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(RESEARCH ARTICLE)

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The production and evaluation of wine prepared from *Aloe Vera* by using *Saccharomyces Cerevisiae*

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Abstract

In view of the increasing demand of value added herbal products, an attempt was made to produce a functional fermented *Aloe Vera* based herbal wine. *Aloe Vera* gel supplemented with sugar found to be a good medium for the growth of *Saccharomyces cerevisiae* for making the *Aloe Vera* wine. Disease preventing the potential of herbs like *Aloe Vera*, Amla, Ginger, Cranberry, and Blueberry have given new dimension to the non-grape wine or fruit wine. *Aloe Vera*, a multifunctional herb, is being increasingly used in beverage applications including wines. Gas chromatography FID analysis reveals presence of alcohol in *Aloe Vera* wine. The major difference between white and red wine is that the juice is fermented without the skins of plant when making white wine. White wine is generally fermented at lower temperatures than red wine, to preserve its fresh, fruity flavours, and can be done in stainless steel or oak barrels. Juice was extracted from *Aloe Vera* pulp with was inoculated with Baker's yeast *(Saccharomyces cerevisiae)* Aloe and naturally grown mycelium and held at 30 ± 2 °C for seven days. The physical chemical parameters was determine during the fermentation using a standard procedures like pH, specific gravity, alcohol content and Alcohol content of wine is increase with time.

Keywords: Fermentation; Alcohol content; *Saccharomyces cerevisiae*; Detoxification

1. Introduction

Wine represents one of the examples of functional fermented foods. Wine, considered as a Phyto-complex to which ethanol has been added following fermentation, contains different constituents with strong antimicrobial properties, such as low pH (ranging from 3.0 - 4.0), relatively high ethanol concentrations (10 - 15%) and some bioactive components.

There are several types of wines available around the world based on the type of fruit used, source of yeast inoculum, alcohol content, presence of carbon dioxide, color, processing of fruit prior to fermentation etc. To produce wines, a variety of raw materials including fruits, sugar, acid, nitrogen source, clarifying enzymes etc. are required.

The fruit constitutes the most important raw material. Most of the wines are generally prepared from grapes but commercial wines are also made from several fruits other than grapes. *Aloe Vera* (*Aloe barbadensis*) is one such herbal plant with enormous medicinal value.

It is an excellent example of functional food that plays a significant role in protection from oxidative stress.[1] reported that *Aloe Vera* has become a subject of interest because of its beneficial effects on human health.

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Aloe Vera synthesizes aromatic substances / alkaloids including phenols, tannis and their derivatives which serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. These aromatic substances, herbs are useful for the maintenance of health in humans.[2]

Aloe Vera is also popular in both traditional Chinese and Ayurvedic medicine (India). *Aloe Vera* is used internally as a laxative, antihelminthic, hemorrhoids remedy and uterine stimulant (menstrual regulator). The bitter yellow exudates from *Aloe Vera* leaves contain 1,8 dihydrooxyanthroquinine derivatives and their glycosides. *Aloe Vera* gel consists of about 99.5% water, the remaining 0.5 - 1% solid material consists of a range of compounds including water soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids.[3] Observed that Wine is a complex matrix that includes components with different chemical natures, the volatile compounds being responsible for wine aroma quality.

Objective

The main and specific objective of the research is to produce wine from *Aloe Vera* at lab scale with minimum cost and to evaluate qualities of the wine.

Plant Profile

Figure 1 *Aloe Vera* plant

- Scientific name: *Aloe barbadensis miller.*
- Family: *Liliaceae*

1.1. Content and active ingredients of *Aloe Vera*

- *Aloe Vera* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids.
- Vitamins: It contains vitamins A (beta-carotene), C and E, which are antioxidants. It also contains vitamin B12, folic acid, and choline.

