Scientific Review
ISSN(e): 2412-2599, ISSN(p): 2413-8835
Vol. 2, No. 7, pp: 84-89, 2016
URL: http://arpgweb.com/?ic=journal&journal=10&info=aims

Ethanol by Volume Produced From Waste Pod of Fluted Pumpkin (*Telfairia Occidentalis*) Using African Giant Snail (*Archachatina Marginata*) Slime and Yeast

Akwukwaegbu, P. I. Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria

Peters, D. E.* Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria

Wegwu, M. O. Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria

Abstract: Ethanol by volume produced from waste of fluted pumpkin pod (*Telfairia occidentalis*) using African giant snail (*Archachatina marginata*) slime and yeast was investigated. Varying weights of 250g, 500g and 750g of solid waste were chosen for the 1st, 2nd and 3rd determinations respectively. A total of seven groups labeled A-G were set up; group A (Pp alone); group B (snail slime (SL) plus Pp); group C (yeast (Y) plus Pp); group D- D1, D2, D3 (25ml of (SL) plus 7.5g of (Y)); group E- E1, E2, E3 (50ml of SL plus 15g of Y); group F- F1, F2, F3 (75ml of SL plus 22.5g of Y) and group G- G1, G2, G3 (100ml of SL plus 30g of Y). All groups were fermented under anaerobic condition at (37oC) for 24hrs, 48hrs and 72hrs. The pH of the solution before and after centrifuging was 5.4 and 5.2. Cellulose content of Pp waste was determined, contents of all treated groups were distilled and percentage of ethanol (Et) determined and characterized using Gas chromatographic (GC) technique. Result of the cellulose content was (20.56±0.58%). The Et by volume produced increased significantly ($p<0.05$) with increase in fermentation time, however, there was no Et produced in group A. Also, increase in concentration of snail slime and yeast brought about corresponding increase in Et by volume. The maximum bioethanol production was obtained in group G (G3). There was a significant difference ($p<0.05$) in the Et by volume of group D-G and groups A, B and C. Characterization of Et using GC showed (group B 1.09%; group C 2.99%; group D-(D1) 13.58% and D2 35.72%). In conclusion, pumpkin pod waste could be a promising source of Agro waste for producing bioethanol using African giant snail slime and yeast.

Keywords: Ethanol; Pumpkin pod; Fermentation.

1. Introduction

Bioethanol is widely recognized as a unique transportation fuel with powerful economic, environmental and strategic attributes. The environmental properties of bioethanol result in a net release of no carbon dioxide and very little sulphur, due to a higher octane number, higher flame speed and evaporation heat, and broader limits for flammability. These lead to a higher compression ratio and a shorter burning time as well as leaner burn engine, which result in better efficiency in internal combustion engines compared to petrol. Their high octane number gives the ability to operate at higher compression ratio without preignition [1] its greater latent heat of vaporization gives a higher charge density [2] and its higher laminar flame speed allows it to be run with leaner, or more dilute, air/fuel mixtures [3, 4]. In addition, alcohol fuels generally yield lower criteria pollutant emissions than gasoline [5, 6] and lower evaporative emissions due to somewhat lower vapor pressures [7]. Currently, the fossil resources are not regarded as sustainable and questionable from the economic, ecology and environmental point of views [8]. The burning of fossil fuels is a big contributor to increasing the level of CO_2 in the atmosphere which is directly associated with global warming observed in recent decades. The adverse effects of greenhouse gas (GHG) emissions on the environment, together with declining petroleum reserves, have been realized. Therefore, the quest for sustainable and environmentally benign sources of energy for our industrial economies and consumer societies has become urgent in recent years [9]. Consequently, there is renewed interest in the production and use of fuels from plants or organic waste. 21st Century is looking for a shift to alternate industrial feedstock and green processes to produce these chemicals from renewable biomass resources [10].

*Corresponding Author

According to Demirbas [11] bioethanol as an alternative fuel can be used either as a gasoline additive or substitute and can be produced from wood, straw, crops and household waste by the alcoholic fermentation of the sugars which are produced by hydrolysis of the biomass.

