

Production and evaluation of wine prepared from banana (*Saccharomyces Cerevisiae*)

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Abstract

The article presents review on potential of wine production from Banana. Traditional alcoholic beverage has become common because of economic issue. This work was aimed to improve production process of alcoholic beverage based banana extract and to evaluate sensory parameters of the obtained alcoholic beverage.

Juice was extracted from banana (*Musa sapientum*) pulp with was inoculated with Baker's yeast (*Saccharomyces Cerevisiae*) and naturally grown mycelium and held at 30+2°C for 14 days. Banana, a wonderfully sweet fruit with firm and creamy flesh that come prepackaged in a yellow jacket, available for harvest throughout the year consists mainly of sugars and fibers which make it a source of immediate and slightly prolonged energy.

When consumed, reduces depression, anemia, blood pressure, stroke risk, heartburns, ulcers, stress, constipation and diarrhea.

The physical chemical parameters was determine during the fermentation using a standard procedures like pH, type of alcohol (Luca's Test) specific gravity, alcohol content, preliminary phytochemical screening and reducing sugar. Alcohol content of wine is increase with time at specific temperature as increase in sugar concentration.

Keywords: Wine; *Saccharomyces Cerevisiae*; Banana; Alcohol; Fermentation etc

1. Introduction

Banana (*Musa sapientum*) is an important staple starchy food and good source of sugars and fibers, contents making it a good source of energy. To forestall huge economic loss due to rapid deterioration of riped banana, production of banana juice from pulp of riped banana became a subject of research. Banana wine is a fruit wine made exclusively from banana (Blocker et. al., 2001 and FAO, 2012). Highly acceptable wines can be made from practically all fruits (Rajković, et. al., 2007). In addition, any application to produce a marketable, value-added product will improve banana farming economies and eliminate the large environmental problem presented by banana waste. (Lee et. al., 2006).

Industrial banana wine manufacture is challenging due to high cost of equipment required for to carry out production, but the processes involved are relatively straight forward (Amerine et. al., 1980). Reports on tropical fruit wines have been mainly on exotic species such as banana, pineapple, citrus, mango, pawpaw, apple and strawberries among others (Maldonado et. al., 1975). Wine represents a safe and healthful beverage; it also provides calories and vitamins. During period when life was often strenuous, it offered relaxation and relief from pains (FAO, 2012).

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Banana could then compete in the market, either as banana juice or as mixtures with other juices because of its flavor and aroma. Banana wine is a sweet-smelling homemade beverage with a light fruit flavor, honey color, and a unique taste. The main ingredients needed are ripe bananas. It can be made sweet or dry depending upon the recipe being used and you can blend it with other wines for more body and flavor. Banana wine is an excellent health tonic that also aids in digestion. This unique tasting fruit wine is loaded with vitamins, potassium, and manganese. It is a favorite among health-conscious people. *Saccharomyces Cerevisiae* is the most important yeast species involved in wine fermentation. Traditionally, this species has been used as a starter culture to conduct the alcoholic fermentation due to its optimal fermentative properties. For this reason, nowadays *S. cerevisiae* is commercialized as an actively yeast and used in wineries worldwide to improve the fermentative processes and wine quality.

Objective of the study

The general objective is to produce wine from banana and the specific objectives is to evaluate the qualities of the wine

1.1. Micro-Organism profile

Saccharomyces Cerevisiae (family- Saccharomycetaceae) is a species of budding yeast and it is also known by name like Brewer's yeast, Ale yeast, Top-fermenting yeast, Baker's yeast Budding yeast etc. It is one of the major types of yeast used in the brewing of beer and wines. It contains protein, soluble fiber, some minerals, Saturated fatty acid, vitamins etc. *Saccharomyces Cerevisiae* (*S. cerevisiae*) is a unicellular fungus, possessing a nuclear genomic DNA of 12068 kilobases (kb) organized in 16 chromosomes(Goffeau A, Barrell BG, Bussey H. et al.)



