E-ISSN No: 2456-6713 Print ISSN No: 3048-6459

International Educational Applied Research Journal

Peer-Reviewed Journal-Equivalent to UGC Approved Journal

A Multi-Disciplinary Research Journal

Making Wine in vitro by using *Sacchromyces cerevisiae* and Grape Juice

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Paper Rcevied date

05/06/2025

Paper date Publishing Date

10/06/2025

DOI

https://doi.org/10.5281/zenodo.15651032

IMPACT FACTOR 5.924

ABSTRACT

The article presents review on potential of wine production from grape juice. Fermentation can take 10-30 days to convert natural sugar to alcohol. The free run wine and press wine, always from the same source are mixed together in appropriate ratios to obtain the desired balance. Sugar and acids that naturally react with yeasts. .To study the fermentation process, sample was drawn after an interval of 5 days and microbiological and biochemical characterization was done. For microbial investigation, Wine grape production is particularly sensitive to temperature. Various wine classes such as grape wine, fruit wine, berry wine, vegetable wine, plant wine, raisin wine etc can be made in vitro. Here, we will discuss about grape juice wine production. The fermentation recorded total viable yeast count from 2.5x 106 to 13.0 x 106 cfu/ml. Sensory evaluation of the wine revealed acceptable aroma/flavor and taste. This study indicates that *Saccharomyces cerevisiae* strain from the grape wine have good fermentative performance which suggested that it could be used for fruit wine production and other industrial applications preceded by further experiments. Wine was prepared after 21 days of fermentation

Keywords: *Sacchromyces cerevisiae*, Grape Juice, Wine Production

Introduction:

Fruits are among the most important foods of mankind as they are not only nutritive but are also indispensable for the maintenance of health. India is one of the largest producers of fruits in the world. The conversion of grape juice to wine is a biotechnological tradition dating back to the

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dawn of civilization. A large diversity of microbes is inherent to winemaking including various yeasts, bacteria and fungi. Prominent in this process are Saccharomyces species (predominantly S. cerevisiae), which dominate the alcoholic fermentation, and the lactic acid bacteria (LAB), which carry out the malolactic conversion. Non-availability and relatively high cost of obtaining effective commercially alcoholic fermentative Saccharomyces cerevisiae strain is a major constrain in development and sustaining local industrial fermentation process. Hence, there is need for search of indigenous strains that could be used as an alternative.

White wine can be straw-yellow, yellow-green, or yellow-gold. Grape proteins are responsible for the appearance of haziness in white wines during storage. Fermentation of the non-colored grape pulp produces white wine. The fermentation may be initiated using a starter culture of *Saccharomyces cerevisiae*, in which case the juice is inoculated with populations of yeast.

Freshly expressed grape juice consists of 70 to 80% water and many dissolved solids. These soluble solids include numerous organic and inorganic compounds. The important group of compounds, from the winemaking point of view, includes the following: sugars, organic acids, phenolic compounds, nitrogenous compounds, aroma compounds, minerals, and pectic substances.

Sugars: In grapes, a large portion of the soluble solid is sugars. Glucose and fructose are the main sugars in the juice. Glucose and fructose are fermentable sugars. During the course of fermentation, the yeast converts these sugars to alcohol and carbon dioxide. The amount of alcohol produced is related to the amount of sugar initially present in the juice.

Organic Acids:

Next to sugars, organic acids are the most abundant solids present in grape juice. They are a very important component of juice and wine. They are responsible for the tart taste and have a marked influence on wine stability, color, and pH. The principal organic acids found in grapes are tartaric, malic, and to a small extent, citric.

Phenolic Compounds:

Phenolic compounds are important constituents of grapes and wine. Phenolic compounds are a group of substances that are structurally diverse and are present in various amounts. They play a vital role in determining the wine's color and flavor. They are involved in browning reactions in grapes and wines and also play a key role in the aging and maturation of wines.

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Nitrogenous Compounds:
Grapes contain various nitrogenous compounds. These include ammonium cations and organic nitrogenous compounds: such as amino acids, peptides, and proteins. The nitrogen content of the grape varies with variety, climate, soil, fertilization, and other cultural practices.
Aroma Compounds: Grapes contain numerous flavor compounds. Some of these compounds have been reported to give a variety their distinct varietal character.

Minerals:
Minerals are taken up by the vine from the soil. They usually make up approximately 0.2 to 0.6% of the fresh weight of the fruit. The important mineral compounds include: potassium, sodium, iron, phosphates, sulfate, and chloride.

