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RESEARCH ARTICLE
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ISOLATION AND SCREENING OF YEAST STRAINS FOR BIOETHANOL PRODUCTION USING SUGAR MOLASSES AND LIGNOCELLULOSE BIOMASS

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ABSTRACT

Ethanol is a highly useful hydrocarbon which is used extensively in major industrial and manufacturing processes. It is also used as fuel. Ethanol is mainly sourced from the fermentation of carbon-based feedstock by microorganisms such as yeasts, which can be found almost everywhere in the environment. In view of the anticipated shortage of the traditional supplies of fossil fuels, there is a great interest in the production of ethanol as an alternative biofuel in recent years. The main objective of this research work was to isolate and characterize stress tolerant and high potential ethanol producing yeast strains from various fruit juice. The potential yeast isolates capable of producing ethanol were isolated from rotten fruit juice samples of sugarcane, sweet lemon, grapes and guava and grown in YEPD medium. Six yeasts isolate S1, S2, Gr, M1, M2, and Gu were characterized based on morphological and physicochemical characters. Based on the morphological appearance of vegetative cells under microscope, colony character and physicochemical characters, the isolates were identified as yeast isolates. Selected yeast strains were checked for alcoholic fermentation using sugarcane molasses and organic kitchen wastes media. The fermentation of molasses was optimized with respect to pH, temperature and ethanol concentration. The results revealed the optimum condition was found as a 30°C, pH 6.0 and 5% ethanol for fermentation. Stress tolerance tests showed that the strains S1, S2 and Gr were found highly thermo-, pH- and ethanol-tolerant. Pycnometer and refractometer were used for estimating percentage of ethanol. Under optimized conditions, S1, Gu, Gr, S2, M1, and M2 produced 85% ,57%, 87%,47%, 60% and 70% of ethanol by using molasses. Kitchen- wastes resulted 53%, 25%, 43%, 42%, 59% and 37% ethanol production after 48 hours of fermentation by using yeast isolates S1, Gu, Gr, S2, M1, and M2. It was concluded that indigenous yeast isolates could be used for high production of bioethanol, spirit and industrial alcohol.

Key words: Yeast Strains, Bioethanol Production, Sugar Molasses, Lignocellulose, Biomass

INTRODUCTION

Ethanol is a renewable energy source produced by the fermentation of sugars. Its production is companioned by greater environmental advantages as a result lower emissions of carbon monoxide (CO) , volatile organic compounds and sulfur oxides on burning as compared to the burning of typical fossil fuels (Jain et al., 2014, Soares et al., 2002). For bioethanol production, yeast is one of the most suitable microbial organisms. Bioethanol can be used as a renewable substitute for fossil fuels (Jeffries, 2007, Piotrowski et al., 2014). Bioethanol has been utilized as a raw material in various food and pharmaceutical industries. Recently, the interest to utilize biofuel is a potential option in contrast to petroleum derivatives which cause environmental and economic disadvantages (Gaurav et al., 2017). Another circumstance that forced us to use bioethanol as a reliable and possible alternative source of fuel is the increase in global warming, consumption of oil resources and the significant expense of gasoline (Volynets et al., 2017). Air pollution, acid rain, and global warming are dangerous environmental issues produced by the overuse of petroleum products. (Kiran et al., 2014). For survival and to fulfil the increasing energy need, biofuel can be used as an alternative to petroleum derivatives. The carbon is found in every biomass, and the biomass is a transitory storage area for sunlight. Environmental carbon is a very important source of energy. Over the most recent couple of years to produce biofuels and chemicals from biomass used as an alternative to petroleum is now increasing in demand. For maximal production of bioethanol fermentation is more competent procedure. Along with these, using microorganisms for fermentation of agriculture biomasses could be our substitute route for the production of biofuels. For commercial fermentation yeasts are famous for bacteria due to cell wall's thickness, requirements of the nutrition's which are less demanding, large in sizes, contamination chances are less, and can grow in Low PH (acidic) solutions. Pichia stipitis and Saccharomyce cerevisia are models which seem very true in utilization of carbohydrate substrates for methods of fermentation in view of their range to convey ethanol in a very high proportion. Through the fermentation procedure these microorganism can utilize various substrates that contain adequate quantity of carbohydrates. Presently the main focus should be towards lignocellulosic biomass, which must have half cellulose 30 to 31% and hemicellulose 20 to 40%, and can be easily changed over into the form of xylose and glucose after pretreatment of enzymatic reaction of hydrolysis. Naturally, second most found sugar which is xylose and it could give supply of a substitute fuel source because of these abilities to use industrially fermented into the form of bioethanol.

At the modern level S. cerevisiae considered as the primary and the most fundamental source for bioethanol production. To ferment xylose the several attempts were actualized to engineer the S. cerevisiae because in light of the fact that normally it doesn't ferment xylose. In addition, xylose-metabolizing yeast like P. stipitis can upgrade genetic engineering to modify their fermentative activities. In this research, the main force on yeasts which are genetically modified to improve its abilities for the bioethanol generation, and talking about the factors that effects positively/negatively on the fermentation method. The strategies of bioethanol production with the use of starch, sugarcane, and microbial fermentation of sugar substrates considered as the primary cause to abstain the nonrenewable energy fuel which causes notable damages for our earth. Lignocellulose, as it is another choice and promising path for bioethanol generation in our future research, so specialists who work in the field of fermentation give more thoughtfulness regarding about lignocelluloses biomasses (Xie, 2017).

