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Bioethanol Production from Decaying Fruits Peel Using *Saccharomyces cerevisiae*

Abambagade Abera Mitiku* and Tanje Mada Hatsa

Department of Chemistry, College of Natural Sciences, Arba Minch University, Ethiopia

**Corresponding author*

Abstract

The rising demand for renewable energy sources induced the development of new technologies to produce bioethanol. In this research work, commonly available large volume-fruit peels (Banana, Mango and Papaya) were investigated for bio-ethanol production via fermentation by yeast, *Saccharomyces cerevisiae*. The fruit peels were crushed and mashed to a fine powder (0.40 mm).The optimized hydrolysis conditions are 10 % biomass/substrate load, 1 % H_2SO_4, 98 ^oC hydrolysis temperature and 24 hours of hydrolysis time. The optimized fermentations conditions are pH(5.0-5.5), 30 ^oC of fermentation temperature, 72 hours of fermentation time and 2 g/L of yeast load. The highest purity of the bioethanol obtained for each fruit (Mango, Papaya and Banana) peel was 95.05 %, 96.11 % and 95.49 % respectively. Findings from this research work suggest that fruit peel wastes like Mango, Papaya and Banana can be used to produce bioethanol rather than discarding in to environment. The work also proved that the waste product of fermentation may be used as animal feedstock or fertilizer to enrich the soil for plant growth.

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Bioethanol, Fruit peel waste, Hydrolysis, Substrate *Saccharomyces cerevisiae*,

Introduction

Energy is one of the most important factors to global prosperity. The improvement of living standard urges the hunt for sustainable energy in order to meet energy consumption across the world. Energy used for heating and cooling, lights our cities, powers our rockets, commercial vehicles (like large trucks and construction vehicles), mass transit (like trains, air planes and buses), warms our homes, and cooks our food, powers machinery in factories and tractors on a farm[1]. Energy does things for us. Everything we do is connected to energy in one form or another. As the world progresses, more energy is required to get along with everyday changes.

Globally our main dependency for the energy needs is on the non- renewable resources like as oil, coal and gas for over 80%[2]. Global warming, environmental pollution, urban pollution, oil reserves depletion and high cost of fossil fuel, have been the driving forces for current research on the use of alternative energy sources, particularly those deriving from agricultural biomass[3].

The search for green energy (environmentally friendly), renewable energy, efficient, cost effective, convenient, safe and sustainable alternatives involving locally available and renewable resource is one of the main concerns of governments, researchers, industries, energy sector, scientists, and business people of worldwide due to economic, social, environmental and health benefits[4].

Renewable energy like bioethanol is now capturing a good share of the worldwide headlines because of concerns about declining supplies of fossil fuels, escalating population, industrialization, triggering ever-increasing demand of fuels and necessarily to minimize problems related to fossil fuels and environmental pollution[5, 6].

Bio-ethanol feedstock's can be divided into three major groups: sucrose-containing feedstock's/sugar crops (e.g. sugar cane, sugar beet, sweet sorghum), starchy materials /starchy crops (e.g. corn, Milo, wheat, rice, potatoes, cassava, sweet potatoes and barley), and lignocelluloses biomass (e.g. agricultural waste, wood, paper, straw, and grasses)[7].The drawback in producing bio-ethanol from sugar or starch is that the feedstock tends to be expensive (because used as food or as feed) and demanded by other applications as well. Lignocelluloses biomass is envisaged to provide a significant portion of the raw materials for bio-ethanol production in the medium and long-term due to its low cost, high availability, vast distribution and it is not competitive with food and feed crops [8].

Bioethanol production from lignocellulosic materials recently attracted huge attention in different countries all over the world because of its renewability, sustainability, availability, regional development, rural manufacturing, job opportunity, reduction of greenhouse gas emissions and its biodegradability[9].

