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An integrated approach to optimization of fermentation conditions for bioethanol production from local leftover Injera waste using central composite design

Abreham Bekele Bayu*, Desalegn Abdissa Akuma, Ketema Beyecha Hundie

Environmental and Process Engineering, School of Chemical Engineering, Jimma Institute of Technology, Jimma University, Jimma, Ethiopia

Abstract

Background: Bioconversion of lignocelluloses to biofuel from cheap non-edible materials such as local leftover Injera waste for renewable energy is very important and minimizes environmental pollution. Local leftover Injera is an abundant, inexpensive, reusable waste to the environment, containing a sufficient amount of carbohydrate material, which is the best source of fermentable sugars.

Methods: In this study, local leftover Injera was treated followed by drying, acidic hydrolysis, and alcoholic fermentation. Besides, the optimization of the fermentation process was done using a central composite box Behnken design. The process included physical and chemical pre-treatment of biomass, which was then followed by acid hydrolysis as a potential step. The scarification and fermentation methods were analyzed to acquire the maximum yield of ethanol. The local leftover Injera waste was pretreated with sulfuric acid and sodium hydroxide solutions. The effect of temperature, substrate concentration, as well pH on bioethanol production was optimized and studied. The optimization process was performed under special condition (temperature=25-40°C, pH=3-5, and substrate concentration=50-200 mg/L).

Results: The maximum product of ethanol was achieved at a temperature of 32.718°C, substrate concentration of 125 g/L, and a pH of 4 with a maximum ethanol yield of 42.598%.

Conclusion: According to the results, the optimum fermentation conditions for bioethanol production from local leftover Injera waste are the points where the maximum product of ethanol was achieved at a temperature of 32.718°C, substrate concentration of 125 g/L, and a pH of 4.

Keywords: Bioethanol, Biomass, Environmental pollution, Fermentation

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***Correspondence to:**
Abreham Bekele Bayu,
Email: abreham.bekele@ju.edu.et

Introduction

One of the foremost challenges of the 21st century is to meet a clean environment and to apply natural wellbeing methodologies and alternatives. Furthermore, energy and environmental issues are very interrelated and the energy demand for heating, transportation, and industrial processes increases in the world. To supply crude materials for chemical businesses in economical ways, it is vital to consider the utilization of squanders (1). Subsequently, biofuels have been developed as a perfect elective to meet these prerequisites in a feasible approach (2). Biofuels are of significance among accessible elective vitality sources in their common compatibility with existing fluid transport fuel (3). Bioethanol can be produced by using different technologies regardless of raw materials (4). One of the most important technologies, fermentation, produces bio-ethanol through biological transformation of natural starch and sugar resources such as energy-rich food biomasses and lignocellulosic biofuels (5). Large-scale generation of fuel ethanol is for the most part based on sucrose from sugarcane in Brazil or starch, primarily from nearby remaining nourishment squander, within the United States (6). Current ethanol generation based on sugar substances, neighborhood Injera, and starch may not be needed due to their nourishment and bolster esteem (7). However, waste biomass can be converted to energy rather than polluting the environment. Sugar cane contains large amounts of sucrose that can be fermented. Starchy materials such as local leftover Injera waste containing polysaccharides that can be hydrolyzed to obtain sugars suitable for fermentation. Also, lignocellulosic biomass contains a complex of several polysaccharides that can similarly be broken down into fermentable sugars (range from paper to wood) (8). Lignocellulosic biomass wastes constitute a significant renewable substrate for bioethanol production that does not compete with animal feed and

food production (9). Currently, the second-generation bio-products such as biodiesel, bioethanol, biohydrogen, and methane from biomass are highly produced from wastes which can be a reference for current studies (10). Jimma University has an annual production potential of over 680 tons of food waste. Thus, the use of food waste such as local leftover Injera waste and others to produce biofuels is mostly interested due to the current environmental health problem (11,12). Local leftover Injera waste has a large amount of starch material, which is the best important source of fermentable sugars (13). Air pollution caused by the combustion of fossil fuels can affect the environment seriously, which leads to the problem of global warming (14). Due to this reason, finding alternative energy that is environmentally and commercially feasible is becoming a critical global issue. The use of food wastes (e.g., local leftover Injera waste) for biofuel production may mitigate environmental pollution (15). In different parts of the world, several agricultural and food wastes are abundantly available (16). For example, local leftover Injera waste is used as animal feed and thrown simply to the environments in some parts of the Ethiopian rural areas, restaurants, and hotels. Converting this local leftover Injera to bio-ethanol using different technology is better for environmental management and to become economically efficient. A few studies have been done on bioethanol production from local leftover Injera without optimizing fermentation conditions and characterizing the product properties. However, in this study, optimization of fermentation conditions and product characterization to obtain optimal points to get the maximum amount of yield during ethanol production were investigated. The optimum fermentation parameters were obtained based on the central design method using response surface methodology (RSM). RSM involves a concerning manipulation of numbers-mathematical procedure useful for optimizing the parameters change response and related to a number of the designed experiments (17,18). One of the main advantages of RSM by the central composite design is to obtain the optimum conditions for removal of pollutants based on the laboratory experiments (19). The results were optimized using the regression equation of RSM (Design Expert 11) based on the central composite design.

The general aim of this research was to optimize the fermentation conditions that enable the production of the maximum product of ethanol from local leftover Injera. For this purpose, the proximate composition of local leftover Injera was determined and the optimum operating parameters like substrate concentration, temperature, and pH were determined during the fermentation process of the local leftover Injera waste. Furthermore, proximate analysis was done for the final product like density, pH, viscosity, flash point, and functional group. The importance of this study is that all energy sources affect the environment. Since local leftovers are widely sold in Ethiopia and provide an alternative to ethanol production, it can solve energy security issues; promote rural development by creating jobs, promoting environmental conservation, and reducing greenhouse gas emissions. The methodologies used in this study were proximate analysis of local leftover Injera, composition determination of local leftover Injera, physical pretreatment, dilute-acid hydrolysis, fermentation, and distillation.