1.2. Health benefits

- Treating constipation
- Providing vitamin C
- Staying hydrated
- Reducing inflammation
- Controlling blood sugar levels
- Preventing stomach ulcers

1.3. Micro-Organism Profile

Figure 2 *Saccharomyces cerevisae*

Figure 3 Baker's yeast

1.4. Species

Budding yeast

1.5. Other Names

- Brewer's yeast
- Ale yeast
- Top-fermenting yeast
- Baker's yeast
- Budding yeast

1.6. Top-Fermenting Yeast

Saccharomyces cerevisiae is known as a **top-fermenting yeast**. It is one of the major types of yeast used in the brewing of beer and wines.

1.7. Contents

- protein
- soluble fiber
- some minerals
- Saturated fatty acid
- Vitamins

2. Methodology

2.1. Cultivation of Micro- Organism

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

2.2. Preparation of Culture Media

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.

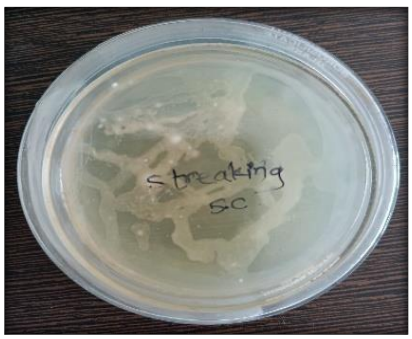


Figure 5 Streaing S.C.

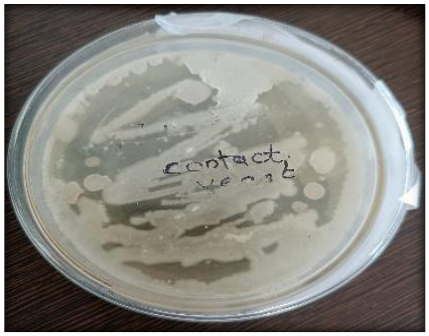


Figure 6 Contact Yeast

2.3. Identification of Fungi

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

2.4. 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.

Figure 7 Flock solution

2.5. *Aloe Vera* Juice Preparation

Must was prepared for *Aloe Vera*. The *Aloe Vera* were washed thoroughly with distilled water and then remove the peels.

Take 10 to 12 leaves of *Aloe Vera*. Then grind the juice in grinder. Then sample was transferred into a cleannew transparent bucket and mixed with distilled water (1:1 w/v). Exactly 4 gm of Sugar was added to the must followed by vigorous stirring.

Sodium metabisulphate serve as a sterilizer and prevents fermentation before the addition of the yeast starter. The sugar concentrations were measured and the must the must were filter through muslin cloth and store in container. [5]

Figure 8 *Aloe Vera* juice

2.6. Alcohol Secretion

The quantity yeast is remain same but the concentration of *Aloe Vera* juice is changes accordingly. Sugar is added to each flask accordingly. Theresulting solution is allow to stand at temprature37 °C, 50 °C and 70 °C of different ratios. The mouth of conical flaskis cover with cotton and aluminum foil to avoid the loss of alcohol which will secreted during fermentation.

The flask of 1:2 concentration will shows change in colour after 2 to 4 days at 50 °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 *Aloe Vera* must.

Saccharomyces cerevisiae could act as a bio-control agent producing killed toxins during wine-making process. Killed stains are able toinhibit 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. Thisphenomenon- the fermentation.

on 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 veryhigh rate of efficiency, fructose tastes twice as sweet as glucose.[6]

Table 1 Alcohol Secretion

Sr.no.	Ratio (yeast extract)	Sugar (gm)	Temp-I 37 °C	Temp-II 50 °C	Temp-III70 °C
1.	1:1	2	No colour change	No colour change	No colour change
2.	1:2	4	No colour change	colour change	No colour change
3.	1:3	6	No colour change	No colour change	No colour change

