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COMPARATIVE ANALYSIS AND OPTIMIZATION OF BIOETHANOL PRODUCED FROM ORANGE AND GRAPE PEELS

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ABSTRACT

This study was aimed at carrying out a comparative analysis on the amount of bioethanol produced from orange and grape peels. The samples were collected, dried, grinded and sieved to obtain particle size not reater than 0,6mm. The sieved samples were acid-hydrolyzed and fermented to produce ethanol. Design of experiment and Response Surface Method (RSM) was used to randomize the independent variable to obtain different runs conditions of acid concentration (0.5 to 1.0%v/v), and hydrolysis time (5 to 25 minutes). A total of 13 runs were carried out for orange peels and grape peels at the same conditions for both samples. At the end of experiment the quadratic model gave R-Values of (0.998 and 0.974) for orange and grape peels respectively. It was observed that optimum condition for producing ethanol was at acid concentration of 0.75%v/v and hydrolysis time of 15 minutes keeping pH and hydrolysis temperature constant at 4.5 and 120 °C respectively and the optimum amount of bioethanol obtained at these conditions were 17 wt. % and 15 wt. % for orange and grape peels respectively. There was 6.25% more bioethanol produced at optimum conditions from orange peels than grape.

1. INTRODUCTION

With the increase in the consumption of fruits and industrial production of fruit juices and other value added products from fruits such as sweet oranges (citrus sinensis), grape orange(citrus paradisi), lemon (citrus limon) and other fruits, a great problem of the disposal of the waste is faced by the industries and fruit sellers who deal with these fruits (Kaur, 2017). Wastes from citrus fruit processing industries which usually are the peels, the seeds and the pulps are always in bulk as they are wasted

on daily to yearly bases from the industries which processes them. These wastes are of adverse effects to the ecological condition of the environment and companies spend more to dispose these wastes. The global depletion of fossil fuels, rising fuel prices, environmental concerns, and pressures for oil independence, are creating a strong and fast growing market for biofuels (Gashaw, 2014).

A biofuel is a fuel that is produced through contemporary biological processes, such as agriculture and anaerobic digestion, rather than a fuel produced by geological processes such as those involved in the formation of fossil fuels, such as coal and petroleum, from prehistoric biological matter. Biofuels can be derived directly from plants, or indirectly from agricultural, commercial, domestic, and/or industrial wastes. Other renewable biofuels are made through the use or conversion of biomass (referring to recently living organisms, most often referring to plants or plant-derived materials). This biomass can be converted to convenient energy-containing substances in three different ways: thermal conversion, chemical conversion, and biochemical conversion. This biomass conversion can result in fuel in solid, liquid, or gas form. This new biomass can also be used directly for biofuels. Biofuels have the potential to be domestically and globally available for energy security, with most being carbon neutral (introducing no additional carbon to the global carbon cycle) or potentially carbon negative (if coupled with carbon sequestration) and supportable within the current agricultural infrastructure (Shilpa et al., 2013).

Increase on world's energy demand and the progressive depletion of oil reserves motivate the search for alternative energy resources, especially for those derived from renewable materials such as biomass. Global concern about climate change and the consequent need to diminish greenhouse gases emissions have encouraged the use of bioethanol as a gasoline replacement or additive (Diasa et al., 2009). Liquid bio-fuels are receiving increasing attention worldwide as a result of the growing concerns about oil security of supply and global climate change. In most developing countries like Nigeria and Brazil, the emerging bio-fuels industry is perceived as an opportunity to enhance economic growth and create or maintain jobs, particularly in rural areas. The liquid bio-fuels market is shared mainly between bio ethanol and biodiesel, with more than 85% market share for the former in 2005. The main advantage of bio ethanol is the possibility to blend it in low proportions with gasoline (5 to 25% bio-ethanol by volume) for use, without any significant change, in internal combustion engines (Osunkoya and Okwudinka, 2011).