Figure 1 *Saccharomyces Cerevisiae*



Figure 2 Baker's yeast

S. cerevisiae produces and accumulates ethanol—which is toxic, or static, for most other microbial species able to compete with it for the sugar compounds- and thus eliminate competition. After *S. cerevisiae* has cleared the particular ecological niche from most of its competitors, it then proceeds in the consumption of the produced ethanol, thus promoting its own growth.(Hagman et al. 2013).

1.2. Banana profile

Banana is a general term embracing a number of species or hybrid in the genus *Musa* of the family Musaceae. Banana is one of the most important food crops of the world which is consume extensively throughout the tropics which it is grown and also valued in the temperate zone for its flavor, nutritional value, and availability throughout the year.

The scientific name of Banana is *musa sapientum*. (family- Musaceae.) It has 20% of starch in the fresh fruit. Ascorbic acid (vitamin C) 10 to 20 mg/100 g in the fresh fruit. Amines: serotonin (28 g/g fresh fruit). Tyramine (7 g/g fresh fruit) dipamina (8)g/g fresh fruit), noradrenaline (2 g/g fresh fruit) Acids: citric acid and malic acid. The banana fruit contains at least 200 individual volatile compounds, the most important being isoamyl acetate, amyl acetate, amyl propionate and amyl butyrate. Bananas" flavor is due, amongst other compounds, to isoamyl acetate which is one the main constituents of banana oil.

Banana has a lot of health benefits. The fruit contains good amount of health benefiting anti-oxidants, minerals and vitamins and are rich in calories but very low in fats. Banana pulp is composed of soft, easily digestible with simple sugars like fructose and sucrose that when eaten replenishes energy and revitalizes the body instantly; thus, for these qualities, banana are being used by athletes to get instant energy and as supplement food in the treatment plan for underweight children. The starch prevents formation of stomach ulcers and reduces cholesterol. It is also used for digestive discomfort, fight diarrhea. Is indicated for dyspepsia, gastrointestinal diseases, diabetes, diarrhea,

hypertension, scurvy and gout. The roots have been used as anthelmintic. It has excellent nutritional properties. The Banana has anti-asthmatic properties, anti- tuberculosis and anti-cancerous and proper functioning of the heart etc. Bananas are eaten raw as dessert fruit and are eaten deep fried, baked in their skin in a split bamboo, or steamed in glutinous rice wrapped in a banana leaf. Bananas can be made into jam, pancakes and chips produced from sliced dehydrated or fried banana. (Akubor, et al. 2005)



Figure 3 Banana Wine

2. Material and methods

2.1. Raw material

The various raw materials and chemicals that are required for the wine production are given as:

Banana fruit, Yeast (*saccharomyces cerevisiae*), Distilled water, Spirit or Acetone, Sucrose and Maltose, Agar and Nutrient broth, Preservative or antioxidant (sodium metabisulphate), Potatoes, dextrose, Incubator, Hot air oven, Autoclave, Refrigerator, Microscope, Conical flask, Petri plate, Measuring cylinder, Beaker, Nichrome wireloop, Burner etc. etc.

2.1.1. Cultivation of Micro- Organism

Saccharomyces Cerevisiae is cultivated naturally and also Baker's yeast (artificial yeast) is used.

2.1.2. Preparation of Culture Media

Culture media, also known as growth media, are specific mixtures of nutrients and other substances that support the growth of microorganisms such as bacteria and fungi (yeasts and molds). Culture media is prepared to promote the growth of micro-organism . And also for identification of micro- Organism.

To prepare a culture media Add 2.4 gm nutrient broth, 100 ml distilled water and 3.5 gm agar in clean conical flask. Sterilized the prepared flask in autoclave at temperatures 121 °C for 30 mins at 15lbs. After sterilization the media is pour in Petri plate with aseptic condition to avoid contamination of micro-Organism. After 24 hrs, the media get solidify. Streak the mycelium of naturally grown fungi on media. Spread the Baker's yeast in small amount on another Petri plate containing media. Seal the all Petri plates with parafilm to avoid contamination. Place the Petri plate in Incubator at 37 °C for 2 days. After 2,3 days the of micro-Organism grown rapidly on media.

