The fermentation capacity of the pulp of *Magifera indica*, *Carica papaya* and the peel of *Magnifera indica* in the absence and presence of additives. The highest % yield of ethanol, furnished by a fruit, *Carica papaya*.

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Abstract

The pulp of *Carica papaya* (papaya), *Magnifera indica* (mango) and the peel of *Magnifera indica* as substrates were fermented for ethanol using *Sacchaomyces cervisiae*. Experiments were carried out in triplicates in the presence and absence of additives (potassium phosphate monobasic) at specific concentration and conditions to study the effects on the yield of ethylalcohol It was found that *Magnifera indica* (mango) in the presence of additives produced the highest mean yield of ethanol of 25.16%. *Magnifera indica* (mango) without additives produced the second highest with an average of 20.56%, while *Carica papaya* (papaya) without additives having the third highest average yield of 7.537% and *Carica papaya* (papaya) with the additives present, having the least average ethanol content of 6.69%. The peel of *Magnifera indica* (mango) in the absence of additives produced an average ethanol content of 1.33% and the reference (glucose), without additives produced an average ethanol content of 11.3%. The mean ethanol content for *Magnifera indica* with additives, is the highest value reported for any fruit to date and exceed the 15% reported in the literature.

Keywords: *Carica papaya, Magnifera indica, fermentation, Sacchaomy cescervisiae*, potassium phosphate

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1.0.Introduction

With, a view to decrease dependence on fossil fuel, as a result of depletion, increasing global fuel price, increasing population and increasing global warming, there has been increased interest in the use of alternative renewable energy sources of which bioethanol is one [1,2,3]. 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[4]. 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 through Fermentation has been one of the world most significant approaches to aid in the Advancement of Commercial Industry.

Ethanol doesn't have significant environmental impact as fossil fuel combustion [3]. It has low air polluting effect and low atmospheric photochemical reactivity, further reducing impact on the ozone layer[6]. It contributes little net CO_2 accumulation to the atmosphere and thus should curb global warming [1-2], [6-10].

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]

Guyana likewise, 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. This ethanol came from a Bio-ethanol E-10 plant at the Albion Estate. The plant is capable of producing fuel blends with 5%, 10%, 15%, 20% and 25% ethanol. The plant is focused on mixing gasoline with ethanol at 10% to produce E-10 blend that is compatible with vehicles in Guyana such as the Toyota Corolla[11].

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, [4,6-29]. Also, acid hydrolysis of lignocellulose material followed by subsequent fermentation [3,6, 21] . Sugar rich sources include ripe fruits [9-29] etc. Other sources include biodegradable fraction of products, waste and residues from agriculture like vegetables and animal origin [9-12, 13-29] etc. The percentage yield of ethanol, ranging from 4.0 -10.0 v/v) have been reported [9-12, 13-29]. Fruits that are high in sugar concentration are favourable to the fermentation process, since they can produce high percentage volume of ethanol [22]

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 was documented 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 [24]. Ethanol fermentation is anaerobic pathway carried out by yeast in which simple sugars are converted to ethanol and carbon dioxide [9,12, 13-29]

There are several reports of fruits and fruit peel used as substrates for fermentation. A few can be cited. The production of ethanol from *Carcia papaya* (pawpaw) agricultural waste, using dried active baker's yeast strain , *Sacchromyces cerevisiae* was investigated. The fermented pawpaw fruit waste produced ethanol contents 2.82-6.60% (v/ v). The rate of alcohol production via fermentation of pawpaw fruit waste by baker's 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 [9-29]

The production, optimization and characterization of wine from papaya using *Saccharomyces cerevisiae* has been noted in the literature. At the optimum conditions, the predicted value of ethanol production was found to be 11- 12% [24]. The possibility of producing wine from *Carica* *papaya* using simple, cheap, and adaptable technology with biochemically characterized yeast strains was investigated [25]. After fermentation for one month, a mean ethanol content of 11.59% alcohol was noted. Ethanol production, via the fermentation of the pulp of " Boko" mangoes were also investigated [26.]