There are limitations to efficient production of ethanol from fruit residues. These limitations include the close physical and chemical association that exists between the lignin and plant cell wall polysaccharides together with cellulose crystallinity (Singh, 2014). Lignin forms a major protective shield around the sugar which can be fermented to yield ethanol. The cellulose must be readily available for cellulosic enzyme activities. Thus by removing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into ethanol during fermentation. In other to achieve this, a pre-treatment is usually done to degrade the lignin in the peel residue thereby decrease cellulose crystallinity and increase the surface area for enzymatic activity.

2. MATERIALS AND METHODS

2.1. Material Collection

Two different citrus peels were obtained from New Benin market. The peels obtained were sweet oranges (*Citrus sinensis*) and grape orange (*Citrus paradisi*) of equal amount. The following apparatus and equipment were used for the experiment: A digital weighing balance, heating mantle,

digital pH meter, crusher, orbital shaker, hot air oven and a sieve. The following chemical reagent were also used for the experiment: 98.8% Sulphuric Acid (H_2SO_4) – used for the pretreatment and hydrolysis of fruit peels, Sodium Hydroxide (NaOH) – used to adjust the pH of soluble cellulose and hemicelluloses before fermentation, Yeast extracts (Agar) – used in media preparation, Urea – used in media preparation, Dextrose sugar – used in media preparation, $MgSO_4.7H_2O$ and Yeast (Saccharomyces cerevisiae).

2.2. Samples Preparation

The samples that were acquired and sun dried for a period of 4 days, milled and sieved to obtain particle size of not greater than 0.6 mm. The samples were kept at low temperature until the next stage of experiment.

2.3. Pretreatment

Milled and screened peels were treated inside Hot air oven, every sample was treated separately using glass vessel. Pretreatment of all samples were carried out at a temperature of 121^{o}C. First, the milled citrus fruit peels were treated in batches with every batch containing 50 g of material with 1:10 (w/v) ratio of water to the sample. The treatment was allowed to proceed for 15 minutes after which the mixture was allowed to cool before the separation of the soluble from the non-soluble portion was done.

2.4. Acid Hydrolysis

For the acid hydrolysis, 1 litre of 0.5% to 1% (v/v) dilute sulphuric acid was added to the non-soluble component from the pretreatment step and allowed to soak for 24 hours. The citrus fruit peels were hydrolyzed in the Hot air oven for 5 to 25 minutes. After hydrolysis, newtralization was done with 10M NaOH until the pH became 4.5. Solid particles were separated from the liquid in the hydrolysate by filtration (to remove the non-fermentable lignin portion). After separation, the solid part was washed with distilled water. The washing was done in other to extract all soluble sugar from the solid citrus peel materials. The soluble component was mixed with the previously filtered solution from the pretreatment steps.

2.5. Fermentation

The fermentation was carried out under anaerobic condition at ambient temperature with the help of aluminium foil, cotton wool and tapes pH 4.5 with 150 rpm stirring condition on an orbital shaker for 3 days. Before conducting fermentation, yeast media was prepared. The following nutrients in their proportion were present in the media for a 100 ml: 10g of dextrose sugar, 0.2g of yeast extract, 1.0g of urea, 100ml of distilled water and 1.0g Mg $SO_4.7$ H_2O. To the above 100 ml media, 0.5g of yeast, (*Sccharomyces cerevisiae*) (instant premium) was added in a 250 ml conical flask. The conical flasks were properly covered with cotton wool and aluminum foil secured with tapes. The conical flask was then placed on an orbital shaker for 24 hours, at ambient temperature (Room temperature) and at a speed of 150rpm. After 72 hours of fermentation, the samples were taken out and alcohol formed was determined through approximate method.

2.6. Determination of Alcohol Produced

Ethanol samples were measured for initial specific gravity and the final specific gravity using the specific gravity bottle. This involves measuring the weight of the empty specific gravity bottle at room temperature and then measuring a known mass of the sample made to the mark of the specific gravity bottle also at room temperature. The initial and final specific gravity were calculated using Equation (1).

$$\text{S. G of sample} = \frac{(W2-W1)}{(W3-W1)} \quad (1)$$

Where:
W_1 is weight in (g) of empty specific gravity bottle
W_2 is weight in (g) of specific gravity bottle + ethanol sample
W_3 is weight in (g) of specific gravity bottle + water

The percentage bioethanol was approximated using Equation (2) (Abebe et al., 2015 and Hadeel et al., 2011).

$$\text{Alcohol wt. percentage } (\%) = 126.582\frac{\text{S.G2} - \text{S.G1}}{\text{S.G1}} \quad (2)$$

SG_1 and $S.G_2$ are initial specific gravity of the ethanol solution sample before the fermentation media was added and the specific gravity of the sample after fermentation respectively.