Demirbas defines any biofuel as a"non-polluting, locally available, accessible, sustainable and reliable fuel obtained from renewable sources" [12] which makes them and especially bioethanol interesting in the future for the industry.

Utilization of biowastes as energy with high efficiency and rationality not only meets the demands for energy, but also provides a basis for environmental protection and sustainable development of the society [13, 14].

For second-generation biofuel production, utilization of renewable biomass resources has received major focus in the world. Renewable 'plant biomass' refers particularly to cheap and abundant non-food lignocellulose-rich materials available from the plants. Biomass to bioethanol process could help in mitigation of global climate change by reducing emissions (mainly CO_2) as well as decreasing dependence upon fossil fuels. Thus, deployment of biomass resources has been projected to play an important role in sustainable development. The second-generation biofuels include hydrogen, natural gas, bio-oils, producer gas, biogas, alcohols and biodiesel. In countries like Nigeria and India, agricultural production of various crops like fluted pumpkin, cotton, mustard, chilli, sugarcane, sorghum, sweet sorghum, pulses, oilseeds, etc. results in generation of huge amounts of wastes that do not find any alternative use and are either left in the fields or are burned. Hence, these could be used as good alternative resources to generate biofuels such as bioethanol in an environmentally friendly manner. Use of agricultural residues helps in reduction of deforestation by decreasing our reliance on forest woody biomass. Moreover, crop residues have short harvest period that renders them more consistently available to bioethanol production [15-17]

The aim of this paper was to determine the ethanol by volume produced from waste of fluted pumpkin pod (*Telfairia occidentalis*) using African giant snail (*Archachatina marginata*) slime and yeast.

2. Materials and Method

2.1. Sample Collection

Pumpkin pod waste was collected from Obodo Ahiara community situated at Ahiazu-Mbaise L.G.A of Imo State, Nigeria while African giant snail was bought at Umuapu in Ohaji Egbema L.G.A of Imo State. The snail shell was broken and the slime extracted mechanically. Approximately 100g of solid waste was weighed using electronic weighing balance and used for cellulose content determination. The remaining solid waste was subjected to fermentation and distillation for ethanol extraction and determination in varying proportions.

2.2. Experimental Design

Pumpkin pods were first collected from storage bans and ground into a fine mixture using mortar and pestle. Varying weights of 250g, 500g and 750g of solid waste were chosen for the 1st, 2nd and 3rd determinations respectively. A total of seven groups labeled A-G were set up as explained below. Group A (pumpkin pod (Pp) alone); group B (snail slime (SL) plus Pp); group C (yeast (Y) plus Pp); group D- D1, D2, D3 (25ml of (SL) plus 7.5g of (Y)); Group E- E1, E2, E3 (50ml of SL plus 15g of Y); group F- F1, F2, F3 (75ml of SL plus 22.5g of Y) and group G- G1, G2, G3 (100ml of SL plus 30g of Y). All groups were fermented under anaerobic condition at 37^{o}C for 24hrs, 48hrs and 72hrs. The pH of the solution before and after centrifuging was 5.4 and 5.2 respectively [18].

2.3. Estimation of Ethanol by Soxhlet Distillation

Each sample container was squashed, filtered with cheese filter cloth to obtain the fermented liquid samples and centrifuged at 2500rpm for 15 minutes to obtain 200ml of supernatant. Afterwards, the sample was decanted and distilled at a temperature of 78oC (boiling point of ethanol) for 1hr. The ethanol by volume content of distillate was determined by correlation with the specific gravity measurements, from correlation tables; Reference: Laboratory manual in food quality control [19].

2.4. Determination of Specific Gravity

Density bottle was used to determine the density of the samples. A clean and dry bottle of 50ml capacity was weighed (W_0) and then filled with the sample. The stopper was inserted and reweighed to give (W_1). The sample was substituted with water after washing and drying the bottle and weighed to give (W_2) [19].