Based on the literature review, most of the previous research focused on the production of bioethanol from different food waste. However, there was no previous research that was done on the optimization of fermentation conditions for bioethanol production from leftover Injera waste. Thus, this study aimed to find the specific parameters condition where a high yield of bioethanol can be obtained from the Injera leftover waste.

Materials and Methods

Equipment

The equipment used in this study include plastic bags to collect and transport samples to the laboratory, knives to cut waste on-site into pieces, ovens to dry samples, mills to grind dried samples, and sieves used to sieve samples. Samples were locally ground to a particle size of 2 mm and vacuum-weighed to weigh the samples. A digital pH meter was used to measure the pH of the hydrolyzate prepared before fermentation, thermostat was used to control the temperature of the sample prepared in the isothermal experiment at the set-point, sample holder, and additives for graduated hydrolysis flasks of different volumes for volume measurement, autoclave for sterilization and hydrolysis, pycnometer used for density measurement, stirrer used to shake the sample and its additives and fermenters and distilleries for fermentation and distillation, respectively.

Chemicals

Sulfuric acid (H_2SO_4, (98%, UK) was used as pretreatment and for the hydrolysis of local waste, NaOH(with minimum dose of 98%) was used. To adjust the pH of soluble cellulose and hemicelluloses before fermentation, Benedict's solution was used for the determination of reducing sugars, yeast extract (Agar), urea, dextrose sugar, $MgSO_4.7H_2O$, and yeast (*Saccharomyces cerevisiae*) were used for media preparation.

Sample collection

A sample of the remaining local bread was collected from the student cafeteria at Jimma University, located in the southwest-west of Ethiopia. Sample preparation includes manual sizing and sample grinding after sample collection. Four kilograms of local waste was used for the experiment. Local waste was reduced to 125 **µm**. The sample was dried in an oven at 100°C for 1 hour to obtain a pulverized material. After drying, the sample was ground. The maximum particle size of the local remnant samples

was 125 **µm**. Samples with a particle size greater than 125 µm were ground several times until all the particle sizes became 125 **µm.** The sample was kept at a low temperature until the next step of the experiment. Grinding of the in situ residues into a powder increases the surface area of the sample, which improves the contact between the hemicellulose and the cellulose with dilute acids to reduce the crystallinity of the cellulose. Local Injera and sample of the remaining waste are shown in Figure 1.

Characterization of local leftover Injera

Determination of moisture content

The remaining 2 g local sample of the pulverized Injera waste was placed in the crucible after heating the crucible and weighing. Moisture content was determined by drying in an oven at 105°C for one hour until a constant mass was obtained (20)including the leaves, pseudostem, stalks, rejected and rotten fruits and the fruit peels. This study focuses on the characterization of banana peels to yield banana peels vinegar (BPV. Samples were taken from the oven and cooled in a desiccator, then, digitally balanced. The percentage moisture is determined by the following formula as shown in equation 1 below.

$$\text{Moisture percentage (\%)} = \frac{w_1 - w_2}{w_1} \quad (1)$$

Where W_1 is weight of the sample before drying and W_2 is weight of the sample after drying.

Determination of volatile content

After emptying the crucible, 1.5 g of sample was added. The sample crucible was placed in a 950°C muffle furnace for 7 minutes (21).

The crucible was removed from the oven, placed in a desiccator to cool, and then, weighed again. The percentage of volatility was determined using the formula given in equation 2 below.

$$\text{Volatile content (\%)} = \frac{W_1 - W_2}{W_1} * 100 \quad (2)$$

Where *W1* is original weight of the sample and *W2* is sample weight after cooling.

Determination of ash content

After emptying the crucible, 3 g of locally retained Injera sample was added and placed in a 550°C temperature-controlled oven for approximately 2 hours for proper ashing. The crucible was removed from the oven, placed in a desiccator, cooled, and reweighed. The ash content was determined using the formula according to equation 3.

$$\text{Ash content (\%)} = \frac{W_2}{W_1} * 100 \quad (3)$$

Where W_1 is original weight of the sample and W_2 is weight of the sample after cooling.

Determination fixed carbon content

Solid carbon content is the residue left after water, volatiles, and ash are discarded. This is done by reducing the percentages of water content, volatiles content, and ash content from 100. The fixed carbon content (FC) is given as in Equation 4.

Figure 1. (a) Local leftover Injera with yellow color, (b) local leftover Injera with reddish color, (c) local leftover Injera with gray color, (d) local leftover Injera waste sample

FC = 100 – (%moisture + %volatile matter + % ash) (4)

Determination of the chemical composition of local leftover Injera

Extractive's content

2.5 g of dry raw local leftover Injera waste was loaded into a cellulose thimble. With the Soxhlet extractor set up, 150 mL of acetone was used as the extraction solvent. The dwell times for the boiling and ascending stages were carefully set to a 4 hour run time at 70°C, a heating mantle of 25 minutes, respectively. After extraction, the sample was air-dried at room temperature for a few minutes. A constant weight of the extracted material was achieved in an oven at 105°C. The % extract content (w/w) was assessed as the weight difference between the raw extractable locally residual Injera and the extract-free locally retained Injera waste, as shown in equation 5 below.

$$\text{Extractive content (\%)} = \frac{M1-M2}{M1}*100 \quad (5)$$

Where M_1 is weight of the sample (g) and M_2 is weight of the sample after oven-dried.

Hemi cellulose

One gram of the extracted dry local leftover Injera waste was transferred to a 250 ml Erlenmeyer flask. 150 mL of 500 mol/m^3 NaOH was added. The sample mixture was boiled in distilled water for 3.5 hours. After boiling, the sample was filtered, then, cooled by vacuum filtration, and washed to neutral pH. The residue was dried in an oven at 105°C to a constant weight. The difference in sample weight before and after this treatment is the hemicellulose content (% w/w) of the dry biomass using equation 6.

$$\text{Hemi cellulose content (\%)} = \frac{M1-M2}{M1}*100 \quad (6)$$

Where M_1 is mass of oven-dried before extraction in gram and M_2 is mass of oven-dried after extraction in gram.