Now different methods and techniques are used for obtaining bioethanol. Fermentation of molasses extracted from sugar cane, biomass of lignocellulosic, strips of vegetable or wastes of many sustenance can also be utilized as a very efficient and easy method for production of bioethanol. The use of bioethanol obtained from cellulosic materials is increasing in countries like Canada, Brazil and USA. Brazil is using the ethanol on a very large scale as a fuel in transportation. Ethanol is not used in very pure form, but blended in with some other fuel and apart from this ethanol is being used in

many other works and everyday requirement. As per applied science survey of World Vitality report from 2018 (Dudley, 2018), the normal increment in worldwide oil utilization inside the year 2017 was 1.8% or 1.7 million and after 10 years it become double. So there is need to develop an alternative fuel to meet the increased world demand. The idea was that the most prominent alternative fuels for replacing fossil fuels in internal combustion engines would be biofuels (biodiesel, bioethanol, bio-methanol, etc.). Biofuels that are obtained from vegetable oil resources seem to be a very good substitute for fossil fuels; their production is rather simple; they are cleaner, biodegradable, nontoxic, recyclable, and benzene-free (Niculescu et al., 2018, Storch et al., 2015, Turner et al., 2011).

MATERIALS AND METHODS

Sample collection

The rotten fruit juice samples of sugarcane, sweet lemon, grapes and guava were collected from different markets of Lahore.

Isolation and morphological examination of yeast isolates

One ml of rotten fruit juice sample was suspended in 9 ml of sterile distilled water and mixed well. The samples were serially diluted in test tubes up to 10^6. An aliquot of 50 µl was spread on YEPD agar media (1 g yeast extract, 2 g peptone, 2 g dextrose and 1.5 g agar in 100 ml of distilled water, the media was autoclaved at 121°C and 15 psi and poured on a petri dish and cooled) by spreading technique and incubated for 48 hours at 28°C. The plates were examined for alcohol smell and the colonies suggestive of yeasts were picked from plates. These colonies were further purified on fresh YEPD agar plates and identification was carried out by studying cell morphology texture, color and surface of colonies and budding characteristics under microscope according to the method of (Lorenz et al., 2000).

Determination of physiological characteristics

Fermentation of different sugars

The yeast extract, peptone broth was used for examination of selected isolate yeast strain on the bases of the ability to ferment different types of sugars. The different types of carbohydrate utilized to glucose, galactose, maltose, sucrose and lactose. Phenol red (0.01%) was added in yeast fermentation broth containing 50 ml of YP media (1 g yeast extract and 2 g peptone). The medium was sterilized in autoclave at 121°C and 15 psi for 20 min. Filtered sterilized sugar (1g) was taken in each tube and incubated at 30°C for 72 h. Ability to ferment five different carbohydrates was examined anaerobically. The color change of broth from dark reddish brown to yellow and the appearance of liquid medium become hazy due the process of fermentation and formation of carbon dioxide was examined (Tarek et al., 2017).

Stress tolerance test

Thermo tolerance

The growth of different yeast strains was examined at different temperature in YEPD broth. The quantity of 15 ml of liquid medium was distributed into test tubes and inoculated by the 48 hours grown yeast culture. The medium which was not inoculated with yeast strain used as blank and before incubation the initial optical density of all the test tubes was recorded by spectrophotometer at 600 nm. All cultures were incubated at different temperatures, 25°C, 30°C, 37°C, and 44°C for 2 days. The growth and tolerance at different temperature of isolated yeast strain was monitored and the change in the value of OD with respect to blank confirmed as the increase in growth of yeast (Promon et al., 2018).

pH sensitivity

YEPD liquid medium was used for detecting the ability to grow at different pH. The medium was autoclaved at 121 °C and 15 psi and cooled. YEPD broth was prepared at pH 2-10. Each test tube

contained 15 ml of YEPD media with different pH and blank media was used as a control. Then each was inoculated by half loopful of yeast cell and measured the initial optical density at 600 nm and incubated at 30°C for 48 h. After 48 h cell density was further recorded at 600 nm for growth.

Ethanol tolerance

YEPD broth was prepared containing 5%, 10%, 15%, 20% and 25% of absolute ethanol. Each test tube contained 15 ml of YEPD liquid media with appropriate concentration of ethanol and blank media was used as a control. Then each test tube was inoculated by half loopful of isolated yeast cell and measured the initial optical density at 600 nm and incubated at 30°C for 48 hrs. After 48 hrs cell density was further recorded at 600 nm (Promon et al., 2018).

