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STUDY OF *SACCHAROMYCES CEREVISIAE* 3282 FOR THE PRODUCTION OF TOAMATO WINE

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ABSTRACT

Wine production, wine consumption and the health risks and benefits associated therewith provide an opportunity to explore a topic, which encompasses climatological and clinical considerations. The complexities of wine-grape cultivation and wine production are influenced tremendously by climate. Tomatoes are consumed through out the world and it has number of medical benefits.

The color of wine is attractive and tomato which is acidic in taste produce sour wine. By adjusting the brix we can get the alcohol percent 7.88% which is acceptable as compare to standard wine. It can be produce in commercial form.

Keywords: tomato, *Saccharomyces cerevisiae*, wine, grape cultivation.

Introduction

Wine production, wine consumption and the health risks and benefits associated therewith provide an opportunity to explore a topic, which encompasses climatological and clinical considerations. The complexities of wine-grape cultivation and wine production are influenced tremendously by climate. As a result of the cultivation of the wine grape *Vitis vinifera*, mankind has the opportunity to benefit from these climatic influences through wine ingestion, which results in numerous physiologic effects, some of which may accrue clinical benefit. It is thus of interest to recognize that there is a critical interplay of climatological factors [sunshine, wind, rainfall] and local soil characteristics [including water retention] which affect viticulture [grape growing for wine production] [1] Currently, several physical processes are available for producing wines containing less alcohol, all of them involving the selective extraction of ethanol based on volatility or diffusion [2] Despite their efficacy, these procedures are expensive and difficult to perform, and they can also affect the flavor balance through the loss of aroma compounds. One biological alternative would

be to use yeast strains that give low ethanol yields, a method that promises to be both faster and less expensive. Several attempts have been made through genetic engineering

to reduce the ethanol yield of *Saccharomyces cerevisiae* by diverting sugar metabolism into by-products other than ethanol. For example, yeast strains producing more glycerol and less ethanol have been obtained by over expressing *GPD1*, a gene that codes for glycerol 3-phosphate dehydrogenase (GPDH) [3-6]. Sensu strictu species of the genus Saccharomyces, as their scientific name implies, are yeast specialized for growth on sugar. In comparison to other yeasts, Saccharomyces favor aerobic fermentation over respiration in the presence of high concentrations of sugar [7].

The first use of *S. cerevisiae* is likely to have been for the production of wine, rather than bread or beer [8, 9]. *S. cerevisiae* has been associated with winemaking since 3150 BC, based on extraction of DNA from ancient wine containers [10], and the earliest evidence for winemaking is to 7000 BC from the molecular analysis of pottery jars found in China [11]. The idea that *S. cerevisiae* was first used to produce wine rather than beer or bread is further supported by the fact that the production of wine requires no inoculum of yeast [12]. Glycerol is the most abundant by-product of alcoholic fermentation after ethanol and carbon dioxide [13-15] and contributes to the sensory characteristics of wine, particularly smoothness and overall body [16-18].

Analysis of microbiological samples from fermentation processes in the beverage industry (beer, wine, and cider) by traditional indirect, culture-based standard methods is time consuming, and the methods do not produce direct information about the physiological state of the microorganisms. Moreover, the plate-counting method detects only cells able to form colonies under the conditions of the medium that is used, ignoring the presence of cells that do not form colonies but are nevertheless metabolically active [18].

Fermentation of grape juice into wine is a complex microbial reaction. Yeasts are primarily responsible for the alcoholic fermentation of musts, while many vines undergo another fermentation process mediated by lactic acid bacteria [19]. Growth of yeasts on velum surfaces produces important changes in the

Characteristics of the wine due to the oxidative metabolism of the yeasts [20-21] various microbiological studies on the

fermentation [22-26] and aging [27-29] processes in the elaboration of sherry wines have been carried out. However, although some of these studies analyze the dynamics of yeast strains during the specific steps of sherry wine making [20]

Human perception of flavor involves integration of multiple chemical stimuli from taste and olfactory receptors. Whereas taste receptors respond to a limited set of cues, olfactory receptors respond to thousands of chemicals and provide the diversity of unique food flavors. For example, there are ≈15–20 volatile compounds that, together, constitute the unique flavor of fresh tomatoes [30–34]. These volatiles are derived from various precursors, including fatty acids, carotenoids, and amino acids. Several of the most important tomato aroma volatiles, including 2-phenylacetaldehyde and 2-phenylethanol, are derived from phenylalanine [31]

