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ASSESSMENT OF AFRICAN STAR APPLE (*CHRYSOPHYLLUM ALBIDUM*) FRUIT PULP AS A POTENTIAL FEEDSTOCK FOR BIOETHANOL PRODUCTION

***Ogbodo, N. J.[1], Esonye, C.[1] and Omotioma, M.[2]**
1 Department of Chemical Engineering, Alex Ekwueme Federal University,
Ndufu-Alike (AE-FUNAI), Ebonyi State, Nigeria.
2 Department of Chemical Engineering, Enugu State University of Science and Technology (ESUT),
Enugu State, Nigeria

Corresponding Author: nkirukaogbodo@yahoo.com

ABSTRACT

This study focused on production of bioethanol from ripped African star apple (Chrysophyllum albidum) pulp through hydrolysis and fermentation processes. The present work determines the potentials of African Star apple fruit pulp as a precursor for bioethanol production. The pre-treated African star apple fruit pulp was characterized by proximate analysis and Fourier transform infrared (FTIR) spectroscopic analysis. Diluted sulphuric acid was used to hydrolyse the dried pulp and the corresponding substrate was fermented under various conditions. Yeast was added which provided enzymes for the conversion of the glucose into ethanol. Effects of pH, yeast dosage, incubation temperature and fermentation time on the ethanol yield were determined. The fermentation process was optimized using Response Surface Methodology. The analysis showed that African star apple fruit pulp contains 54.97% carbohydrate. Predominant functional groups of the sample include; N-H stretch, O-H stretch, C≡C stretch, C-H out of plane, C=O stretch, N-H deforming, S=O stretch, N=O stretch and C-F stretch. The optimum ethanol yield of 9.89g/cm^3 was obtained at pH of 5, yeast dosage of 4.5 %wt/v, incubation temperature of 35^{o}C and fermentation time of 72 hrs. A quadratic model adequately described the relationship between the ethanol yield and the production process parameters.

***Keywords**: Bioethanol, African star apple, Chrysophyllum albidum hydrolysis, fermentation*

1.0 INTRODUCTION

Exploration for alternative source of energy is on the increase with the intention to overcome the challenges of over dependent on fossil fuel. It has been established that one of the ways to reduce energy insecurity and environmental pressure is by diversifying energy sources, hence research efforts are being made to generate biofuels from biomass. Biomass-based fuels (biofuels) are eco-friendly fuels derived from plant biomass and they are seen as a potential way to reduce energy insecurity and environmental pressure as well as diversifying transportation energy sources (Vertes et al., 2009; Kuhad et al., 2011). In recent times, bioethanol has recently attracted enormous attention in different countries throughout the world as the most promising biofuel for transportation, hence increasing research and development efforts have been conducted on the commercial production of bioethanol due to the global demand to reduce oil importation, thereby contributing towards boosting rural economies and improving air quality (Demirbas and Karslioglu, 2007). Accordingly, recent research works are concentrated on renewable energy from crops with lignocellulosic residues for bioethanol production (*Naik* et al., 2010; Oiwoh et al., 2018) because their availability and easy conversion into bioenergy is feasible without high capital investments.

Bioethanol(ethanol) is a biofuel made by microbial fermentation, mostly from carbohydrates rich in sugar or starch-bearing plants such as corn, sugarcane, sweet sorghum or lignocellulosic biomass and more recently from microalgae (Anyanwu et al., 2022). It is biodegradable, less toxic and oxygenated (35% oxygen). Thus, it provides the possibility of reducing automobile greenhouse gas emissions (generated in fossil fuel combustion) and lessening dependence on petrol diesel (Hahn-Hagerda et al., 2006; Farrell et al., 2006; *Zhang and Smith, 2007*; Ibeto et al., 2011; Ye et al., 2017). Numerous biomass resources have been investigated for bioethanol production such as: sugar juice, starchy crops, and lingocellulosic biomass (Balat, 2009; *Randor et al., 2010;* Summoogum et al., 2015; Silva et al., 2017). They

are broadly classified into: a) first generation, which involves the use of sugarcane, sugar beet, wheat, fruits, corn, potato, rice, sweet potato or barley (Cheng et al., 2007; Abdulkareem et al., 2015; Elemike et al., 2015); b) second generation, which involves lingo-cellulosic biomass (Segui et al., 2018); c) third generation comes from micro and micro algae biomass (Kumar et al,, 2018).

Bioethanol production involves a fermentation process that transforms carbohydrates into ethanol and among the second generation bioenergy crops used for bioethanol production, agricultural wastes and over ripped fruits such as African star apple (*Chrysophyllum albidum*) comprise up to 50% of agricultural production (Lafond, 2009), Citrus processing industry uses mainly citrus peel as their major solid by-product which comprises around 50% of the fresh fruit weight and can be utilized as substrate for bioethanol production (Alemayehu, 2014). Ethanol as a grain alcohol can be blended with gasoline and used in regular motor vehicles at a concentration of up to 10 percent. Gasoline- ethanol blended fuel reduces production energy and total greenhouse gas emission hence total hydrocarbon and nitrogen oxide emissions are decreased up to 34.5% - 35.6% with increased oxygenated fuel. This is one of the best tools to fight vehicular pollution, as it contains 35% oxygen that helps in the complete combustion of fuel which reduces harmful tailpipe emissions (Lee & Park, 2020; Khan, 2013).

