

## CONVERSION OF ORANGE PEEL TO ETHANOL BY FERMENTATION PROCESS AND ITS CHARACTERIZATION

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**Abstract:** Increasing world population has led to increase in the demand for fossil fuels and its derivatives for both domestic and industrial uses. Hence, the demand and cost of global non-renewable energy resources like natural gas and fossil fuels is on the increase daily. Consequently, the search for alternative sources of energy to reduce dependency on fossil fuels has been attracting worldwide attention as this has become necessary. Agricultural waste causes environmental pollution and fruit processing industries produce large amount of waste material, which poses considerable disposal problems leading to environmental pollution. Therefore, converting the agricultural wastes to wealth should be considered. Orange peel wastes (OPW) was used as a substrate to produced ethanol through material pre-treatment, biological hydrolysis, fermentation, filtration and distillation process. Enzymes *saccharomyces cerevisiae* was utilised in the fermentation of the substrate before distillation process. The production yielded 0.035ml/g of ethanol and the results obtained from the analysis indicated that the density (0.787 g/ml), specific gravity (0.802), kinematic viscosity (1.70 cst), flash point (14 °C), fire point (18 °C), cloud point (-103 °C) and pour point (-112 °C) are within The American Society for Testing and Materials (ASTM) standard. The result obtained showed that, the ethanol produced can be used as fuel or fuel additive for gasoline in internal combustion spark ignition engine. This will consequently reduce fossil fuel dependence, greenhouse gas emission and environmental effluence of toxic emissions.

**Keywords:** Orange peel waste, *saccharomyces cerevisiae*, fermentation, distillation, ethanol, characterization

### 1.0 INTRODUCTION

Bioethanol from waste reduced the environmental pollution, convert waste to wealth, create more job opportunity to improve the nation's economy and national energy security. The bioethanol from biological source will reduce the cost expended on fossil fuel and reduce carbon monoxide released, thereby reducing the greenhouse gas emission. The ethanol produced from agricultural wastes by biological conversion can be used in different applications to replace conventional fuels, treat wastes and reduce air pollution. Biofuels have the potential to be domestically and globally available for energy security, with most being carbon neutral and supportable within the current agricultural infrastructure [18, 2 and 6]. Previous researches have shown the potential of obtaining ethanol from lignocellulosic wastes conversion [10, 12, 8, 25 and 14]. Hence, ethanol production from agricultural waste and lignocellulosic materials from orange, plantain, mango, babana, cassava, maize, pineapple, sugarcane and other solid wastes could be explored. This would reduce drastically the solid wastes that will eventually constitute environmental pollution over time due to accumulation and improve environmental health. Fruit wastes are known to be the cheapest and easiest available source for the production of bioethanol which is a possible energy source, from which ethanol can be obtained. These wastes that are usually thrown away have a good potential for green fuel and as well reduce environmental pollution. The ethanol has an important application as an industrial ingredient that is widely used as a base chemical for other organic compounds, medical wipes, antibacterial hand sanitizer gels, preservative agent, industrial solvents, cleansing agents and an antiseptic; it can also be used as motor fuel and fuel additive [16 and 6]. The citrus peel material includes a mixture of peel, segment membranes, and seeds rich in pectin, cellulose, and soluble sugars while orange peel is an excellent example of a waste resource [8]. Production of bioethanol from fruit waste is getting more awareness especially in the developing countries, considering the shortage of energy resources, increasing disposal costs and its major role in reduction of greenhouse gas emissions and to improve their economy [12]. Due to depleting reserves and competing industrial needs of petrochemical feed-stocks, there is global emphasis on ethanol production by microbial fermentation process [10 and 25]. Consequently, the result

of the study may increase the value of agricultural wastes by ensure a clean environment, create more job opportunities, reduce seasonal losses of the fruits and serve as a substrate for production of bioethanol. Thus, the study would consider bioethanol production from resources that do not contend with food production and determine the ethanol properties.

The first generation biofuels production used sugarcane, grains, soybeans and corn as substrate and the major producers are Brazil, USA and China [16, 11 and 14]. Ethanol is an organic compound with one or more hydroxyl (OH) groups attached to a carbon atom. Ethanol is clear, colourless, volatile, liquid with characteristic odor, in dilute aqueous solution, it has a fairly sweet flavor and has a burning taste in more concentrated solutions but concentrated ethanol has a strong, burning taste. It is soluble in water and organic solvent but poorly soluble in oils and fats.

