THE ETHANOLIC CONTENT OF PAPAYA (*Carica papaya*) VERSES SAPODILLA (*Manilkara zapota)* VIA FERMENTATION

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Abstract

The fermentation of sapodilla (*Manilkara zapota)* and papaya (*Carica papaya*) was successfully achieved. The mean ethanol content of sapodilla (14.91, v,v) was found to be significantly higher than that of papaya (1.964, v/v). The former equals the highest yield of ethanol, recorded for a fruit to date. In both cases, the final brix was zero, indicating that fermentation has proceeded to completion within the 72 hrs. Also, there was a further decrease in the specific gravity of the fruit. The acidity of the fermenting matrix was found to increase as fermentation proceeded. Compared to the reference compound, glucose, the mean ethanol content of both fruits were lowered. Our research shows that the sapodilla fruit can be used as an attractive fruit substrate 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.

Keywords: Fermentation, sapodilla, papaya, mean ethanol content, specific gravity and Agro Sector.

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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 [1,2,3]. 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[6-9].

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, [4,6-28]. Also, acid hydrolysis of lignocellulose material followed by subsequent fermentation [8-28] . 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 [3-28]. Fruits that are high in sugar concentration are favourable to the fermentation process, since they can produce high percentage volume of ethanol [20]

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 by Zhang *et al.* 2012 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 [23]. Ethanol fermentation is anaerobic pathway carried out by yeast in which simple sugars are converted to ethanol and carbon dioxide [7-28]

This paper reports the fermentation of sapodilla (*Manilkara zapota*) 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 [22].

There are several reports of fruits and fruit peel used as substrates for fermentation. A few articles can be cited for the use of sapodilla pulp and peel as a fermenting matrix. Rotten fruits serve as potential feedstock for bioethanol production due to high sugar content and cost effective substrate. Results indicate that amongst five fruits rotten sapota [23] (Manilkara zapota) produced highest amount of bioethanol 9.40% on 5th day of incubation [23].

The production of Biodiesel from *Manilkara zapota* (chikoo or Sapodilla) seed oil and performance characteristics study on single cylinder CI engine has been reported [24]

The production of ethanol from *Carcia papaya* (pawpaw) agricultural wastes, 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 [25]

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% [26]. The possibility of producing wine from *Carica papaya* using simple, cheap, and adaptable technology with biochemically characterized yeast strains was investigated [27]. 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 [28.] This paper, thus reports the fermentation capacity of the pulp of *Sapodilla (Manikara zapota)* and *Carica papaya* and the peel of *Magifera indica* in the absence and presence of additives, with a view to increase the ethanol content beyond 15% reported in the literature.

2.0 Materials and Method:

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

Fully ripe samples of sapodilla (*Manikara zapota)* and papaya 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. In addition to the above a control and reference experiment was conducted to validate research results. All results collected were expressed as mean values.

(b) 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 SAPODILLA

Tests	Jar 1	Jar 2	Jar 3	Average
Weight of fruit (g)	500	500	500	500
Initial Volume of H_2O, V_i (ml)	360.0	360.0	360.0	360.0
Final Volume of Water, V_f (ml)	780.00	800.00	780.0	786.66
Volume of H_2O displaced by fruit (ml)	420	440	420	426.66

Density of fruit (g/ml)	1.19	1.14	1.19	1.17
Ph				
Initial	5.15	5.17	5.55	5.29
Final	3.86	4.50	4.06	4.14
Concentration of sugar brix (%)				
Initial	3.685	4.316	5.495	4.498
Final	0.00	0.00	0.00	0.00
Specific Gravity				
Initial	1.01441	1.01691	1.02163	1.01765
Final	0.98515	0.98204	0.96198	0.97639
Mean Alcohol (% v/v)	12.0,5.73,3.12	15.36, 10.62, 8.95	30.43,30.59, 17.35	14.90
Temperature (ºC)	20	20	20	20
Mean alcohol % per gram of fruit (ml)				0.0298

