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Studies on the Potentials of *Citrullus lanatus* (Watermelon) Fruit Peels Using Acid for Bioethanol Production

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Abstract: Bioethanol was produced from watermelon fruit peels using acid hydrolysis. The peels were pretreated by Steam expulsion method at 121 ^{o}C for 1 hour. Hydrolysis was carried out using 0.5%, 1%, 1.5%, 2% and 2.5% sulphuric acid with varying temperature and time. Dried active baker's yeast strain (*Sacchromyces cerevisiae*) was used for fermentation. The concentration of the bioethanol produced was determined using Potassium Dichromate method. After hydrolysis, the reducing sugar concentration was determined using the Dinitrosalicylic acid (DNS) colorimetric method for 5 days; 1.04±0.02 g/L was the highest yield of reducing sugar after pretreated samples were hydrolyzed with 2.5% sulfuric acid at 60^{o}C for 30 minutes. The results revealed that there is significance difference (p<0.05) in the yields of the reducing sugar produced after optimizing the samples. Base on the above findings, the highest bioethanol yield after hydrolysates were fermented with *Sacchromyces cerevisiae* was 0.13 ± 0.017 mg/L. This Study revealed that watermelon fruit peels are suitable agricultural wastes product for Bioethanol production.
Keywords: Bioethanol, Watermelon fruit peels, Sulfuric acid, Baker's yeast *Saccharomyces cerevisiae*.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a vine-like flowering plant originally from southern Africa [1]. It is a worldwide economically important member in family Cucurbitaceae. It has been cultivated for a long time in Africa, the Middle East and Egypt [2, 3]. In Nigeria watermelon peels are fermented, blended and consumed as juice [4]. Water melon seeds contain cucurbocitrin which help in lowering of blood pressure and improve kidney function [5].

It is a good source of fiber which helps to improve bowel regularity and works to prevent colon and rectal cancer. The peels contain phytochemicals such as saponins, phenol, flavonoids and alkaloids. These phytoconstituents have biological effects like anticancer, anti-diabetic, anti-inflammatory, which are used for therapeutic effect, medicinal values or can be used in the pharmacological production of drugs, reducing the risk of chronic disease and to meet nutrient requirement for optimum health [6].

Bioethanol is considered as an alternative to fossil fuel, and its production has become a great issue in the world. In concern of energy-related environmental pollution, Bioethanol has been proposed as a clean and efficient energy carrier. It is already used as a cleaner-burning alternative to gasoline and it is the only fuel whose oxidation products do not contain much carbon dioxide and do not contribute to ozone depletion or acid rain.

Industrial production of fuel bioethanol started in Brazil during the oil crisis in the 1970s, and has ever since been widely important there. Today bioethanol is produced from all sorts of biomass rich in sucrose, starch and cellulose. These are easily hydrolyzed by acids or enzymes to C-6 sugars (mainly glucose), which are fermented into ethanol and CO_2. The types of feedstock used today for production of bioethanol are primarily based on edible bio-resources, which can also find use as food for humans and animals [7].

Second generation bioethanol or cellulosic bioethanol as it is also called is made from biomass like agricultural waste, straw and wood. These consist of large amounts of cellulose, hemicelluloses and lignin (termed lignocellulose) and much less starch and sugar compared to the feeds for first generation bioethanol [8]. This study will evaluate the potentials of watermelon peels as substrate for bioethanol production.

MATERIALS AND METHODS

Collection of samples

Watermelon fruit peels were collected in new clean polythene bags from Kasuwar Daji Market, Sokoto State where it was transported to the laboratories of Microbiology and Biochemistry Departments, Usmanu Danfodiyo University, Sokoto for the research purpose.

Experimental Design of the Research

The experimental design used to determine the effects of the acid concentration, hydrolysis time and temperature on the efficiency of reducing sugar yield is as follows. A total of 5 experiments were carried out for optimization purpose where the effect of each factor was analyzed by using lower and higher values from optimized conditions.

Table-1: Experimental Design of Acid Hydrolysis for Reducing Sugar Determination

Sample	Factor 1	Factor 2	Factor 3
	A: Acid conc. (% v/v)	B: Temp. (^{o}C)	C: Time (min)
A	0.5	100	70
B	1.0	90	40
C	1.5	70	60
D	2.0	80	50
E	2.5	60	30

Preparation of the Sample

About 10kg of watermelon fruit peels were washed under tap water and chopped into small pieces of about 3-5 cm length using a stainless steel knife. The peels were then dried in hot air oven for 3 days at 60^{o}C to completely remove the water content. Next, the sample was taken out of the drier once they were dry enough to be grind. The cut pieces were milled using a grinder. The maximum particle size was 2 mm. The ground sample was kept at low temperature until the next step of experiment [9].