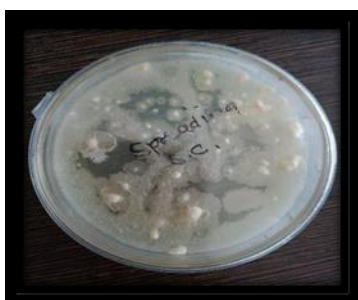


Figure 4 Spreading S.C.



Figure 5 Streaking S.C.



Figure 6 Contact yeast

2.1.3. Preparation of Potato Dextrose Agar (PDA)

- Boil 200 gm unpeeled sliced potatoes in 1 liter distilled water for 30 mins.
- Filter the extract through muslin cloth and add 20 gm of dextrose and 15 gm agar in extract.
- Autoclave the resultant mixture at 121 °C for 30 mins.
- Pour the mixture into Petri plates in aseptic condition and allow it to solidify. After that streak the micro-organism on media.

2.1.4. Identification of Fungi

Identification of micro-organism is done by using microscope. The sample is collected from the Petri plate with the help of Nichrome wire loop and observe under microscope i.e. *Saccharomyces Cerevisiae*.

2.1.5. Preparation of Flock Solution

After the identification of fungi, the flock solution of fungi is prepared. Add 200 ml of distilled water in each 2 conical flask is taken and add 4.8 gm of Nutrient broth in each conical flask Autoclave the both flask for 30 mins for 121 °C at 15 lbs and cool after completing the sterilization. Add naturally grown and artificial yeast in conical flask. Cover the mouth of flask with cotton and parafilm. Place the conical flasks in incubator and shake continuously until the solution shows the formation of flock.

2.2. Preparation of wine

2.2.1. Banana Juice Preparation

In manufacturing wine, the major steps include the must (juice) preparation (crushing and extracting the juice), fermentation, aging and storage, clarification and packaging. (Pederson, C.S, 1979).

Must was prepared for banana fruit. The fruits were washed thoroughly with distilled water and then peeled. Exactly 600 gm of banana were weighed. This was then chopped into smaller pieces using a clean knife before transferring them quantitatively into laboratory blender for crushing. The crushed sample was transferred into a clean new transparent bucket and mixed with distilled water (1:1 w/v). Exactly 0.39 gm of Sugar was added to the must followed by vigorous stirring. Exactly 2.4 g of sodium metabisulphate was dissolved in 400 ml of water and poured in 100 ml aliquots to each of the mixtures and stirred properly. Sodium metabisulphate serve as a sterilizer and prevents fermentation before the addition of the yeast starter. (Ogodo et al.) The sugar concentrations were measured and the must the must were filter through muslin cloth and store in container

2.2.2. Estimation of alcohol secretion

The yield of alcohol varies with the yeast strain, composition of must, fermentation temperature, amount of mixing through stirring and the design of the fermenter particularly the surface area to volume ratio (Kunkee and Amerine, 1972). To estimate the alcohol secretion, the different quantity of sugar at different temperatures with different ratio of Yeast and juice is taken. The quantity of yeast is remain same but the concentration of Banana juice is changes in yeast : banana ratio. Sugar is added to each flask with increased in quantity accordingly. The resulting solution is allow to stand at three different temperature such as 37 °C, 50 °C and 70 °C. The mouth of conical flask is cover with cotton and aluminum foil to avoid the loss of alcohol which will secreted during fermentation and also to prevent contamination of wine. The alcohol content is measured by using hydrometer.

The flask of 1:1 concentration shows change in colour after 2 to 4 days. At 70 °C that will consider to secretion of alcohol. The aseptic condition should be maintained during this process.

Saccharomyces Cerevisiae metabolism produce ethanol and other compounds during fermentation of Banana must. *Saccharomyces Cerevisiae* could act as a bio-control agent producing killed toxins during wine-making process. Killed stains are able to inhibit the growth of spoilage microorganisms that affect wine quality.