Lignin

0.3 grams of dried, extracted raw topical residual Injera was weighed into a glass tube and 3 mL of 72% H_2SO_4 was added. The sample was left at room temperature for 2 hours and gently shaken at 30-minute intervals to completely hydrolyze. After the first hydrolysis, 84 mL of distilled water was added. The second hydrolysis step was performed in an autoclave at 121°C for 1 hour. The slurry was then cooled to room temperature. The hydrolyzate was vacuum filtered using a filter crucible according to equation 7.

$$\text{Lignin content (\%)} = \frac{M2}{M1}*100 \quad (7)$$

Where M_1 is mass of the oven-dried sample before hydrolysis in gram and M_2 is mass of the oven-dried sample after hydrolysis in gram.

Cellulose

The cellulose content (% w/w) was calculated by the difference using equation 8 assuming that the extract, hemicelluloses, lignin, and cellulose are the only constituents of the total biomass.

Cellulose content (%) = 100-extractive-hemicellulose-lignin (8)

Methods

Acid treatment of local leftover Injera powder

According to the study of Singh (22), pretreatment by dilute acid hydrolysis for biofuel production of 0.2-2.5% H_2SO_4 (w/v) at 120-220°C was used for 2-90 minutes. In this study, a 1.1% concentration of dilute sulfuric acid and 80 g of locally residual Injera powder with a sample to solution ratio of 1:10 (w/v) was used and pretreated in an autoclave at a temperature of 130°C for 60 minutes. The sample was then cooled and filtered using a filter vacuum. The residue was washed 4 times with distilled water to remove sulfuric acid until the pH reached 55.5. This is within the recommended interval during preprocessing.

Procedure

Eighty grams of crushed local leftover Injera waste sample was placed in a 1000 mL Erlenmeyer flask and diluted sulfuric acid with a concentration of 1.1% was added to the sample. Then, aluminum foil was used to close the Erlenmeyer flask. The sample was heated in a vertical autoclave to a temperature of 130°C for 45 minutes. The sample was then removed from the autoclave and cooled after the specified time and temperature. The soluble moiety was separated from the insoluble moiety by filtration. The filtrate was stored in a separately prepared Erlenmeyer flask and stored for fermentation.

Measurement of reducing sugars

Benedict's solution for determining the glucose concentration

In this study, the total reducing sugar content of the hydrolysis process using Benedict's solution method was investigated. The total reducing sugar concentration of the hydrolyzate obtained from the hydrolysis was determined using a digital spectrophotometer by measuring the absorbance vs. sugar concentration at a wavelength of 540 nm. Quantitative Benedict solutions and standard glucose solutions were used in the assay to record the calibration curve. The prepared Benedict solution should demonstrate the presence of reducing sugars.

Standard preparation

A standard stock solution of 0.01 g/mL glucose was prepared by dissolving 1 g of glucose in 100.0 ml of distilled water. Working standards were created by pipetting 5, 4, 3, 2, 1, 0.5, and 0%. Six test tubes were made with 5 mL of distilled water containing a glucose solution. The

sample was then shaken until the glucose was completely dissolved in distilled water. Six other tubes containing 2 mL of Benedict solution were prepared for each tube. Then, 1 mL of each standard solution was pipetted and placed in a test tube containing Benedict's solution. After rapid cooling, the mixture was kept in a water bath at a temperature of 90°C for 5 minutes. Next, a UV-Vis spectrophotometer was used to check the absorbance (540 nm) of the filtered solution. The sugar concentration of each sample was read from the calibration curve of the standard glucose solution.

$$Y=mx+b \quad (9)$$

Where Y is absorbance, m is the slope, x is the concentration, and b is the intercept.

$$\text{CTRSUS} = \frac{(\text{absorbance of unknown sample}) - (\text{Y} - \text{intercept})}{\text{slope}} \quad (10)$$

Where *CTRSUS* is concentration of total reducing sugar in an unknown sample. The yield of total reducing sugar can be obtained using the following Equation (11).

$$Y=C*\frac{V}{M}*100 \quad (11)$$

Where Y is yield of total reduced sugar, V is liquid volume, and M is amount of biomass.

Fermentation

The fermentation process was carried out in a shaking incubator for 72 hours at different temperatures and stirring speeds of 175 rpm. All assays were performed with 10% v/v inoculums. The hydrolyzate produced was adjusted to pH values under various conditions optimal for saccharomyces cerevisiae using a 2 M sodium hydroxide solution.

Media preparation

The medium was prepared in 250 ml test tubes consisting of (g/L) yeast extract (10). Glucose (20); Urea (5); Mg $SO_4 7H_2O$ (5); Peptone (20).

Medium preparation procedure

The medium was sterilized at a temperature of 121**°C** for 15 minutes. After cooling the medium, 0.50 g of saccharomyces cerevisiae in 100 ml of preparation medium in a 250 mL Erlenmeyer flask was added. The Erlenmeyer flask was appropriately covered with aluminum foil and placed in an incubator shaken at 30°C and 200 rpm for 24 hours.

The Procedure for Fermentation

Hydrolyzed samples were adjusted to three different temperature ranges: 25, 30, and 35°C. These temperatures were suitable for fermentation by *S. cerevisiae*. To create favorable conditions for *S. cerevisiae*, the pH of the sample was adjusted using 2M NaOH to adjust the solution pH to 35. Hydrolyzed samples containing 10% inoculum were placed in a shaking incubator at 25, 30, 35°C, 175 rpm for 3 days. After 72 hours of fermentation, samples were taken and sent to distillation to separate hydrous ethanol.

$$C_6H_{12}O_6\,(l) \rightarrow 2\,C_2H_5OH\,(l) + 2CO_2\,(g) \quad (12)$$

$$A \rightarrow 2B + 2C \quad (13)$$

Where *A*, *B*, and *C* are glucose, ethanol, and carbon dioxide, respectively.

Ethanol separation

Distillation

After the fermentation has stopped, the purity of ethanol should be high. Distillation is one of the most important steps in the purification process.