Isolation and characterization of Microorganisms

Baker's Yeast (*Saccharomyces cerevisiae)* was reactivated and used in this studies according to the methods describe by Alkasrawi, [10]. One (1) Gram of Baker's yeast was put in a flask containing a solution of warm distilled water, Glucose broth media and yeast extract. The solution was then subjected to incubation for 24 hours. The reactivated yeast was then inoculated in to Potato Dextrose Agar (PDA and incubated at 28^{0}C for 5 days. After 5 days, the growth observed was used to form a pure culture. This was done to avoid inhibition of some important inoculums in the yeast.

Pretreatment of the Fruit Peels Powder

Pre-treatment of the watermelon fruit peels powder was carried out by steam expulsion method. In this method, 10 g each of grounded fruit peels were put in to 15 conical flasks of size 250 ml and diluted each with 100 ml of distilled water. The conical flasks were then cover with aluminum foil paper and capped with rubber plugs and subjected to autoclave at 121^{0}C for 1 hour. After autoclaving, the samples were allowed to cool. The insoluble biomass was made to hydrolyze in the next step which is hydrolysis [9].

Acid Hydrolysis of the Samples

Acid hydrolysis was carried out according to the methods describe by Rabah *et al.* [11]. Exactly, 0.5, 1, 1.5, 2.0, and 2.5 ml of sulfuric acid was added to the treated samples and were covered with cotton wool, wrapped in aluminum foil, and heated in water bath from 60, 70, 80, 90 and 100 ^{o}C for 30 , 40, 50, 60 and 70 minutes respectively. The flasks were removed from the water bath and allowed to cool. Reducing sugar determination was carried out for five days. The samples were then filtered using Whattman filter paper No. 1 and the filtrates were used for fermentation.

Determination of Reducing Sugar

The method of Abdul *et al.* [12] was used. One (1) ml of each samples filtrate was taken into test tubes and 1ml of DNS reagent was added in the each of the test tubes. A blank was prepared containing 1ml distilled water and 1ml DNS reagent. The test tubes were boiled in a water bath for 10 minutes and allowed to cool to developed red brown color. The color developed fully after 20 minutes. One (1) ml of 40% sodium potassium tartarate was added to stabilize the colour and the absorbance was read at 540nm wave length using ultraviolet (UV- VIS) spectrophotometer. A standard curve of glucose determination was prepared to calculate the percentage of the reducing sugar.

pH Adjustment

Before addition of yeast to the above prepared samples, the pH of the samples was adjusted to 5.0, to avoid denaturing of the yeast in hyper acidic or basic state. A highly concentrated NaOH solution and concentrated HCl was prepared to adjust the pH and was regularly checked using a digital pH meter [13].

Sterilization

All the hydrolysate samples were sterilized at 121^{o}C for 15 minutes prior to fermentation [14].

Fermentation of the Sample

The hydrolysates were aseptically inoculated with 1ml suspension (6.0×102cfu/ml) of the reactivated yeast. The flasks were then covered with cotton wool

and wrapped in with aluminum foil paper. Fermentation was carried out at room temperature for 7 days [15].

Fractional Distillation of the Sample

Fractional distillation was used to extract the produced bioethanol, with the temperature adjusted to 78^0C as described by Wayne *et al.* [16].

Determination of Percentage Concentration of the Bioethanol Produced

Percentage concentration was carried out using acid potassium dichromate reagent according to the methods of [17]. One milliliter (1ml) of standard ethanol was diluted with 99 ml of distilled water to give a concentration of 1 %. Then 0, 2, 4, 6, and 8 ml each of the 1% ethanol was diluted to 10 ml of distilled water to produced 0, 0.2, 0.4, 0.6, and 0.8 ml of the ethanol. To each of the varying ethanol concentrations, 1mls of acid potassium dichromate was added and allowed to stand for an hour for color development. The absorbance of each concentration was measured at 580 nm using UV-VIS spectrophotometer and the reading was used to develop standard ethanol curve [18].