Previously, production of bioethanol from some agro-based products such as African star apple (*Chrysophyllum albidum*) has not been fully explored. African star apple is from the *Sapotaceae* family, an order of *Ericales* (Adekanmi and Olowofoyeku, 2020). The family comprises seventy (70) genera and eight hundred (800) species. Nigeria has seven of the species which includes *albidum* and *africanum* which are widely found within the country (Omotioma et al., 2013; Bello et al., 2015). In Nigeria, the common name for African star apple includes *cherry, agbalumo* (Yoruba), *udara* (Ibo) and *ehya* (Igala) (Akubor et al., 2013). The ripe fruits are mostly available in the market between the periods of December and April every year. Amusa et al., (2003) reported that the deterioration of fruit pulp begins after about five days under ambient conditions and this leads to loss of huge amount of the fruits yearly during gathering, transportation and storage. Significantly, different parts of the *Chrysophyllum albidum* tree serve a number of important uses; the wood can be used to produce charcoal, the seed oil has proven to be a good precursor for industrial biodiesel production (Onukwuli et al., 2021), its pulp contains high amount of sugar that can be valorized for the production of a product with high added value such as bioethanol and the leaves are used for medicinal purpose (Odugbemi et al., 2007), hence the improved variety can strive in any agricultural sector if properly harnessed. Despite its multipurpose character, this species remains underutilized in the country and consequently, there is need to use over-ripen African star apple as raw materials for fermentation, enzymatic hydrolysis using microbial enzymes which could be a possible solution to reduce the input costs in bioethanol production (Hammond, et al., 1996). Mostly, for the production of bioethanol in commercial quantities, baker's yeast (*Saccharomyces cerevisiae*) can be used because of its high ethanol tolerance, rapid fermentation rates, insensitivity to temperature and substrate concentration (Linden, T & Hahn-Hägerdal, 1989).

Previously, Ureigho, (2010), Okwu *et al. (*2018) studied the organoleptic variation of species of African Star Apple fruits in contrast with their proximate parameters. From their research, the fruits have mean values of 73.33, 2.64, 3.61 and 16.99% for the moisture, ash, fibre and protein contents respectively. The seed shell pericarp has higher crude protein of (8.66%) and fat (8.07%) content while the fruit pulp has more carbohydrates (66.45%) and the fruit skin is copious in crude fibre (15.57%) (Aboaba & Olide, 2006; Ibrahim *et al.*, 2017). They also confirmed that the pulp was abundant in protein and carbohydrate content (4.1 and 60.1%). Ylitervo, (2008) used dried orange peels to produce bioethanol by enzymatic hydrolysis with the application of the *Mucor Indicus* fungus, hydrolyzing and converting into sugars, in the bioethanol production by two fermentable sugar process, and obtained a yield of 0.36g/g after 24 hrs. Azevedo, (2007) evaluated the production of bioethanol from the persimmon juice and observed that the factors of the initial concentration of inoculum, soluble solids and initial pH did not influence the alcoholic fermentation of the organic matter. Lima, (2015) in his study presented in terms of efficiency and yield of the process of obtaining cellulosic ethanol by means of alcoholic fermentation of the hydrolyzed liquors with the use of the yeast *Saccharomyces cerevisiae*. These authors obtained maximum yield and efficiency values of 0.445g of ethanol/g of bagasse and 87.1% for the hydrolyzed liquor with the addition of cashew juice. Bello & Henry, (2015) studied the effects of storage on the proximate composition of *Chrysophyllum albidum* under ambient conditions and reported an increase in moisture content and crude fibre in the pulp after 5 days from 36.45 to 46.69% and 2.54 to 2.89% respectively. This result

validates the findings of (Adeboyejo *et al.*, 2019) and suggest that fruits stored at ambient conditions for long loose nutritive value and become softer increasing their susceptibility to deterioration.

In order to harness and add more values to the sugar content of the over ripen African star apple fruit pulp, this research work focused on assessing the potentials of the fruit pulp as a second-generation bioethanol production so as to improve its valorization and evaluate it as a precursor for industrial production of bioethanol. For the possibility of maximizing bioethanol yield and minimizing the cost of bioethanol production, this work further optimized the process parameters such as pH, incubation temperature, fermentation time and yeast dosage. It is important to note that if proper attention is given to the use of African star apple as an alternate biofuel feedstock; it will become economically valuable, the greenhouse gas emissions in the environment and scarcity of fuel will be reduced drastically.

2.0 MATERIALS AND METHODS

2.1 Materials

The materials used in the fermentation of bioethanol include Dried African Star Apple fruit pulp, dilute Sulphuric acid (H_2SO_4), Hydrochloric acid (HCl), Baker's yeast (*Saccharomyces cerevisiae)*, sodium hydroxide. The chemicals used in this study were purchased from a local chemical shop in Enugu, Nigeria and were used without any pre-treatment.

