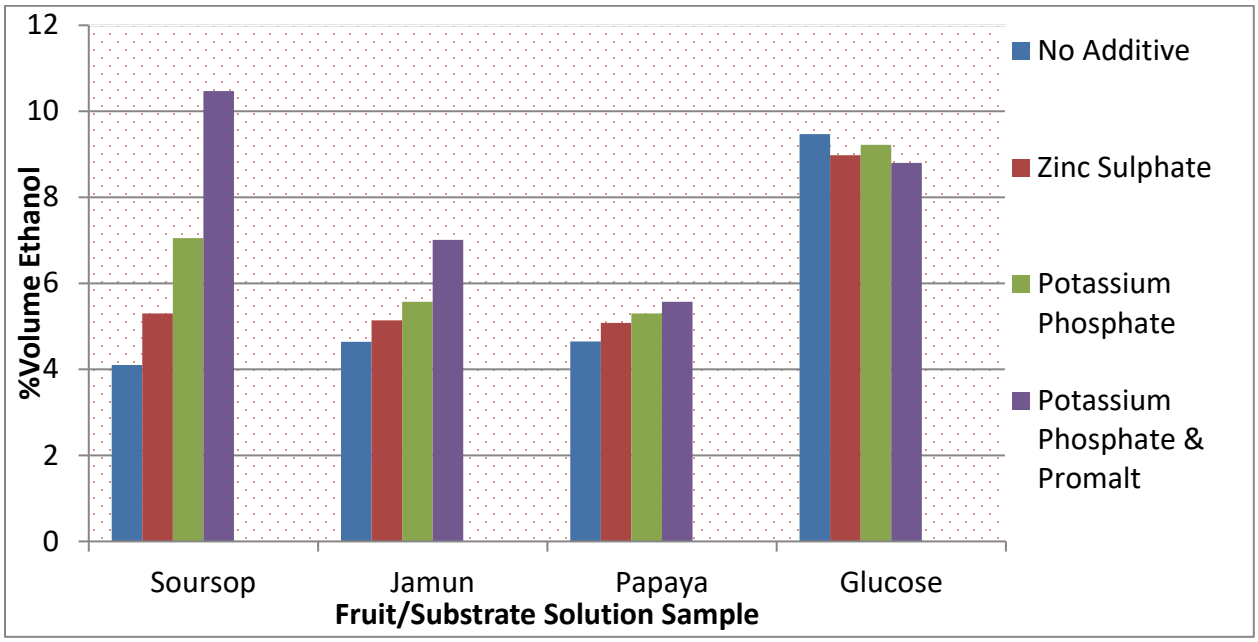
Bar graph 2.0 below showing average % v/v of ethanol that was obtained for Jamun *(Syzigium Cumini)* using selected additives



Bar graph 3.0 below Showing average % v/v of ethanol that was obtained for Papaya*(Carica Papaya)* using selected additives



Bar graph 4.0 below showing the average % volume of ethanol that was obtained for the selected fruits and reference glucose, with and without additives.



8.0. Discussion

The main objective of this research was to produce ethanol and to see what effects the variables in metal salts such as $ZnSO_4$, K_3PO_4 and promalt with 0.5% K_3PO_4 will have on the mean percentage yield of ethanol. The initial ^{0}Brix (initial total soluble solids), is a measurement of the amount of sugar/sucrose that are available for fermentation. Of the three fruits, soursop displayed the highest initial ^{0}Brix value (16.55), followed by jamun (14.0) and the least was papaya (11.3). Hence, its was anticipated that soursop produced the highest yield of ethanol, followed by jamun and papaya. As fermentation proceeds, the Brix content is expected to decrease. As an indication of complete and efficient fermentation, the Final Brix value is expected to be zero. From Table 1.0, it was noticeable that the final brix value was not zero. The final Brix value range from (1.237 to 2.625), indicating that some more hours were necessary for complete fermentation. For soursop, the initial brix was 16.55 and the final brix was 1.433. For the reference compound, glucose, it was noticeable that the final brix was zero, indicating that fermentation has proceeded to completion and was highly efficient. In addition, Fehling's reducing sugar test indicates that there was still some fermentable sugar left after the prescribed fermentation period. Hence, some more time was necessary for fermentation.