2.7. Central Composite Design (CCD)

The central composite design (CCD) which is a tool under the Response Surface Methodology (RSM) in the Design Expert 7 was used to randomize the experimental conditions and this helped to be able to determine various conditions to carry out the experiment.

Table 1: Experimental conditions for central composite design

Variable	Symbol	Unit	-Alpha	+ Alpha
Acid concentration	A	% v/v	0.5	1
Hydrolysis time	B	min	5	25

3. RESULTS AND DISCUSSION

Equation (1) and (2) were obtained from the Design Expert software to predict the percentage alcohol that can be produced from the given experimental condition. Equation (1) represent the predicted percentage alcohol from orange peel and Equation (2) represent that of grape peel. Values obtained from the equations were compared with thoses from actual experiment.

$$\text{Ethanol yield} = -121.41 + 299.83\text{A} + 3.78\text{B} - 2.4\text{AB} - 180.44\text{A}^2 - 0.065B^2 \quad (1)$$

$$Ethanol\ yield = -125.68 + 303.54A + 3.54B - 1.70AB - 187.48A^2 - 0.072B^2 \quad (2)$$

From the Table 2, it is seen that the predicted values obtained from Equations (1) and (2) are closed to the values obtained from the experiments.

Table 2: Comparison of predicted and experimental results

Runs	Independent variables		% Ethanol (Orange peels)		% Ethanol (grape peels)	
	A (%v/v)	B (min)	Actual	Predicted	Actual	Predicted
1	0.93	7.93	10	9.47	6	5.47
2	0.75	15	16.9	16.84	15	14.28
3	0.75	15	16.5	16.84	14.9	14.28
4	1	15	3.5	3.86	2	1.77
5	0.75	25	10.5	10.44	8	8.08
6	0.75	15	17	16.84	13	14.28
7	0.57	7.93	6	5.82	4	2.29
8	0.93	22.07	4	3.54	2.5	2.58
9	0.5	15	7.5	7.27	2	3.36
10	0.75	5	10	10.19	5	6.05
11	0.75	15	16.8	16.84	14	14.28
12	0.75	15	17	16.84	14.5	14.28
13	0.57	22.07	12	12.10	9	8.05

From Figures 1 and 2, it was observed that the plots of predicted ethanol versus actual ethanol produced had the points clustering around the 45^{o} straight line which indicates that data fits into the model used. The analysis of variance results for the model used for both samples are shown in the Tables (3) and (4).

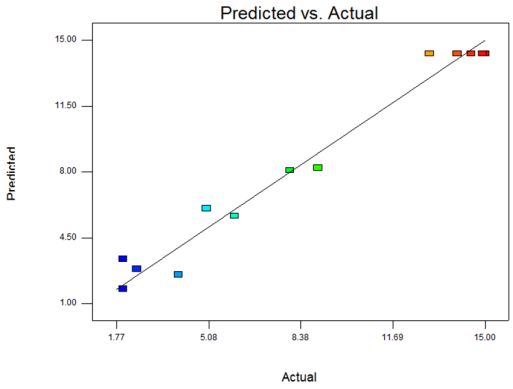

Figure 1: Predicted vs actual (orange peel)

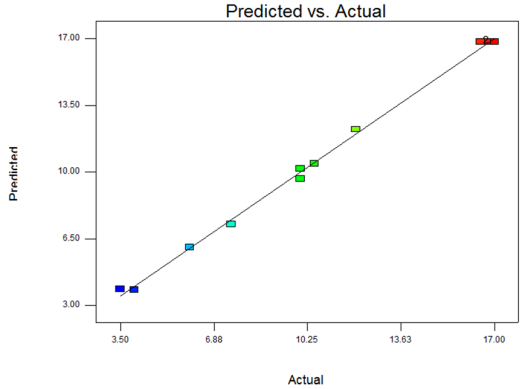

Figure 2: Predicted vs actual (grape peel)