2.2 Sample collection and preparation

The method employed by (Onukwuli et al, 2021) was used for the sample preparation. The over ripped African star apple were collected from local market in Ugwogo Nike, Enugu state of Nigeria and were washed to remove dirt and sand, the fruit pulps were carefully peeled to remove the seeds. The pulps were cut and chopped into the small slice. The pulps were air dried for seven (7) days to reduce the moisture content and to promote effective grinding with electric grinding machine into smaller particles before being sieved to obtain a finer particle size of 400µm. The residual moisture in the sieved grounded fruit pulp was removed by further sun drying for a period of five (5) days and stored in an air tight container until use.



Figure 1a: African Star Apple Fruit pulp and seed.



Figure 1b: African Star Apple Tree.

2.2.1 Determination of Moisture Content

Association of Official Analytical Chemist (AOAC method, 1990) was used for the proximate analysis of

the sample. For determination of moisture content, 5g of the sample was placed into weighed crucibles and then put inside the oven set at 105^{o}C for 4 hrs. The sample was removed from the oven, cooled and weighed. The drying continued until a constant weight was noticed. Then, the moisture content was calculated using Equation (1).

$$\% \text{ moisture} = \frac{A-B}{A} \times \frac{100}{1} \quad (1)$$

where A is the original weight of sample while B is the weight of dried sample.

Standard method (Kjeldahl method) was used in determination of the crude protein content. This method involves the estimation of the total nitrogen in the waste and the conversion of the nitrogen to protein with the assumption that all the protein in the sample is present as nitrogen. Using a conversion factor of 6.25, the actual percentage of protein in the sample was calculated using

% crude protein = % Nitrogen x 6.25

Titration was carried out using 0.01M standard HCl to first pink colour.

$$\% \text{ Nitrogen} = \frac{Titration\ vol. \times 0.014 \times M \times 100x\ 100}{wt.ofsample \quad 10} \quad (2)$$

where M is the molarity of standard HCl.

2.2.2 Determination of Crude Fibre Content

5g of the sample was weighed into a 500ml beaker and 150ml of preheated 0.128M H_2SO_4 was added to it. It was heated at 70°C for 30 minutes, filtered and washed with hot distilled water. The residue was then transferred to a beaker and was place in a heating mantle at 70°C for 30 minutes with 150ml of preheated 0.223M KOH. It was filtered and washed with hot water until the washings are no longer alkaline. The residue was washed three times with acetone and dried in an oven at 105°C for 1.5 hrs. It was then cooled in a desiccator, weighed and heated in a muffle furnace at 500°C for 4 hours. The ash obtained was cooled in a desiccator and weighed.

$$\% \text{ Crude fibre} = \frac{W_2 - W_3}{W_1} \times \frac{100}{1} \quad (3)$$

where W_1 is the weight of sample, W_2 is the weight of dry residue and W_3 is the Weight of ash.

For the determination of fat content, 5g of the ground sample was transferred into a rolled filter paper and then placed inside the extraction thimble. The thimble was placed inside the extractor. 180 ml of petroleum ether was poured inside the extraction flask. The condenser and the flask were connected to the extractor. The whole unit was placed on a heating mantle for 3 hrs after which the petroleum ether was recovered. The oil collected in the flask was dried in an oven at 105°C. It was then weighed and the percentage fat calculated using Equation (4):

$$\% \text{ fat} = \frac{C - A}{B} \times \frac{100}{1} \quad (4)$$

C is weight of flask + oil, A is weight of empty flask and B is weight of original sample.

In the determination of ash content of the sample, 5 g of the finely ground sample was weighed into porcelain crucibles which have been washed, dried in an oven at 100°C, cooled in a desiccator and weighed. It was then placed inside a muffle furnace and heated at 600°C for 3 hrs. It was then removed and cooled in a desiccator and weighed. The ash content was determined using Equation (5):

$$\% \text{ Ash} = \frac{A - B}{C} \times \frac{100}{1} \quad (5)$$

where A is the weight of crucible + ash, B is weight of crucible and C is the weight of original sample.

Total carbohydrate content was calculated Equation (6):

Total carbohydrate =100- (protein + fat + crude fiber +ash + water) (6)

2.3 Determination of the functional groups

Fourier transform infrared (FTIR) spectrophotometer (Cary 630, Agilent Technologies USA) was used to determine the functional groups of the African star apple fruit pulp. FTIR spectra were collected in transmission mode using the Br pellet method. In the process, Fourier transform converted raw data into actual spectrum (with various peaks). Analysis of the FTIR produced peaks were carried out so as to identify the appropriate functional groups (Onukwuli and Omotioma, 2016).

2.4 Experimental Design and Statistical Analysis

A standard response surface methodology (RSM) design using central composite design (CCD) was applied to develop the standard combinations of the process conditions for maximum yield of bioethanol from the African star apple fruit pulp. The three levels, four factors process conditions used for the optimisation are as follows:− Ph level: 4.0, 5.0 & 6.0; Incubation temperature, °C: 25, 35 & 45; Fermentation time, hrs: 24, 72 & 120; Yeast dosage, %w/v: 3.5, 4.5 & 5.5.