Ethanol is one of the most advanced liquid fuels because it is environment friendly [5]. It is a renewable resource of energy and is potentially cleaner alternative to fossil fuels [2]. Renewable resources such as fuel ethanol provide numerous benefits in terms of environmental protection, economic benefit and national energy security [24]. This research discussed the pretreatment, hydrolysis, fermentation and distillation process for ethanol production from agricultural waste.

### **1.1 Agricultural and Industrial Waste**

The Agricultural waste and industrial fruits waste generated has a problem of disposal in our environment, thus caused environmental pollution. The citrus-processing industry generated huge amounts of orange peels as a byproduct from the industrial extraction of citrus juices in the Mediterranean area [22]. Therefore, the conversion of agricultural waste to bioethanol will reduce environmental pollution and improve environmental health [8]. Conventional crops such as corn and sugarcane are unable to meet the global demand of bioethanol production due to their primary value of food and feed; therefore, lignocellulosic substances such as agricultural wastes are attractive feed stocks for bioethanol production. Agricultural wastes are cost effective, renewable and abundant [2]. The conversion of orange peels into bioethanol is currently been investigated, Lignocellulosic biomass is seen as an attractive feedstock for renewable fuels, particularly ethanol. Lignocellulosic feed stocks include agricultural residues; wood, municipal solid waste and dedicated energy crops which have significant advantages over first generation feed stocks for ethanol production [8 and 34]. Hence, the current research considered the bioethanol production from resources (orange peel) that do not compete with food production. [37] Experimentally investigated production of ethanol by yeast isolated from apple, orange and banana. Also, [21] investigated ethanol production from corn, potato peel waste and its process development. Dried orange peels have a high content of pectin, cellulose and hemicellulose, which make it suitable as fermentation substrate when hydrolysed [8]. Increasing petroleum prices and the threat to fuel security are strong drivers in the search for sustainable fuel alternatives. [25] carried out review on biofuel from agricultural waste which considered solid biofuel as agricultural waste such as wood, domestic waste, fodder waste and dried manure like animal dung. Leading bioethanol producers like Brazil and United states diverted the surplus harvest in agricultural crops like corn in US and sugarcane in Brazil towards bioethanol industrial production. Corn was used as substrate for production of ethanol due to its higher yield and potato peel waste which consist of about 58% of dry weight as starch [21].

Dried orange peels have a high content of pectin, cellulose and hemicellulose, which make it suitable as fermentation substrate when hydrolysed [8]. [25, 8 and 14] in their investigations observed that the production of ethanol processes involves two steps, hydrolysis of cellulose in the lignocellulosic biomass for production of reducing sugars and fermentation of the sugars to ethanol.

### **1.2 Ethanol**

Ethanol is the most widely used liquid biofuel and is produced as a result of fermentation process from sugars, starches or cellulosic biomass including fruit wastes [14]. Ethanol is an eco-friendly alternative to petroleum based fuel as it has fewer greenhouse gas emissions [2]. Ethanol (C<sub>2</sub>H<sub>5</sub>OH) is a high performance, biomass fuel considered the most suitable alcohol to be used as an alternative fuel for spark ignition engines [29]. In India, first generation ethanol is mainly produced from whole grain while corn grains is the most commonly used feedstock in the western countries like Brazil and USA [21].

In the last decade, there has been an increasing interest in the conversion of non-food, cheap and renewable biomass into new green biofuels and bioproducts, to be used for industrial applications in place of those of traditional fossil source [22]. [15] carried out research on bio-ethanol production from banana, plantain and pineapple peels by simultaneous saccharification and fermentation process, [26] investigated on butanol production by fermentation of fruit residues and [4] reported the production of ethanol from maize cobs and groundnut shells. [5] made a comparative study of bioethanol production ability of *Bacillus subtilis* and *Saccharomyces cerevisiae* using Banana and Orange Peels. Also, [28] carried out research on enhanced production of ethanol from grape waste in which different biofuels such as ethanol, methanol and others are produced by fermentation of agricultural wastes, fruit wastes, municipal and industrial wastes using *Saccharomyces Cerevisiae* as food for the micro-organisms. However, the physical properties of ethanol show that it is corrosive on rubber and plastics in car engines [25]. The industrial ethanol produced is used by chemical, pharmaceutical industries and hospitals and also used to produce Ethyl Tertiary-Butyl Ether (ETBE) [21].

### **1.3 Ethanol Application**

Ethanol is used in the production of liquid detergents, drugs manufacture, plastics, polishes, plasticizers, perfumes, cosmetics, rubbers, accelerators, an anti-freeze, cellulose nitrate and the possibility to blend it in low proportions with gasoline for use, without any significant change in internal combustion engines as fuel [2 and 4].