Current bioethanol production processes depend on biomass feedstocks (availability of substrate and the ease of its formation)[10]. The main structural components of lignocellulosic biomass are cellulose, hemicelluloses, and lignin [11]. The process bioethanol production consists of the following parts: pretreatment (due to the association between the three major components of plant cell wall (cellulose and hemicelluloses fractions and lignin) (to remove lignin, reduce cellulose crystallinity, sterilize the lignocellulosic waste biomass and increase the porosity of the materials), hydrolysis (to converts polysaccharides in the lignocellulosic feedstock to fermentable monomeric sugars), fermentation (hexoses and pentoses are converted to ethanol by fermenting microorganisms), ethanol separation and purification/distillation to remove the bioethanol and to meet fuel specifications[12].

Production of bioethanol from decaying fruits which are discarded as waste and that are readily available in the country in large quantities cause real environmental problems, these can be used as a low-cost potential feedstock to generate energy can reduce problems (attractive alternate) associated with waste management (disposal of the polluting residues) such as pollution, greenhouse gaseous emissions and fossil fuels use and this could also be an attractive alternate for disposal of the polluting residues[13, 14].

Banana, Mango and Papaya peels are known to contain high concentrations of inhibitory substances i.e. lignin which inhibits complete fermentation of the inherent cellulose and hemicelluloses, and it is difficult to degrade [15]. In order to break down the hemicellulose and cellulose to sugars, the basic structure of the biomass must be attacked. Once the structure of the biomass is disrupted, the hemicellulose and cellulose can be converted /hydrolyzed to sugars. This can be done by the use of acid known as acid hydrolysis or by enzymes known as enzymatic hydrolysis[16].

Lignocellulosic biomass (like fruit waste) is the nearest future feedstock for ethanol production because of its low acquisition cost and its huge availability. Using nonfood raw materials, food security is not affected by this industry improving its social and environmental impacts[17].These agricultural waste fruits (Banana, Papaya and Mango peels) generate solid waste and economic losses to farmers both in the farm and the market places, therefore, the use of these wastes for bioethanol production shall reclaim the farmer's economic loss and rid the environment of the negative impact of these waste[18].

The most commonly used microorganism for bioethanol production (used in fermentation) from its history is the yeast, especially, *Saccharomyces cerevisiae* (also widely known as brewers' yeast)[19]. *Saccharomyces cerevisiae* is preferred due to its long history of utilization for both ethanol production and baking, and the fact that it has GRAS (Generally Recognized as Safe) status. *Saccharomyces cerevisiae* has extremely high ethanol yield, high ethanol tolerance, high selectivity, low accumulation of by-products, high fermentation rate, high fermentation rate, good tolerance to substrate concentrations, aptitude to grow in simple, high inhibitors tolerance, low nutrient requirement, robust growth with simple requirements allowing for the use of inexpensive media, tolerance to acidic pH or high temperatures in order to retard contamination and can use a wide range of hexoses and disaccharides[20].

Reforming of renewable biomass feedstock such as bioethanol is biodegradable, non-toxic, simple to use, suitable substitute for fossil fuels, essentially free of Sulphur and aromatics and capable to reduce greenhouse gas, CO_2 and NOX, emissions[21].

Bioethanol is currently used in the fuel industry as an additive for petrol. It is a high octane number/fuel (which allows a higher engine compression ratio to be used, which leads to improved thermal efficiency and increased power, thereby reducing somewhat the difference in fuel consumption) and has replaced lead as an octane enhancer in petrol.

Blending ethanol with petrol oxygenates the fuel mixture so that it burns completely and reduces harmful emissions of particulate matter, hydrocarbons and carbon monoxide[22]. And the products from bioethanol incomplete oxidation (acetic acid and acetaldehyde) are less toxic in comparison to other alcohols. The most common blend is 90% petrol and 10% ethanol.