2.5 Bioethanol production and Characterization

Bioethanol was produced from African star apple fruit pulp by acid hydrolysis and enzymatic fermentation process. 15g of dry sample of African star apple fruit pulp was weighed into 250 ml conical flask. 200ml of 5% dilute sulphuric acid (H_2SO_4) was used to hydrolyse the samples. The flask was covered with cotton wool and wrapped with aluminium foil, and then heated at 50°C for

30 minutes. The sample was allowed to cool and filtered using whatman 42 filter paper. The pH value of the sample was adjusted with sodium hydroxide before addition of yeast to the hydrolysed sample. Baker's yeast (*Saccharomyces cerevisiae*) dosage was added to the flask containing the sample and stirred thoroughly. The substrate was fermented under various conditions. Effects of pH, yeast dosage, incubation temperature and fermentation time on the ethanol yield were determined. The added yeast provided enzymes (invertaze and zymaze) for the conversion of the sample into ethanol and carbon dioxide. The bioethanol yield was optimized using response surface methodology (RSM). In designing the experiment, central composite design tool of design expert software as used by Omotioma and Onukwuli (2015) was employed. At the end of fermentation, the produced bioethanol was distilled out at 78 ºC. The bioethanol was characterized to ascertain its properties in terms of specific gravity, flash and smoke points, viscosity, refractive index, cloud and pour points and sulphur.

3.0 RESULTS AND DISCUSSIONS

3.1 Characterisation of the Samples

3.1.1 Proximate Analysis

Results of proximate analysis of the sample are presented in Table 1. For the analysis, 20g of star apple fruit pulp was used. The moisture content of the dried African star apple fruit pulp was within the acceptable limit of not more than 10% for long term storage of flour (Akubor et al, 2013; Onimawo and Akubor, 2012). The low moisture content would enhance its storage stability by preventing mould growth and reducing moisture dependent biochemical reactions (Onimawo and Aklubor, 2012). It contains 54.97% carbohydrate which is close to the value of 60.01% obtained by (Asare et al, 2015). Crude fibre content of 6.03 is higher than that recorded by Bello et al (2015). Ash content of 5.12% was recorded, which indicates high mineral content of the African star apple fruit pulp.

Table 1. Proximate analysis of African star apple fruit pulp.

Composition (%)	Values
Ash	5.12
Crude fat	15.43
Crude fibre	6.03
Moisture content	7.85
Protein	10.6
Carbohydrate	54.97

3.2 Fourier transform infrared (FTIR) spectroscopy

The functional groups present in the sample of African star apple as determined by the use of FTIR spectrometer and the spectrum of the sample as determined by Fourier transform infrared spectroscopy is presented in Figure 1. The peaks as represented in brackets shows the functional groups of the sample (Onukwuli and Omotioma, 2016). The predominant functional groups of the African star apple fruit pulp as shown in Figure 1 confirms the presence of N-H (3295.0) stretch, O-H(2922.2) stretch, C≡C(2109.7) stretch, C-H(1990.4) out of plane, C=O(1729.5)stretch, N-H(1617.7) deforming, S=O(1375.4) stretch, N=O((1244.9) stretch and C-F(1025.0) stretch. Presence of the heteroatoms portrays that the African star apple fruit pulp is a potential source of bioethanol.

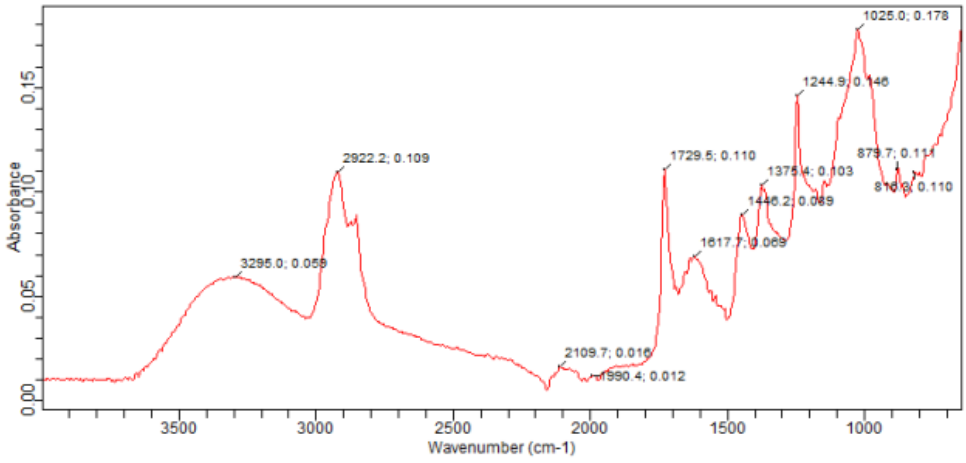


Figure 1: Spectrum of the functional groups of African star apple pulp

3.3 Characteristics of the bioethanol

Experimental values of various properties of produced bioethanol according to American Society for Testing and Materials standards (ASTM) methods using standard apparatus for the measurement are shown in Table 2.

The refractive index of the bioethanol produced indicates that the refractive index of the produced ethanol is 1.352 which is a little lower than the set limit of 1.36. The essence of measuring the refractive index of fuels is to verify the purity of the fuels, it can be deduced from the results obtained that the ethanol produced is pure bioethanol. Flash point is described as the lowest temperature at which the ethanol will ignite when exposed to an ignition source. The flash point of the produced bioethanol is 15.30. It is a bit lower compared to the set limit of 18.60, which implies that the bioethanol produced is within the standard bioethanol flammability. Also, ash content of bioethanol produced of 0.2% is within the acceptable limit. Fuels with high sulphur content impact negatively on environment. The bioethanol has sulphur content of 0.01%, which makes the bioethanol suitable for various applications. The specific gravity obtained was 0.858 which corroborates the results obtained in literature for bioethanol produced from fruits.

