Studies on the Production of Bio-ethanol from Brown Guinea Corn (*Sorghum bicolor l.*), Pearl Millet (*Penisetum typhoides*) and Sweet Potato (*Ipomea batatas*) using Modified Method

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Abstract- This study determined mean volume distillate, percentage purity and specific gravity of bio-ethanol produced by the modification of the methods of Benue Brewery Limited (BBL), Makurdi and that of Mathewson using guinea corn, pearl millet and sweet potato as feedstocks. The modified reaction pathway yielded bio-ethanol of significantly ($P < 0.01$) higher mean volume distillate and percentage purity. Pearl millet feedstock yielded the highest mean volume distillate (98.00 cm^3) while guinea corn produced the highest mean volume distillate (92.17cm^3) in the unmodified BBL method. Sweet potato feedstock yielded the lowest mean volume distillate of 79.00 cm^3 and 56.17cm^3 in the modified reaction pathway and BBL method respectively. Pearl millet bio-ethanol also recorded the highest percentage purity (91.08%) by modified route and 88.64% by BBL method. Specific gravity measurements of the bio-ethanol showed that BBL method produced higher values with all the feedstocks. The highest value (0.8582) was recorded with guinea corn bio-ethanol produced while the least value (0.8268) was recorded with pearl millet bio-ethanol produced by the modified reaction pathway. This finding suggests that addition of hitempase (α-amylase) at onset and end gelatinization temperatures of starch improves the completeness of fermentation process with corresponding increase in volume distillate and percentage purity of bio-ethanol.

Keywords- Bio-ethanol, Feedstocks, Fermentation, Hitempase, Reaction pathway.

1. Introduction

The dwindling global oil reserves, increasing cost of production of petroleum products leading to price increase in many developing countries and the adverse effects of oil spillage and emission of obnoxious gases from fossil fuels have made it imperative to explore alternative energy sources. Bio-fuels; bio-ethanol, bio-diesel and biogas obtained from agricultural products (bio-mass) are increasingly becoming alternative sources of energy globally because they are replenishable and have environmentally friendly potential [1]. It has been reported that the global demand for liquid bio-fuels was more than triple between 2000 and 2007 and that future targets and investment plans suggest strong growth in bio-fuels demands in the near future [2].

Beyond energy benefits, the development of bio-fuels can: enable job creation and food security; reduce volume of imports and generate savings of foreign exchange [3]. This will avert crises such as “occupy Nigeria” which occurred recently in the country resulting to loss of lives and exacerbating the socio- economic problems of the people when subsidy on gasoline was removed.

The crops used in producing bio-fuels; sugarcane, millet, maize, sweet potatoes, cassava, guinea corn, etc thrive well in tropical and subtropical climate and demonstrate tropical comparative advantage in production. In developing countries like Nigeria where the productivity of land is poor to medium due to poor management, and only 42% of the arable land is cultivated, agricultural production is basically at the subsistence level [4, 5]. If farmers become energy farmers who sell their crops on international market: their socio-economic status would be boosted due to availability of suitable market and competitive prices [6]. Their improved economic status would enable them manage the soils better for improved productivity. Bio-ethanol production from grain; millet, guinea corn, maize and tubers; cassava, sweet potatoes, utilizes only the starch, broken down by amylases, the remaining components (Proteins, Minerals and Vitamins) are used to produce a variety of highly valuable by- products that can be used as feeds to livestock [6].

The study explores the production of bio-ethanol from brown guinea corn commonly used in making local alcoholic beverage "burukutu", millet and sweet potatoes using a modified method. The areas studied produce a lot of crops including those selected for this study and contributes greatly to the name "Food basket of the Nation" for which Benue state is popularly known in Nigeria.

2. Materials and Method

2.1. Study Area

The Local Government Areas of Benue state selected for this study include Gwer West located between latitude 7°25' to 7°40'N and longitude 8°00' to 8°23'E, Konshisha; latitude 6°45' to 7°24'N and longitude 8°25' to 8°52'E, Vandeikya; latitude 6°33' to 7°03'N and longitude 8°48' to 9°04'E, Gboko; latitude 7°13' to 7°35'N and longitude 8°30' to 9°03'E as well as Buruku located between latitude 7°07' to 7°45'N and longitude 8°50' to 9°08'E. Based on the item sampled, the areas were designated as sampling stations and labelled as Am (millet), Ds (Sweet potatoes) and Fg (Guinea corn) as summarized in Table 1 below.