Table 2.0. shows the mean % ethanol ± SD for the different fruit substrates in the absence and presence of additives. The mean ethanol content v/v) in the absence of additives range from (4.100 ±0.245 v/v) to (4.650 ±0.255 v/v) ethanol. The highest percentage yield of (4.65 ±0.00, v/v) was produced by fermented papaya. Thus, the mean ethanolic content decrease in the order: papaya > jamun > soursop. All the additives produce an increase in the mean % of ethanol, v/v to that without. This can be discussed with reference to each fruit. However, for additives, increasing the percentage concentration of the additive, didn't always increase the mean % of ethanol yield, v/v).

Some showed an increase at the 0.5% and then a decrease at 1%, whereas others showed a decrease at 0.5% and then an increase at 1%. For example, consider soursop, as a typical example, with $ZnSO_4$ as an additive, there was an increase in ethanolic content at 0.1% i.e from (4.100 ± 0.245 v/v to 4.65 ± 0.10, v/v). At the 0.5% concentration of $ZnSO_4$, this decrease to 4.520 ±0.08, v/v) and then increase to 6.74 ±0.01, v/v) at the 1% concentration. For the K_3PO_4 additive, there was an increase at the 0.1% concentration, (4.78 ±0.091, v/v). There was also a further increase of value to (8.690 ±0.0095, v/v) at the 0.5% concentration. However, at the 1% value, there was a decrease to (7.690 ±0.055, v/v). Likewise for jamun, there was an increase in ethanolic content from (4.64 ± 0.00, v/v to 5.340 ± 0.11, v/v) at 0.1 % level with K_3PO_4. At the 0.5% concentration, the value increase to (5.740 ± 0.065, v/v) and then decrease to (5.630 ± 0.081, v/v) at the 1% level. For the soursop with promalt additive, and 0.5% K_3PO_4, the mean ethanolic content increase from (4.100 ± 0.245, v/v to 14.163 ±0.017 , v/v) at the 0.1% level, then to (9.87 ±0.05, v/v) at 0.5% level and then decrease further to (7.430 ± 0.026, v/v) at the 1% level.

The additive increased the ethanolic content to varying percentage and this is dependent on the fruit type. Comparing the 0.1 % addition, the highest increase was seen for soursop with promalt's addition and 0.5% K_3PO_4 (14.163 ± 0.017, v/v) and the lowest at the 0.1% addition of $ZnSO_4$ on soursop (4.650 ± 0.101, v/v). The lowest mean % of ethanol was registered with $ZnSO_4$ on papaya (4.780 ± 0.043, v/v) at the 1.0% level. At the 0.5% percentage, the highest percentage yield of ethanol of (9.870 ± 0.05, v/v) was produced by soursop with promalt additive and 0.5% K_3PO_4, whereas the lowest of (4.520 ± 0.08, v/v) was induced by soursop with 0.5% $ZnSO_4$. .

At the 1% concentration, the additive that produced the highest % yield of ethanol was K_3PO_4 on soursop (7.690 ±0.05, v/v) and the lowest was induced by papaya with $ZnSO_4$ at the same percentage. Bar graph 1.0, 2.0 and 3.0 shows the average % v/v of ethanol that was obtained for

soursop (*Annona muricata*), Jamun (*Syzigium cumini*) and papaya (*Carica papaya*) with and without additives. Bar graph 4.0 shows the average % volume of ethanol that was obtained for the selected fruits and reference glucose, with and without additives.

Other than the production of lactic acid (which is due to the fact that fermentation is an anaerobic process), acetic acid is also produced if filtrate is exposed to oxygen, Acetobacter bacteria will convert some of the ethanol into acetic acid. This process is known as the acetification and can be determined by titration, in which 25ml of each filtrate was titrated against a 0.2N sodium hydroxide to determine how much acetic acid was produced. From Table 1.0, fermented jamun produced the highest acidity (168-170 (g/ml) followed by soursop (68-70g/ml), then papaya (64-65g/ml) and lastly glucose (55- 56g/ml). Accordingly, at normal fermentation, the range of acetic acid is around 300-400mgs/L. Yeasts need low pH to replicate, but higher acidity that the normal range can inhibit chain elongation which affect DNA replication