Table 2. Properties of ethanol produced from African star apple fruit pulp.

Parameters	Values
Refractive index	1.352
Specific gravity	0.858
Flash point (ºC)	15.30
Viscosity (mPas)	1.71
Sulphur content (%)	0.01
Ash content (%)	0.10
Cloud point (ºC)	19
Pour point (ºC)	4.82

3.4 Result of the Response Surface Methodology

The Interactive effects of process variables; pH, yeast dosage, incubation temperature and fermentation time on the ethanol yield are presented in Table 3. Highest value of the ethanol yield was recorded at the mid-points of the process variables. Maximum bioethanol yield occurred at the mid-points of the variables. This is an indication that the interactive effect of the variables on the ethanol yield is in parabolic form (Tripathi, 2018). Further analyses of the data are required for proper interpretation of the data. Such analysis include; analysis of variance, mathematical modeling, graphical analysis and optimization. Ethanol yield of 10.14 g/cm^2 was obtained from African star apple at pH of 5, yeast dosage of 4.5 %wt/v, temperature of 35ºC and fermentation time of 72 hrs.

Table 3. Interactive effects of process variables on the ethanol yield.

Std	Run	Factor 1 A: pH	Factor 2 B: Yeast dosage %wt/v	Factor 3 C: Incubation Temp. ºC	Factor 4 D: Fermentation Time Hr.	Response 1 Ethanol Yield g/cm^3
29	1	5	4.5	35	72	10.14
14	2	6	3.5	45	120	5.71
28	3	5	4.5	35	72	10.14
1	4	4	3.5	25	24	1.75
3	5	4	5.5	25	24	2.34
18	6	6	4.5	35	72	9.53
24	7	5	4.5	35	120	9.92
26	8	5	4.5	35	72	10.14
7	9	4	5.5	45	24	2.64
21	10	5	4.5	25	72	8.93
12	11	6	5.5	25	120	8.95
2	12	6	3.5	25	24	3.32

5	13	4	3.5	45	24	1.78
16	14	6	5.5	45	120	9.93
4	15	6	5.5	25	24	5.86
11	16	4	5.5	25	120	3.09
10	17	6	3.5	25	120	4.61
22	18	5	4.5	45	72	9.86
6	19	6	3.5	45	24	4.21
19	20	5	3.5	35	72	5.74
8	21	6	5.5	45	24	6.63
25	22	5	4.5	35	72	10.14
20	23	5	5.5	35	72	9.63
23	24	5	4.5	35	24	7.41
30	25	5	4.5	35	72	10.14
17	26	4	4.5	35	72	4.96
13	27	4	3.5	45	120	2.19
9	28	4	3.5	25	120	2.55
27	29	5	4.5	35	72	10.14
15	30	4	5.5	45	120	4.25

3.4.1 Fit summary of the model of the ethanol yield

Fit summary of the model of the ethanol yield is presented in Table 4. The experimental data was tested with linear, 2 factor indicators (2FI), quadratic and cubic models. Quadratic model is suggested as best fitted model because the predicted R^2 of 0.9366 is in reasonable agreement with the adjusted R^2 of 0.9748. The difference is less than critical value of 0.2 which is within the acceptable R^2 range for a suitable model.

Table 4. Fit Summary of the Model of the Ethanol Yield.

Source	Sequential p-value	Adjusted R^2	Predicted R^2	Remarks
Linear	**0.0280**	**0.2373**	**0.0661**	
2FI	**0.9834**	**0.0459**	**-1.3251**	
Quadratic	**< 0.0001**	**0.9748**	**0.9366**	**Suggested**
Cubic	**0.1220**	**0.9860**	**0.5571**	**Aliased**

3.4.2 Analysis of Variance (ANOVA) of the Quadratic Model of Ethanol yield

ANOVA of the quadratic model of the ethanol yield is presented in Table 5. The model F-value of 81.26 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant (Onukwuli and Omotioma, 2016). In this case A, B, C, D, AB, AD, BD, A^2, B^2, D^2 are significant model terms. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 23.451 indicates an adequate signal. This model can be used to navigate the design space.

Table 5. Analysis of Variance of the Model

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	**292.86**	**14**	**20.92**	**81.26**	**<0.0001**	**Significant**
A-Ph	**61.24**	**1**	**61.24**	**237.87**	**<0.0001**	
B-Yeast dosage	**25.59**	**1**	**25.59**	**99.39**	**<0.0001**	
C-Incubation temperature	**1.87**	**1**	**1.87**	**7.26**	**0.0166**	
D-Fermentation time	**12.94**	**1**	**12.94**	**50.26**	**< 0.0001**	
AB	**5.61**	**1**	**5.61**	**21.77**	**0.0003**	