The specific gravity (sp. gr) was calculated as;

$$\mathbf{Sp.gr} = \frac{\textbf{mass of sample}}{\textbf{mass of equal volume of water}}$$

$$= \frac{\mathbf{W_1 - W_0}}{\mathbf{W_2 - W_0}}$$

2.5. Cellulose Content

A 10g sample was weighed into 50ml glass centrifuge tubes. The sample was suspended in water, centrifuged, and the supernatant decanted. The sample was resuspended in 12.5ml glacial acetic and 2.5ml of conc. nitric acid (HNO_3) and digested in a boiling water bath for 20 minutes during which the sample was again resuspended. The cellulose was transferred to a Gooch crucible labeled (W_1), washed successfully with hot alcohol, 10ml of 90% benzene and 60% of ether. It was dried and weighed (W_2), then finally ashed and reweighed as (W_3) [20].

$$\textbf{Cellulose content} = \frac{\mathbf{W_3 - W_1}}{\mathbf{W_1}} \times \frac{\mathbf{100}}{\mathbf{1}}$$

2.6. Statistical Analysis

All data collected were subjected to descriptive and one way analysis of variance using Statistical Package for Social Sciences (SPSS), Inc.20.0 software. Multiple comparisons of data were done using post hoc turkey. All data were represented in mean±standard deviation (M±S.D). Confident level of determination (P=0.05).