TABLE 2.0. FERMENTATION OF PAPAYA

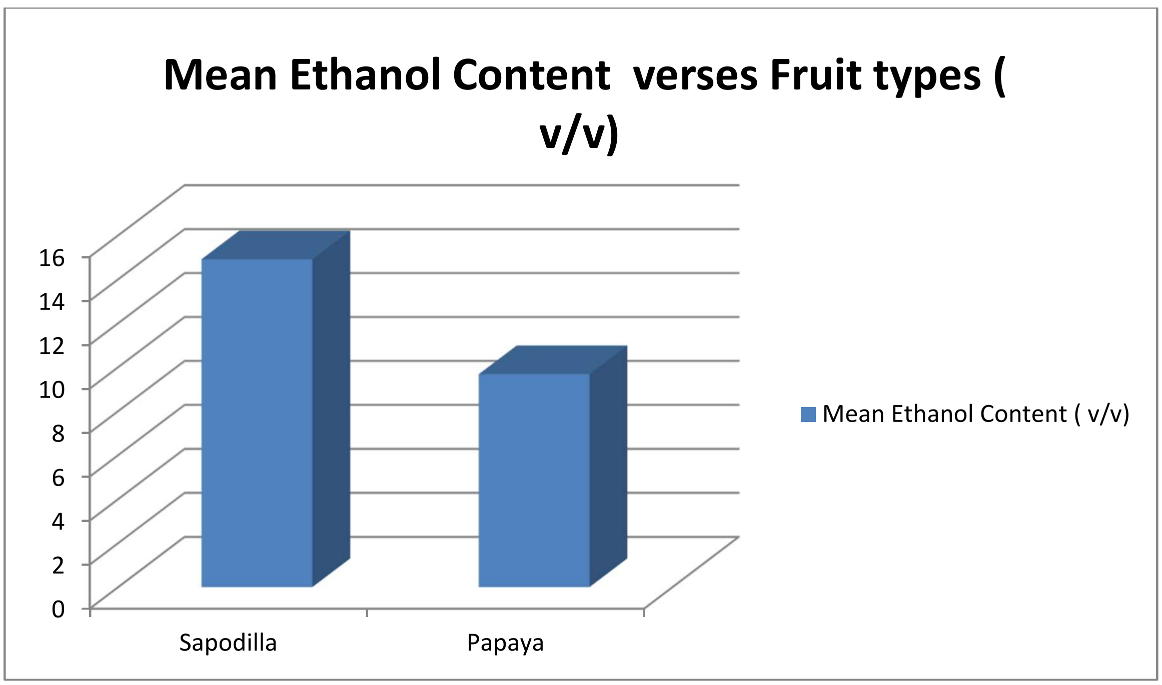
Tests	Jar 1	Jar 2	Jar 3	Average
Weight of fruit	500	500	500	500
Initial volume of H_2O, V_i	360	360	360	360
Final Volume of H_2O (ml), V_f	720	700	720	713.33
Volume of H_2O displaced by fruit (ml)	360ml	340ml	360	353.33

Density of Fruit (g/ml)	1.39	1.47	1.38	1.42
Ph				
Initial	5.45	4.93	4.75	5.04
Final	4.08	4.17	4.10	4.12
Concentration of sugar, % brix				
Initial	2.137	1.603	2.153	1.964
Final	0.00	0.00	0.00	0.00
Specific gravity				
Initial	1.00831	1.00441	1.00838	1.00703
Final	0.99882	0.99942	0.98124	0.99316
Mean alcohol (% v/v)	7.33, 5.43, 4.82	8.28, 7.38, 6.30	30.71, 10.16, 6.71	9.68
Temperature (ºC)	32	32	32	32
Mean alcohol % per gram of fruit (ml)				0.01936

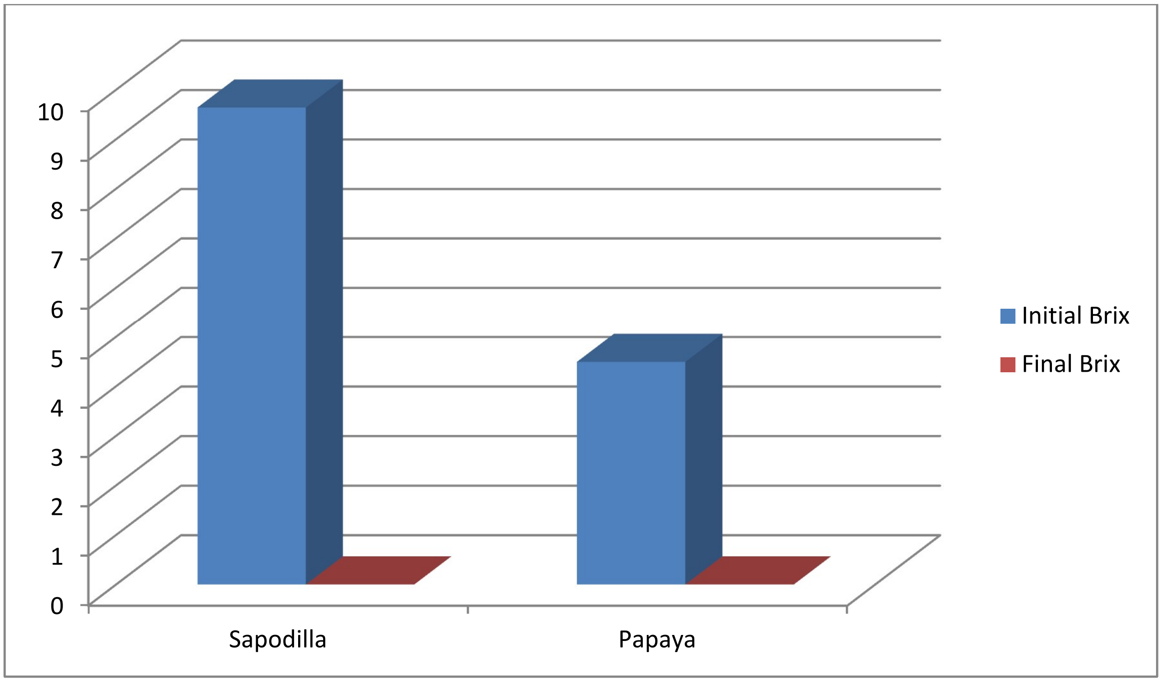
TABLE 3.0. SHOWING THE FERMENTATION OF GLUCOSE