### **1.4 Micro-Organisms**

Agricultural raw materials can be converted into fuel components by fermentation. The sugar present in the plants is fermented to ethanol by micro-organisms called *saccharomyces cerevisiae* [15]. The species of *saccharomyces cerevisiae* commonly called Baker's yeast is the organism used for ethanol production due to its high ethanol yield [21, 30 and 31]. Also, agricultural waste is used for the production of bioethanol using *saccharomyces cerevisiae* [8, 23 and 36]. Therefore, ethanol produced from agricultural waste materials by biological conversion can be used in different applications for replacing fossil fuel products, treating wastes and reducing air pollution.

### **1.5 Fermentation**

Fermentation is a bio-chemical process used to obtain commercial products through the activity of micro-organism. It is directly caused by the life processes of minute organisms. Micro-organisms, which include; bacteria, yeast and moulds, feeds on the organic materials that is of utmost interest to the producer of ethanol [3].

Basically, fermentation is a process in which microorganisms like yeasts convert simple sugars to ethanol and carbon dioxide. Fermentation of biomass is a process where microbes use sugars as food and simultaneously produce alcohols as a product of their metabolism [25, 32 and 1]. Fermentation is one of the processes used in making a variety of products including foods, flavorings, beverages, pharmaceuticals and chemicals from a range of products such as grain, molasses, fruit, cobs and shell [20, 11 and 4]. Starchy materials are first hydrolysed to fermentable sugars and subsequently fermented with the required yeast species to produce ethanol [9]. Bioethanol is obtained as product of fermentation of simple sugars in the biomass with help of microorganisms performing enzymatic digestion [25]. Hydrolysis of starch is much simpler and more cost effective than that of cellulosic materials and can be achieved by acid or enzyme or a combination of both [19].

### **1.6 Distillation**

Distillation is the process of separating mixtures of liquid with different boiling points (temperature) which is used for the refinement of the fermented product to a fuel grade ethanol after fermentation [10 and 12]. The fermentable products are taken in batches for distillation process to produce ethanol which involves heating and cooling under convective flow conditions and the distillate dehydration for absolute ethanol [28 and 17].

## 2.0 MATERIALS AND METHODS

The fermentation process is used in the production of ethanol and was carried out between 25°C and 35°C to maximize ethanol production and prevent heat-inactivation of the yeast [27 and 16].

Fruit waste (Orange peel), sucrose, urea, distilled water, stirrers, foil paper and baker's yeast (*saccharomyces cerevisiae*) strain purchased from the local markets were used as materials for this research. Equipment used includes electronics weighing machine, autoclave, Incubator, centrifuge, Alchometer Hydrometer, Waltmann No 1 filter paper, Thermometer, Distillation unit, heating mantle, Magnetic stirrer with hot plate and water bath.

The production of ethanol was carried out at the Pharmaceutical Chemistry Organic Laboratory, Pharmaceutical department, University of Ibadan, Ibadan, Nigeria.

### 2.1 Pretreatment

Orange peels, an agricultural waste, were used in this study as substrate for bio-ethanol production. The orange peels were collected from the orange seller from the local market. The peels was separated, sorted out and air drying according to [12 and 22]. The dried orange peel was grinded with electric kitchen blender (grinder) and kept in air tight polythene. Pretreatment exposed the material surfaces to enhance quality of fermentable carbohydrate. Physical pretreatment used was size reduction of material by grinding into smaller parts. Biological pretreatment of the substrate was used for the hydrolysis of the materials to open its surface area for micro-organisms to performing enzymes activities with addition of yeast for fermentation [10,12, 23 and 24].

### 2.2 Inoculums preparation

50 gm of orange peel waste taken was put in 500ml beaker, washed with 5% potassium permanganate and thereafter flush with distilled water. A separate beaker used contain 1.5gm of urea, 50gm of sucrose and 10gm of *Saccharomyces cerevisiae* was thoroughly mixed before adding 150ml warm distilled water and mixed together with stirrer to form inoculums. Also, 50g of orange peel (substrate) mixed with 250g of distilled water in another baker. The substrate placed inside a 2000ml conical flask was mixed with inoculums vigorously for 5 minutes and covered with foil paper for fermentation process to start. *Saccharomyces cerevisiae* yeast was used as micro-organism in fermentation and urea used as food for the micro-organisms as describes by [16 and 13]

### 2.3 Fermentation process

The fermentation process started after preparation of the substrate thoroughly mixed with inoculums. Ethanol fermentation was carried out in 2000ml conical flasks. The mixture covered with foil was left on the Laboratory bench and the specific gravity measurement taken periodically by using hydrometer till constant value is obtained. During the fermentation process, enzyme from *saccharomyces cerevisiae* changed the basic sugars into ethanol and CO<sub>2</sub>. When the specific gravity achieves a constant value, it signifies the end of the fermentation process according the procedures describes with little modification by [12, 13 and 7]. In the process of fermentation, the sucrose is converted into glucose and fructose which is converted into ethanol and carbon dioxide. The fermented material was filtered.