Pectic Substances:
Pectin substances are cementing agents present in the cell wall. Chemically, they are complex polysaccharides made of galacturonic acid molecules linked together. During ripening, pectin is hydrolyzed by naturally occurring pectolytic enzymes, which renders the berry softer as it ripens.

Classification of Wine:
A typical wine contains ethyl alcohol, sugar, acids, higher alcohols, tannins, aldehydes, esters, amino acids, minerals, vitamins, anthocyanins, minor constituents like flavouring compounds etc. Depending upon the various attributes such as cultivar, stage of ripening of fruits, chemical composition of juice, use of additives to the must, vinification techniques and ageing of wine, the alcohol and sugar. The wines are classified as natural wines (9-14% alcohol) and dessert and appetizer wines (15-21 % alcohol). The most famous types of wines are red and white wines.

White Wine:
White wine is not exactly white; it is often yellow, gold or straw coloured, depending on whether it includes the skin of the grape or just the juice. White wine can be made by the alcoholic fermentation of the non-coloured pulp of green or gold coloured grapes or from selected juice of red grapes. White wines often taste lighter, crisper and more refreshing than a red wine and so they often gain popularity during warmer months of the year.

Red wine:
Red wine is made from red grapes, which are actually closer to black in color. There are many different types of red wines. This is considered to be the most classic in the kingdom of wines,

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mixing the delicious red grapes with a wide range of aromas, from oak to eucalypti, chocolate or even mint hints. The juice from most black grapes is greenish-white; the red colour comes from anthocyan pigments present in the skin of the grape. Table 1 gives the characteristics of different types of red wine.

Apple cider and wine:

Apple (Malus domestica) fruit is used to prepare mild alcoholic beverages which are more nutritious than distilled liquors. The apple fruit is more associated with cider than any other alcoholic beverages. Cider is a low alcoholic drink produced by fermentation of apple juice and is believed to have been produced for over 2000 years. Cider is known by different names around the world such as cidre (France), sidre (Italy), sidra (Spain) and apfel wein (Germany and Switzerland). Cider can be sweet or dry. Depending upon the alcohol content, cider is categorised into soft cider (1-5%) or hard cider (6-7%).

In the case of wines made from grapes, pre-fermentation involves crushing the fruit and releasing juice. In case of white wine, juice is separated from the skin. Then yeast is added to the clarified juice to initiate fermentation. In red winemaking, the pulp, skins and seeds of grapes are kept together after crushing and during all or part of the fermentation. This is done to extract color and flavor. Fermentation involves a reaction that converts the sugars in the juice into alcohol and carbon dioxide. Yeasts utilize the sugars during the fermentation period.

Materials and Method:

Microorganism used:

Saccharomyces cerevisiae .

Collection of sample:

Fresh grapes were collected from local market, sucrose as a supplement.

Equipment

Graduated cylinder, Test tube, Erlenmeyer flasks or bottles, Rubber stoppers

Method:

a. **Extraction of juice from grapes:** Some grapes were taken and skin of grapes was peeled out. Then the grapes were crushed and diluted with distilled water.

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b. **Preparation of Must:** The grapes are crushed and the liquid is separated. Sterilized this must by pasteurization.

c. **Inoculation of an organism in juice:** Inoculate *Saccharomyces cerevisiae*, in 500 ml grape juice kept in the flask. Keep this flask on orbital shaker for 3 days. Add sucrose in a sufficient amount which is utilized by yeast as its growth supplement.

d. **Incubation of suspension**: Incubate the Erlenmeyer flask containing desired inoculum at 25^0C for 21 days. Add sucrose in a class interval of 2 to 4 days for proper fermentation.

e. Ageing: After 21 days of incubation, wines is prepared and incubate more for 1-5 months for aroma and flavor development.

Flow chart of Wine Production

Selection of grapes (Mature and undamaged grapes)

Crushing (Traditionally manual but now crushers are used)

Removal of skins (By standing, filtration or centrifugation)

Clarification

Fermentation

Ageing

Conclusion:

Fruits both in fresh as well as in processed form not only improve the quality of our diet but also provide essential ingredients like vitamins, minerals, carbohydrates etc. Fruit wines are undistilled alcoholic beverages usually made from grapes or other fruits. Wine contains most of the nutrients present in the original fruit juice. The nutritive value of wine is increased due to release of

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amino acids and other nutrients from yeast during fermentation. This study therefore indicates that *Saccharomyces cerevisiae* strain isolated from the locally tapped palm wine has good fermentative performance and can therefore be used to make fruits wines. However, process optimization and scale up will be required; and hence starter culture obtained to augment for the more expensive and non-available commercial wine *Saccharomyces cerevisiae* for better application in wine making.

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