Determination of the properties of ethanol

Density and specific gravity test

An empty pycnometer was weighed. The pycnometer was filled with a sample (ethanol), the excess was removed, the weight was recorded accordingly and the density was calculated using the following formula: density (g/mL) = (mass)/(Volume) or Density = (M2Mo)/(M1Mo) where M2 = (g) empty bottle mass, M1=(g) empty bottle mass + water specific gravity is calculated by dividing density of ethanol to density of water.

Viscosity test

Fifty milligrams of ethanol was placed in the arm of the U-tube capillary viscometer through the opening to the set point. A suction device was used to lift the sample to the set value up to the arm of the capillary. A stopwatch was used to adjust the time. Next, a viscosity calibration curve was used to convert the viscosity in seconds to centimeter stokes.

Flashpoint test

The beaker in the device was dried. Fifty milligrams of sample (produced ethanol) was placed in a brass beaker and touched the predetermined mark in the beaker. Next, the cover was attached according to the position on the cup. A Bunsen burner was used to heat the bottom of the device. The heating was adjusted to provide a temperature rise of approximately 7°F/min and the sample was continuously agitated. When the sample approached the temperature of the flash, the injector burner was turned on, and then, injected into the sample at intervals of approximately 12 seconds until a clear flush was obtained in the container and injector burner. At this point, a thermometer was used to record near the flashpoint. Next, the flashpoint was recorded by pH test. To standardize the device, a pH meter was first placed in the buffer. Then, the measurements were taken.

The yield of Bioethanol from each fermented sample was determined as follows:

$$\text{Yield} = \frac{\text{sample weight}}{\text{mass of sample distillate}} * 100 \quad (14)$$

FT-IR determination of bio-ethanol

For bioethanol produced from localized residual Injera waste, functional groups were analyzed using an IR correlation diagram through the Prinks-Elmer spectrum 65FTIR method. The IR spectrum was reported as transmittance. The wavenumber range of the analysis was 4000400 cm^1 (mid-infrared region).

Data analysis

The experiment was designed to determine the effect of fermentation conditions on the yield of ethanol production from localized residual Injera. A fully randomized design of experiments was performed to determine the optimal points. Randomization ensures that the conditions of execution do not depend on the conditions of the previous execution and do not predict the conditions of subsequent executions. Temperature, substrate concentration, and pH were used as experimental factors. Analysis of variance (ANOVA) was run using a test version of Design Expert® V.11.0.0. RSM is widely used for experimental data analysis. This model anticipates experimental changes such as changing operating conditions and various processing steps, and ultimately, helps design experimental setups. The experiment was performed by a design expert using a combination of the values of the actual design elements at each level. The answer surface method was used to provide room for improvement and optimization of design answers that are affected by various variables. The response variables are fitted to the following quadratic polynomial model. This model can generally describe the relationship between the response and the independent variable, as shown in equation 15.

$$Y = \beta 0 + \beta 1A + \beta 2B + \beta 3C + \beta 11\ A2 + \beta 22\ B2 + \beta 33\ C2 + \beta 12\ AB + \beta 13\ AC + \beta 23BC \quad (15)$$

Where Y is predicted response, A, B, and C are temperature, substrate concentration, and pH, respectively. $\beta 0$ is the intercept, $\beta 1$, $\beta 2$, and $\beta 3$ are the linear coefficient, $\beta 11$, $\beta 22$, and $\beta 33$ are the squared coefficients, $\beta 12$, $\beta 13$, and $\beta 23$ are interaction coefficients.

Data analysis was performed by Design Expert software@11(Box-Behnken) to assess the impact of process variables. Temperature, pH, substrate concentration. The response variable was ethanol yield after fermentation. This design of experiments helps optimize process parameters using RSM. The significance of the results was determined by ANOVA.

Temperature

To determine the optimum temperature for maximum yield of bioethanol production by selected isolates after the hydrolysis process, each flask containing a 100 mL sample of hydrolysate was inoculated with 10% (v/v) yeast isolates and incubated at a different temperature between 25 and 40 under stationary conditions. A 10% yeast concentration was selected with a stirring rate of 175 rpm. To determine the optimum pH for maximum yield of bioethanol production by selected isolates after the hydrolysis process, each flask containing 100 mL sample of hydrolysate were inoculated with 10% (v/v) yeast isolates and incubated over a selected point of temperature with a pH in between 3 and 5 under stationary conditions. A required fermentation process for 10% yeast concentration was achieved at a stirring rate of 175 rpm.

pH

To determine the optimum pH for maximum yield of bioethanol production by the selected isolates after the hydrolysis process, each flask containing 100 mL sample of hydrolysate were inoculated with 10% (v/v) yeast isolates and incubated over a selected point of temperature with a pH in between 3 and 5 under stationary conditions. A required fermentation process for 10% yeast concentration was achieved at a stirring rate of 175 rpm.

Substrate concentration

To determine the optimum substrate concentration for maximum yield of bioethanol production by the selected isolates after hydrolysis process, each flask containing 100 mL sample of hydrolysate were inoculated with 10% (v/v) yeast isolates, incubated over a selected point of temperature and pH with different substrate concentration between 50 hours and 200 g/L at a stationary condition. A required fermentation process for 10% yeast concentration was achieved at a stirring rate of 175 rpm. The summary of factorial designs and the minimum and maximum values of a factor is summarized as a type of study is response surface, first design Box-Behnken, model design, quadratic polynomial, number of 17 runs, and no block. The minimum and maximum values of temperature were 25°C and 40°C, respectively. Similarly, the minimum and maximum values of substrate concentration were 50 and 200 mg/L, respectively. For pH, the minimum and maximum values were 3 and 5, respectively.

Results

Characterization of local leftover Injera

Proximate analysis

Moisture and volatility content determination of local leftover Injera

The moisture content of the remaining Injera waste was determined to be 7.4 according to equation 1 by continuously placing the measured sample in the oven drying position until it reached a certain weight. The volatility level was determined according to Equation 2 and was found to be 73.2%.