AD	**1.97**	**1**	**1.97**	**7.64**	**0.0145**
BC	**0.1502**	**1**	**0.1502**	**0.5833**	**0.4569**
BD	**1.41**	**1**	**1.41**	**5.48**	**0.0335**
A^2	**14.82**	**1**	**14.82**	**57.57**	**<0.0001**
B^2	**9.87**	**1**	**9.87**	**38.34**	**<0.0001**
C^2	**0.1513**	**1**	**0.1513**	**0.5878**	**0.4552**
D^2	**2.45**	**1**	**2.45**	**9.50**	**0.0076**
Residual	**3.86**	**15**	**0.2574**		
Lack of Fit	**3.86**	**10**	**0.3861**		
Pure Error	**0.0000**	**5**	**0.0000**		
Cor Total	**296.72**	**29**			
Std. Dev.	**0.5074**	**-**	**R^2**		**0.9870**
Mean	**6.55**	**-**	**Adjusted R^2**		**0.9748**
C.V. %	**7.74**	**-**	**Predicted R^2**		**0.9366**
			Adeq Precision		**23.4507**

3.4.3 Mathematical model of the yield

Model of the ethanol yield (Y) in terms of coded and actual factors are expressed in Figures (7) and (8) respectively. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients (Omotioma and Onukwuli, 2019). The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor.

$$Y = +9.89 + 1.84A + 1.19B + 0.3222C + 0.8478D + 0.5919AB + 0.3506AD + 0.2969BD - 2.39A^2 - 1.95B^2 - 0.2417C^2 - 0.9717D^2 \quad (7)$$

$$Y = -88.93464 + 22.00080pH + 15.01347Yeast\ dosage + 0.067889Incubation\ temperature + 0.009980Fermentation\ time + 0.591875pH * Yeast\ dosage + 0.007305pH * Fermentation\ time + 0.006185Yeast\ dosage * Fermentation\ time - 2.39167pH^2 - 1.95167Yeast\ dosage^2 - 0.002417Incubation\ temperature^2 - 0.000422Fermentation\ time^2 \quad (8)$$

3.4.4 Graphical RSM result

Predicted versus actual ethanol yield is displayed in Figure 2. The points clustered along the line of best fit, showing that the generated model adequately predicted the experimental data. This assertion is in agreement with the report of Oiwoh et al (2018). 3-D plots of the interactive effects of the variables on the ethanol yield are shown in Figures 3 -8. Each plot showed a parabolic curve, which is typical of quadratic model. Also, optimum ethanol yield of 9.89 g/cm^3 was obtained at pH of 5, yeast dosage of 4.5 %wt/v, incubation temperature of 35^{o}C and fermentation time of 72 hrs. This value competes favourably with the report of Oiwoh et al (2018).