Tomato plants are dicots, and grow as a series of branching stems, with a terminal bud at the tip that does the actual growing. When that tip eventually stops growing, whether because of pruning or flowering, lateral buds take over and grow into other, fully functional, vines. [35]

Energy	**75 kJ (18 kcal)**
Carbohydrates	4 g
Sugars	2.6 g
Dietary fiber	1 g
Fat	0.2 g
Protein	1 g
Water	95 g
Vitamin C	13 mg (22%)

Table1. Nutritional value (per 100 g)

Tomatoes are now eaten freely throughout the world, and their consumption is believed to benefit the heart among other things. They contain lycopene, one of the most powerful natural antioxidants. In some studies lycopene, especially in cooked tomatoes, has been found to help prevent prostate cancer [36] but other research contradicts this claim.[37] Lycopene has also been shown to improve the skin's ability to protect against harmful UV rays [38]. Natural genetic variation in tomatoes and their wild relatives has given a genetic treasure trove of genes that produce lycopene, carotene, anthocyanin, and other antioxidants. Tomato varieties are available with double the normal vitamin C (Doublerich), 40 times normal vitamin A (97L97), high levels of anthocyanin (P20 Blue), and two to four times the normal amount of lycopene (numerous available cultivars with the high crimson gene).

Tomato consumption has been associated with decreased risk of breast cancer [39], head and neck cancers [40] and might be strongly protective against neurodegenerative diseases [41-43].

MATERIALS AND METHODS

In the present study one microbial species was used. *Saccharomyces cerevisiae* 3282.This strain was obtained from the National Collection of Industrial Microorganisms (NCIM) NCL, Pune. The cells were preserved on Yeast Extract potato Dextrose Agar (YEPDA) at 4°C.

Composition	**Quantity**
Yeast extract	0.3%
Malt Extract	0.3%
Peptone	0.3%
Dextrose	0.5%
Agar	1.0%
Distilled water	100ml
pH	6.4-6.8

Table 2.Media composition of YEPDA.

i.) Microscopic observation of yeast cells:

The suspension of yeast cells were prepared, loop full of culture were stained with lacto phenol cotton blue on sterile glass slide observed under 45X objective microscope. The cells are 4.5X5.1mm approximately, the cells contain prominent nucleus. The yeast cells were also showing the budding condition. Yeast cells were characterized as Growth on carbon sources, ethanol tolerance and alcohol production.

ii) Preparation of starter culture:

One loop full of inoculums of yeast culture was inoculated to test tube containing sterile YEPDB 10 ml. Then it was kept overnight at 26^0C on rotary shaker.

iii) Tomato Juice Preparation:

Selection of well graded tomato, then crushing of tomato later on removal of seeds and outer layer by filtration of pulp by using sterile masculine cloth, then pasteurized the juice at 80^0C for 2-3 mins. We also sterilized by addition of 1% potassium metabisulphate. We blended the juice with sugar so that to adjust the brix of 22^0.Total volume of tomato juice was 500ml.

iv) Fermentation:

The process of fermentation tomato carried out by following mina four steps: Primary fermentation, Secondary fermentation, stabilizing and clarification /bottling.

v) Primary fermentation: Sterilization of container was done; 500 ml of prepared tomato juice was poured under aseptic conditions. Before inoculation the microbial

load was detected, then inoculated with 10 ml of starter culture of *Saccharomyces cerevisiae 3282* .The juice was added with pectic enzyme to increase rate of fermentation and alcohol content. It was kept for fermentation in anaerobic condition for 5 days at 26^0C.

vi)Secondary fermentation: For secondary fermentation, the juice fermented for 5 days was transferred into another sterilized container so that solid particles get settled at the bottom which has to be separated from fermented juice. The separation was done by centrifugation and filtration with Whatman filter paper in order to obtain clear fermented juice. Centrifugation was carried out at 10,000 rpm for 15 min. the process was repeated many times until clear juice was obtained. Fermentation process will stop at this stage.

vii) Stabilization: Potassium sorbet was added as preservative and to prevent the fermentation process. The juice was stirred vigorously to release any excess of co_2 gas.

viii) Clarification/bottling: Wine container was kept at low temperature or in refrigerator for 5 to 12 days to settle the solids. Then the wine was filtered for clarification. It was kept for aging for minimum 3 months in refrigerator.