8.5-10% (w/v) of ethanol was obtained from the fermentations of mango juices without adding any nutrients [27]. The mango peel contained good amount of reducing sugars up to 40% (w/v). Direct fermentation of mango peel extract using *Saccharomyces cerevisiae* gave only 5.13% (w/v) of ethanol. [28].

A study was carried out to ascertain the behaviour and fermentation performance of mixed yeasts in mango juices of three varieties [29]: *Saccharomyces cerevisiae*, MERIT ferm and *Williopsis saturnus var. mrakii* NCYC500 at a ratio of 1:1000 were simultaneously inoculated into juices of three mango (*Mangifera indica L.*) varieties (R2E2, Harum Manis and Nam Doc Mai). Nam Doc Mai wine possessed the highest aroma intensity with winey, yeasty, fruity and floral notes attributed to higher amounts of alcohols, acetate esters and ethyl esters. These findings may help develop different styles of mango wine.

This paper, thus reports the fermentation capacity of the pulp of *Magifera indica* and *Carica papaya* and the peel of *Magifera indica* in the absence and presence of additives, with a view to produce ethanol for commercial use and to increase the ethanol content beyond 15% as reported in the literature [9-12, 13-29].

2.0 Procedure

(a) Fermentation of the pulp of *Carica papaya and Magifera indica* in the absence and presence of additives.

Fully ripe samples of papaya and mango were purchased from a vendor at a market. The fruit were washed with distilled water, dried, weighed, the seed removed and cut into smaller pieces and then added to the fermentation glass jar. Experiments were done in triplicates. The volume of the fruit was determined with help of graduating measuring cylinder. The fruit was introduced in a cylinder filled three quarter of the way with water. The difference of volume before and after total immersion of the fruit corresponds to the volume of the fruit (V_f). Specific gravity of the fruits was calculated as the ratio between the mass of the fruits (M_f) and its volume (V_f). The inoculated Yeast, *S. Cerevisiae* (6 grams), wine strain, capable of existing up to a temperature of 35-45 degree Celsius in the presence of 30% sugar and 18% of ethyl alcohol was to the medium. In other experiments, additives such as specific salts/salt, amino acids/urea, vitamins at specific concentration was added to the medium to study their effects on the yield of ethylalcohol. The requisite common pH 4.5 was maintained using citric acid. The fermentation process was monitored at specific intervals. At the end of 72 hours, the contents of the mixture were filtered and the filtrate distilled using a vigreux column. The composition of the filtrate was tested for the presentation yield of ethanol using a pictometer and HPLC at BANKS DIH Inc, a brewery in Guyana. In addition to the above a control and reference experiment was conducted to validate our research results. All results collected were expressed as mean values.

(b) Fermentation to *Magnifera indica* peel

Mango peel (400g) was added in triplicates to sample jars. These were then subjected to acid hydrolysis for 24 hours to remove lignin which is an hindrance to the fermentation process. *S.Cervesiae* was then added and the mixture fermented for 72 hrs after which the contents were filtered. The filtrate was distilled and the ethanol content of selected fractions determined.

(c) Preparation of 12% yeast solution

44 ml of deionized water was added to a 100ml beaker. 6g of dry yeast was added and left to incubate during 90 minutes at 27 degree Celsius to achieve a solution of 12% yeast.

3.0 Results

TABLE 1.0 FERMENTATION OF PAPAYA WITHOUT ADDITIVES

Tests	Jar 1	Jar 2	Jar 3	Average
Weight of fruit (g)	500g	500g	500g	500g
Volume of H_2O displaced by fruit (v)	360ml	380	380	373.33
Density of fruit (g/ml)	1.38	1.32	1.32	1.34
Ph				
Initial	4.55	4.46	4.56	4.528
Final	4.23	4.16	4.31	4.233
Concentration of sugar brix (%)				
Initial	5.688	6.295	5.542	5.8416
Final	0.99	0.16	0.91	0.6866
Specific Gravity				
Initial	1.38888	1.31578	1.31578	1.340146
Final	0.99642	0.99687	0.99662	0.99672
Mean alcohol (% v/v)	5.12, 5.11, 5.13	10.2 , 10.2,10.2	7.29, 7.30,7.29	7.537
Temperature (ºC)	32	32	32	32
Mean alcohol % per gram of fruit, v/v				0.015