As much as ethanol is a major end product of fermentation, there are also other by products produced such as CO_2, other alcohol isomers, various forms of acids etc. The jar used to ferment each substrate was modeled in such a way as to create an anaerobic environment and also to let the excess carbon dioxide produced to be released. The presence of carbon dioxide inhibits fermentation[31] and it was important to have this carbon dioxide released, creating a more fermentable environment. As the yeast goes through its growth stages, it has to then compete with the production of ethanol, and scarcity of nutrients. Thus, a step was taken further to analyze the distillates on a gas chromatogram by the use of External method, the problem with this method is that the ethanol peak could not have been quantified, but this was the best available option with limited time at hand. Due to the fact that the filtrates were distilled using simple distillation it meant that the distillate would not have been pure ethanol because during fermentation process

other side reactions also take place resulting in the production of other alcohol isomers. To confirm that ethanol was in the samples, absolute ethanol was analyzed on the GC and was used as a reference to superimpose the retention time at which ethanol peak comes off, and that large peak came off at a retention time from 4 to 6 minutes because its purity being 99.99% pure absolute ethanol. The profile came back as null when all of the other external standards were compared. Keeping this in mind all the other distillates were analyzed and at a similar retention time, the largest peak came off that was no able to be quantified based on the calibration of the instrument. This large peak was concluded to be the ethanol peak. There were other forms of isomer that were able to be quantified and the area under each of these peaks was integrated by the use of a calculation factor that is done automatically by the Gas chromatogram. This maintained for each gas chromatograph profile produced.

Glucose, which was used as a fermentation reference, was analyzed on gas chromatogram. At a 9.030%(v/v) ethanol as determined by the density meter, the profile produced by this run shows that a large peak came off at 4.473 retention time and this peak was not quantified was concluded to be the ethanol peak. Also, traces of acetaldehyde, 1-propanol, Ethyl Acetate, Isobutyl Alcohol, Amyl Alcohol, and 2 Methyl-1-butanol and the retention times were 2.877, 7.176, 7.943, 9.098, 11.842, and 11.916 minutes respectively. Second to ethanol was iso amyl alcohol (161.69509g/100L of absolute alcohol) and the least abundance form of isomer in this sample was that of 1-propanol(21.01589 g/L of absolute alcohol). The total volume of the isomers quantified by the gas chromatogram was 393.67259g/100L of absolute ethanol. The GC profile for the soursop distillate (14.16%v/v ethanol) , showed a that a large peak came off at 4.407(min) retention time and this peak was not quantified and was concluded to be the ethanol peak. Also were traces of acetaldehyde, methanol ,1-propanol, Ethyl Acetate, Isobutyl Alcohol, an unidentified isomer,

1-butanol Amyl Alcohol, and 2 Methyl-1-butanol, 1-pentanol and furfural and the retention times were 2.876, 3.088, 7.194, 7.94, 9.110, 10.087, 11,859, 11.932, 12.753, 14.428 minutes respectively. Methanol was 348.78311g/100L of absolute alcohol in this sample was more seen to be the most abundant isomer other than ethanol, secondly iso amyl alcohol which was 99.86300g/100L of absolute ethanol. And the total volume of isomers quantified by the gas chromatogram was 643.51377g/100L of absolute ethanol. For jamun distillate(7.53%v/v ethanol), there was a large peak at retention time 4.414 which was concluded as the ethanol peak. Also there were traces of acetaldehyde, 1-propanol, ethyl acetate, isobutyl alcohol, iso amyl alcohol and methyl-1 butanol, the corresponding retention times were 2.873, 3.076, 7.151, 7.928, 9.085, 11.838, 11.913 minutes respectively. In this sample iso amy alcohol(175.24066g/100L of absolute alcohol) was the second most abundant isomer present and the least abundant was that of methanol(32.73031g/100L of absolute alcohol). The total volume isomers quantified by the gas chromatogram was 575.00674g/100L of absolute alcohol.