Tables 3 and 4 show a p-value less than 0.0001 for the models chosen and lack of fit of p value 0.1347 and 0.1581 respectively for orange and grape peels which were insignificant. The implication of these details is that the model chosen was able to correctly represent the experimental data. Having a significant lack of fit is an indication that the data does not fit correctly into the model used. The data obtained were further justified with the help of the normal plot of residuals for both orange and grapes which are shown in the Figures 3 and 4. Figures 3 and 4 are an indication that data do not deviate from normality which can be seen from the way the data of the experiments clusters along the straight line of the plot. This further help to confirm how accurate the model used is.

Table 3: Analysis of variance for response surface quadratic model for orange peel

Source	Sum of squares	df	Mean square	F-Value	p-Value
Model	314.14	5	62.83	722.29	<0.0001
A-Acid conc.	11.66	1	11.66	134.01	<0.0001
B-Hydrolysis time	0.063	1	0.063	0.72	0.4247
AB	36.00	1	36.00	413.86	<0.0001
A^2	221.19	1	221.19	2542.80	<0.0001
B^2	74.10	1	74.10	851.89	<0.0001
Residual	0.61	7	0.087		
Lack of fit	0.44	3	0.15	3.39	0.1347
Pure error	0.17	4	0.043		
Cor. Total	314.75	12			

Table 4: Analysis of variance for response surface quadratic model for grape peel

Source	Sum of squares	df	Mean square	F-Value	p-Value
Model	320.76	5	64.15	51.79	<0.0001
A-Acid conc.	2.53	1	2.53	2.04	0.1959
B-Hydrolysis time	4.12	1	4.12	3.33	0.1109
AB	18.06	1	18.06	14.58	0.0066
A^2	238.78	1	238.78	192.77	<0.0001
B^2	90.60	1	90.60	73.14	<0.0001
Residual	8.67	7	1.24		
Lack of fit	6.00	3	2.00	3.00	0.1581
Pure error	2.67	4	0.67		
Cor. Total	329.43	12			

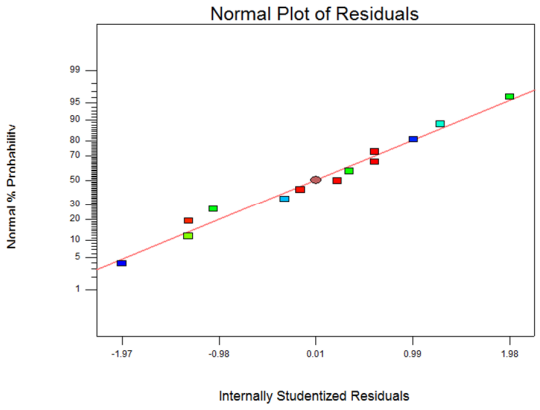

Figure 3: Normal plot of residuals (orange peel)

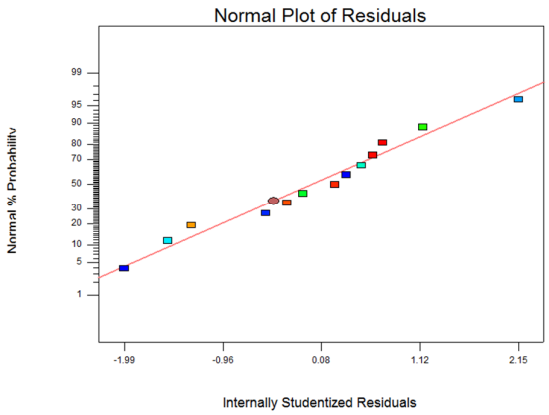

Figure 4: Normal plot of residual (grape peel)