TABLE 2.0 SHOWING FERMENTATION OF PAPAYA WITH ADDITIVE

Tests	Jar 1	Jar 2	Jar 3	Average
Weight of fruit (g)	500g	500g	500g	500g
Volume of H_2O displaced by fruit (ml)	280	300	260	280
Density of fruit (g/ml)	1.79	1.67	1.92	1.79
		Ph		
Initial	5.45	4.93	4.75	5.04
Final	4.08	4.17	4.10	4.11
		Concentration of sugar brix (%)		
Initial	7.543	5.672	5.998	6.4043
Final	1.7857	1.666	1.923	1.7915
		Specific Gravity		
Initial	1.7857	1.7857	1.92307	1.83149
Final	0.99687	1.02232	1.0022	1.00713
Mean alcohol (% v/v)	5.9, 5.8, 5.9	7.64, 7.65, 7.65	6.56, 6.56, 6.56	6.69
Temperature (ºC)	32.0	32.0	32.0	32.0
Mean alcohol % per gram of fruit, v/v				0.01338

TABLE 3.0. FERMENTATION OF MANGOES WITHOUT ADDITIVES

Tests	Jar 1	Jar 2	Jar 3	Average
Weight of fruit (g)	450g	450g	450g	450g
Volume of H_2O displaced by fruit (ml)	280	300	260	280
Density of fruit (g/ml)	1.61	1.5	1.73	1.61
		pH		
Initial	5.45	4.93	4.75	5.04
Final	4.08	4.17	4.10	4.12
Concentration of sugar, % brix				
Initial	7.23	6.78	6.392	6.8006
Final	0.3	0.5	0.6	0.46
Specific gravity				
Initial	1.607	1.5	1.7307	1.6125
Final	0.9987	0.99982	0.99642	0.9987
Mean alcohol (% v/v)	23.34, 23.35, 23.35	18.83, 18.83, 18.83	19.5, 19.6, 19.5	20.56
Temperature (°C)	32	32	32	32
Mean alcohol % per gram of fruit, v/v				0.0444

TABLE 4.0. FERMENTATION OF MANGOES WITH ADDITIVES

Tests	Jar 1	Jar 2	Jar 3	Average
Weight of fruit	450g	450g	450g	500g
Volume of H_2O displaced by fruit	380	360	340	360
Density of fruit (g/ml)	1.18421	1.25000	1.323529	1.252579
pH				
Initial	4.16	3.78	3.98	3.97
Final	4.23	4.16	4.31	4.23
Sugar Concentration, % brix				
Initial	7.6	7.8	8.0	7.8
Final	-10.0	0.3	0.4	-3.1
Specific Gravity				
Initial	1.18421	1.25000	1.323529	1.252579
Final	1.02968	0.99600	0.99542	1.00703
Mean Alcohol (% v/v)	22.67, 22.67, 22.66	25.6, 25.6, 25.6	27.2, 27.3, 27.2	25.16
Temperature (ºC)	32	32	32	32
Mean alcohol % per gram of fruit				0.05032