Production of ethanol using sugar molasses

Pretreatment of sugar molasses

Sulfuric acid is used to convert calcium salts in molasses to calcium sulfate salts. Calcium acts as an inhibiting agent during fermentation of molasses by yeast. Sulfuric acid decreases the fermentation medium pH that controls bacterial contamination. Sugar molasses contained various nutriments for fermentation. In ethanol production, urea was added in the sugar molasses as a nitrogen source. Diluted sugar molasses was pretreated with 0.20 ml conc. (H_2SO_4) and 0.11 gm of urea (Shafkat, 2013).

Preparation of yeast cell suspension

Aseptic loop was used to pick up the selected yeast isolates inside biosafety camber and transferred into the test tube (15 ml) contained sterile sugar molasses fermentation media and label the test tubes. All test tubes placed in a rotary incubator at 30°C in under shaking (180 rpm) to form a homogeneous suspension.

Sugar molasses fermentation method

Fermentation was carried out in Erlenmeyer conical flasks. 250 ml fermentation media was taken into 500 ml Erlenmeyer flasks and added the homogenous suspension of yeast was inoculated into the media in an aseptic condition. The flask was cotton plugged and incubated at different temperatures in an incubator at 30°C under shaking condition for 7 days.

Kitchen waste (vegetable peels) fermentation media

Pretreatment of kitchen waste

Vegetables peels were gathered from various house discards. Peels of potatoes, papaya, pumpkin, cucumber, woman's finger, and carrot were used. Solid wastes (200 gm) were sliced, pounded and put in 1000 ml distilled water. Blended by used of a mechanical blender. The complete breakage of solid substances can only ensure the proper nutrient availability for the microorganism in medium.. Bacterial contamination in the fermentation medium decreased by the used of HCl through reduction the pH value. The chemical hydrolysis of plant deposits through HCl in a boiling temperature and converted plant carbohydrate units of Cellulose and starch into simple form of carbohydrates (Shafkat, 2013).

Vegetable peels fermentation method

Erlenmeyer conical flasks were used for this procedure. 200 ml of sterile vegetables peels media was taken in 500 ml conical flasks and under sterilized condition added the homogenous suspension of selected isolated yeast into the media. The flasks were covered with aluminum foil and incubated at the shaking condition at 28 ℃ for 7 days. (Shafkat, 2013).

Distillation

Ethanol recovery from the broth was done through fractional distillation process. The separation of two liquids on the basis of their boiling points. Boil the fermented media in a flask attaches with the condenser at 78°C. The vapors of the ethanol condensed and collected in reservoir (Bakiet and Mahmoud, 2015).

Ethanol was chemically detected

According to the following method (Bakiet and Mahmoud, 2015).

Iodine test

Two ml of distillate was taken in a test tube and added 4ml of iodine solution. And then added 4 drops of sodium hydroxide (NaOH). The test tube was heated then cooled in water bath. The white precipitate was appeared.

Potassium permanganate test

Two ml of distillate was taken in a test tube and added 1 ml of potassium permanganate ($KMnO_4$), the color of distillate turn to purple and shake well. After some time the solution became colorless.

Potassium dichromate

Potassium dichromate is an oxidizing agent. Two ml of distillate was taken in a test tube and added 1 ml of 2% potassium dichromate ($K_2Cr_2O_7$) followed by the addition of 1 ml of concentrated sulphuric acid H_2SO_4. The color of distillate changed to green or blue.

Estimation the percentage of ethanol

Determination the ethanol concentration by specific gravity

Concentration of ethanol determined through measured the specific gravity of the sample obtained after distillation. Determined the weight of empty dry pycnometer (25ml) taken as m1. Add water in the pycnometer in that way a capillary hole in the stopper is completely filled with water. Dry the spare water that leaks through the capillary hole with a filter paper and measure total weight taken as m2. Rinse it once with the solution (obtained after distillation) whose density you are going to determine next. Fill pycnometer with the unknown solution and measure the weight taken as m3. Specific gravity= m3- m1/ m2- m1, this equation was used to measure the value of specific gravity of the unknown sample. Measured the concentration of ethanol in a sample using the S.G table for water –ethanol mixture vs Specific gravity at various temperature. .

Determination the refractive index of distillates sample

Measured the refractive index of the distillate samples using a particular monochromatic light source, the apparatus is calibrated with water as the liquid. Adjust the micrometer screw to focus the boundary between the bright and dark regions. Placed a sample on the prism; while looking through the eyepiece, the control knob is turned until the shadowline is centered in the crosshairs, the reading is taken where the vertical line crosses the scale (Shi long et al., 2019).

RESULT

Identification of selected yeast strains

Examination on growth medium

The plates were examined for alcohol smell and the colonies suggestive of yeasts were picked from plates. These colonies were further purified on fresh YEPD agar plates and identification was carried out by studying cell morphology texture, color and surface of colonies and budding characteristics.