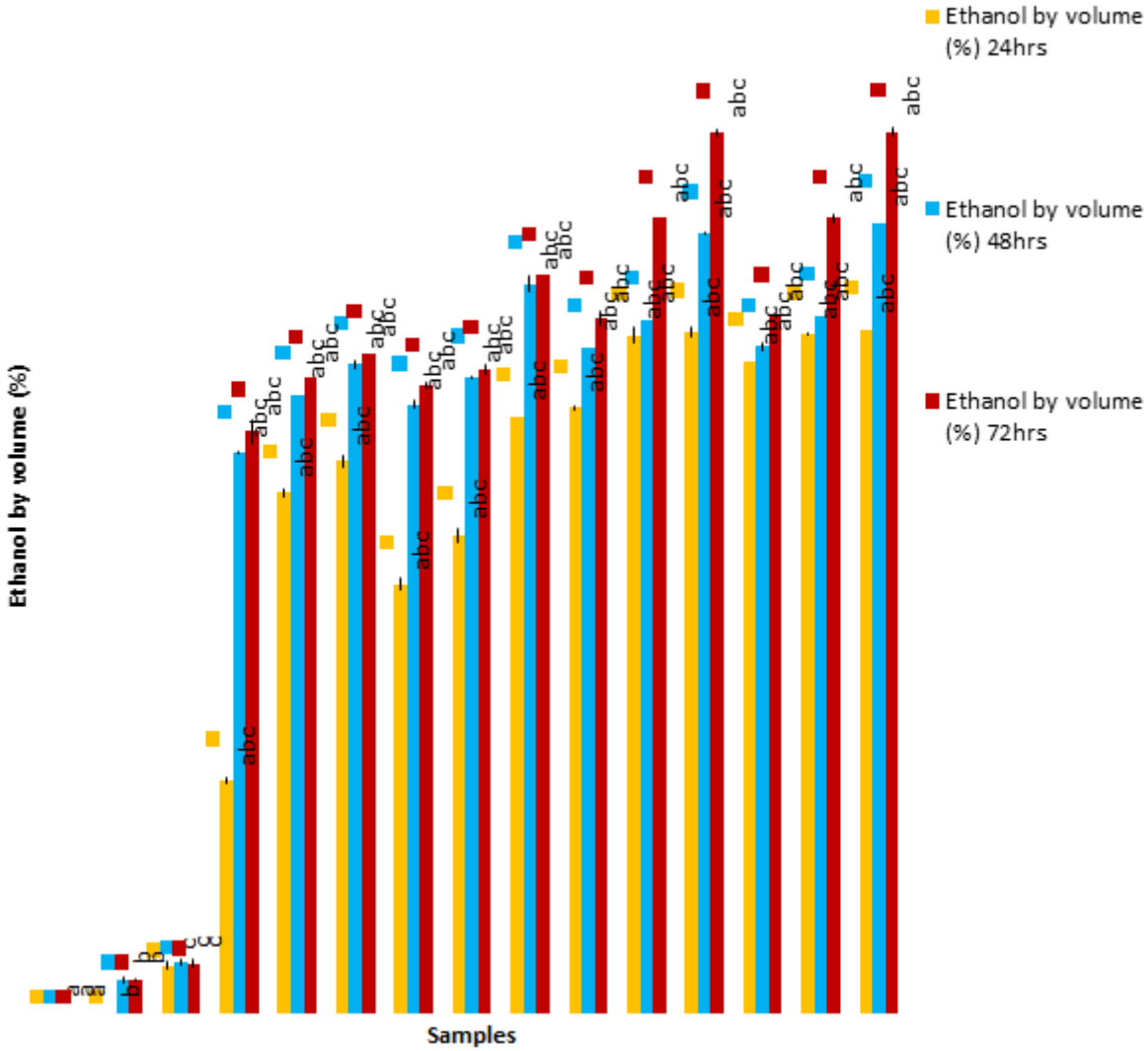
3. Results

Table-1. Cellulose content of fluted pumpkin (*Telfairia occidentalis*) pod waste

Cellulose content	Pumpkin pod waste (%)
Cellulose	20.56±0.58

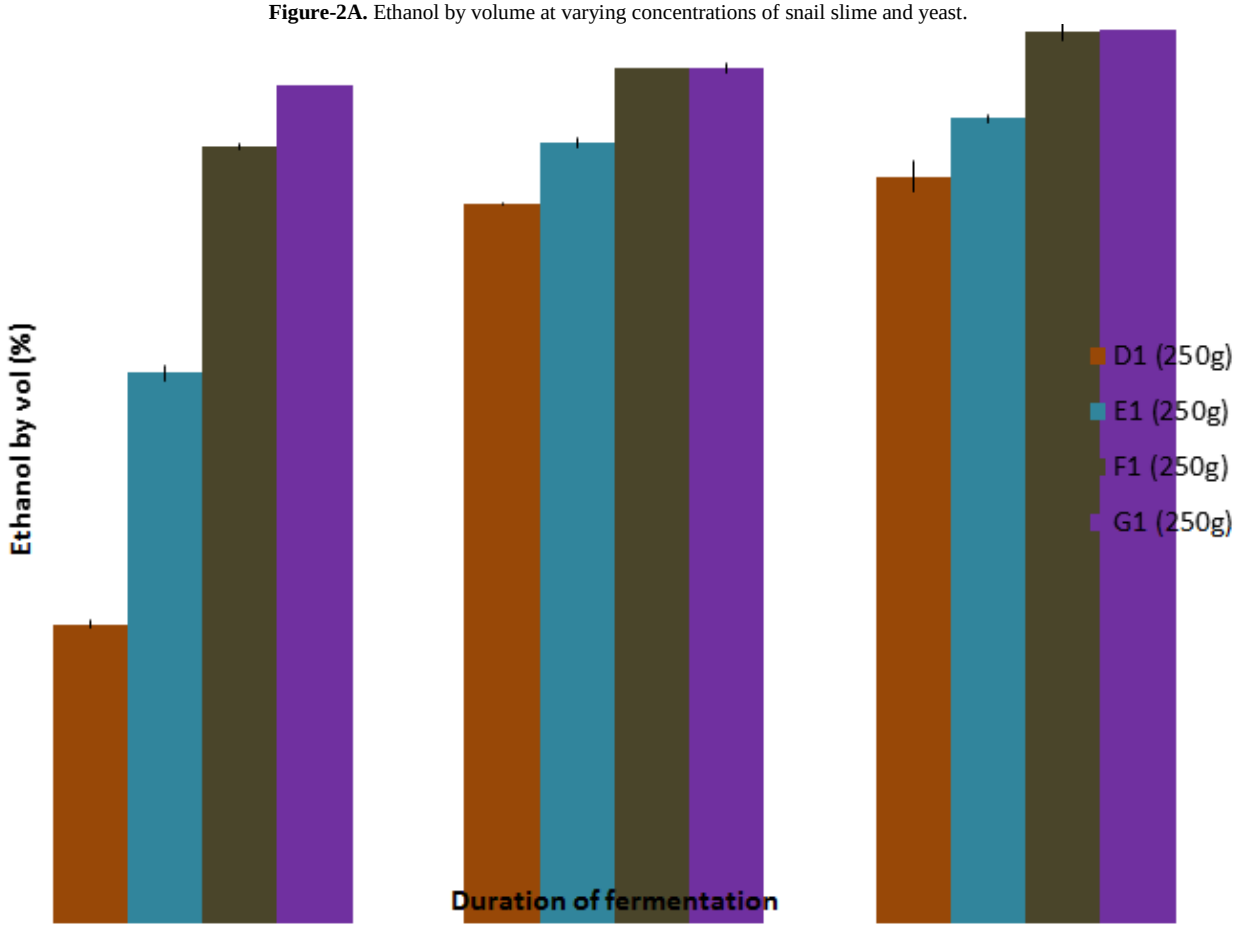
Value is mean ± standard deviation (M±S.D) of triplicate determination (n=3).