S. cerevisiae nevertheless ferments glucose in the presence of oxygen when this sugar is abundant in the growth medium. This phenomenon- the fermentation of sugar under aerobic conditions- is referred to as the Crabtree effect. *Saccharomyces* (whose very name translates to “sugar fungus”) preferentially consumes glucose; as a result, residual sugar is typically composed of 60 to 70% fructose, though this varies according to grape variety and yeast strain. Because fructose molecules interact with the sweetness receptors on our taste buds at a very high rate of efficiency, fructose tastes twice as sweet as glucose.

A wine with too much suspended matter will appear cloudy and dull, even if its aroma and flavor are unaffected; wines therefore generally undergo some kind of clarification. Here, the solution of wine is centrifuge in centrifuge machine which allow the heavy particles, deadly yeast, bacteria and other impurities to settle down. The supernatant liquid is remove and the remaining settle matter is discarded. Fining agents such as bentonite added to the must in order to promote the eventual agglomeration and settling of colloids.

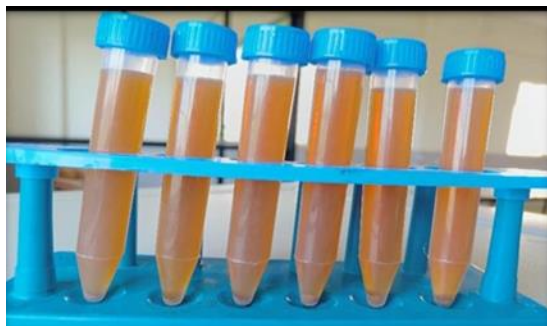


Figure 8 Wine after clarification

2.4. Purification

Bentonite a.k.a volcanic clay is a natural clarifying agent for beverages. Bentonite comes from natural volcanic clay from the mines. Furthermore, it's processing does not involve any chemicals or additives. It is made from aluminum silicate montmorillonite and hence, is a naturally occurring VEGAN product.

2.4.1. Rehydration Of Bentonite

Re-hydrate the bentonite powder by vigorously mixing 2 teaspoons with 1/2 cup water at 60 °C (140 °F). The powder will have a tendency to clump together as it absorbs the warm water. Break up as many clumps as you can. We can call this mixture as slurry.

Store the bentonite slurry in a sanitized and airtight container for at least four hours. This allows the bentonite to become fully hydrated. The maximum amount of time you let bentonite hydrate is debatable. Some sources say hydrate for at least 24 hours some say 48 hours. Other resources say don't let it sit for more than 24 hours. Whereas, I found 4 hours worked just fine. Add the slurry to your wine at a rate of 1 – 2 tablespoons per gallon of wine. Accordingly, use one tablespoon per gallon for mild cloudiness and two per gallon for wines with a thicker haze.

Stir the bentonite slurry in your wine vigorously though not so vigorous that you introduce oxygen into your wine. Degassing tools are perfect for this job. Re-attach your airlock and let it stand for four to seven days or until clear. Most wines take about a week, however, heavy hazing can take longer to clear. The cooler your wine is kept the quicker it will clear.

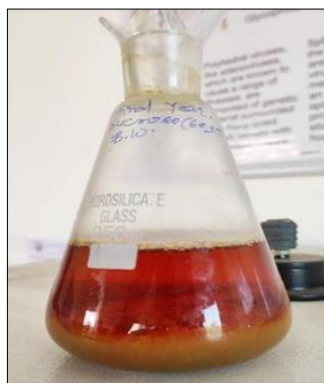


Figure 9 Matter settled down during Purification of wine

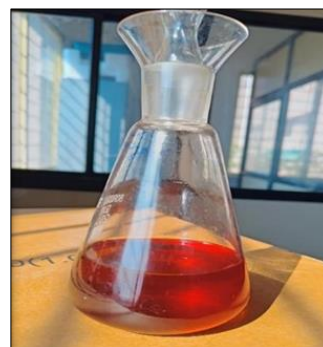


Figure 10 Purified wine

2.5. Evaluation

2.5.1. pH determination

- The pH meter was standardized with buffer solution.
- The buffer solution was prepared with pH buffer powder of pH 4.00 at 25 °C dissolved in 250ml distilled water.
- The electrode of the pH meter was immersed in a glass beaker containing the sample.
- Readings were obtained from the photo-detector of the pH meter.