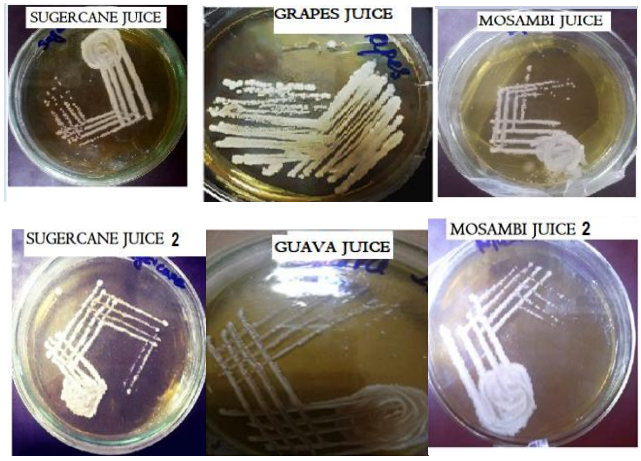


Figure 1: Colonies of different isolated strain of yeast on YEPD agar .

Morphological characterization According to the method of (Lorenz et al., 2000) pure culture of yeast was examined based on the morphological appearance and physiological characteristics. Morphology of vegetative cell of yeast was examined on YEPD agar (Growth on a solid medium).The physiological characteristic identified under the (40x) compound microscope.

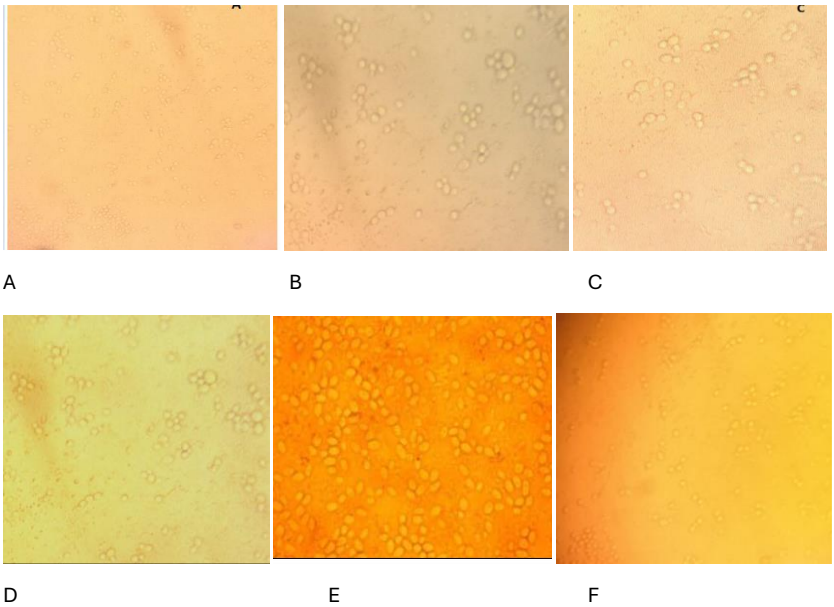


Figure 2: Isolated yeast strains cell morphology of (a) S1, (b) S2, (c) M1, (d) M2, (e) Gu and (f) Gr.

Table I: The morphological characterization of selected yeast isolates**.**

Yeast isolates	Source	Colony color	Colony nature	Elevation	Margin	Shape
S1	Sugarcane	White cream	Smooth and dry	Raised	Entire	Round
S2	Sugarcane	White cream	Smooth and dry	Raised	Entire	Round
M1	Sweet Lemon	Off white	Dry	Raised	Entire	Spherical
M2	Sweet Lemon	Off white	Dry	Raised	Entire	Spherical
Gr	Grapes	White cream	Smooth and shiny	Raised	Entire	Oval
Gu	Guava	Off white	Smooth	Flat	Entire	Ellipsoidal

Physiological characterization

The fermentation of different sugar

In this experiment, different isolates yeast strains showed their ability to utilize different types of sugar. Different isolate yeasts strain S1, S2 ,M1, M2, Gu and Gr were able to ferment the glucose, galactose, maltose, and sucrose except the lactose.

Table 2: Fermentation of different sugar by yeast isolates.

Yeast isolates	Sugar	Color before fermentation	Color after fermentation	Gas formation
S1	Glucose	Reddish brown	Yellow	+
S2	Glucose	Reddish brown	Yellow	+
M1	Glucose	Reddish brown	Yellow	+
M2	Glucose	Reddish brown	Yellow	+
Gr	Glucose	Reddish brown	Yellow	+
Gu	Glucose	Reddish brown	Yellow	+
S1	Galactose	Reddish brown	Yellow	+
S2	Galactose	Reddish brown	Yellow	+
M1	Galactose	Reddish brown	Yellow	+
M2	Galactose	Reddish brown	Yellow	+
Gr	Galactose	Reddish brown	Yellow	+
Gu	Galactose	Reddish brown	Yellow	+
S1	Maltose	Reddish brown	Yellow	+
S2	Maltose	Reddish brown	Yellow	+
M1	Maltose	Reddish brown	Yellow	+
M2	Maltose	Reddish brown	Yellow	+
Gr	Maltose	Reddish brown	Yellow	+
Gu	Maltose	Reddish brown	Yellow	+
S1	Sucrose	Reddish brown	Yellow	+
S2	Sucrose	Reddish brown	Yellow	+
M1	Sucrose	Reddish brown	Yellow	+
M2	Sucrose	Reddish brown	Yellow	+
Gr	Sucrose	Reddish brown	Yellow	+
Gu	Sucrose	Reddish brown	Yellow	+
S1	Lactose	Reddish brown	-	-
S2	Lactose	Reddish brown	-	-
M1	Lactose	Reddish brown	-	-
M2	Lactose	Reddish brown	-	-
Gr	Lactose	Reddish brown	-	-
Gu	Lactose	Reddish brown	-	-