Interest in the use of bio-fuels worldwide has grown strongly in recent years due to the limited oil reserves, dependence on petroleum-based fuels, concerns about climate change from greenhouse gas emissions, taking advantage of the higher octane number and higher heat of vaporization and the desire to activate the grass-root economy by stabilizing the income of farmers and generating employment in the local community [23].

Bioethanol has several attractive features as an alternative fuel. As a liquid it is easily transported and it also can be blended with gasoline to increase the octane rating of the fuel. The huge fluctuations in the price of petroleum have made commercial production of fermentation ethanol a more attractive. Bio-ethanol has a higher octane number, broader flammability limits, higher flame speeds and higher heats of vaporization. These properties allow for a higher compression ratio and shorter burn time, which lead to theoretical efficiency advantages over gasoline in engine[24, 25].

The question of sustainable and cleaner energy resources has become prominent in the past few decades. Thus, security of petroleum supply or other sources of energy which can replace petroleum is critical for the world to diversify the energy mix. The process of utilizing the solid waste (like Banana, Papaya and Mango peels) those are very rich in cellulose, hemicellulose and lignin, gives zero waste generation techniques.

Objective of the research work

To produce bioethanol from Banana, Papaya and Mango peels waste, without interfering with food security using *Saccharomyces cerevisiae*.
Determine the optimum operating conditions in hydrolysis/fermentation.
To compare the ethanol purity for each substrates.
To protect the environment from fruit peel wastes and there by generating financial revenue from waste.

Materials and Methods

Study area

Arba Minch town is one of the emerging towns of Ethiopia which is located in Southern Nations Nationalities and Peoples Regional (SNNPR) State of Ethiopia. The name Arba Minch was derived from the "forty springs" which means a collection of more than forty springs which are located in the Arba Minch natural forest. It is found in Gamo zone and used as a zonal capital town. It is located at about 454 km south of Addis Ababa. Astronomically Arba Minch is located at 6°04′ North Latitude and 36°40′ East Longitude. Around Arba Minch Mango, Banana and Papaya fruit are harvested. Banana, Mango and Papaya peels were collected from SNNPR, namely Gamo Zone, Arba Minch town.

Experimental site

The experiments for synthesis of bioethanol and its characterization were conducted in Arba Minch University chemistry research laboratory, Ethiopia.

Chemicals

All chemical reagents utilized in this work were analytical grade chemicals. Chemicals used for the production of bioethanol are sulfuric acid (H_2SO_4), peptone, urea, $MgSO_4.7H_2O$, sodium hydroxide (NaOH), potassium dichromate ($K_2Cr_2O_7$) and distilled water.

Plant material: Banana, Mango and Papaya peels.
Micro-organism: Yeast (*Saccharomyces cerevisiae*)

Instruments and equipments

Plastic bags, Knife, Digital ovens, Crushers, Balances, Digital pH meter, Thermometer, Vessels, Graduated cylinders, Autoclave, Pycnometer, Fermentation and Distillation set ups.

Identification and collection of banana, mango and papaya peels

The fruit peels were identified and authenticated by a botanist working in the Department of Biology, using the standard morphological characteristic features. Fruit wastes were collected from SNNPR, Gamo Zone, Arba Minch town. Fruit peels were collected in plastic bags and transported to chemistry laboratory for bioethanol production.

Substrate preparation and physical pretreatment

Lignocellulosic residues of raw materials (Banana, Mango and Papaya peels) were washed with distilled water and their outer coats are removed, reduced to 1-2 cm long pieces to make it easier to handle and kept under shade at room temperature for few days and then kept in an oven at 65°C for 24 hours and the oven dried fruit peels was grinded to form powder (to mesh size of 40,0.40 mm) using electronic grinder, then the powder packed in polyethylene bags separately and stored at 4ºC in refrigerator prior to use[26,27].Five hundred gram of each (waste peel of papaya, mango and banana) was used for the substrate preparation.