Table 1. Sampling Guide

S/N	Sampling Stations	Sample	No of Samples
1	Gwer West (Am)	Millet	3
2	Konshisha (Ds)	Sweet potatoes	3
3	Vandeikya (Ds)	Sweet potatoes	3
4	Gboko (Fg)	Guinea corn	3
5	Buruku (Fg)	Guinea corn	3
		Total	15

2.2. Sample Collection

A total of three samples were collected from the major markets in each sampling station between April and November, 2010. They were identified by a botanist in the Department of Biological Sciences, Benue State University, Makurdi. The grains were kept in a dry place while the sweet potatoes were processed immediately after collection and pre-treatment.

2.3. Samples Preparations

All enzymes, yeast and other reagents were provided by Benue Brewery Limited (BBL), Makurdi, Benue State, the maker of 'More' lager beer. The Brewery's Laboratory was also used for the analyses.

The cereal grains (guinea corn and millet) were milled while sweet potato was washed, peeled and grated and 454.0g of each flour were subjected to liquefaction, saccharification and fermentation. The methodology employed in this work was a modification of the methods employed by Benue Brewery Limited (BBL) and Mathewson, [7]. In the present method, hitempase (α-amylase) (19%) was added at the onset gelatinization temperature and another (81%) added at the end gelatinization temperature. Furthermore, the modified route was designed to target maximum production of bio-ethanol by employing optimum conditions for enzyme activity. Fractional distillation of the fermented wort was carried out and the bio-ethanol collected at 78.0°C. Specific gravity, drying and percentage purity of the bio-ethanol were determined according to the procedures described by Matthewson [7].

2.4. Liquefaction of Guinea corn flour

800 cm^3 of hot brewing water was measured into a 3L round-bottom flask and 0.681g of $CaSO_4$ was added, then stirred properly. A known amount (454.0g) of the flour was weighed and added into the flask and stirred after which 0.7718g of soybean flakes was added and the mash stirred. The pH of the mash was checked and corrected to 6.1 (by adding small quantity of lime). The flask was heated with constant stirring in a thermostatic water bath to a temperature of 50°C. Then, 0.3178g of bio-protease (proteolytic enzyme) was added and the temperature maintained at 50°C with constant stirring for 30mins and raised to 75°C (onset gelatinization temperature of guinea corn starch, [6]. The pH of the mash was again checked and adjusted to 5.6 (by adding few drops of 25% H_2SO_4). Then, 0.0850g (19%) of hitempase (α-amylase) was added and the mash stirred. The temperature was maintained at 75oC for 30mins and raised to 95°C; the temperature at which cereal starches would have geltinized [8]. This was followed by the addition of 0.3686g (81%) of hitempase. The mash was again stirred thoroughly and heated at that temperature for 1hr after which it was cooled to 60°C and 0.2134g of fungamyl added, then stirred.

The mash was kept undisturbed at that temperature for 30mins after which some aliquot was taken and tested with

iodine solution. The mixture turned reddish-brown indicating complete liquefaction.

2.5. Liquefaction of Millet flour

The procedure used for the liquefaction of the millet flour was similar to the liquefaction of guinea corn flour except that the 0.0850g (19%) of hitempase was added at 60^{o}C (onset gelatinization temperature of millet starch [6].

2.6. Liquefaction of Sweet Potato

700cm^3 of hot brewing water was measured into a 3L round-bottom flask and 0.681g of $CaSO_4$ was added, then stirred properly. A known amount (454.0g) of the grated sweet potato was weighed and added into the flask and stirred after which 0.7718g of soybean flakes was added and the mash stirred. The pH of the mash was checked and adjusted to 6.2 (by adding small quantity of lime). Then, it was heated with constant stirring in a thermostatic water bath to a temperature of 50^{o}C and 0.3178g of bio-protease was added and the temperature maintained with constant stirring for 30mins. The temperature was raised to 70^{o}C (onset gelatinization temperature of sweet potato starch [6]. The pH of the mash was again checked and adjusted to 5.6 (by adding few drops of 25% H_2SO_4) after which 0.0850g (19%) of hitempase was added and the mash stirred. The temperature was maintained at 70^{o}C for 30mins and raised to 95^{o}C followed by the addition of 0.3686g (81%) of hitempase.