Fig-1. Ethanol by volume at varying fermentation times



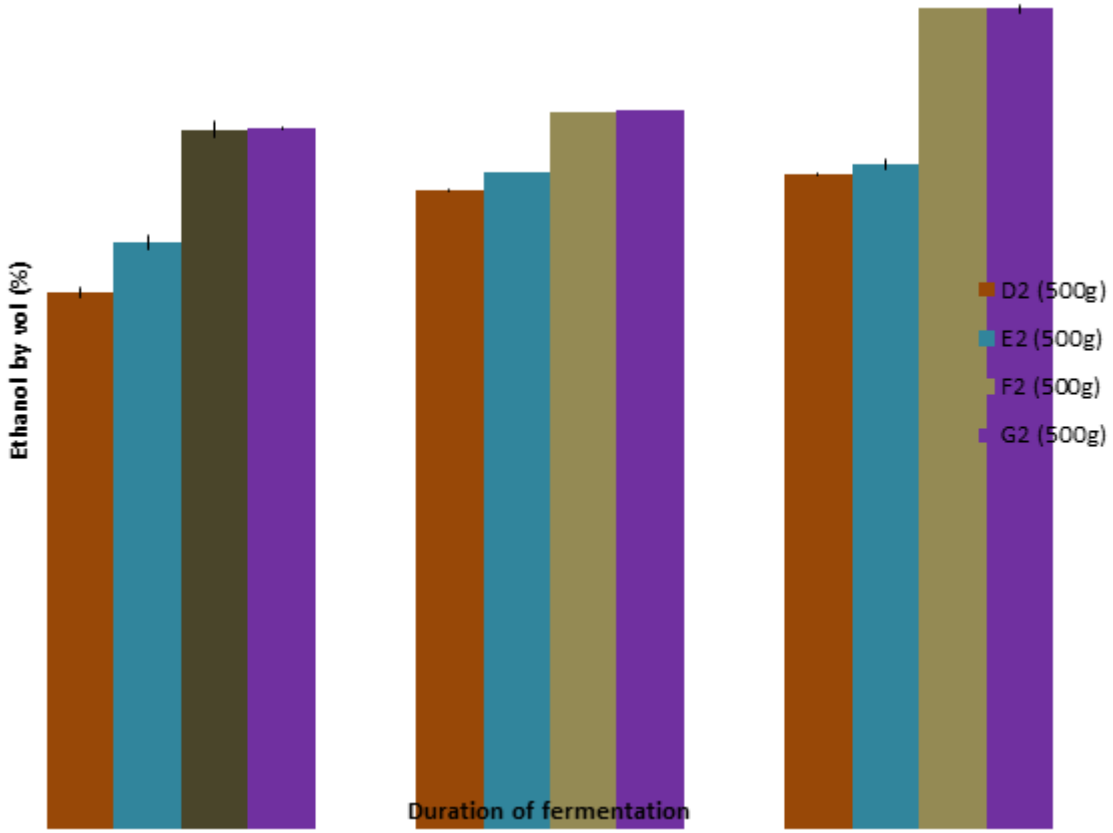
Data are mean ± standard deviation (M±S.D) of triplicate determinations. Superscripts a, b, c indicate significant difference (P<0.05) when compared to groups A, B and C respectively.