3. Result

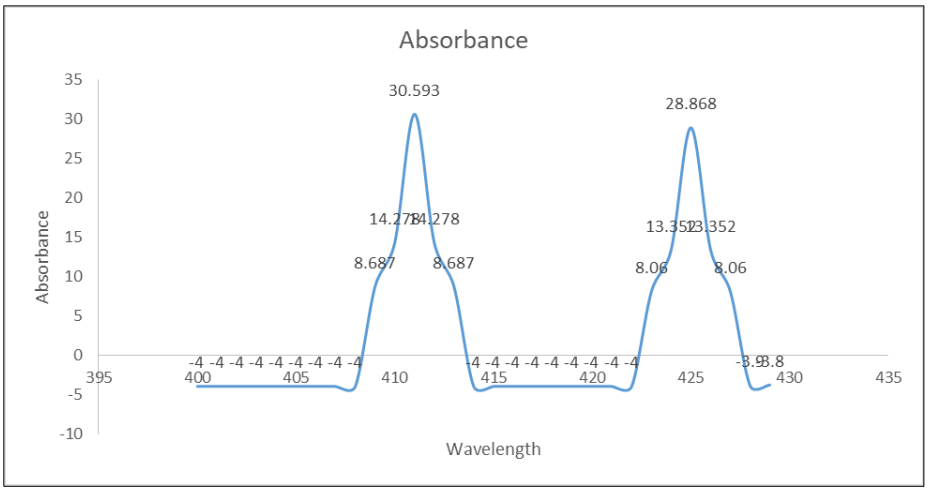
- Ratio of yeast and must shows the fermentation at 50 °C so, this concentration is carried out in next process.
- Ratio of yeast and must shows the fermentation at 50 °C so, this concentration is carried out in next process. The derivatives of sugar are used along with sucrose and maltose are used with large quantity such as 40 gm and 60 gm.

Now, 2 conical flasks are taken. The 100 ml of yeast containing flock solution is added to 100 ml of must in each flask. Sucrose and fructose are added to each flask with 40 gm and 60 gm respectively. The flasks are allowed to put in hot air oven at 50 ° C The same process is carried out for maltose. The fermentation process is takes place and alcohol is secreted by organism. The alcohol content is determine by hydrometer. The alcohol content increase with period of time.

The solution showing highest alcohol content is being used for the clarification and purification process. The increasein sugar content shows the increase in alcohol with period of time.

3.1. UV Absorbance Graph of Sucrose (60 gm)

The above sample are proceed for UV absorbance. The sample of sucrose of 60 gm shows the peak value at 30.59 and 28.86 at 411 and 425 nm having absorbance 0.507 and 0.532 Therefore, the sucrose containing 60 gm of sugar sample is used. The sample is then carried out to next process of clarification and purification for final result.



Sucrose (60 gm) shows the peak value 30.59 and 28.86 at 411 and 425 nm. Respectively. Actual absorbance is found to be 0.507 and 0.532 respectively.

Figure 9 UV absorbance of sample sucrose (60 gm)

3.2. Clarification

In winemaking, clarification and stabilization are the processes by which insoluble matter suspended inthe wine is removed before bottling. This matter may include dead yeast cells (lees), bacteria, tartrates, proteins, pectins, various tannins and other phenolic compounds, as well as pieces of grape skin, pulp, stems and gums. Clarification and stabilization may involve fining, filtration, centrifugation, flotation, refrigeration, pasteurization, and/or barrel maturation and racking**.** Wine is considered "clear" when there are no visible particles suspended in the liquid and, especially in the case of white wines, when there is some degree of transparency. 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.

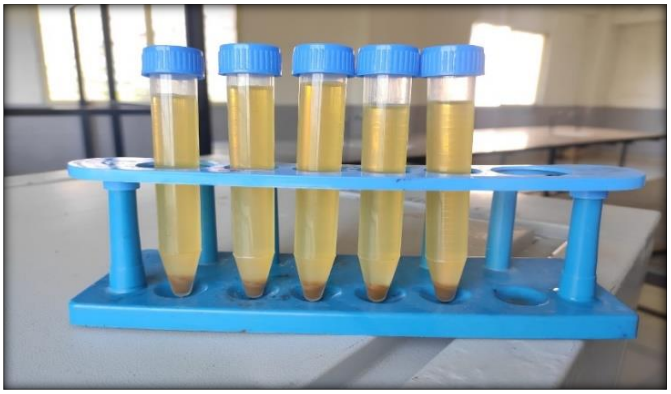


Figure 10 Clarification of Wine after Centrifugation

3.3. 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.