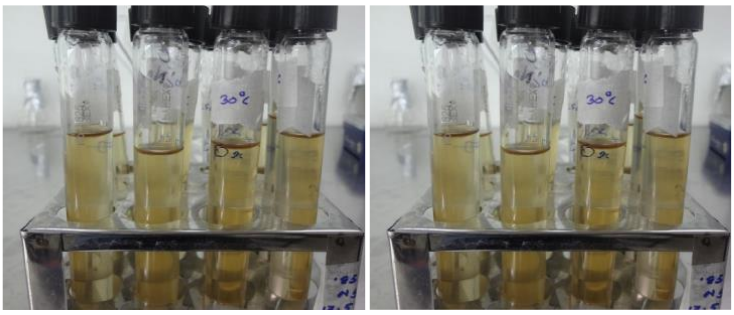
A B

Figure 3: The color of liquid medium (a) before incubation. (b) after incubation.

Determination the tolerance of yeast isolates under stress environmental conditions

Thermo tolerance

The growth and tolerance of different yeast strains were examined at different temperatures such as 25°C, 30°C, 37°C, and 44°C for 2 days. Isolated yeast strains S1, S2, M1 , M2, Gu and Gr were able to grow from 25°C to 37°C and unable to grow at 44°C. Selected yeast strains showed maximum growth at 30°C.



A B

Figure 4: The liquid medium growth **(a)** before incubation**. (b**) after incubation.

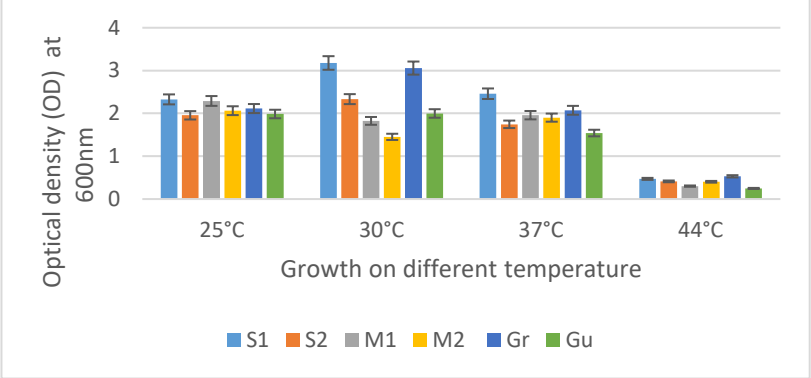


Figure 5: The growth and tolerance of yeast isolated at different temperature.

Ethanol tolerance

The growth and tolerance of different yeast strains were examined at different ethanol concentrations of (5%, 10%, 15%, 20% and 25%). Selected yeast strains S1, S2, M1, M2, Gu and Gr showed growth up to 20% ethanol concentration. The maximum growth of S1, S2, M1, M2, Gu and Gr strain seen on 5% ethanol concentration. Yeast strain Gr strain showed maximum growth at 15% except all strains.

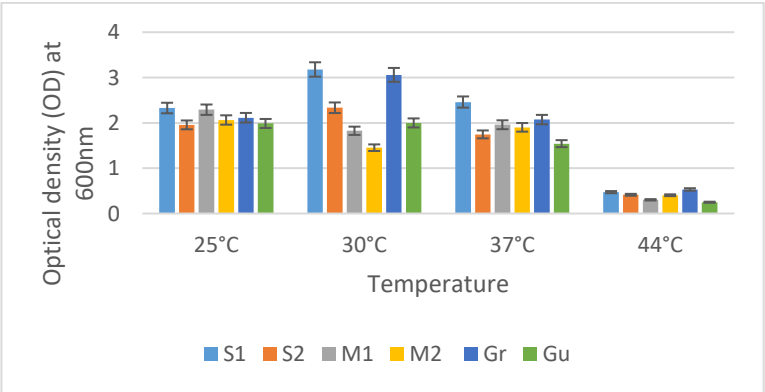


Figure 6: The graphical representation of growth and tolerance of yeast isolates at different ethanol concentration.

Growth at different pH

The growth of different isolated yeast strains was examined at different pH values. The isolate shown growth up to 10 pH. The maximum growth of S1, S2, M1, M2, Gu and Gr strain was seen at 6 pH.