Determination of Quantity (Volume) of Bioethanol produced

The methods of Amenaghawon *et al.* [19] was used to determine the volume of Bioethanol produced. To determine the quantity of the bioethanol produced, the distillate collected was measured using a measuring cylinder, and express as the quantity of bioethanol produced in g/l by multiplying the volume of the distillate collected at 78^oC by the density of bioethanol (0.8033gl/ml).

Statistical Data Analysis

The data obtained was statistically analyzed using analysis of variance (ANOVA). The results are expressed as Mean ± Standard deviation. Duncan multiple range tests were used in mean comparison and $p< 0.05$ will be considered as the level of significance.

RESULTS AND DISCUSSION

Concentration of Reducing Sugar Produced from Acid Hydrolysis of Treated Watermelon Peels from Day One (1) to Five (5). The highest yield of reducing sugar of 1.04 g/l was obtained from acid hydrolysis after using 2.5% sulfuric and third day of hydrolysis at 60^oC for a period of 30 minutes. But when the sulfuric acid concentration was reduced to 0.5% concentration at 100 ^{o}C for a period of 70 minutes and first day of hydrolysis, a lowest reducing sugar yield of 0.48 g/l was obtained. This shows that acid concentration have more effects than the temperature and time. Statistical analysis using One- way Analysis of variance (ANOVA) reveals that there is significant difference at ($p< 0.05$) in the percentage mean concentration of reducing sugar across the days of hydrolysis and effects of acid concentration or combination of both acid concentration and days of hydrolysis on the substrate. But with effects of temperature and time only or combination of both, there is no significant difference at ($p< 0.05$).

Table-2: Concentration of Reducing Sugar Produced from Acid Hydrolysis of Treated Watermelon Peels from Day One (1) to Five (5) Concentration of reducing sugar produced (g/l)

Days	A	B	C	D	E
1	$0.48 \pm 0.02^{a(y)}$	$0.56 \pm 0.02^{a(y)}$	$0.58 \pm 0.03^{a(y)}$	$0.71 \pm 0.01^{a(x)}$	$0.83 \pm 0.02^{b(y)}$
2	$0.64 \pm 0.02^{a(x)}$	$0.67 \pm 0.02^{a(x)}$	$0.79 \pm 0.11^{b(y)}$	$0.76 \pm 0.03^{b(y)}$	$0.94 \pm 0.01^{b(x)}$
3	$0.67 \pm 0.02^{a(x)}$	$0.71 \pm 0.02^{a(x)}$	$0.81 \pm 0.03^{b(y)}$	$0.89 \pm 0.02^{b(x)}$	$1.04 \pm 0.02^{bc(y)}$
4	$0.62 \pm 0.01^{a(y)}$	$0.69 \pm 0.01^{a(x)}$	$0.72 \pm 0.01^{a(x)}$	$0.80 \pm 0.02^{b(y)}$	$1.00 \pm 0.01^{bc(y)}$
5	$0.58 \pm 0.01^{a(y)}$	$0.62 \pm 0.01^{a(y)}$	$0.65 \pm 0.02^{a(x)}$	$0.74 \pm 0.02^{b(y)}$	$0.97 \pm 0.02^{bc(x)}$

a,b,c,d,e means within a column with different superscripts are significantly different at ($p<0.05$)
x,y,z within a row with different superscripts are significantly different at ($p<0.05$)

Percentage Concentration of Bioethanol produced after Hydrolyzed by Acidic hydrolysis. The results revealed that the highest bioethanol yield of 0.13 % after using 2.5% dilute sulfuric acid at 60^oC for 30 minutes after 24 hours of fermentation. However, a lowest yield of 0.07 % was obtained after using 0.5% dilute sulfuric acid at 100^oC for 70 minutes after 24 hours of fermentation.

Table-3: Percentage Concentration and Volume of Bioethanol Produced after Acidic Hydrolysis

A	0.07 ± 0.005	A	55.00 ± 1.2
B	0.08 ± 0.012	B	37.33 ± 2.5
C	0.08 ± 0.058	C	41.00 ± 1.7
D	0.10 ± 0.058	D	42.30 ± 1.1
E	0.13 ± 0.010	E	38.00 ± 0.4

Sample (Conc. of Bioethanol produced (mg/l)) Sample (Volume Bioethanol produced (ml))

Volume of Bioethanol produced after Hydrolyzed by Acidic hydrolysis. The results revealed that 55 ml was the highest volume produced by sample A after using 2.5% sulfuric acid at 60^oC for 30 minutes.