Acid hydrolysis

The aim of hydrolysis is to further degrade the polysaccharides present in the pretreated lignocellulosic biomass of papaya, mango and banana peels into monosaccharides subunits[28]. The monosaccharides that will be produced upon hydrolysis will enhance the fermentation process by *Saccharomyces cerevisiae*. Acid hydrolysis was done using H_2SO_4. For acid hydrolysis, different amount of each pretreated fruit peels waste were mixed with various H_2SO_4 concentrations (0 %, 0.5%, 1%, 2% and 3% v/v), different hydrolysis temperature (60 ^oC, 70 ^oC, 80 ^oC, 90 ^oC, 100 ^oC and 110 ^oC) and different hydrolysis times (3, 6, 12, 18, 24, 36, 48) hours, in order to optimize the productivity of fermentable sugars.

pH Adjustment

Pretreated and hydrolyzed sample were mixed, shaken substrate primarily checked for pH using a digital pH meter. Mixed samples (pretreated and hydrolyzed) were acid hydrolyzed in the range of 5.0-5.5[29].To determine the effect of pH, the pH of the medium in the inoculated flasks was adjusted appropriately using 1 M NaOH.

Sterilization

After hydrolysis, the flasks containing the hydrolyzed samples were covered with cotton wool, wrapped in aluminium foil, autoclaved for 15 minutes at 121 °C and allowed to cool at room temperature[30].

Microorganism and fermentation medium

The yeast *Saccharomyces cerevisiae* (instant premium) was purchased from Arba Minch market. Before using in fermentation, the yeast was activated. Bake's yeast, *Saccharomyces cerevisae* used for fermentation was cultured on yeast extract agar.

The following nutrients were mixed in their correct proportion. About 2 g of dry yeast was added in a 250 ml conical flask containing 30 ml of 5% sterilized glucose solution, 4 g peptone, 1 g urea and 1g $MgSO_4.7\ H_2O$. Then distilled water was added up to mark, 250 ml. And then the prepared media were activated at 38ºC for 1h, cooled from 38ºC to 30ºC and then used in the experiment [31]. Next the conical flasks were properly covered with aluminum foil. Temperature, revolution per minute and culturing duration of shaker incubator was adjusted. The fermentation flasks were incubated in the dark on a shaking incubator (200 rpm) at 30 °C for 24 hours. And the culture was placed for 24hours and the pH was adjusted.

Fermentation

Batch fermentations are generally run on a smaller scale compared to others and utility requirements are therefore considered lower than their counterparts[32]. The fermentation was carried out at varying temperature (22 to 43 °C), pH (4.5 to 6), amount of yeast added (0.5 to 3.5 g/L) and fermentation time (24 to 96 hours). The prepared hydrolysates samples (papaya, mango and banana peels) and media were mixed in the 500ml Erlenmeyer flasks with the ratio of 10 % (1% media with 10% sample). Then after this the Erlenmeyer flasks were covered using aluminum foil. Then, it placed on shaking incubator at a temperature of 30^oC and at 200 rpm for 3 days. And after 72 hours of fermentation, the samples was taken out and distilled. During that the ethanol concentration was determined every day.

Distillation

Distillation is the final step in the production of bioethanol from Mango, Banana and Papaya peel

waste[33]. After fermentation, the broth was centrifuged at 6000rpm for 10 minutes. The supernatant was collected and fed into a simple distillation column. The boiling temperature of ethanol is 78°C hence distillation was carried out around that temperature to facilitate the evaporation of ethanol. The vapour was collected and got condensed by means of the circulation of cold water around the column. The distillate having ethanol was recovered in a conical flask at the other end of the column[34].

Dichromate test

The produced bioethanol was examined by standard dichromate test[35]. About 1ml of K_2Cr_2O7 (2%), 2ml H_2SO_4 and 3ml of the distillate sample were added together.