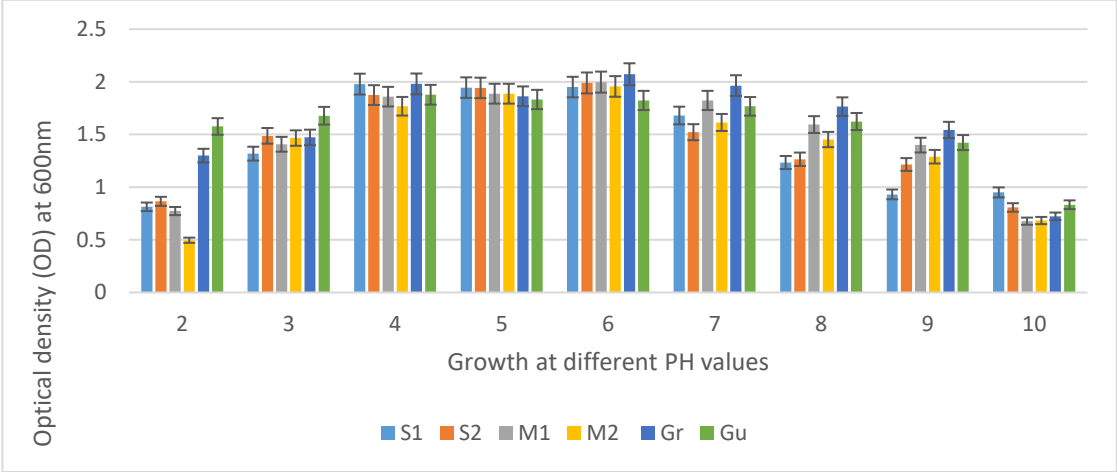


Figure 7: The graphical representation of growth and tolerance of different yeast strain at different pH values.

Preparation of fermentation media

Using kitchen waste

For ethanol production, kitchen waste of 250 gm was diluted in 1000 ml distilled water and distributed into six flasks. Each flask was inoculated by individual selected yeast strains (S1, S2, M1, M2, Gu and Gr) and incubated under the shaking condition of 180 rpm with a pH-6 and at 30℃ for one week.

Using sugar molasses

For ethanol production, sugar molasses of 250 gm was diluted in 1000 ml distilled water and distributed into six flasks. Each flask was inoculated by individual selected yeast strains (S1, S2, M1, M2, Gu and Gr) and incubated under the shaking condition of 180 rpm with a pH-6 and at 30℃ for one week.

(a) **(b)**

Figure 8: Sugar molasses fermentation under shaking condition using **(a)** Sugar molasses. **(b)** Vegetable peels.

Distillation of ethanol

After the incubation of vegetable peels and sugar molasses fermented mediums, the mediums were examined for ethanol smell. The distillation of pure ethanol obtained with different concentration media to boil the media at 78 °C by using a distillery set.

Estimation of ethanol percentage by pycnometer

Kitchen waste

Using kitchen waste media, the ethanol (%) was obtained by different yeast isolates as S1 53%, Gu 26%, Gr 43% , S2 49% M1 59% and M2 37%. Maximum ethanol (%) was obtained from M1 and minimum from Gu strain.

Table 3: Different ethanol concentration obtained from yeast Isolates using vegetables peels media.

Yeast isolates	Density	Concentration of Ethanol
S1	0.906g/ml at 20 °C	**53%**
Gu	0.96 g/ml at 20 °C	26%
Gr	0.93g/ml at 20 °C	43%
S1	0.92g/ml at 20 °C	42%
M1	0.893g/ml at 20 °C	59%
M2	0.94g/ml at 20 °C	37%

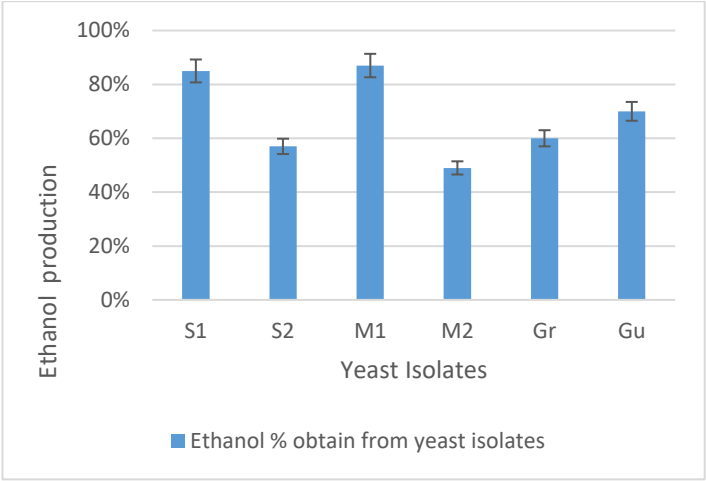


Figure 9: Using kitchen waste media the ethanol concentration in distillate samples obtained from different yeast isolates measured by pycnometer

Sugar molasses

Using sugar molasses the ethanol (%) was obtained by different yeast isolates as S1 85%, Gu 57%, Gr 87%, S2 49%, M1 60% and M2 70%. Maximum ethanol (%) was obtained through Gr and minimum Gu t strain. The result is shown in Table 4.

Table 4: Different Ethanol concentration obtained from yeast Isolates using sugar molasses.