However, the lowest volume of 37 ml was produced by sample B. after using 0.5% sulfuric acid at 100^{o}C for 70 minutes.

An experimental design of acid hydrolysis for reducing sugar determination was carried out in this study. Biothanol production can be affected by many parameters starting from sample preparation to distillation. The best way of showing the effects of this parameter for the yield of ethanol are to generate an experimental design. The effect of acid concentration and temperature has effect on the yield. When the levels of temperature increased, hydrolysis resulted in higher yield of Bioethanol. However, increase in acid concentration also increases the yield. Hence there were well defined optimums operating conditions. As hydrolysis temperature increases at lower level of acid concentration and as increase level of acid concentration. The findings are similar to the finding of Oyeleke *et al.* [20].

The percentage mean concentration of reducing sugar produced from watermelon fruit peels after using sulfuric acid to hydrolyze the hemicellulose, cellulose and lignin was also studied. The highest yield of reducing sugar of 1.04 g/l was obtained after using 2.5% sulfuric and third day of hydrolysis at 60^{o}C for a period of 30 minutes. But when the sulfuric acid concentration was reduced to 0.5% at 100 ^{o}C for a period of 70 minutes and first day of hydrolysis, a reducing sugar yield of 0.48 g/l was obtained. This means that acid concentration have more effects than the temperature and time. This shows that sulfuric acid can hydrolyze the substrate in to fermentable sugars as reported by Rabah *et al.* [21]. Statistical analysis using One- way Analysis of Variance (ANOVA) revealed that there is significant difference at $p< 0.05$ in the percentage mean concentration of reducing sugar across the days of hydrolysis and effects of acid concentration and combination of both acid concentration and days of hydrolysis on the substrate. But with regards to the effects of temperature and time only or combination of both, there is no significant difference at $p< 0.05$. Conclusively, acid hydrolysis using 2.5% sulfuric acid at 60^{o}C for 30 minutes and a third day of hydrolysis produced the highest sugars of 1.04 g/L through optimization.

The percentage concentration of bioethanol produced after hydrolysates were fermented using baker's yeast Saccharomyces cerevisiae was studied. The highest concentration of Bioethanol obtained was 0.13 % after 24 hours of fermentation and after using 2.5% sulfuric acid at 60^{o}C for 30 minutes for hydrolysis. The results revealed a higher production by Sample A is because 2.5% sulfuric acid for hydrolysis tends to facilitate the breaking down of the watermelon fruit peels as reported by Oyeleke, *et al.* [20]. All these might be responsible for high bioethanol produced from the hydrolysates. The highest bioethanol yield (0.13%) obtained was lower than 0.5% reported by Fish and Burton [7]. They found that Fermentation of watermelon juice at pH 5 after hydrolysis with 2.5% sulfuric acid produced the highest yield. This may be associated to environmental factors and differences in methods of hydrolysis. Conclusively, the percent yield of Bioethanol obtain from Sample A of acid hydrolysis is the highest among all the samples. This is as a result of the ability of the acid concentration to expose the composition of sugars in the substrate. This was done with reference to the research reported by Zhao *et al.* [22] who stated that further hydrolysis of substrate will produce more bioethanol. These studies revealed that Bioethanol can be produced from watermelon fruit peels with maximum yield obtained using 2.5% sulfuric acid at 60^{o}C for 30 minutes for hydrolysis.The results of volume of Bioethanol produced by acidic revealed that Bioethanol produced by sample A has the highest volume of 55 ml where sample B has the lowest volume of 37 ml bioethanol. This might be as a result of average acid and temperature used for sample A during acid hydrolysis. Akin-Osanaiye *et al.* [23] used 20kg of the whole watermelon fruits plant and produced 10 ml concentrated Bioethanol.

CONCLUSION

At the end of this study, it was found that Pretreatment of watermelon fruit peels by steam expulsion method enables the accessibility and ease of hydrolysis of the peels. This study had shown that 2.5% sulfuric acid at 60^{o}C for 30 minutes produced the highest reducing sugars of 1.04 g/L from sample A than rest of the samples. This study concluded that bioethanol produced by sample A is higher with an average yield of 0.13 mg/L than sample B and C which are the least with an average of 0.06% respectively.

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