The mash was again stirred thoroughly and heated at that temperature for 1hr after which it was cooled to 60^{o}C by the addition of 200cm^3 of brewing water at 4^{o}C (BBL). Then, 0.2134g of fungamyl was added, stirred and allowed to stand undisturbed for 30mins after which liquefaction test was conducted to ensure completeness.

2.7. Saccharification of the mashes

The pH of each mash was checked and adjusted to 4.5 and 0.227g of amyloglucosidase (AMG) (glucoamylase) was added and stirred. The mash was allowed to stand at 60^{o}C for 45mins [9]. Some aliquot of each mash was taken and tested with iodine solution. The mixture turned pale yellow indicating that saccharification was complete (BBL). The temperature was then raised to 70^{o}C and the mash heated for 15mins. Thereafter, the mash was brought down, filtered and sparged with 600cm^3 hot brewing water.

2.8. Fermentation

The pH of the wort was checked and adjusted to 5.1 (by adding few drops of lime). Then, 100cm^3 of yeast (Saccharomyces uvarum) was added and the wort allowed to ferment for 84hrs (with specific gravity checked 12 hourly and wort aerated regularly). Thereafter, the apparent gravity of the fermented wort was checked and recorded (BBL).

2.9. Fractional Distillation

The fermented wort was filtered and the apparent gravity of the beer was checked and noted. Then, the beer was subjected to fractional distillation and the bio-ethanol collected at 78^{o}C [6].

2.10. Determination of Specific Gravity of the Bio-ethanol

The density of water was determined by measuring 50cm^3 of water in a density bottle and cooled to 20^{o}C and weighed (BBL). The same procedure was carried out with the bio-ethanol produced and the specific gravity was determined by calculating the ratio of the mass of bio-ethanol to the mass of water, both at 20^{o}C [8].

2.11. Drying of Ethanol

The water containing bio-ethanol was mixed with lime (CaO) at the ratio of 3.49:1 (lime:water) for each 1ml of water to be removed and allowed to slake for 24hrs with occasional stirring. Simple distillation was then used to separate the bio-ethanol from calcium hydroxide. During distillation, the temperature was maintained at the boiling point of pure ethanol. The specific gravity of the dried bio-ethanol was determined as in above [7].

2.12. Determination of Purity of Ethanol

The percentage purity of the bio-ethanol was determined by comparing the specific gravity with the standard values given by Official Methods of Analysis of the Association of Official Analytical Chemists [10].

3. Results and Discussion

The results presented in Table 2 showed the specific gravity, volume distillate and percentage purity of bio-ethanol produced from guinea corn using Benue Brewery Limited (BBL), Makurdi production methods and the modified hitempase reaction pathway respectively. Against the bulk addition of hitempase only once after heating mash to a temperature of 70^{o}C in the BBL method, the modified reaction pathway involves the addition of hitempase; 0.0850g (19%) at 75^{o}C; onset gelatinization temperatures of guinea corn starch and 0.3686g (81%) at 95^{o}C; the temperature at which cereal starch would have completely gelatinized (broken down into a mixture of polymers in solution) [6, 11]. The unmodified BBL method produced guinea corn bio-ethanol with a mean volume distillate of 92.17cm^3 while the modified pathway recorded mean volume distillate of 96.33cm^3. The mean percentage purity revealed that BBL methods yielded guinea corn bio-ethanol of 81.66% while the modified route yielded guinea corn bio-ethanol of 90.50% purity. The mean specific gravities of the bio-ethanol recorded were 0.8582 and 0.8288 by BBL method and the modified reaction pathway respectively.

The results of similar studies on bio-ethanol produced from millet and sweet potato revealed similar trend. BBL method yielded mean volume distillate of 78.67cm^3, mean purity of 88.64% and mean specific gravity of 0.8348 of bio-ethanol obtained with millet feedstock (Table 3) while the modified route yielded millet bio-ethanol of 96.00cm^3 mean volume distillate, 91.08% mean percentage purity and 0.8268 mean specific gravity. Bio-ethanol produced with sweet potato as feedstock recorded 56.17cm^3 mean volume distillate, 88.44% mean percentage purity and 0.8356 mean specific gravity using the BBL method while the modified reaction pathway produced sweet potato bio-ethanol of 79.00cm^3 mean volume distillate, 90.69% mean percentage purity and 0.8281 specific gravity (Table 4). T-test analyses of the variations in the quality characteristics of the bio-ethanol produced from the different feedstocks by BBL and modified methods were statistically significant ($p < 0.01$).