Yeast isolates	Density	Concentration of Ethanol
S1	0.834 g/ml at 20 °C	85%
Gu	0.896 g/ml at 20 °C	57%
Gr	0.826g/ml at 20 °C	87%
S1	0.92g/ml at 20 °C	49%
M1	0. 893g g/ml at 20 °C	60%
M2	0.86g/ml at 20 °C	70%

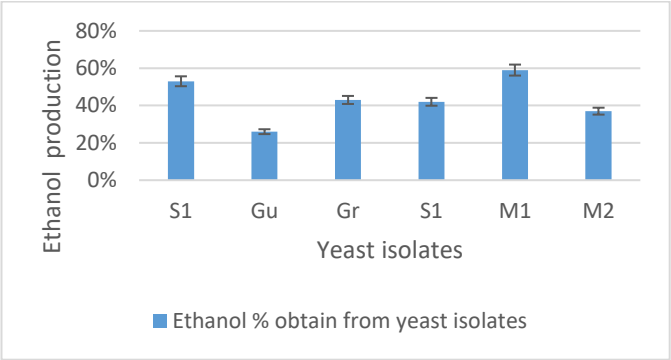


Figure 10: Using sugar molasses the ethanol concentration in distillate samples obtained from different yeast isolates measured by pycnometer.

Estimation of refractive index by refractometer

Using kitchen waste media

Maximum refractive index was obtained through S1 and minimum Gu yeast strain. The result are shown in Table 5.

Table 5: Refractive Index value of distillate samples obtained from yeast isolates using kitchen wastes.

Yeast isolate	Refractive index value
Standardized sample of Ethanol	1.361
S1	1.355
Gu	1.341
Gr	1.350
S1	1.351
M1	1.356
M2	1.348

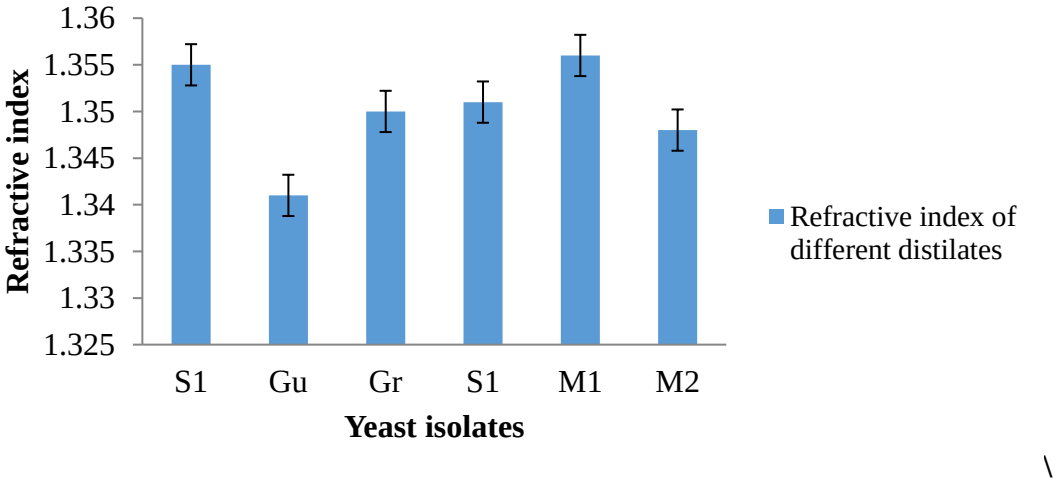


Figure 11: Refractive Index value of distillate samples obtained from yeast isolates by using vegetables peel.

Using sugar molasses

Maximum refractive index was obtained by Gr and minimum by Gu yeast strain. The result are shown in Table 6.

Table 6: Refractive Index value of distillate samples obtained from yeast isolates using sugar molasses.

Samples	Refractive Index value
Standardized sample of ethanol	1.361
S1	1.357
Gu	1.348
Gr	1.358
S1	1.353
M1	1.352
M2	1.356

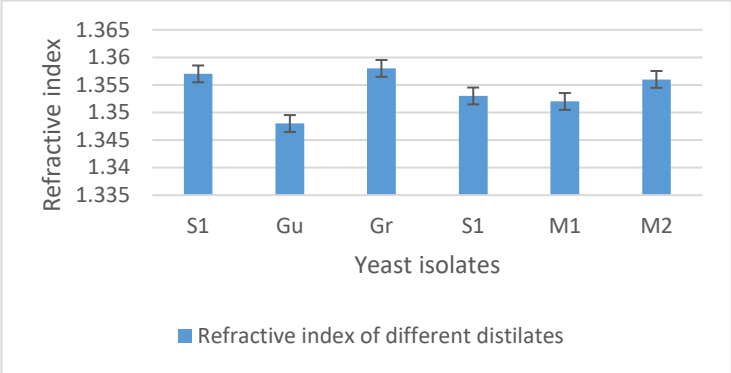


Figure 12: Refractive Index value of distillate samples obtained from yeast isolates using sugar molasses.