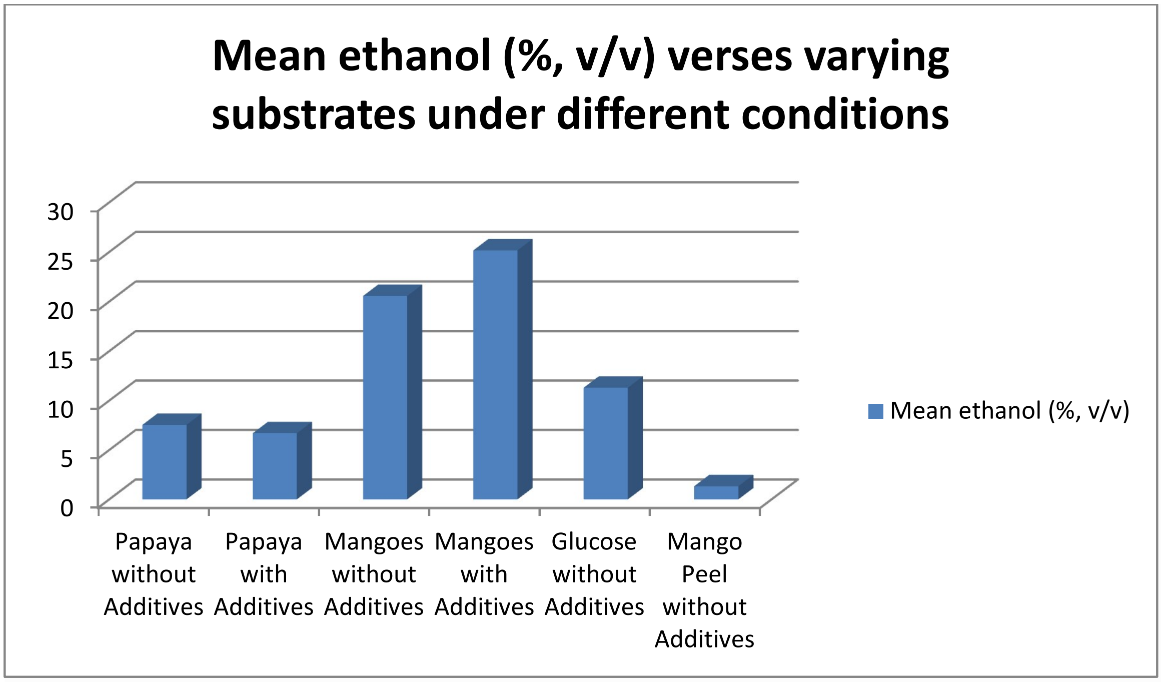
TABLE 5.0. SHOWING THE FERMENTATION OF GLUCOSE WITHOUT ADDITIVES

Tests	**Jar 1**	**Jar 2**	**Jar 3**	**Average**
Weight of fruit (g)	10g	10g	10g	10g
Volume of H_2O displaced by fruit (ml)	7.0	7.0	7.0	7.0
Density of fruit (g/ml)	0.7	0.7	0.7	0.7
pH				
Initial	2.37	4.68	2.21	3.086
Final	2.12	3.66	2.06	2.61
Sugar concentrations, % brix				
Initial	5.7	3.6	4.5	4.6
Final	1.4	0.3	1.0	0.9
Specific Gravity				
Initial	0.7	0.7	0.7	0.7
Final	0.00214	0.54321	0.0034	0.1829
Mean Alcohol (% V/V)	10.9, 10.9, 10.89	10.7, 10.6,10.7	12.3, 12.3, 12.3	11.3
Temperature (°C)	32	32	32	32
Mean alcohol % per gram of fruit				1.13

TABLE 6.0. SHOWING THE FERMENTATION OF MANGO PEEL WITHOUT ADDITIVES

Tests	Jar 1	Jar2	Jar 3	Average
Weight of fruit (g)	350g	350g	350g	350g
Volume of H_2O displaced by fruit (ml)	200 ml	200 ml	200 ml	200 ml
Density of fruit (g/ml)	1.75	1.75	1.75	1.75
pH				
Initial	1.06	0.98	0.96	1.0
Final	0.96	0.78	0.84	0.86
Sugar concentrations, % brix				
Initial	11.0	11.5	12.0	11.5
Final	9.8	10.0	9.5	9.76
Specific Gravity				
Initial	1.75	1.75	1.75	1.75
Final	1.029542	1.02968	1.02868	1.05126
Mean Alcohol (% v/v)	1.5, 1.4, 1.5	1.3, 1.3, 1.3	1.2, 1.2, 1.1	1.33
Temperature, C	32	32	32	32
Mean alcohol % per gram of fruit				3.8×10^{-3}

(a) A Plot of Mean Ethanol Content (%, v/v) Verses Varying Fermentable Substrates