Specific gravity measurements using pycnometer

The ethanol concentrations of the samples were measured by using specific gravity. The final products were evaluated for their ethanol content by measuring the corresponding density using Specific gravity bottle (pycnometer) at room temperature[36]. The 25 ml pycnometer was cleaned and dried first and then weighed (W_0), then after the bottle was filled with ethanol, stopper inserted and reweighed to give (W_1). The ethanol was substituted with water after washing and drying the bottle and weighed to give (W_2).The formula for specific gravity is[37]:

$$\text{Specific Gravity} = \frac{(W2 - W1)}{(W3 - W1)} = \frac{\text{Weight of bioethanol}}{\text{Weight of distilled water}}$$

Where: W_0- weight (g) of empty bottle
W_1- weight (g) of bottle + sample (ethanol)
W_2 - weight (g) of bottle + water

Determination of purity of bioethanol

The sample percentage of bioethanol was calculated by the specific gravity method. The sample percentage purity of the bioethanol produced was calculated by comparing the ratio of the specific gravity of ethanol extracted and ethanol in its purest form [38].

$$\%\ \text{purity} = \frac{\text{Specific Gravity of pure ethanol}}{\text{Specific Gravity of produced bio ethanol}}$$

Results and Discussion

Biomass size reduction

The physical treatment of fruit peel waste is most important for rate of hydrolysis because the mixing is related with the size, so that rate of hydrolysis is affected by biomass size. As the particle size decreases the surface area available for the hydrolysis reaction is more, which affects porosity, maximizing the contact between the material and acid to increase hemicellulose hydrolysis, producing maximum glucose units that are possible eventually it is all fermented to ethanol[39].

Effects of different parameters on hydrolysis

The carbohydrate polymers in lignocelluloses materials need to be converted to simple sugars before fermentation, through a process called hydrolysis. Hydrolysis is carried out at high temperature (90–110 °C); however, at low temperatures, it is possible and can contribute to energy savings [40].

There are two different types of hydrolysis processes that involve chemical hydrolysis either acidic (sulfuric acid) or enzymatic hydrolysis. Acid hydrolysis is considered as the oldest and most commonly used method. In this research work acid hydrolysis was done. Hydrolysis temperature, residence time, sulfuric acid concentration and biomass concentration variables influence the hydrolysis of different cellulosic materials.

The effect of biomass concentration on hydrolysis

This study was done by using biomass concentration (5 %, 7.5%, 10%, 12.5% and 15%w/v). The highest sugar content was obtained at 10% biomass (papaya, mango and banana peels) concentration. Amount of bioethanol produced increase gradually with increasing biomass amount from 5 % to 10 % and began to decline above 10 % biomass amount.

The increase of bioethanol production with increasing biomass amount was due to availability of carbon source. As the solid loading increased beyond the maximum (10% w/v) sugar release decreases due to increase in viscosity which might lead to restrict the hydrolysis[41].

Effect of acid concentration on hydrolysis

This study were done by dilute acid hydrolysis using (0 %, 0.5%, 1%, 2% and 3% v/v) H_2SO_4.It was observed

that 1 % sulfuric acid hydrolysis produced high amount of bioethanol. The result showed that the amount of sugar obtained increases as the acid concentration increases from 0-1 % and decreases as the acid concentration increases from 1-3 %.This suggests that maximum sugar yield could be obtained at 1% H_2SO_4 (low to moderate acid concentration). Application of high concentrations of H_2SO_4 resulted in browning or charring of hydrolyzate occurred with increasing acid concentrations and also tend to formation of undesirable by-products along with sugar such as furfural and 5-dihydroxymethyl furfural, which are known to inhibit fermentation and toxic for Saccharomyces cerevisiae in fermentation [42, 43].