Figure-2A. Ethanol by volume at varying concentrations of snail slime and yeast.



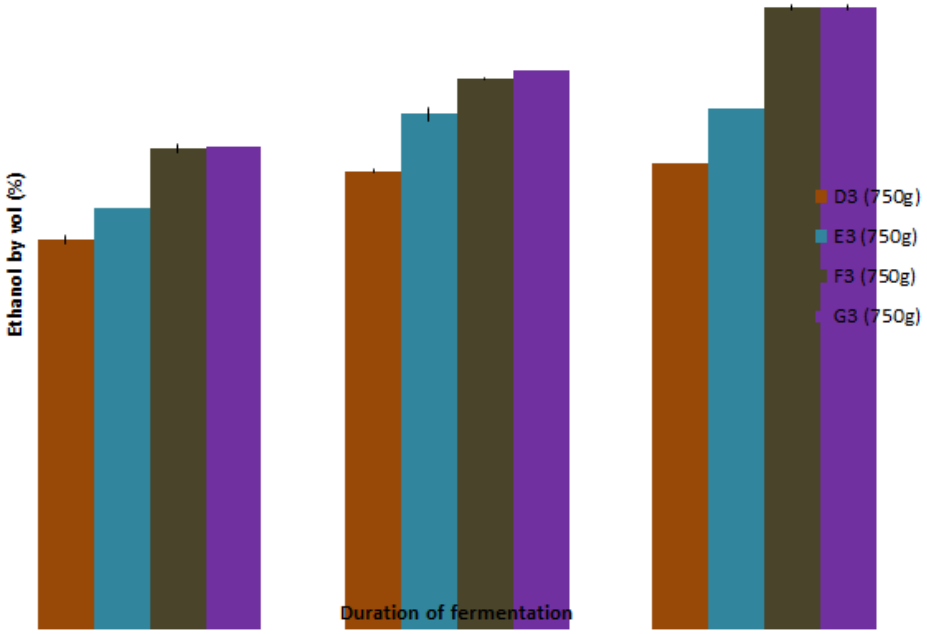
Data are mean ± standard deviation (M±S.D) of triplicate determinations.

Figure-2B. Ethanol by volume at varying concentrations of snail slime and yeast.



Data are mean ± standard deviation (M±S.D) of triplicate determinations.

Figure-2C. Ethanol by volume at varying concentrations of snail slime and yeast.



Data are mean ± standard deviation (M±S.D) of triplicate determinations.

Table-2. Characterization of ethanol produced using GC technique for some selected samples

Sample	Percentage of ethanol (%)
Group B	1.09
Group C	2.99
Group D (D1)	13.58
Group D (D2)	35.72

4. Discussion and Conclusion

The cellulose content in fluted pumpkin waste was found to be 20.56% (Table 1). The cellulose contents together with the fibre content of fluted pumpkin provide substrate for cellulose action. Snail slime was able to degrade pumpkin pod waste because of the substantial amount of cellulose and this to a large extent influenced our choice of lignocelluloses waste.