Determination of ash and fixed carbon content of local leftover Injera

The ash content was determined to be 2.4% according to Equation 3. The solid carbon content was determined to be 17% according to formula 4.

Chemical composition of local leftover Injera

Determination of extractives and hemicellulose

The amount of extract was determined to be 21.5% according to equation 5. The amount of hemicellulose was determined to be 24% according to equation 6.

Determination of lignin and cellulose

The amount of lignin was determined to be 18.33% according to Equation 7. The amount of cellulose was determined to be 36.2% according to Equation 8. The concentration and absorbance values for the unknown sample from the standard curve were determined to be 0.809315 g/ml using Equation 9.

Characterization of bioethanol produced

In this study, cultures of saccharomyces cerevisiae were used to estimate the viscosity, pH, density, flash point, and functional groups of bioethanol produced by separate hydrolysis and fermentation.

Density measurement

Cultures of Saccharomyces cerevisiae were used to estimate the density of bioethanol produced by separate hydrolysis and fermentation. Recorded observations showed that the specific gravity of the bioethanol produced was 0.809 g/mL at a temperature of 19.9°C. At room temperature, it is denser than 785 kg/m^3 of ethanol.

Viscosity

Ethanol was able to flow through the capillaries over time. When ethanol reaches the indicated mark, use a viscometer. In a fuel-injection combustion chamber system, the viscosity of the fuel must be considered. This property is a measure of the flow resistance of a substance (mainly a liquid). The kinematic viscosity was determined to be 1.2324 * 106m2s1.

Flashpoint

This is an important property in determining the flammability of a fuel. The flashpoint is the minimum temperature at which the applied ignition source ignites the fuel vapor. Therefore, the sample tends to form a flammable mixture. The flashpoint of the produced ethanol was 17°C. This is shown near 1213°C as described in the literature. The difference may be due to personal mistakes.

Optimization of operating process variables in the fermentation process using RSM

Based on the above-mentioned analysis, the best maximum ethanol yield was 42.592% at substrate concentration of 125 g/mL, temperature of 324.536°C, and pH of 4, with the desired value of 90.6%.

Discussion

Proximate analysis

The water content of locally retained Injera was determined by (23), and it was 5.1%, which is lower than that reported in this study. This may be due to a personal error or a device error. Moisture content analysis to determine the proportionality of solid-liquid ratio to increased water content in pretreatment and hydrolysis methods affects product quality. Local residue Injera samples with high water content require more heat for water evaporation. The ash content of locally retained Injera investigated by (24), was 8.5% higher than that reported in this study. Ash is a measure of local residual Injera mineral contamination. Using the method proposed by (25), 21.3% higher ash content was produced than that in this study. In this study, the low ash content of the localized residual Injera component reduced sludge formation during ethanol production. From this, it can be concluded that Injera waste is a good source for bioethanol production. Finally, solid carbon is the carbon found in the material that remains after the volatiles have been expelled. There was a slight difference in the content of cellulose, hemicellulose, and lignin compared to the study by (26), which were 33.7%, 31.9%, and 6.1%, respectively. This difference is expected to be because comparisons made between local raw materials are different, in different geographic locations, and in different weather conditions where the Injera production-based crops are grown. The relationship between glucose concentration and absorbance is shown in Figure 2.

FTIR characterization of the produced bio-ethanol

By creating an infrared retention region using Fourier Transform Infrared (FTIR) spectroscopy, chemical bonds in the molecule of ethanol made from locally remaining Injera waste were detected. The spectrum produces a profile of the sample. This is a specific molecular fingerprint that can be used to screen and scan samples in a wide range of segments (27). Fourier Transform Infrared is an operational analysis tool for distinguishing functional groups and characterizing covalent data, as shown in Figure 3 and Table 1.

As shown in Figure 3, the peak at 3303 cm^{-1} corresponds to the OH stretching vibration and indicates the presence of hydroxyl groups. On the other hand, around 2844 cm^{-1} represents the C stretch corresponding to the presence of alkanes. Picks in the range of 1000 and 600 cm^{-1} are related to C-O stretch and C-H bending, respectively.

Characterization of bioethanol produced

In this study, viscosity, pH, density, flash point, and functional group of bioethanol produced by separate

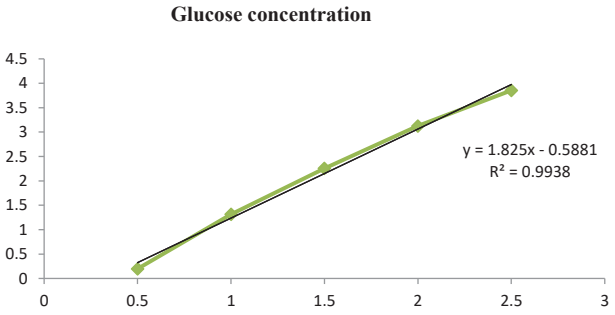

Figure 2. Glucose concentration vs absorbance.

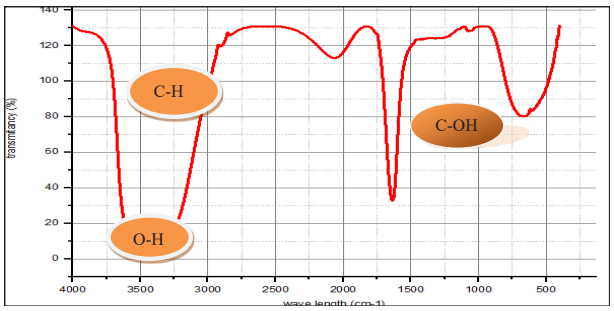

Figure 3. FTIR results of ethanol yield at a temperature of 32.718°C, for a substrate concentration of 125 g/mL and pH of 4.

hydrolysis and fermentation using the culture of Saccharomyces cerevisiae, were estimated.