2.5.2. Specific gravity

- Specific gravity of wine is determined by using hydrometer.
- Pour the sample liquid into the hydrometer jar.
- Place the hydrometer in the jar and give it a quick twirl to dislodge any air bubbles.
- Once the hydrometer has settled, take the reading from the appropriate scale at the lowest level of the liquid's surface.

2.5.3. Luca's test

- Primary, Secondary and Tertiary Alcohols are classified based on their reactivity with the Lucas reagent.
- Primary, secondary and tertiary alcohols react with Lucas reagent (zinc chloride and concentrated hydrochloric acid) at different rates. This reagent forms a cloud-like appearance on reacting with alcohols.
- Tertiary alcohols react immediately; secondary alcohols react slowly to form a cloud-like appearance after 10 to 15 minutes. However, there is no reaction with primary alcohols. Alcohols are soluble in Lucas reagent, so a clear solution is obtained, while alkyl chlorides being insoluble results in cloudiness in the solution.
- Take a very small quantity of the given sample in a test tube. Now add ~2ml of the Lucas reagent in the test tube containing the given sample and mix them and allow to stand for 10 to 15 mins.

2.5.4. Identification of *Saccharomyces Cerevisiae*

- Wet mounts were prepared for all the 3 wine samples by suspending a portion of culture or fermenting wine (1 drop) in a drop of water.
- One drop of Gram's iodine was added and covered with coverslip and watch under Microscope.

2.5.5. Phytochemical tests

- Test for alkaloids (Wagner's test)
 - Few drops of freshly prepared Wagner's reagent were added to 1 ml of the sample, a brown precipitate was observed if alkaloids were present.
- Test for tannins (lead acetate test)
 - Few drops of lead acetate (1%) were added to 1ml of the sample, appearance of white gel precipitate indicating tannins were present.
- Test for flavonoids (ammonium hydroxide test)
 - 3 ml of extract in 10% NH₄OH solution development of yellow fluorescence indicates presence of flavonoids.
- Test for amino acid (Million's test)
 - To 1 ml of sample, add 1 ml of Million's reagent and heated for 3 minutes. Then 1% sodium nitrate is added. Red colour formed.
- Test for carbohydrates (Molisch test)
 - To 2 ml of filtrate, two drops of alcoholic solution of α -naphthol is added. The mixture is shaken well and 1 ml of concentrated sulphuric acid is added slowly along the sides of the test tube, the test tube is cooled in ice water and allowed to stand. A violet ring at the junction of two liquids indicates the presence of carbohydrates.

2.5.6. . *Determination of Ash in Wine*

- The dried sample from moisture determination (3.2-5) was placed into a previously ignited and weighed crucible.
- This was placed in a muffle furnace (pre heated to 550 °C) for about 5 hours. The crucible was removed, cooled in a dessicator and then weighed.
- The experiment was done in triplicate and the mean value of ash calculated (gm/100ml).

2.5.7. *Alcohol content*

The potential alcohol content in the wine was determined by means of a wine hydrometer.

2.5.8. *Determination of Total Dissolved Solids*

- A crucible was dried in the oven at 105 °C for 1 hour.
- It was placed in a desiccator after one hour and allowed to cool. The crucible was weighed.
- 20ml of the sample was filtered using 0.45 membrane filter paper. The filtrate was measured and poured into a weighed crucible. The crucible containing the filtrate was dried in the oven for 1 hour.
- After one hour, the crucible was placed in desiccator and allowed to cool for 1 hour. The crucible containing the dried sample was re-weighed. The total dissolved solids were calculated.