Weight of Glucose	10g	10g	10g	10g
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 (ºC)	32	32	32	32
Mean alcohol % per gram of glucose (ml)				1.13



Graph 1.0. A plot of the mean ethanol content verses fruit types.



Graph 2: A Plot of initial verses Final Brix per fruit type

Discussion

The fermentation of sapodilla (*Manilkara zapota)* and papaya (*Carica papaya*) was successful. The fermentation of sapodilla yielded a mean ethanol content of 14.90%, v/v), whereas the fermentation of papaya yielded a mean ethanol content of 9.68% (v/v), Graph 1.0, Table 1.0 and Table 2.0. The mean ethanol content per gram of sapodilla and papaya was 0.029ml and 0.019 ml respectively. The mean ethanol content for sapodilla, 14.90 %, v/v) is comparable with the highest value, recorded in the literature [8-28].

It was noticeable for both fruits, there was a decrease in the pH as fermentation proceeded. This is due to the production of CO_2 which combines with H_2O to yield carbonic acid. For example, for the sapodilla, the average initial pH was 5.29, whereas the average final pH was 4.14. For the papaya, the average initial pH was 5.04 whereas the average final pH was

4.12. It was also noticeable that the Final brix in all both cases was zero. This indicates that all the fermentable sugar was fermented within the 72 hrs period. For example, for the sapodilla, the initial average Sugar Brix was 4.498, whereas the final Brix was zero. Likewise, the initial final brix for papaya was 1.964, whereas the final brix was zero. The initial Final brix of Sapodilla was higher than that of papaya, indicating that sapodilla has a higher fermentable sugar content, Graph 2.0. Literature do revealed that sapodilla has a higher carbohydrates content (19.96g/100g of fruit) compared to papaya (8% gram per 100g of fruit) [29]. There was also a decrease in the specific gravity for both fruits. For example, for sapodilla, the Initial average Specific gravity was 1.01765, whereas the Final average Brix was 0.97639. For the Papaya, the Initial Final Average Specific gravity was 1.0073, whereas the final is 0.9931. A decrease in specific gravity is due to a decrease in fermentable mass as fermentation proceeded, Table 1.0 and Table 2.0. The density of the sapodilla and papaya used was found to be 1.17 g/ml and 1.42g/ml respectively.

The fermentation of glucose was also studied as the reference compound. The results also indicate that there was also a decrease in the Brix content, showing that fermentation has occurred. However, the average final brix is not zero (0.9), showing that fermentation wasn't completed within the 72 hrs period. There was also a decrease in the specific gravity of glucose from an average value of 0.7 to 0.1829. This again is due to a decrease in fermentable sugar. The mean ethanol content was found to be 11.3 %, v/v) and the mean ethanol content per gram of glucose was 1.13, significantly higher than that of either fruits, sapodilla (0.0298g/ml) and papaya (0.01936, v/v), Table 3.0, Graph 1.0 and Graph 2.0.

Anova analyses [30-33] with two factor replication, indicates a p value of 0.24 ($P > 0.05$), indicating that there is no statistical significance difference in the ethanol content of both fruits.

Since sapodilla produce a relatively high ethanol content comparable to other fruits in the literature[8-28], it should thus be an attractive fruit to cultivate in the Agro sector of the country for the production of ethanol for commercial uses.

Conclusion

The fermentation of sapodilla and papaya was successfully achieved. The mean ethanol content of sapodilla (14.91, v,v) was found to be higher than that of papaya (1.964, v/v). The former equals the highest yield of ethanol, recorded for a fruit. In both cases, the final brix was zero, indicating that fermentation has proceeded to the maximum. The acidity of the fermenting matrix was found to increase as fermentation proceeded. Compared to the reference compound, glucose, the mean ethanol content of both fruits were lowered. Our research shows that the sapodilla fruit can be used as an attractive fruit substrate for the production of ethanol and hence a boost to the Agro Sector of the country and also as a source for the blending with gasoline to produce gas alcohol of E-10 blend that is compatible with vechicles in Guyana.

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