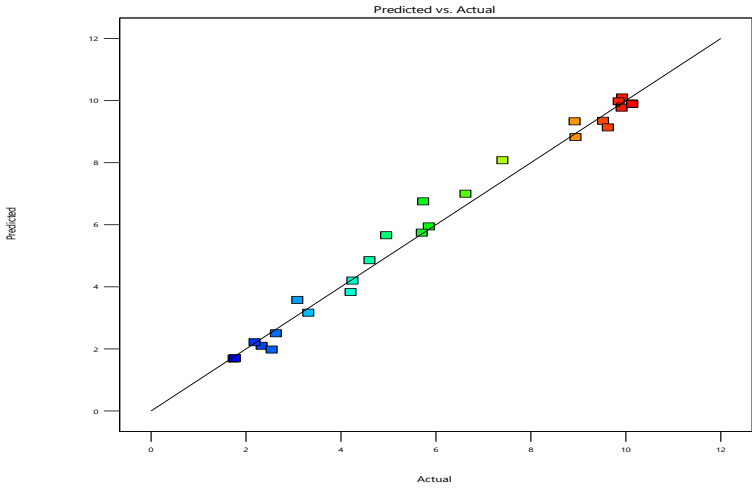


Figure 2: Predicted versus actual ethanol yield

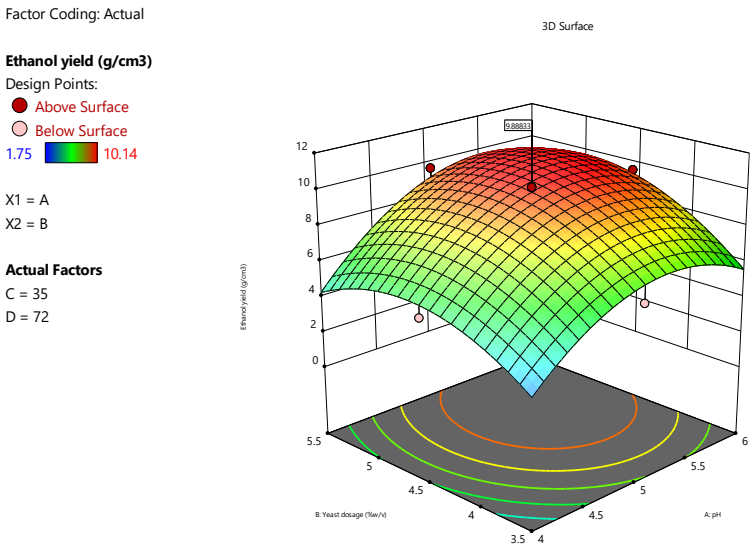
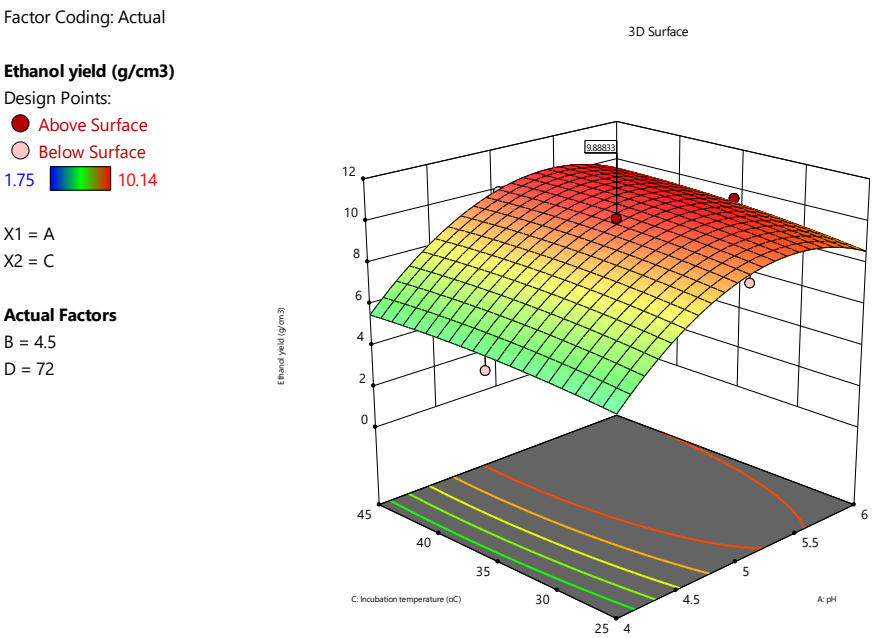


Figure 3: Ethanol yield versus pH and yeast dosage



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Figure 4: Ethanol yield versus pH and incubation temperature