### 2.4 Filtration

After fermentation process for a specific reaction time, the mixtures in the bottles were then filtrated using a beaker covered with a piece of folded cheese cloth. The liquid obtained inside the glass was the raw bioethanol. The volume of the raw bioethanol was measured using the measuring cylinder and it was then transferred into a plastic bottle and labeled. Weight of the residues was also checked as described by [33].

### 2.5 Distillation

Distillation is a method mostly used for the purification of the fermented product to a fuel grade ethanol and it is a separation process for a mixture of liquids. It relies on differences in the boiling points of the component liquids to be separated. The mixture to be separated is added to a distilling pot where it is heated to the boiling point. A lower boiling component (ethanol of 78<sup>0</sup>C) was preferentially vaporized

first. The resulting vapor passes into a distilling head and then into a condenser. Within the condenser the vapor cooled and it liquifies. The resulting liquid is then collected in a receiving flask. Initially, lower boiling components are collected in the receiving flask. As the distillation proceeds, these components are depleted from the distilling pot and higher boiling components begin to distill over [10 and 13]. The fermentable products are taken in batches for distillation process to extract ethanol which involves heating and cooling under convective flow conditions and the distillate de-watering for pure ethanol. The distillation unit consisted of three components: a reboiler, condenser pipe and a receiving flask. The filtered sample of 500ml per batch was transferred into the reboiler and heated to boil with heating mantle. The vapors started to rise into the still head and passed through condenser pipe. The condensed liquid enters the still receiver and the distillate collected was tested for its ethanol presence by using iodine test to check the distillate content and the temperature of the distillate was also measured. Other parameters tested are pH, Temperature, Specific gravity according to [16, 28 and 17].

## 2.6 Ethanol Production chart

Size reduction - Pretreatment

Biological Hydrolysis - Fermentation

Distillation - Ethanol The test carried out at Pharmaceutical Chemistry organic Laboratory, University of Ibadan, Ibadan was to determine the values of density and specific gravity of ethanol produced.

Materials and Equipment used in the production of ethanol are showing in the figures 1 – 8.



*Fig. 1. Orange fruits (sweet orange)*



*Fig. 2. Grinded orange peels waste*



Fig. 3. Fermentation process in plastic bottles



Fig. 4. Filtrate (supernatants) collected



Fig. 5. pH Meter (PHS-3C, 220v, 5w) used to determine the pH of the extract



*Fig. 6.1<sup>st</sup> Distillation process of supernatants using heating mantle*



*Fig. 7. 2<sup>nd</sup> Distillation process of ethanol using water bath*



*Fig. 8. Extract (Ethanol) from distillation process*

### 3.0 RESULTS AND DISCUSSION

The ethanol produced from orange peel through fermentation methods yielded 0.035ml/g of ethanol. About 10kg orange peels were used to produce 350 ml of ethanol.

*Table 3.1 Comparison of ethanol produced with other species*

S/N	Substrate	Enzymes	Time	Ethanol % (v/v)	Remark
1	Sweet orange	<i>S. cerevisiae</i>	72	2.77	Produced ethanol
2	Sweet orange	<i>S. cerevisiae</i>	72	2.85	Produced ethanol
3	Sweet orange	<i>S. cerevisiae</i>	72	2.57	Produced ethanol
1	Orange	<i>C. albicans</i>	72	1.54	Kavitha et al., (2014)
2	Orange	<i>C. albicans</i>	72	1.54	Kavitha et al., (2014)
3	Orange	<i>S. cerevisiae</i>	72	1.25	Kavitha et al., (2014)

Source: A Comparative study of ethanol production from various fruit wastes [42].

Table 3.1 presented ethanol from sweet orange with a maximum yield ethanol of 2.85% (v/v) by fermentation process using *saccharomyes cerevisiae* compared to 1.54 % (v/v) ethanol yield from orange using *Candida albicans*, which indicates that large scale production is feasible with *saccharomyces cerevisiae* yeast .