Ethanol by volume at varying fermentation times is represented in figure 1. This expresses more of the purity of ethanol. It was observed that the ethanol by volume increased with increase in fermentation time. There was no ethanol produced in group A while very little ethanol were produced in group B and C. There were significant differences ($p<0.05$) in the ethanol by volume from group D-G when compared to groups A, B and C respectively (Fig 1) which indicate that ethanol produced was affected by fermentation time. Also maximum ethanol was produced in group G-(G3) after 72hr fermentation. The maximum ethanol produced in group G-(G3) and second to highest ethanol produced in group F-(F3) are similar to values obtained for cornstraw (58.6GI) and sugarcane bargasse (51.3GI) lignocelluloses wastes respectively, though the fermentation conditions varied [21].

Results in figures 2A, 2B and 2C for ethanol by volume at varying concentrations of snail slime and yeast further highlights the need for extended time of fermentation to bring about greater yield of ethanol. There was an increase in ethanol by volume with increase in concentration of snail slime and yeast. However, there was no marked change in the ethanol by volume in groups F and G which could be that the active sites of the enzyme were fully occupied.

Characterization of the ethanol produced by gas chromatographic technique confirmed the presence of ethanol in the following groups as follows; (group B 1.09%; group C 2.99%, group D (D1) 13.58% and group D (D2) 35.72%) (Table 2). Peak representations (shown in the Appendix) indicate that there were other products (toluene, ethyle acetate and methanol) produced alongside ethanol at these conditions. The percentage of ethanol by volume obtained in these peaks corresponded to the percentage of ethanol (group B 1.09%; group C 2.99%, group D (D1) 13.58% and group D (D2) 35.72%) in Table 2 for the selected groups and samples. This shows that enzyme activity and action of yeast at optimum conditions are required for optimum yield of ethanol. Cellulase from snail slime is required to act on the lignocellulosic waste to breakdown the carbohydrate content to glucose before the glucose is fermented to ethanol and carbon dioxide (CO_2) in anaerobic condition [22].

The percentage bioethanol yield from pumpkin pod waste system was very poor generally when compared to the E 85 (85% ethanol) used in Brazil and China that reduces GHG, particulate and sulfate emissions by 10, 20 and 80 %, respectively. The poor yield of ethanol may be due to acidic pH of the solution. The pH was measured over the time of analysis. The pH before and after centrifuging was 5.4 and 5.2 respectively. Though the acidic pH was necessary to prevent the formation of microorganisms in the containers which could have inhibited the fermentation

process, however, it contributed to lower percentage yield of ethanol from the waste. Acids and bases are known to delignify plant cell structures. Findings from research by Zhao, *et al.* [23] indicated that highest level of de-lignifications was shown in the treatment of agricultural residues with alkaline sodium hydroxide solution. It could as well be as a result of high content of indigestible fibre waste (hemicelluloses and lignin) among others in fluted pumpkin waste pod, which are structural polysaccharides and very difficult to biodegrade [24]. Cellulase enzyme degrades cellulose to glucose to generate ethanol while hemicelluloses are fermented to pentoses and require acid pretreatment first before fermentation can take place.

From this study, it can be concluded that pumpkin pod waste could be a promising source of Agro waste for bioethanol production with the synergistic action of snail slime and yeast. Furthermore, increasing the concentration of the snail slime and yeast brought about corresponding increase in ethanol production from the waste. Maximum ethanol by volume was obtained in group G (G3) on the third day of fermentation.

5. References

[1] Radwan, M. S., 1985. "Performance and Knock Limits of Ethanol-Gasoline Blends in Spark-Ignited Engines." SAE Paper850213.

[2] Dodge, L. G., Shouse, K., Grogan, J., Leone, D. M., Whitney, K. A., and Merritt, P. M., 1998. "Development of an Ethanol-Fueled Ultra-Low Emissions Vehicle. SAE Paper 981358."

[3] Metghalchi, M. and Keck, J. C., 1982. "Burning velocities of mixtures of air with methanol, isooctane, and indolene at high pressure and temperature." *Combustion and Flame*, vol. 48, pp. 191-210.