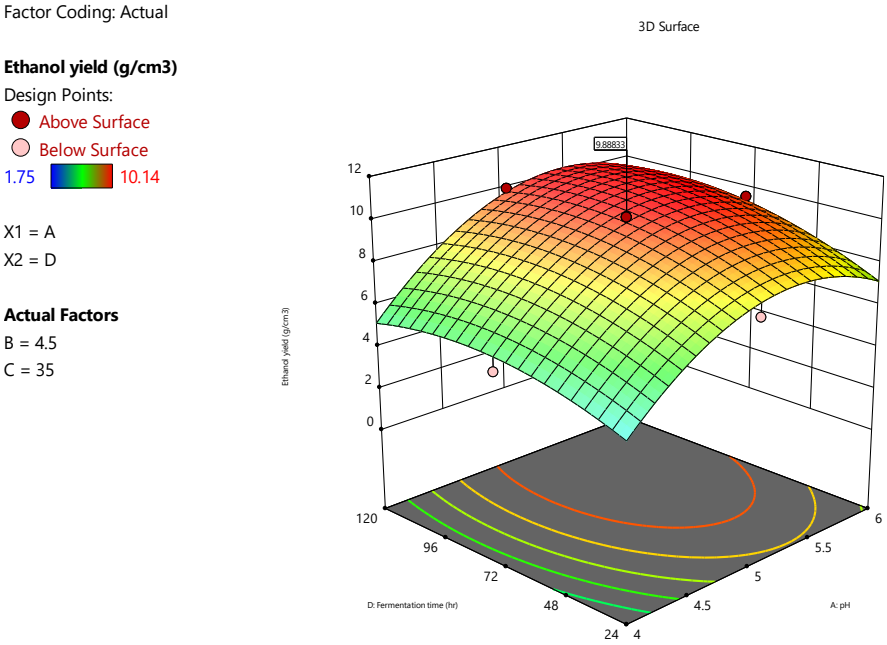


Figure 5: Ethanol yield versus pH and fermentation time

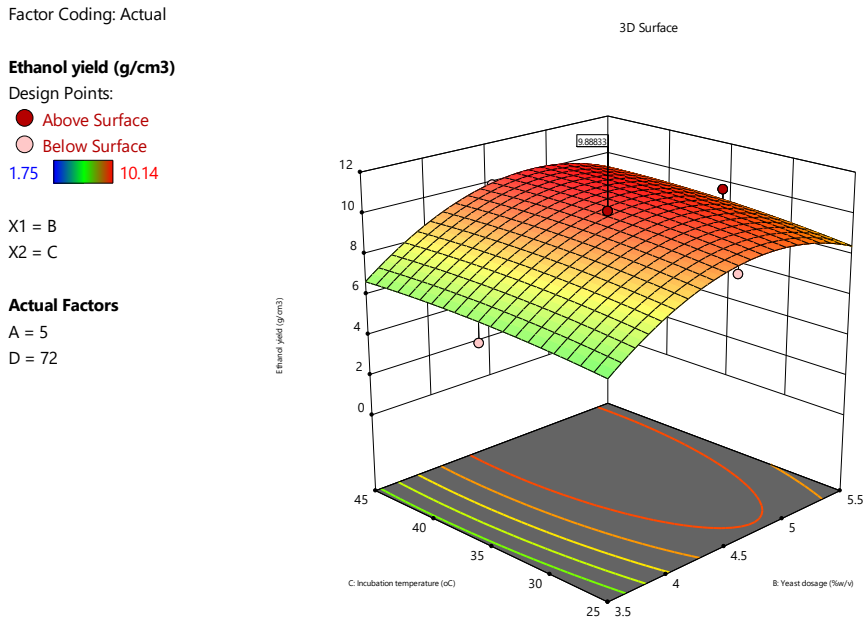


Figure 6: Ethanol yield versus yeast dosage and incubation temperature

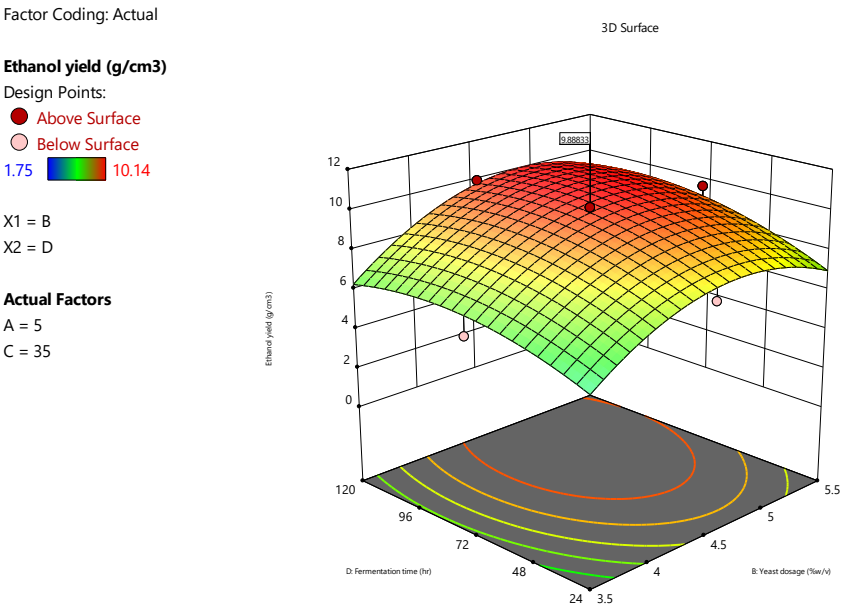


Figure 7: Ethanol yield versus yeast dosage and fermentation time

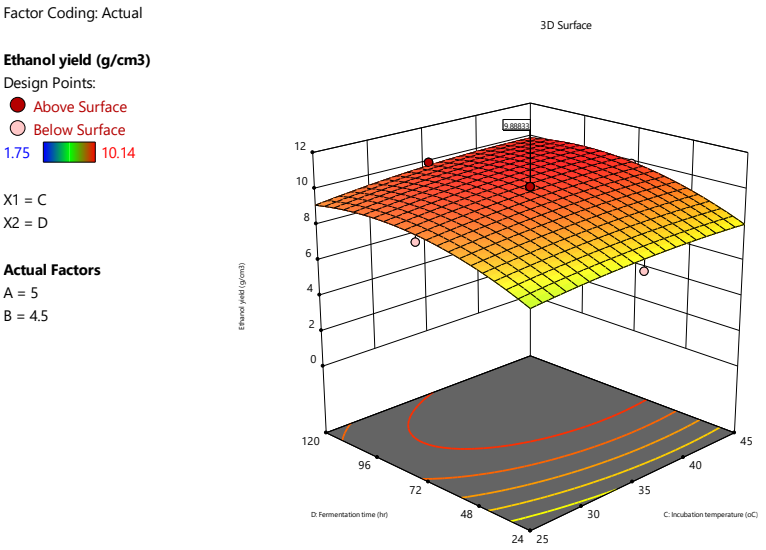


Figure 8: Ethanol yield versus incubation temperature and fermentation time

3.4.5 Validation of the optimum result

Validation result of the optimum ethanol yield is presented in Table 6. Optimum ethanol yield was compared with that of experimental ethanol yield using percentage deviation. The determined percentage deviation of 1.59% is less than 5%, which confirmed that the model adequately predicted the experimental data.

Table 6.

Optimum parameters and the validation.

Optimum pH	Optimum yeast dosage	Optimum incubation temperature (ºC)	Fermentation time (hr)	Optimum ethanol yield% g/cm3	Experimental ethanol yield% g/cm3	Percentage deviation (%)
5	4.5	35	72	9.89	10.05	1.59

4.0 CONCLUSION

Despite the values of the African Star Apple revealed in the literatures, it is yet to find prominence in industrial application due to lack of experimental and statistical

analysis of the sugar content and its application in bioethanol production. From this analysis, it was observed that African star apple contains 54.97% carbohydrate and predominant functional groups of the sample include; N-H stretch, O-H stretch, C≡C stretch, C-H out of plane, C=O stretch, N-H deforming, S=O stretch, N=O stretch and C-F stretch. The presence of the heteroatoms in the structure of African star apple confirms that it is a potential feedstock for bioethanol production. The developed quadratic model adequately described the relationship between ethanol yield and the considered factors of pH, yeast dosage, incubation temperature and fermentation time. The optimum ethanol yield of 9.89 g/cm^3 was obtained at pH of 5, yeast dosage of 4.5 %wt/v, incubation temperature of 35^{o}C and fermentation time of 72 hrs. This study is the first to characterize star apple fruit pulp and to determine its total sugar content for bioethanol production.

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