For papaya distillate, the ethanol peak came off at 4.322(min) retention time, with a strength of 5.360 %, v/v). Acetaldehyde, methanol, 1-propanol, ethyl acetate, isobutyl, and unidentified isomer, iso amyl alcohol, and 2- methyl-1-butanol came off at the retention time at 2.871, 3.076, 7.152, 7.933, 9.092, 9.803, 11.838, 11.912 minutes respectively. The second largest amount of alcohol in this sample was methanol and was quantified as 2129.36989 g/100L of absolutes alcohol, secondly was iso amyl alcohol (130.00957g/100L of absolute alcohol) and the least was ethyl acetate which was quantified as 15.19698g/100L of absolute alcohol that was present in the sample. The total volume of isomers as quantified by the gas chromatogram was 2524.78922g/100L of absolute alcohol.

Gas chromatograph 6.0 shows the profile of the distillate from soursop, with potassium phosphate additive. There was a large peak at retention time, 4.378, 8.494% ethanol, which was concluded to be the ethanol peak. Also, there were traces of acetaldehyde, methanol, 1-propanol, ethylacetate, 2-butanol, isobutyl alcohol, 1-butanol, iso-amyl alchol, 2-methyl-1-butanol, 1-pentanol and 1-furfural. These came off at retention time of 2.870, 3.076, 7.154, 7.930, 8.222, 9.089, 10.067, 11.847, 11.919, 12.743 and 14.43 respectively. The total volume of isomers as quantified by the gas chromatogram was 1213.56128.

When comparing the amount of isomers other than ethanol that were analyzed on the gas chromatograph, it is seen that indeed the distillates were not only comprised of ethanol but other quantified isomers and followed the sequence in terms of percentage abundance: **papaya > jamun >soursop > glucose** and is due to the fact that the fruits fermented had other compounds present, which facilitated other side reactions, whereas glucose was merely glucose, but was able to have had other side reactions taking place also. Side products of fermentation such as glycerol, methanol, higher alcohols (fusel oil), succinic acid, volatile acids, and lactic acid acetaldehyde and hydrogen sulphides may be formed as a result of other side reactions. Methanol is a side product of demethylation of pectins by enzymatic activities.

Based on this, the Anova single factor, using Microsoft excel 2010, Table 4.0., test were done to compare % ethanol for all fruit substrates, without additive and it was seen that the p-value was 0.026416 which is less than 0.05 and also the F value (7.073) was greater than the F-critical value (5.143), and the conclusion was drawn that indeed there was significant differences with the percentage per volume of ethanol produced by the different fruit substrates and this was so because the initial Brix for each fruit varied, and this value is one of the main determinant of the percentage ethanol production. Also when this analysis was done with Anova, two factors with

replication test on Microsoft excel 2010, to compare % ethanol for all fruit substrates with selected additive, it was observed that p-values for the samples (4.88×10^{-47}), the columns (2.265×10^{-47}) and, their interactions (9.97×10^{-47}), were all less than 0.05, and also the F values were larger than the F-critical values. Based on these, the conclusion was drawn that indeed there was significant differences with the percentage per volume of ethanol produced by the different fruit substrates with different additives.

7.0. Conclusion

The fermentation of sugar rich fruits: jamun (*syzigium cumini*), soursop (*Annona muricata*), and *Carica papaya* in the absence and presence of additives was achieved under anaerobic condition at a pH of 4-5. In the absence of additives, ethanol production decrease in the order: **Papaya > Jamun > Soursop,** with papaya producing an ethanolic content of (4.650 ± 0.255, v/v**)**. The additive did increased the ethanolic content to varying percentage and this is dependent on the fruit type. The ethanolic content from the fruit type range from: 4.520 ± 0.08, v/v to 14.163 ± 0.017, v/v). Gas chromatographic analyses were also done on the distillate, from the fermented matrix, without and with additives. It was found that the distillate in most cases consists of ethanol, acetaldehyde, methanol, methylacetate, 1-propanol, ethylacetate, 1-butanol, isobutylalcohol, iso-amyl alcohol, 2-methyl-1-butanol, 1-pentanol and furfural in most cases. Our research shows that all of the selected fruits can be used as attractive substrates for the production of ethanol and hence its cultivation should be encouraged as a boost to the Agro Sector of the country and also, a source for the blending with gasoline to produce gas alcohol. However, future work is necessary to intensify the yield of ethanol beyond the 25% recorded in the literature[34].

9.0 References

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