Density measurement

Cultures of Saccharomyces cerevisiae were used to estimate the density of bioethanol produced by separate hydrolysis and fermentation. Recorded observations showed that the specific gravity of the bioethanol produced was 0.809 g/mL at a temperature of 19.9°C, which is consistent with the results of other studies (6). At room temperature, it is denser than 785 kg/m^3 of ethanol. This is due to the presence of water. About 5% was found in the recovered ethanol due to the formation of an azeotropic mixture in which the gas and liquid phases of the mixture had the same composition at a particular temperature of 78°C. It is important to note that the slight differences in observed densities may be primarily due to differences in the raw materials used, the fermentation process used, and the presence of impurities.

Viscosity

Ethanol can flow through the capillaries over time. When ethanol reaches the indicated mark, a viscometer is used. In a fuel-injection combustion chamber system, the viscosity of the fuel must be taken into account. This property is a measure of the flow resistance of a substance (mainly a liquid). There was a slight deviation compared to the standard value. This may be due to personal and experimental errors. If the viscosity is too low, the fuel will flow more easily. This usually has the disadvantage of not maintaining a lubricating film between the moving and fixing parts of the carburetor or pump. On the other hand, the very high viscosity of the fuel prevents the fuel from atomizing into small droplets, allowing for good evaporation and combustion.

Flashpoint

This is an important property in determining the flammability of a fuel. The flashpoint is the minimum temperature at which the applied ignition source ignites the fuel vapor. Therefore, the sample tends to form a flammable mixture. The flashpoint of the produced ethanol was 13.5°C. This is shown near 12-13°C as described in the literature (29).

Statistical analysis of the experimental results

Analysis of variance

A statistical summary of each model is shown in Table S1. The secondary model with high values of tuned, R^2 predicted, and no use of aliases was proposed in comparison to the tertiary model. The results of using the Design Expert® 11 software are shown in Table S2. It was important to perform an ANOVA to determine if the second model was significant. In Table S3, the probability values (*P* values) were used as an aid in observing the significance of the individual coefficients, which also reflected the strength of the interaction of the individual parameters. The smaller the *P* value, the greater the significance of the corresponding coefficient. According to Table S2, a value of "Prob> F" less than 0.0500 indicates that the model term is significant. In this case, A, B, C, AC, BC, A2 are important model terms. The coefficients of the linear effect of temperature, substrate concentration, and pH were very important. It has also been observed that there is an interaction effect between temperature and substrate concentration.

The F-number is used to compare the variance of the model with the residual (error) variance. It is calculated by dividing the mean square model by the mean residual square. Here, a model F value of 75.32 means that the model is significant. The probability of a model F value is only 0.01%. The large values are due to noise. A value of "Prob> F" less than 0.05 indicates that the model term is significant. The "lack of F value" in 5.91 indicates that the lack of conformance is not as important as the pure error. There is a 7.09% chance that such a large underfit F-value will occur due to noise. The regression coefficients and their corresponding 95% CI elevations are

Table 1. Functional groups and respective frequency (28)

Frequency range (cm^{-1})	Groups	Class of compound
3303	O-H Stretching	Alcohol, phenols
2844	C-H stretching	Alkanes
1589	C=C bending	Aromatic compound
1029	C-OH stretching	Alcohol, phenols, esters
582	C-H	Aromatic compound

shown in Table S4.

The regression coefficients and their corresponding 95% CI (confidence interval) elevations are shown in Table S4. If there are zeros in the high and low 95% confidence intervals, the coefficients have no effect. From the high and low 95% CI of each model term, it can be concluded that temperature, pH regression coefficients, and the temperature-substrate concentration interaction term have a very important effect on ethanol production. Using the constructed experimental data and applying it to Table S5, a model formula for ethanol production from the remaining local Injera waste for fermentation was found using equation 15.

Final equation in terms of coded factors

Ethanol yield = + 42.29 + 0.7687 * A0.9875 * B0.8062 * C + 0.3500 * AB + 1.21 * AC1.07 * BC + 10.27 * A2

The equation can be expressed as an actual coefficient. Response to a particular level of individual factors. Table S5 shows the model validity measures.

The predicted R^2 of 0.9003 is consistent with the adjusted R^2 of 0.9702. It means that the difference is less than 0.2. Model accuracy measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Those ratios of 21.973 indicate a sufficient signal. This model can be used to navigate the design space. This indicates that the regression line fits the data perfectly, as the R2 value is close to 1.0. Similar to this study, the resulting R^2 was 0.9832, close to 1. The results suggest that the predicted values are in good agreement with the experimental values (R^2 = 0.9832 and AdjR^2 = 0.9702), indicating that RSM has been achieved. The goodness of fit of the model was checked using the regression coefficient (R^2). In this case, the values of the coefficients in Table S5 (R^2 = 0.9832) indicate that the developed regression model did not explain only 1.68% of the total variance. The R^2 observations show a good fit for the experimental results. The adjusted coefficient of determination (AdjR^2 = 0.9702) was also sufficient to confirm the importance of the model. Predicted R-squared suggests that the model is likely to explain the high rate of variability in the new data (about 90.03%). "Adeq Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. In this study, 22.403 showed a good signal. A typical plot of residuals is shown in Figure 4a).

According to Figure 4, the normal probability chart shows the residuals, followed by the normal % probability distribution. For these experimental data, the points in the figure fit the straight lines in the figure. The polynomial model meets the ANOVA. That is, as shown in the figure, the error distribution was almost normal. In some cases, if the model found is correct and the assumptions are met, it should be independent of other variables, including the predicted response. A simple system to check is to plot the residuals against the approximate (predicted) values. Plots of residuals and increasing predictive response values test the assumption of constant variance. This figure shows a random variance that does not guarantee changes to minimize personal errors.

Ethanol production can be affected by many parameters. The best way of showing the effects of this parameter for the ethanol yield is to generate response surface plots of the equation.

The effects of interactions, contour lines, and reaction regions are plotted in the figure below as a function of the interaction between any two variables by keeping the other values of the variables centered. The black and red lines of the interaction numbers indicate the low and high parameter.

Figure 5a and b shows the effects of substrate concentration and acid pH on ethanol yield, when the temperature was at the center point, and contour plots of the effects of substrate concentration and pH on ethanol yield. Figure 6 shows the effect of substrate concentration and pH on ethanol yield at mid-temperature.