Fermentation sampling procedures. Samples (approximately 3.5 ml/sample) were taken at appropriate times. Three milliliters of the sample was filtered through a 0.45mm syringe filter into a 5.0-ml sterile test tube with a sealing cap and was frozen for later analysis. The remainder of the sample was used immediately for obtaining total cell count and viable cell count as well as for obtaining an optical density reading.

Viable and total cell concentration procedures. For determining total and viable cell counts, by hemacytometer was used under a light microscope at 400 magnifications. A sample was diluted with water such that when a final dilution with methylene blue at a 1:1 ratio was made, the final dilution would give a minimum of 100 cells and a maximum of 400 cells in the counting area of the hemacytometer. Samples were mixed on a vortex mixer at each stage of the dilution. The methylene blue used was 0.02% wt/vol in a citrate buffer. Cells were allowed to be in contact with methylene blue for at least 1 min, but not more than 5 min. Blue cells were counted as dead, and noncolored cells were counted as live. Five of the 25 squares in the hemacytometer grid were counted, and the result was multiplied by 5 to give a total count

FLOWCHART ILLUSTRATING THE PREPERATION OF TOMATO WINE

Tomato selection

↓

Washing (By salt water)

↓

Squeeze & separation of outer coat

↓

Juice Separation

↓

Addition of all ingredients

↓

Extraction of Juice (Pasteurization at 80^0 for 2-3 mins.)

Addition of starter ⟶ ↓

Culture (10ml) Fermentation for 5 days (At 25 to 26 ^{0}C)

↓

Separation of juice by cheese cloth

↓

Filtration (2-3 times)

↓

Filled in bottle, sealed & label

↓

Storage for aging (cool condition)

Fig. 1 Flow chart of alcohol preparation

ix) PH determination: pH of sample was recorded by using pH meter also detected by pH strips which showing acidic (pH 4.4). Before initial fermentation and after completion of fermentation the pH was recorded.

x) Tomato TA:

This test is intended for measuring the Titrable Acidity of Tomatoes and Tomato Juice. Results were reported as g of citric acid/100 mL, equivalent to % citric acid. Tomato TA is based on the titration of

tomato acids, mainly citric acid, by an alkaline solution. The test minimizes interferences from colored and turbid samples.

xi) Brix determination: By using hand refractometer ^{0}brix was recorded before initial fermentation and after completion of fermentation.

xii) CO_2 content determination: The carbon dioxide content of the wine was estimated by titrating known volume of the wine sample against 0.0227 N NaOH using phenolphthalein as an indicator. The CO_2 was measured in terms of mg/lit.of wine sample.

CO_2 of the tomato wine sample were calculated by using the following formula as

$$CO_2 \text{ in mg/lit.} = \frac{\text{MBR X 0.0227 X 44 X 100}}{\text{Volume of sample taken}}$$

xiii) Alcohol determination by specific gravity method:

a) By using specific gravity bottle specific gravity was calculated using the following formula,

$$\frac{W2-W1}{W3-W1} = \text{specific gravity}$$

Where,

W1: Weight of empty specific gravity bottle.
W2: Weight of sample + specific gravity bottle.
W3: Weight of distilled water + specific gravity bottle.

b) By using Hydrometer:

In order to obtain accurate alcohol content a hydrometer was used. The hydrometer measures the density of the liquid by floating at a given level corresponding to the specific gravity of the fluid being measured. It was read by noting the level at which the surface of the fluid contacts the glass when the hydrometer is floating in the liquid. The first reading was taken before the addition of yeast, and at 26^0 C. The second reading was taken after completion of fermentation, that is, before bottling, and before adding the sugar.