(b) A plot of Initial Brix verses Final Brix against substrates type

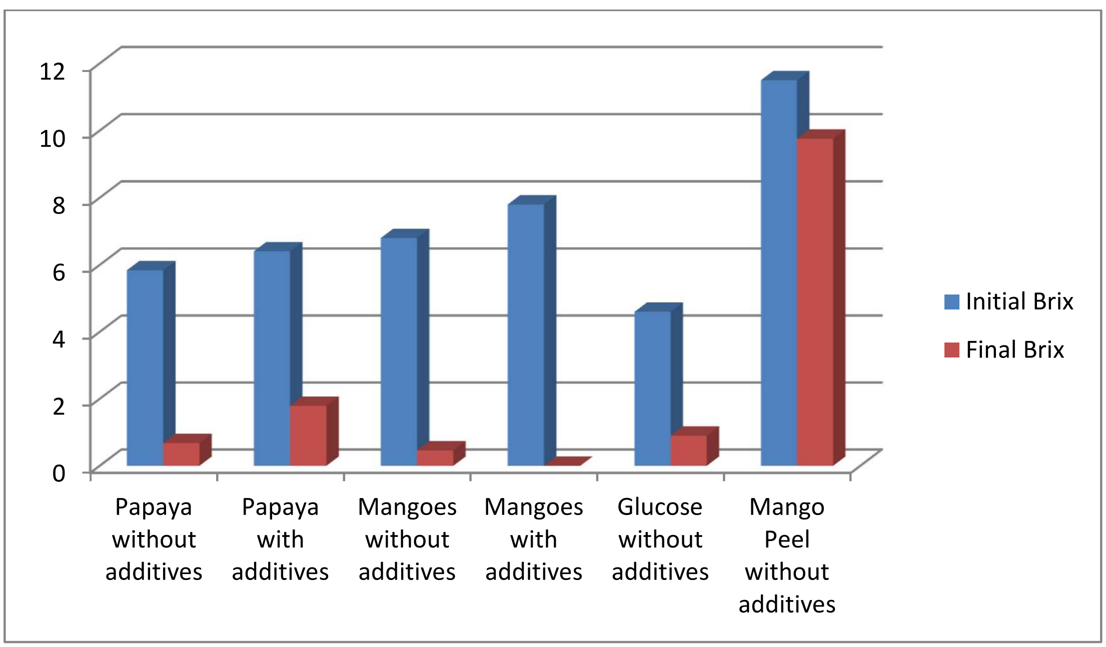


Table 7.0. shows the statistical analyses of the data

Data	Analysis
Papaya with and without additives: p value = 0.1140	p value = 0.1140 (> 0.05) .
Mango with and without additives:	p value = 0.0085 (< 0.05)
Mango and papaya without additives:	P value = 3.74×10^{-10} (< 0.05)
Mango and papaya with additives:	P value = 9.24×10^{-15} (< 0.05)

4.0 Discussion:

The results displayed some common trends. There is a general decrease in pH for all the fruits studied, with the exception of fermentation of mangoes with additive. This indicates that as fermentation proceeded, the fermentation matrix became more acidic as a result of the release of CO_2 which forms carbonic acid with the water. An optimum pH of 4.5 is necessary for the fermentation process. For example, for the fermentation of Papaya without additives, there is a decrease in the average pH from 4.523 to 4. 233. Likewise, the fermentation of mangoes without additives, illustrates a decrease in the average pH from 5.04 to 4.12.

Also, in all cases, there is a decrease in the specific gravity of the fruit. For example, the fermentation of papaya with additive, there is an average decrease in specific gravity from 1.83 to 1.00. In most cases, the concentration of sugar in % brix decrease significantly to less than 1.0, showing that fermentation was almost completed or the concentration of the fermentable sugar decreased significantly. For example, the fermentation of mangoes without additives, see an average decrease in % Brix from 6.8 to 0.46. Likewise, the fermentation of papaya without additive sees a decrease in the average sugar concentration, % average brix from 5.84 to 0.69. For the fermentation of papaya with additive and mango peel without additive, the final % brix was 1.7915 and 9.76 respectively, showing that fermentable sugar was available and that fermentation was incompleted. Thus, the mango peel required a longer period for fermentation to occur, probably greater than 72 hrs. A decrease in brix content is an indication that fermentation has occurred. Ideally, in a fermentation process, 0 % final Brix should be achieved.This indicates that for the above fermentation substrates system, longer period for fermentation is required.