The effects of substrate concentration and pH on ethanol yield are selected with temperature as the focus, and Figures 7 to 8 show the lower and upper limits of substrate concentration and pH value, ethanol production yield, and fermentation medium.

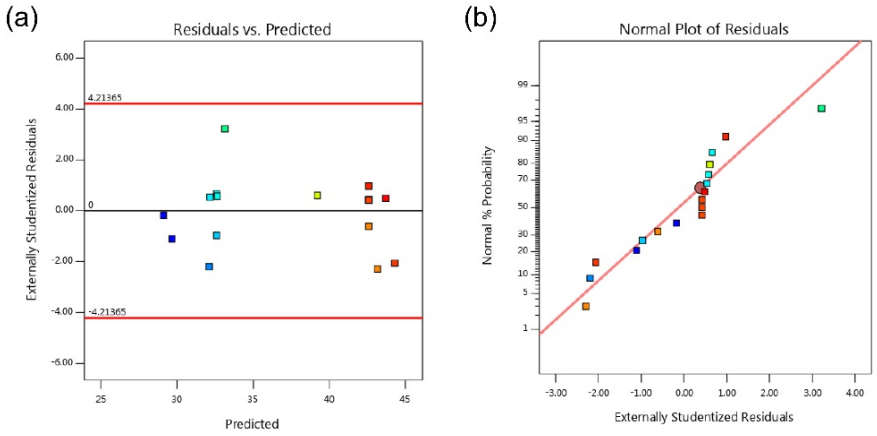

Figure 4. Normal plots of residuals and residual versus predicted values. (a) Normal plots of residuals. (b) Residual versus predicted values

At lower and higher temperatures and substrate concentrations, ethanol yield production is reduced because it affects the fermentation medium. At lower temperatures and substrate concentrations, cellulose may not be converted to ethanol, and at higher substrate concentrations and higher pH levels, cellulose may be converted to other molecules that may not be fermentable. Therefore, both temperature and substrate concentration are closely related to the ethanol yield production. This is shown in Figures 7a and 7b.

Effect of temperature

Figure 7 shows the effect of temperature on ethanol yield at constant pH and central substrate concentration.

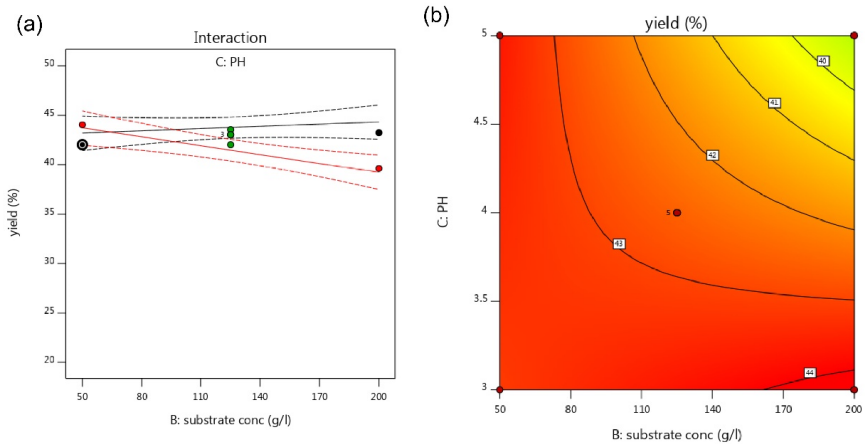

Figure 5. The effects of substrate concentration and acid pH on ethanol yield, when the temperature was at the center point, and contour plots of the effects of substrate concentration and pH on ethanol yield. (a) The effects of substrate concentration and acid pH on ethanol yield. (b) Contour plots of the effects of substrate concentration and pH on ethanol yield

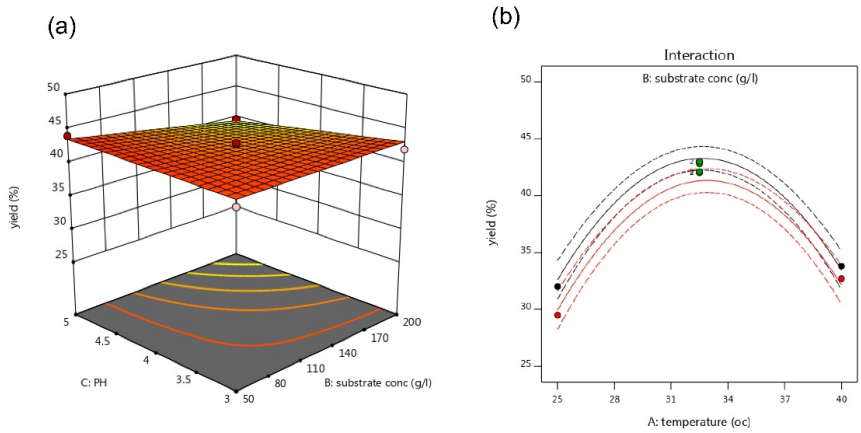

Figure 6. Effect of substrate concentration and pH on ethanol yield when the temperature was at the center point, and the effect of temperature and substrate on ethanol yield when pH was at the center point. (a) Effect of substrate concentration and pH on ethanol yield. (b) Effect of temperature and substrate on ethanol yield.