Effect of Hydrolysis Temperature

To know the optimum temperature for sugar production, the hydrolysis media were kept at (60^oC, 70^oC, 80^oC, 90^oC, 100^oC and 110^oC). The sugar yield increase due to increasing in temperature from 60^o C to 100^o C and maximum at 98^oC. Lower production of sugar/glucose at higher hydrolysis temperatures (above 98 ^oC) is due to formation of toxic inhibitors (not fermentable product) such as furfural from xylose and hydroxymethyl furfural (HMF) from glucose in addition to phenolics and acetic acid[44].

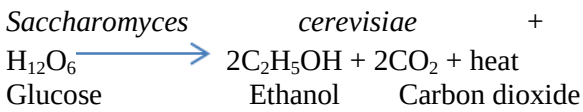
Effect of hydrolysis time

The effect of hydrolysis times (3, 6, 12, 18, 24, 36, 48 hours) on bioethanol yield was investigated under the constant conditions. When the hydrolysis time of the reaction mixture was increased, an increase in sugar content was observed. The maximum bioethanol concentration was achieved at 24 hours hydrolysis time. However, as hydrolysis time increased from 24 hours it resulted in decreasing concentration of bioethanol. The reason for this could be that longer residence time makes the sugars degraded to form inhibitors (furfural and HMF[45].

Effects of different parameters on fermentation of hydrolysates

Fermentation is the processes by fermentable carbohydrates are converted by (bacteria, yeast) into alcohol, carbon dioxide, and numerous. The byproducts have a considerable effect on the taste, aroma, and other characteristic properties of the ethanol[46].

During ethanol fermentation, glucose and other sugars in the substrate are converted into ethanol and carbon dioxide.



Ethanol fermentation is not 100% selective with side products such as acetic acid and glycols. They are mostly removed during ethanol purification. Fermentation takes place in an aqueous solution. In this study, fermentation of hydrolysates obtained from the acidic hydrolysis was carried out by baker's yeast (*Saccharomyces cerevisiae*).

Effect of pH on fermentation

In order to obtain high ethanol yield from fermentation medium, the adjustment of pH to the optimal value is quite important. Each microorganism has its specific pH that enhances specific enzymes to catalyze certain required reactions. *Saccharomyces cerevisiae* contains invertase enzymes that are affected by changes in pH. To determine the effect of pH on fermentation, the production of ethanol was studied by adjusting the pH in range of 4.5, 5.0, 5.5 and 6.0.As the pH increased from 4.5 to 5.5, the bioethanol yield increased to a maximum at pH range 5.0-5.5. Further increase in pH to 6.0 resulted in decrease in the yield of bioethanol. At the correct pH, therefore, ions and nutrients needed by the microorganism are readily used by the microorganism, then growth rate increases and yields of metabolic product is enhanced [47]. Yeast needs a slightly acid environment in order to grow well, with increase in pH, to basic conditions, yeast produces acid rather than alcohol and this lead to the decrease in alcohol production as the pH increases[48].The inhibitory effect of high pH on the bioethanol production could be due to the lower ATP production during the metabolic changes in *Saccharomyces cerevisiae*.

Effect of yeast on ethanol production

Ethanol is produced by microbial fermentation of the sugar. The size of this microbial life activity determines the amount of ethanol that will be produced and this activity is also influenced by several factors. These factors are generally closely related to the supply and use of nutrients that are used to support life. Various quantities of yeast like (0.5, 1, 1.5, 2, 2.5, 3, 3.5 g/L) were analyzed keeping rest of the parameters at their optimal conditions. The yeast concentration of 2g/L

yielded the optimum rate of fermentation and ethanol concentration were highest. The results obtained suggest that the ethanol yield increases with an increase in yeast concentration up to a certain concentration and starts to decrease which is in accordance with the results reported and reasons explained in earlier work (increasing the yeast concentration is associated with activation of glycerol biosynthesis pathways in the yeast)[49]. This may be as a result of more catalyst consuming the limited glucose for self-sustenance, thereby resulting in low yield of bioethanol.