The other statistical obtained from the RSM software for both samples are shown in Table 5. The fitness of the model depends on the coefficient of determination R^2 value. For a good model analysis the R^2 value should not be less than 0.8 and if possible close to 0.9. For this experiment the R^2 values for orange and grape peels were 0.998 and 0.974 respectively. The adjusted R-value for both samples were 0.997 and 0.955 respectively. The adjusted R^2 value helps to check the R^2 value and as such they should be relatively close to each other. It is always better to look at the Adj R value compared to the R^2 value as this is what helps to penalize the values obtained and model used. The "Pred R-Squared"

of 0.858 is in reasonable agreement with the "Adj R-Squared" of 0.955 for grape peels while that of orange peel has "Pred R-Squared" of 0.989 which is in reasonable agreement with the "Adj R-Squared" of 0.997.

Table 5: Statistical information

Parameter	Value	
	Orange peel	grape peel
R-Squared	0.998	0.974
Adj R-Squared	0.997	0.955
Pred R-Squared	0.989	0.858
Adeq Precision	64.987	16.549

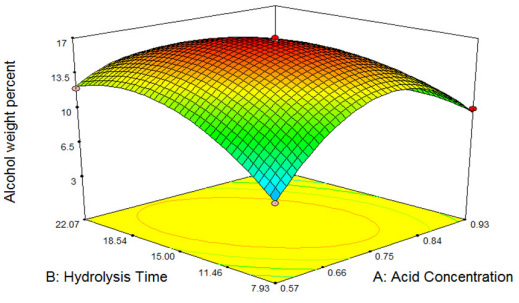

Figure 5: Combine effect of hydrolysis time and acid concentration on amount of bioethanol produced for orange peel

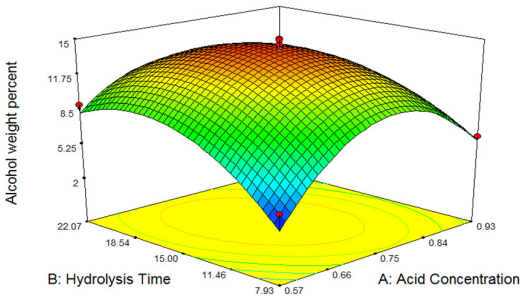

Figure 6: Combine effect of hydrolysis time and acid concentration on amount of bioethanol produced for grape peel

From Figures 5 and 6, it can be seen that at a constant hydrolysis temperature and pH of 120^{o}C and 4.5 respectively, there was a gradual increase in the amount of bioethanol from 7.5 wt.% to 17 wt.% for orange peel while that of grape increased from 2 wt.% to 15wt.% with varying hydrolysis time and acid concentration. It was also observed that as acid concentration gradually increase from 0.5%v/v there was a corresponding increase in alcohol produced up to 0.75%v/v and at acid concentration above 0.75%v/v a decrease in the ethanol produced was observed. Also it can be seen that as the hydrolysis time increases from 7.93 minutes to 15 minutes there is a gradual increase in the amount of bioethanol produced and time after 15 minutes ethanol production reduced. While we can see that the maximum production of alcohol from Figure 5 is 17 wt%, Figure 6 reveals a value of 15% at the same experimental conditions. It was observed that optimum conditions for producing bioethanol were at an acid concentration of 0.75%v/v and hydrolysis time of 15 minutes.

4. CONCLUSION

The effect of hydrolysis time and acid concentration on the amount of bioethanol produced was evaluated using RSM approach. It was observed that the amount of ethanol increases gradually from an acid concentration of 0.5%v/v to 0.75%v/v and time of 7.93 minutes to 15minutes. Conditions outside that resulted in a decrease in the amount of bioethanol produced. From the experimental analysis it was observed that the maximum amount of bioethanol was observed at acid concentration of 0.75%v/v and time of 15minutes. While the maximum attainable bioethanol from orange peel was 17 wt. % that of grape peels was at 15 wt. % when pH and hydrolysis temperature were kept constant

at 4.5 and 120ºC respectively as fermentation were carried out under room temperature. The values obtained gives orange peel about 6.25% more yield of bioethanol than grape peel at optimum conditions with a total of 14.674% more yield of bioethanol from orange peels than grapes for 13 runs at the same condition.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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