The relative mean volume distillate of bio-ethanol yields in the two methods (Fig.1) showed significantly ($p < 0.01$) higher volume distillate with the addition of hitempase (19% and 81%) at the onset gelatinization temperature and the temperature at which all cereal starch would have gelatinized. This finding suggests that completely gelatinizing the starch in starchy feedstocks have significant positive impact on the conversion of starch to fermentable carbohydrate (sugar) with the corresponding increase in the volume of bio-ethanol yield.

The higher percentage purity of the bio-ethanol produced in the modified route (Fig. 2) also suggests that gelatinization may help to sediment some impurities from the gelatinized starch prior to fermentation. Generally, millet feedstock yielded the highest mean volume distillate (98.00cm^3) while sweet potato yielded the lowest mean volume distillate (79.00cm^3) in the modified reaction pathway. Ethanol yields by starchy feedstocks may depend on the availability of fermentable starch in them and this may account for the different mean volume distillate recorded by the three different feedstocks processed under the same method. Moorthy [12], reported that bioavailability of starch differed among grain cultivars and may affect the conversion rate and final yield of ethanol.

Table 2. Quality Characteristics of Guinea corn Bio-ethanol Produced by Modifying BBL

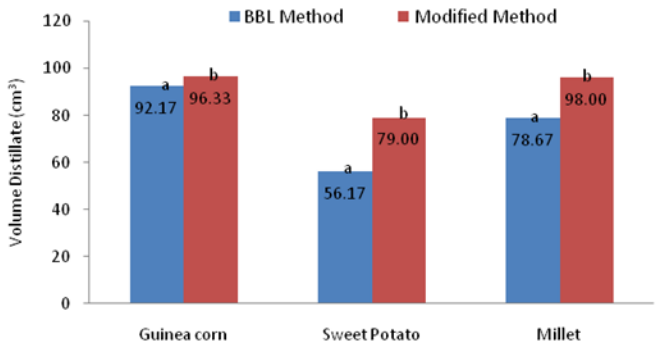
Sample (Guinea corn)	BBL Method			Modified Method		
	Specific Gravity	Volume Distillate (cm^3)	% Purity	Specific Gravity	Volume Distillate (cm^3)	% Purity
Fg1	0.8590	90.00	80.55	0.8290	94.00	90.44
Fg2	0.8588	92.00	80.62	0.8288	96.00	90.50
Fg3	0.8590	96.00	80.55	0.8284	98.00	90.62
Fg4	0.8588	89.00	80.62	0.8286	92.00	90.56
Fg5	0.8566	88.00	81.40	0.8291	100.00	90.41
Fg6	0.8570	98.00	81.26	0.8288	98.00	90.50
Mean	0.8582	92.17	81.66	0.8288	96.33	90.50
S.D	0.0011	4.02	1.94	0.0003	2.94	0.08

Table 3. Quality Characteristics of Millet Bio-ethanol Produced by Modifying BBL Reaction Pathway

Sample (Millet)	**BBL Method**			**Modified Method**		
	Specific Gravity	Volume Distillate (cm^3)	% Purity	Specific Gravity	Volume Distillate (cm^3)	% Purity
Am1	0.8314	76.00	89.71	0.8300	98.00	90.14
Am2	0.8367	82.00	88.06	0.8250	96.00	91.62
Am3	0.8364	78.00	88.16	0.8255	100.00	91.47
Mean	0.8348	78.67	88.64	0.8268	98.00	91.08
S.D	0.0030	3.06	0.93	0.0028	2.00	0.81