Effect of temperature on ethanol production

In order to obtain the desired amount of product, it is important to monitor the temperature, as it is one of the important factors that alters the rate of process and directly affects the final yield. Temperature is a fundamental parameter of the fermentation process. Temperature greatly affects the enzymatic activity and membrane turbidity of yeast cells. Higher temperature may shorten the log phase of yeast cells, subsequent denaturation of enzymes and ribosome, accumulation of toxic results in decrease of yield[50]. Each microorganism has its specific temperature that enhances specific enzymes to catalyze certain required reactions. Temperature greatly affects the enzymatic activity and membrane turgidity of yeast cells and yeasts which are active and tolerant at high temperature are ideal for industrial bioethanol production. As fermentation is an exergonic process, particular attention is required for fermentation temperature control.

Fermentation medium prepared were placed in different temperature (22^0C- 43^0C) to analyze the optimum temperature for ethanol production. Bio-ethanol production increases with the increase in temperature and the temperature range of 30ºC was found to be the optimum temperature at which both rate of fermentation and ethanol concentration were highest. Beyond this temperature (30^oC) the ethanol content decreases significantly, because high temperatures can become a stress factor for micro-organisms. Further increase in temperature reduces the percentage of ethanol production and it is mainly due to the denaturing (ribosomes, proteins and enzymes) of yeast cells, fatty acid composition in yeast cell membrane, problems associated with the membranes fluidity and died yeast cell decanted at the bottom of the bioreactor, and the fermentation reaction stopped [51].

Effect of fermentation time on ethanol production

Fermentation duration must also be chosen to obtain an adequate microbial growth and ethanol yield. Impact of changes in fermentation time on bioethanol production was determined by measuring the bioethanol concentration at different time (24, 48, 72 and 96 hours).Fermentation time (72 hours) was given optimum ethanol concentration as well as good ethanol productivity. However, after 72 hours the concentration decreased significantly.

This could be attributed to loss of ethanol by evaporation and or the utilization of the sugar as carbon source for the growth, energy and metabolic activities of the micro-organism (*Saccharomyces cerevisiae*) or the hydrolyzate does contain significant levels of metabolic inhibitors (e.g., furfural and HMF) (unpredictable reactions) that can interfere with fermentation or longer fermentation time gives toxic effect on microbial growth (risk of contamination) especially in batch mode due to the high concentration of ethanol in the fermented broth [52].

Distillation

For the ethanol to be usable as a fuel, the yeast solids and the majority of the water must be removed. After fermentation, distillation has been carried out using fractional distillation apparatus. In the distillation process, the separation of a mixture is based on the difference in the boiling points of the components[53]. After fermentation, the mash is heated so that the ethanol evaporates. This process, known as distillation, separates the ethanol, but its purity is limited to 95–96% due to the formation of a low-boiling water-ethanol azeotrope with maximum (95.6% m/m (96.5% v/v) ethanol and 4.4% m/m (3.5% v/v) water)[54].

This mixture is called hydrous ethanol and can be used as a fuel alone, but unlike anhydrous ethanol, hydrous ethanol is not miscible in all ratios with gasoline, so the water fraction is typically removed in further treatment to burn in combination with gasoline in gasoline engines. The boiling point of standard ethanol is 78.24°C, but the boiling point of our sample is 78.35 C° and it approaches to the standard value. Since the ethanol has a smaller boiling point (78.24^oC) in comparison with that of water (100 ^{o}C, at standard conditions), the ethanol turns in to the vapor state before the water and can be condensed and separated. Analysis of distillate (produced bioethanol) shows positive test result.