2.5.9. *Residual Sugar Determination*

- A refractometer was standardized with 10%, 20% and 30% w/v glucose solutions.
- A few drops of wine were put on the screen of the refractometer and the percentage sugar content of the wine measured (gm/100ml).

2.5.10. *Organoleptic evaluation*

Taste, colour, odor, texture of wine was evaluated.

3. Results and discussion

The project outcomes involves the evaluation tests carried out which comprises of pH determination, specific gravity, total dissolved solids, phytochemical screening , alcohol content, Residual sugar determination, yeast type , Ash in wine , Luca's test to determine the type of alcohol was also carried out.

pH of wine was gradually decreased to acidic. It was decreased from 6.34 to 4.30. The drop in pH also records the utilization of the sugar present in the must by the yeast. This indicated that the *Saccharomyces Cerevisiae* can be survive in acidic pH.

Most of the sugar in the banana wine is converted to the alcohol during fermentation process. The nature banana sugar that is either unfermented at the end of fermentation process or added back into the wine is called Residual sugar(RS). It was 21.10±0.40 gm / L. Wine sample were evaluated to determine the Yeast type in wine. It was found to be budding yeast. The specific gravity evaluated by using hydrometer was found to be 1.001.

Results obtain in the study records that the alcohol content increases with the period of fermentation. The result of alcohol content of 18% to 21% was recorded. This result is found to conform and observed gradual increase in the alcohol content in the fermentation of plantain.

Luca's test indicated which type of alcohol present in wine. Primary, Secondary and Tertiary Alcohols are classified based on their reactivity with the Lucas reagent. Primary alcohol i.e.1-pentanol present in wine.

The result of the sensory evaluation of the wine sample produced show that the wine produced is clear with red to brown color. The wine sample produced has a sharp sour taste with strong alcoholic odor/aroma. In texture, the wine produced was completely watery at the end of fermentation. This result is similar to that recorded by Amerine et al., (1980) that as fermentation rate proceeded, gas was formed and this rose through the liquid then during active fermentation, forth or foam is formed on the surface. The gas carries the cells through the fermenting must cause it to be cloudy and as a result, a strong odor of alcoholic fermentation developed.

The result of the banana wine produced by the fermenting with dried yeast strains (*Saccharomyces Cerevisiae*) shows high rate of alcoholic production at the end of fermentation. The total dissolved solids decreased as the fermentation period increased. The total dissolved solid was found To be 1 mg / ml. Phytochemical screening results shows the presence of flavonoids, amino acid, tannins , phenols , carbohydrates, alkaloids etc.

4. Conclusion

Banana wine was produced and evaluated successfully with the help of laboratory analysis of main physical parameters and also sugar content, alcohol content, specific gravity, UV absorbance, pH phytochemical screening etc. *Saccharomyces Cerevisiae* was found to be better strain for the production of banana wine. Fermentation of banana juice to obtain alcohol with higher yield.

Fruit wines are undistilled alcoholic beverages usually made from banana or other fruits such as peaches, plums or apricots, elder berry or black current etc. which are nutritive, more tasty and mild stimulants. Being fruit based fermented and undistilled product, wine contains most of the nutrients present in the original fruit juice. The nutritive value of wine is increased due to release of amino acids and other nutrients from yeast during fermentation. The critical quality parameters of Banana wine are within recommended range .

Banana wine has good flavor and aroma and high nutritional value. Its production could be a way of reducing economic losses due to rapid deterioration of ripe banana as the process is easy and does not require expertise and sophisticated equipment.

The other thing is that in many places bananas are one of the cheapest fruits by weight so it makes this banana wine recipe very inexpensive to make. The great thing about making banana wine is that you can do it at any time of year. producing wine from banana will generally satisfy medical needs of people especially elders because fresh Banana fruit has a lot of nutritional and medicinal qualities and, banana wine and all other wines should be drunk moderately.

Compliance with ethical standard

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Disclosure of conflict of interest

We have no conflicts of interest.

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