[4] Marinov, N. M., 1999. "A detailed chemical kinetic model for high temperature ethanol oxidation." *Int. J. Chemical Kinetics*, vol. 31, pp. 183-220.

[5] Sinor, J. E. and Bailey, B. K., 1993. "Current andPotential Future Performance of Ethanol Fuels. SAE Paper 930376."

[6] Kelly, K. J., Bailey, B. K., and Coburn, T. C., 1996. "Federal Test Procedure Emissions Test Results from Ethanol Variable-Fuel Vehicle Chevrolet Luminas." SAE Paper 961092.

[7] Furey, R. L., 1985. "Volatility Characteristics ofGasoline/Alcohol and Gasoline/Ether/Fuel Blends. SAE Paper 852116 "

[8] Kamm, B., Gruber, P. R., and Kamm, M., 2006. "Biorefinery industrial processes and products." *Status and future direction, Weinheim:Wiley-Verlay Gmbtt and Co KGaA*, vol. 1-2.

[9] Mabee, W. E., Gregg, D. J., and Saddler, J. N., 2005. "Assessing the emerging biorefinery sector in Canada." *Appl. Biochem. Biotechnol*, vol. 121–124, pp. 765–778.

[10] Stevens, C. V. and Verhe, R., 2004. *Renewable bioresources scope and modification for non-food application*. England: John Wiley and Sons Ltd.

[11] Demirbas, M. F., 2006. "Current technologies for biomass conversion into chemicals and fuels." *Energy Sour. Part A*, vol. 28, pp. 1181–1188.

[12] Demirbas, A., 2008. " Biofuels sources, biofuel policy, biofuel economy and global biofuel projections." *Energy Conversion and Management*, vol. 49, pp. 2106-2116.

[13] Pimentel, D., 2006. "Environmental energetic and economic comparison of organic and conventional farming systems." *Biosci*, vol. 55, pp. 573-582.

[14] Zeng, X. Y., Ma, Y. T., and Ma, L. R., 2007. "Utilization of straw in biomass energy in China." *Rene. sust. Energy Rev*, vol. 11, pp. 976-987.

[15] Knauf, M. and Moniruzzaman, M., 2004. "Lignocellulosic biomass processing: a perspective." *Int. Sugar J.*, vol. 106, pp. 147-150.

[16] Kim, S. and Dale, B. E., 2004. "Global potential bioethanol production from wasted crops and crop residues." *Biomass Bioenerg*, vol. 26, pp. 361–375.

[17] Limayema, A. and Ricke, S. C., 2012. "Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects." *Prog. Energy Combust. Sci*, vol. 38, pp. 449–467.

[18] Sekeran, V., Balaji, C., and Bhagavathi, P. T., 2005. "Evaluation of effective microorganisms EM) in solid waste management." *Electronic Green Journal*, vol. 21, pp. 1076-7975.

[19] Uzomah, I., 2002. *Laboratory manual in food quality control*. Hamilton: CABI Publishing. p. 51.

[20] Crampton, F. E. and Mayrand, U. C., 1978. *Determination of cellulose content*. Hamilton: CABI Publishing. pp. 34-48.

[21] Sarkar, N., Ghosh, S. K., Bannerjee, S., and Aikat, K., 2012. "Bioethanol production from agricultural wastes: an overview." *Renew Energy*, vol. 37, pp. 19–27.

[22] Demirbas, M. F., Balat, M., and Balat, H., 2011. "Biowastes-to-biofuels." *Energy Conversion and Management*, vol. 52, pp. 1815-1828.

[23] Zhao, L., Wu, Q., Dai, J., Zhang, S., and Wei, F., 2007. "Evidence of cellulose metabolism by the giant panda gut microbiome." *Proc. Natl. Acad. Sci.*, vol. 108, pp. 17714–17719.

[24] Koegel, R. G., Sreenath, H. K., and Straub, R. J., 1996. "Alfalfa fiber as a feedstock for ethanol and organic acids." *Appl. Biochem. Biotechnol*, vol. 92, pp. 105–115.