The average ethanol content range from 1.33% to 25.16 % v/v. The latter value was obtained when mangoes, with additives were subjected to fermentation. The lowest value of 1.33 % v/v was obtained from the fermentation of mango peel without additives. For the fruits pulp, the lowest value of 6.69 % v/v was observed for the papaya with additive . Mangoes with additives showed the highest ethanol content with an average of 25.16 %, v/v. This can be explained by both the % Brix % and the additives. The additives used in this experiment are phosphate compounds. Phosphorus is found in the nuclei of cells where it forms phosphate groups which are crucial for linking the building blocks of DNA together. The additives were added to the mixture to enhance the growth and activity of yeast and act as a nutrient so that the condition can be favorable for *S.cervisease*. Hence the additives aid in the growth of the yeast *S.cervisease* DNA during fermentation which boosts the chances of obtaining a higher yields of ethanol. Mangoes without additives had the second highest ethanol content with an average of 20.56 %. The papaya fruit without additives showed the third highest ethanol content with an average of 7.537%. Thus, the order of decreasing ethanol content per fruit is: Mango with additive > Mangoes without additives > papaya without additives > papaya with additives > mango peel without additives. It seems that the additive was more effective on the mangoes than papaya as the ratio of fruits with additive/fruits without additives were 25.16/20.56 for mango and 6.69/7.537 for papaya. The ethanolic content for mango with and without additives is the highest recorded for any fruit in the literature 7-29.

Fermentation was also done on glucose without additives. It was found that the weight % yield of ethanol per gram of glucose was 1.13 % v/v. This was significantly higher than the fruits with and without additives. For example, mango pulp with additives gave a weight % yield of ethanol of per gram of fruit of 0.05 % (v/v), whereas mango pulp of fruit without additives gave a % yield of ethanol per gram of fruit of 0.0444, v/v).

The data were analysed using the single factor, ANOVA analyses and the results are presented in Table 7.0. It was found that when comparing papaya with and without additives, there was no significant difference in the ethanolic content with a p value of 0.1140 (> 0.05), whilst, when comparing mango with and without additives, it was found that there is a significant difference in the ethanolic content with a p value of 0.0085 ($p < 0.05$) [31-34]. Mango and papaya without and with additives were analysed and the p values are 3.74×10^{-10} and 9.24×10^{-15} respectively i.e < 0.05, indicating significance difference between the ethanolic content, produced via fermentation from both experimental conditions.

5.0. Conclusions

It was found that *Magnifera indica* (mango) in the presence of additives produced the highest mean yield of ethanol of 25.16%, *Magnifera indica* (mango) without additives having the second highest with an average of 20.56%, while *Carica papaya* (papaya) without additives having the third highest average yield of 7.537%, v/v and *Carica papaya* (papaya) with the additives present, having the least average ethanol content of 6.69%, v/v. The peel of *Magnifera indica* (mango) in the absence of additives produced an average ethanol content of 1.33% and the reference (glucose) produced an average ethanol content of 11.3%, v/v. The mean ethanol content for *Magnifera indica*, is the highest value reported for any fruit to date and exceed the 15% reported in the literature [8-29]. In developing countries such as Guyana, the production of organic waste such as the skin, rind, pulp and other materials in the fruit as well as other waste products is dramatically increasing. In 2013, Guyana opened its first biofuel ethanol plant. The one thousand (100) liter a day capacity demonstration plant aimed to find a cheaper source of energy for the country development and also decrease the buildup of organic waste in the environment. Hence the use of black strap molasses as a feed stock, which is essentially a waste stream from Guyana Sugar's Albion Sugar Factory [23]. This project will exceed the ethanol content achieved in the ethanol plant which was found to be %10 when using black trap molasses and it will do so by means of additives. In addition it will also reduce the accumulation of waste in the environment since the peels of the fruits will also be used.

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