### 3.1 Physicochemical properties of ethanol

The physicochemical properties of bioethanol produced are presented in table 3.2.

*Table 3.2: Ethanol fuel properties comparison*

Ethanol parameters	Produce d Values	Engineering Toolbox values
Temperature (°C)	30	25
pH	6.68	6.5-9.0
Specific gravity	0.802	
Density (g/ml)	0.787	0.785
Kinematic	1.70	1.36
Viscosity (cst)		
Flash point °C	14	13
Fire point °C	18	-
Pour point °C	-112	-114
Cloud point °C	-103	-

Source: Engineering toolbox values [38].

Viscometer constant = 0.1 cst/s

Kinematic viscosity = Effluent time x viscometer constant.

The kinematic viscosity was measured in Cannon-Fenske viscometer in accordance with ASTM D445 [40]. Cloud, pour, flash and fire points were determine from the Production Laboratory, Petroleum Engineering Department, and University of Ibadan with apparatus. Cloud and pour point was measured by Petrotest (ASTM D97 Standard). Heat of combustions was measured in record bomb calorimeter according to PARR 1266 standards, France (ASTM D240) [41].

### 3.2 Discussions

Table 3.2 shows the values of produced ethanol at 30°C properties on pH, density, specific gravity, viscosity (kinematic), flash point, fire point, pour point and cloud point which are within the American Society for Testing and Materials (ASTM) standard.

### 3.3 Kinematic Viscosity

The viscosity of the bioethanol produced was important when considering the spray characteristics of the fuel within the engine, since the change in spray could greatly alter the combustion properties of the mixture. From the result obtained in table 3.1, it could be seen that the bioethanol produced from fermentation of orange peels at temperature of 30°C with yeast were in the range of ASTM standard considered according to [35], which were within 1 to 5 centistroke. This would give an indication that ethanol produced from fruits was suitable as a possible biofuel substitute. Moreover, low viscosity value was good for engine and reduced the problems of corrosion to the engine. So, the higher glycerol content could cause higher viscosity to the solution. However, the viscosity obtained was still maintained under ASTM standards, presenting it as a qualified alternative fuel. The results obtained were in the best range 1 to 5 centistroke under The American Society for Testing and Materials (ASTM) standard specification.

### 3.4 Density and specific gravity of fuel

The specific gravity of the bioethanol was determined using the specific gravity bottle. The density of ethanol produced was 0.787g/cm<sup>3</sup> and its specific gravity was 0.802 respectively. The small variation in the value may be due to water content in the ethanol, though it is within density of 0.785 g/cm<sup>3</sup> as reported by [38].

### 3.5 Flash and Fire point

The values of flash and fire points for produced ethanol are 14°C and 18°C respectively.

However, the flash point varies with the engineering toolbox values of 13°C which may be due to fuel volatility and relates to safety precautions that must be taken when handling a fuel.

### 3.6 Pour and Cloud Point

The values of the pour point and cloud point are  $-112^{\circ}\text{C}$  and  $-103^{\circ}\text{C}$  respectively. The cloud point typically occurs between  $5^{\circ}\text{C}$  and  $8^{\circ}\text{C}$  above the pour point. The pour point of  $-112^{\circ}\text{C}$  is closely related to  $-114.1^{\circ}\text{C}$  reported by [38]. Cloud and pour points become important for heavier fuels in the higher boiling range. Thus, the pour-ability of gasoline is not a problem but it was specified in the guideline of fuel properties standards.

The result of this study agrees with the report of [15] and within The American Society for Testing and Materials (ASTM) standard.

### 4.0 CONCLUSION

The fermentation method is significantly dependent on the effectiveness of enzymes *saccharomyces cerevisiae*. The ethanol produced is environmental friendly, economic and transportation advantages. The density of ethanol produced is  $787\text{g}/\text{cm}^3$  and its specific gravity is 0.802 respectively. The values of flash and fire points for ethanol produced are  $14^{\circ}\text{C}$  and  $18^{\circ}\text{C}$  respectively, which varies with fuel volatility and relates to safety precautions that must be considered. The values of the pour point and cloud point are  $-112^{\circ}\text{C}$  and  $-103^{\circ}\text{C}$  respectively are within The American Society for Testing and Materials (ASTM) standard.

It could be concluded, that the present work has clearly shown that fermentation of orange peel waste to ethanol by a mixture of starch digesting enzymes *saccharomyces cerevisiae* is feasible.

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