Ethanol analysis by odor and flame

The produced bioethanol sample was tested for exactness by odour and flame test, both is qualitative test. Produced bioethanol is a transparent and colorless liquid with pleasant odor. This property makes the bioethanol produced from fruit peels (Mango, Papaya and Banana) are of high quality bio-ethanol. Its odour is similar with that of the spirit alcohol and is flammable on fire**.** Bioethanol is entirely comprised of biological products, and hence the combustion of bioethanol results in cleaner (more complete combustion) emissions (carbon dioxide, steam/water and heat).

$$C_2H_5OH + 3O_2 \longrightarrow 2CO_2 + 3\,H_2O + \text{heat}$$

Ethanol analysis by dichromate methods

Potassium dichromate is a strong oxidizing agent. It oxidizes the hydroxyl group of primary alcohol whereas chromium from dichromate reagent gets reduced.

The oxidizing agent used in these reactions is normally a solution of potassium dichromate (VI) acidified with dilute sulphuric acid. When oxidation occurs, the orange solution containing the dichromate (VI) ions is reduced to a green solution containing chromium (III) ions[55]. The orange color of the dichromate was changes to green indicate that the presence of bioethanol (primary alcohol) in the sample. Potassium dichromate is available in high purity, is highly stable (under ordinary conditions of use and storage) up to its melting point and can be used as a primary standard[56]. The theoretical reaction stoichiometry is shown below:

$$2Cr_2O_7^{-2} + 3C_2H_5OH + 16H^+ 4Cr^{+3} + 3CH_3COOH + 11H_2O$$

Bioethanolpurity and yield

The purity of bioethanol was determined and tabulated in table 1.

Table.1 Density and Percentage of ethanol purity/yield from each sample

Substrate/Fruit Peel	Density (g/cm^3)	Specific gravity	% purity
Mango	0.8286	0.8311	95.05
Papaya	0.8196	0.8220	96.11
Banana	0.8249	0.8273	95.49

The density of water at 25 ^oC is 0.997 g/cm^3. The density of produced bioethanol from fruit (Mango, Papaya and Banana peel) waste are 0.8286g/cm^3, 0.8196g/cm^3 and 0.8249 g/cm^3 respectively. The specific gravity of specific gravity of absolute ethanol is 0.79.The specific gravity of produced bioethanol from fruit (Mango, Papaya and Banana peel) are 0.8311, 0.8220 and 0.8273 respectively. The purity of the bioethanol produced was found to be 95.05 %, 96.11 % and 95.49 % for Mango, Papaya and Banana peel waste respectively.

Result showed that, specific gravity of ethanol (0.8220) obtained from Papaya peel waste is very close to the specific gravity of absolute ethanol (0.79) than the other samples. The purity of ethanol produced from papaya peel by *Saccharomyces cerevisiae* was 96.11 %.This result this study shows that ethanol was of high purity even when compared to others ethanol found in the market that had the highest percentages purity of 98 %.Bioethanol produced is a volatile, colorless liquid that has a slight odor. Further work will be carried out to evaluate the economic potential of this process.

It is concluded based on this research work, lignocellulosic waste (Mango, Papaya, Banana peels) is a promising alternative feedstock for bioethanol production in Ethiopia. The optimum conditions for diluted acid hydrolysis were: sulfuric acid concentration of 1 %, hydrolysis temperature of 98 °C, hydrolysis time of 24 hours and biomass load of 10 %. The maximum purity of ethanol (96.11 %) was achieved for Papaya peel waste after 72 hours of fermentation time, at 30°C, at pH of 5.0-5.5 and with 2 g/L yeast load. The choice of newer substrate for the production of ethanol is being a non-seasonal fruit available throughout the year. The waste from the fruit can be efficiently utilized based on overall

economics and energy. The bioethanol purity achieved appears quite attractive and demonstrates that Papaya peel waste have excellent potential as an alternative feedstock to the production of fuel ethanol. Considering the cost-effectiveness, in addition to being a means to control environmental pollution, the use of Banana, Mango and Papaya peel waste for ethanol production is concluded as a worthwhile venture.

Author contributions

The author conducted practical work in laboratory, prepared the research article and approved the final manuscript.

Competing interests

The author has declared no conflict of interest.

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