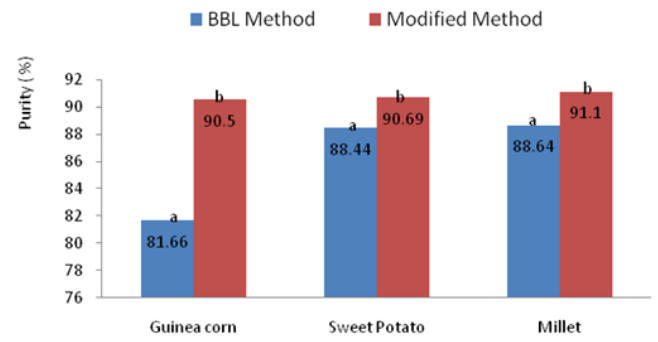
Table 4. Quality Characteristics of Sweet Potato Bio-ethanol Produced by Modifying BBL

Sample (Guinea corn)	**BBL Method**			**Modified Method**		
	Specific Gravity	Volume Distillate (cm^3)	% Purity	Specific Gravity	Volume Distillate (cm^3)	% Purity
Ds1	0.8360	55.00	88.29	0.8290	77.00	90.44
Ds2	0.8309	50.00	89.86	0.8280	79.00	90.73
Ds3	0.8369	63.00	88.00	0.8260	79.00	91.32
Ds4	0.8362	55.00	88.22	0.8288	78.00	90.50
Ds5	0.8365	57.00	88.13	0.8286	81.00	90.56
Ds6	0.8369	57.00	88.00	0.8280	80.00	90.73
Mean	0.8356	56.17	88.44	0.8281	79.00	90.69
S.D	0.0023	4.22	0.70	0.0011	1.41	0.32



*Key: BBL = Benue Brewery Limited, Makurdi

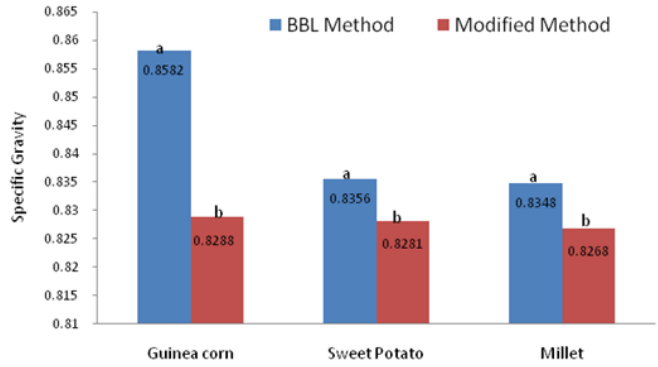
Fig. 1. Mean Yields of Volume Distillate (cm^3) of Bio-ethanol Produced by modifying BBL Methods



*Key: BBL = Benue Brewery Limited, Makurdi

Fig. 2. Percentage mean purity of the bio-ethanol produced by the BBL and modified methods.

The relative mean specific gravity of the bio-ethanol produced by the two methods (Fig. 3) showed that guinea corn bio-ethanol had the highest value of 0.8582 and 0.8288 by BBL and Modified methods respectively while the lowest record (0.8348 and 0.8268) was obtained in millet bio-ethanol. T-test analyses of the variations in specific gravity of bio-ethanol produced by the two methods were statistically significant ($p < 0.01$).



*Key: BBL = Benue Brewery Limited, Makurdi

Fig. 3. Mean specific gravity of the bio-ethanol produced by the BBL and modified methods.

The results of the specific gravities recorded in this study were higher in bio-ethanol produced using the BBL method than the modified reaction pathway. This suggests that the addition of hitempase at the onset and end gelatinization temperatures improve the completeness of the fermentation process. This finding colloborates the report by Ensminger [13], that the decline in specific gravity of alcohol over time informed the brewer about the health and progress of fermentation to completion and that fermentation is complete when specific gravity stops declining.

4. Conclusion

The results of this study revealed that addition of hitempase in stages; at the onset and end gelatinization temperatures of starch of guinea corn, millet and sweet potato feedstocks yielded bio-ethanol of higher volume distillate, higher percentage purity and lower specific gravity. This suggests that the modified reaction pathway improves completeness of fermentation process. Millet feedstock yielded the highest mean volume distillate and mean percentage purity by the modified method. The significant variations in bio-ethanol mean volume distillate among the starchy feedstock could be attributed to the bioavailability of starch which differed among cultivars and affect the conversion rate and final yield of ethanol. Furthermore, this study revealed that brown guinea corn which has been reported to contain high anti-nutritional factor; tannin has high potential for usage as feedstock in bio-ethanol production instead of utilization as raw material for "burukutu" an alcoholic beverage as widely used at present.

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