Detection of ethanol

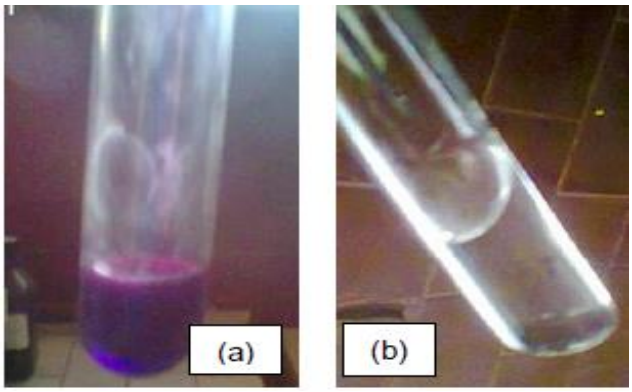


Figure 13; (a) Coloured production (purple) by addition of $KMnO_4$ (b) reduction of $KMnO_4$ and sample become colourless **.**

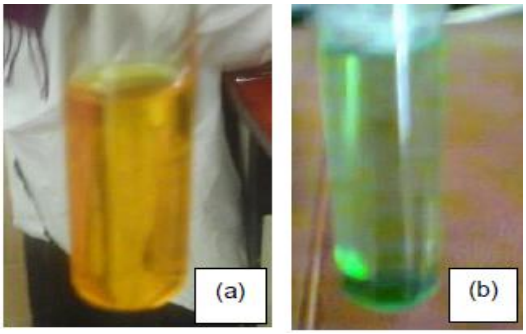


Figure: 14. Coloured production by addition of $K_2Cr_2O_7$ in the sample **(a)** yellow colour (b) green colour**.**

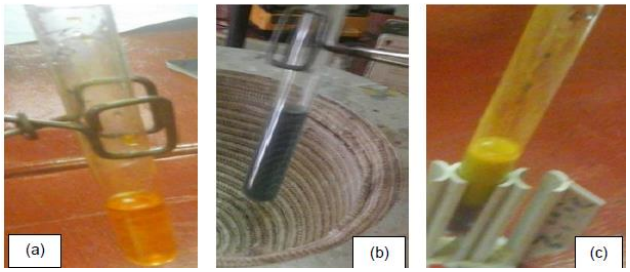


Figure 15: (a) I_2 reagent before adding into the sample.(b) I_2 reagent added into sample and heating the sample the yellow colour of iodine change to blue (c) cool the sample then add the few drops of NaOH in the samples give yellow precipitates**.**

DISCUSSION

Six potential samples were selected which include sugar cane, grapes, mosambi, and guava juices which were aged for different time periods. The isolates were selected after screening the samples using a variety of different physiological and biochemical parameters. Morphological characterization studies of the six samples and microscopic observation seemed to indicate that all of the samples contained yeasts that are members of Saccharomyces cerevisiae. This conclusion was reached on the basis of growth pattern studies in liquid and solid YEPD media; in all cases white and creamy colonies with butyrous colony texture were obtained after subculture (Lorenz et. al., 2000) Microscopic observation further confirmed this, as oval and circular shaped individual cells which reproduced by polar budding were seen in all cases. The isolates were named S1 and S2 (from sugarcane juice), M1 and M2 (from musambi), Gr (from grapes), and Gu (from Guava).

The isolates were tested for fermentation of carbohydrates, isolate yeasts strain S1, S2, M1, M2, Gu and Gr are able to utilize 6 sugars out of the seven (Tarek et al.,2017). Thermotolerance tests also indicated that all isolates (S1, S2, M1, M2, Gu and Gr) grow best at 30°C within a 48 hour incubation period; this is also the optimum growth temperature of Saccharomyces cerevisiae (Alexopoulos, 1962). However the isolates also showed good at 25°C and 37°C.The ethanol concentrations are the major influencing factors during the fermentation process. Extremely high ethanol concentrations in the fermentation cultures have been shown to inhibit or depress the fermentation process. Six yeast isolates were screened for ethanol in YEPD liquid growth media. A slow growth rate was observed at 10-20% ethanol containing media. Normally, members of Saccharomyces spp. can tolerate ethanol concentrations of up to 16.5 % (Teramoto et al, 2005). The optimum pH range for ethanol production of S. cerevisiae was 3.5-6 In our study S1, S2, M1, M2, Gu and Gr isolate can grow in a wide pH range from 2 to 10, but at pH 6 yeast isolates S1, S2, M1, M2, Gu and Gr showed optimum results.

Six isolates produced ethanol by fermenting molasses at 30°C.The production was maximal at 30°c after 72 hours of incubation. Using sugar molasses isolates produce ethanol % were S1 85%, Gu 57%, Gr 87% , S2 49% M1 60% and M2 70%. Maximum % obtained through Gr and minimum % from Gu yeast strain. Another ferment media prepared by using kitchen-wastes. This particular media contain plant organelles and a rich source of cellulose and starch. The ethanol % obtained through different yeast isolates S1 strain 53%,Gu 26%, Gr 43% ,S2 49% M1 59% and M2 37% using kitchen waste. Maxmiun % obtained through M1 and minimum % from Gu yeast strain.

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