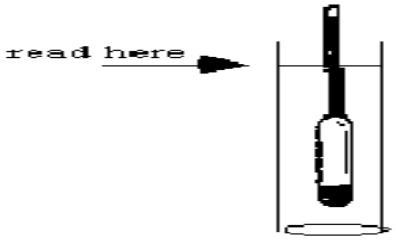

Fig.2. hydrometer

Results and Discussion:

The yeast culture *Saccharomyces cerevisiae 3282* was shown in fig.3.Which was obtained from the National Collection of Industrial Microorganisms (NCIM), NCL, d Pune. Stained by crystal violet stain and was

confirmed the budding yeast cells which is shown in fig.4.as follows,

Fig.3. *Saccharomyces cerevisiae 3282*

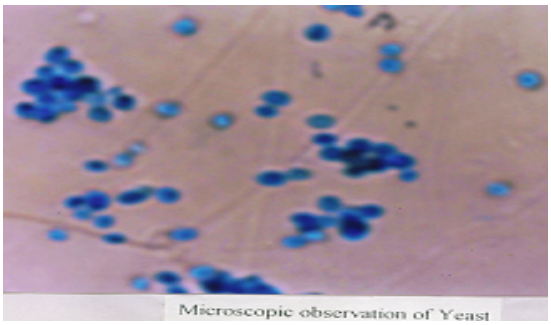

Fig.4 Yeast cells stained by crystal violet

The high grade tomato were selected for the fermentation shown in fig.5.Then those selected tomato crushed to prepare juice which is shown in fig.6

Fig.5: Tomato (Solanum lycopersicum)

Fig.6. Tomato juice.

Fig.7. Tomato wine for aging.

The tomato juice was inoculated with 10 ml of starter culture and incubated for 5 days at 26^0 C anaerobic conditions. After 5 days of fermentation, the fermented juice was analyzed for contaminating organisms, brix, pH, specific gravity, CO_2 content, yeast cell count and alcohol percent.

After completion of fermentation, the juice was filtered many times so as to remove total solids to get clear fermented juice as shown in fig.7.

To determine the alcohol content following calculation was done

O.G. (First reading) = 1.095

F.G. (final or finishing gravity) = 1.035

Subtract final from original. 1.095 - 1.035 = 0.060

Multiplied this figure by 105. 0.060 x 105 = 6.30% a.b.w.

Therefore, tomato wine contains 6.30% alcohol by weight.

To get Alcohol by volume multiplied the figure for % a.b.w. by 1.25

6.30 x 1.25 = 7.88% a.b.v.

-The CO_2 of the tomato wine sample were calculated by using the formula as

$$CO_2 \text{ in mg/lit.} = \frac{\text{MBR X 0.0227 X 44 X 100}}{\text{Volume of sample taken}}$$

The calculated CO_2 before fermentation and after fermentation were recoded in table1

Analysis	**Before fermentation**	**After primary fermentation**
pH	4.4	3.3
Specific gravity	1.095	1.035
Titrable Acidity	0.35 g/100 mL	0.45g/100mL
Brix	22^0	7^0
Alcohol	-----	7.88%
CO_2 content	6.4mg/lit	5.01mg/lit
Viable cell count	$5X10^7$/ml	$3X10^2$

Table 3. Result Analysis of wine

Conclusion: The variety of tomato rich in vitamin C, carbohydrates and less protein which was used for the production of wine is easily and cheaply available throughout India.

As the content of sugar is less than required, we increased it to 22 brix by additional sugar. The production of tomato wine is simple because the fermentation can be carried out at normal pH and temperature conditions. The color of wine is attractive and tomato which is acidic gives sour taste to wine. By adjusting the brix we can get the alcohol percent 7.88% which is acceptable as compare to standard wine. It can be produce in commercial form.

Abbreviations:

1. S. cerevisiae = *Saccharomyces cerevisiae*
2. BC =Before Christ.
3. DNA = Deoxyribose nucleic acid
4. kJ = Kilo joule
5. Kcal = Kilo calorie
6. g = grams
7. mg = Milligrams
8. ml = Milliliter
9. mm = Micrometer
10. mins = Minutes
11. a.b.w. = Alcohol by weight
12. a.b.v. = Alcohol by Volume
13. pH = Hydrogen ion concentration.
14. lit = liter
15. O.G. = Original gravity
16. F.G.= final gravity
17. CO2 = Carbon dioxide
18. wt/vol = weight by volume
19. MBR = mean burette reading
20. N = normality

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