3.3.1. 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.[13]

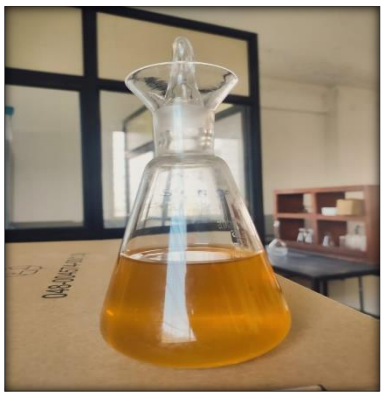


Figure 11 Final purified wine

3.4. Evaluation Test

3.4.1. 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 desiccator and then weighed. The experiment was done in triplicate and the mean value of ash calculated (gm/100ml).

3.4.2. Specific gravity

Specific gravity of wine is determine by using hydrometer.

3.4.3. Determination of total solid content

100ml of sample were evaporated in an evaporating dish placed on a water bath to a syrupy consistency. The residue was then heated for 2-5 hours in drying oven at 105 °C. It was cooled in a desiccator and then weighed as soon as room temperature was reached. The same process was repeated till no change in weight occurred.

The total solids was calculated as follows:

Wt of crucible: x

Wt of crucible Total solids: Y

Gm/100ml total solids- y-x

3.4.4. 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).

3.4.5. 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 5 to 10 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. Solution shows presence of tertiary alcohol.

Figure 12 Luca's test

3.4.6. PH Determination

A pH meter was used to determine the pH of the wine.

Figure 13 pH by using pH meter

3.4.7. Identification of Yeast Type

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.

3.4.8. Determination of Alcohol Content

The potential alcohol content in the wine was determined by means of a wine hydrometer 18%± 2.

Figure 14 Determination by using hydrometer

3.4.9. Phytochemical test

Table 2 Phytochemical tests

Sr.no.	Test name	Result
1.	Molisch test	Violet ring at junction shows presence of Carbohydrates.
2.	Lead acetate test	Bulky white ppt shows presence of tannins and phenols.
3.	Wagner's test	Red ppt shows presence of Alkaloids.
4.	Ammonium hydroxide test	Yellow colour shows presence of flavonoids.
5.	Million's test	Red Colour shows presence of amino acids.

Figure 15 Screening

4. 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 5.25 to 4.42. 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.

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 16% to 20% was recorded.

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. Tertiary alcohol present in wine.

The result of the sensory evaluation of the wine sample produced show that the wine produced is clear with yellow color. The wine sample produced has a slightly bitter with strong alcoholic odor/aroma. In texture, the wine produced was completely watery at the end of fermentation.

The result of the *Aloe Vera* 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.

5. Conclusion

The current work was aimed at exploring the vast alternatives of beneficial herbs and botanical ingredients to further enhance the efficacy and functionality of ever popular health beverage i.e. wine. The work was carried out to develop process methodology for the production of *Aloe Vera* wine. Generally, *Aloe Vera* juice is not often consumed by people because of its taste and odor. On the other side wine gives sweeter taste, fruity smell which can be easily consumed daily which promotes human health and exempts from diseases.

Overall, the present study demonstrated that fermentation of *Aloe Vera* enhances the bioactivity of the *Aloe Vera* active compounds.

The fermentation process itself increases the release of the plant's bioactive compounds, increases bio accessibility and bioavailability, and consequently improves the nutritional and functional properties of the food, with beneficial effects on health.

It's potential use as functional foods or cosmetics to guard human intestinal health, delaying senescence and preventing chronic diseases. This research provides a overview of *Aloe Vera* wines, the medicinal potential of wine for human health.

Compliance with ethical standards

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