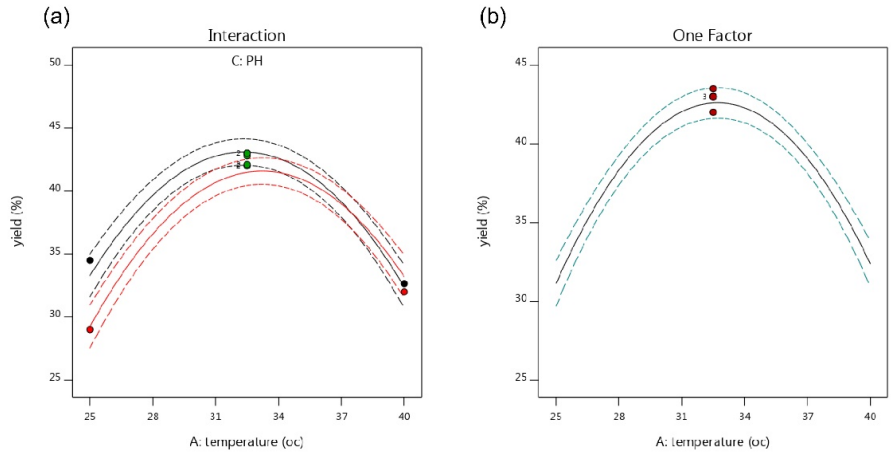

Figure 7. Effect of temperature and pH on ethanol yield when substrate concentration was at the center and the effect of temperature on ethanol yield. (a) Effect of temperature and pH on ethanol yield. (b) Effect of temperature on ethanol yield.

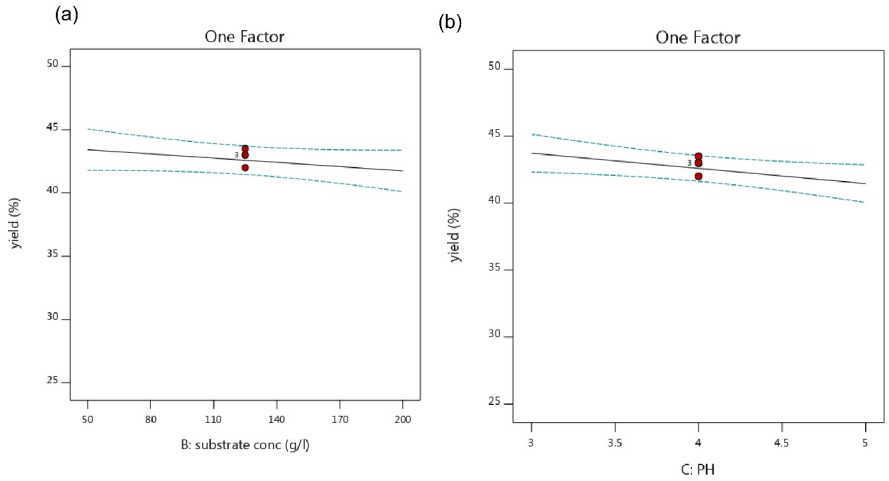

Figure 8. Effect of substrate concentration and pH on ethanol yield. (a) Effect of substrate concentration on ethanol yield. (b) Effect of pH on ethanol yield.

As shown in this figure, the yield of ethanol is highly temperature-dependent. As temperature increased from 25°C to 32.50°C, ethanol yield increased significantly. The optimal ethanol yield was obtained at a temperature of 32.5°C. Ethanol yields are slightly reduced from temperatures of 32°C, which is consistent with the results of other studies (30).

Effect of substrate concentration

Figure 8 shows the effect of substrate concentration on ethanol yield at central constant temperature and pH. As shown in this figure, ethanol yield was slightly affected by substrate concentration, but substrate concentration increased from 50 to 126 g/mL, ethanol yield increased slightly, and at a substrate concentration above 126 g/ml, ethanol yield decreased.

Effect of pH

Figure 8 shows the effect of the hydrolyzed pH value on ethanol yield at constant temperature and median substrate concentration. As shown in this figure, ethanol yield is slightly affected by pH, but as pH increases from 3 to 4, the yield increases slightly. At a pH level above 4, ethanol yield decreases slightly, which is consistent with the results of other studies (31).

Optimization of operating process variables in the fermentation process using RSM

The optimization of fermentation criteria for ethanol production from locally leftover Injera is summarized in Table S6 and Table S7 using RSM.

If the desirability is between 0 and 1, it indicates that the answer is close to its ideal value. If the response is found to be within an unacceptable interval, the desirability is 0, and if the response is found to be within the ideal interval, or if the response reaches the ideal value, the desirability is 1 (32). Based on the above-mentioned analysis, an ethanol yield of 42.592% was found at a substrate concentration of 125 g/mL, a temperature of 324.536°C, and a pH of 4, with the desired value of 90.6%.

Conclusion

Injera, a local leftover, is a solid waste that is thrown into the environment as useless and pollutes the environment. However, it is a promising raw material for the production of bioethanol fuel. This is the most common by-product produced as waste in hotels and restaurants. The study area was Jimma University that has an annual production potential of over 680 tons of food wastes from students Cafeteria. This study examined the possibility of using this local residual Injera waste left from Jimma University students' cafeteria to produce bioethanol by anaerobic fermentation. Characterization of the chemical bonds of ethanol produced from local residual Injera waste was performed by the FTIR analysis. From the results obtained, it was observed that ethanol made from locally retained Injera contained OH, CO, CH_2, and CH_3 functional groups, which makes sure the product is ethanol. The fermentation conditions were optimized, and the effects of variables like temperature, pH, and substrate concentration were investigated using RSM for ethanol production from leftover Injera. Based on the ANOVA, fermentation temperature, pH value, and the interaction of temperature with substrate concentration have a significant effect on ethanol yield. Positive ethanol yields were obtained at both high and low substrate concentrations. The range of conditions tested for temperature, pH, and substrate concentration was 25-40°C, 3-5, and 50-200 mg/L, respectively. Based on the results of RSM optimization at fermentation temperature of 32.718°C, pH 4, and substrate concentration of 125 g/mL, respectively, ethanol yields were 42.598% in all cases. Therefore, work on the further development of ethanol production from the leftover Injera waste should be continued by optimizing various process parameters (fermentation variables) as per the experimental findings of this study. Future research should include optimization of the pretreatment process and distillation process

variables to get the maximum ethanol yield from local residual Injera waste.

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Ethical issues

The authors hereby certify that all data collected during the study are described in the manuscript, and no data from the study have been or will be published separately elsewhere.

Competing interests

The authors declare that there is no conflict of interests.

Authors' contributions

AB contributed as supervisor, KB as data collector and analyser, DA as reviewer and editor throughout the manuscript.

Supplementary files

Supplementary file 1 contains Tables S1-S7.

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