International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 13-03-2022, Accepted: 28-03-2022, Published: 13-04-2022
Volume 4, Issue 1, 2022, Page No. 45-53

Comparative analysis of different grapes verities for red wine production

Milta Vanjare[1], Vrushali Sahane[2], Yogita Gulve[2], Yogesh Chaudhari[1]
[1] Assistant Professor, Department of Microbiology, Karamshibhai Jethabhai Somaiya College of Arts, Commerce and Science Kopargaon, Maharashtra, India
[2] Department of Microbiology, Karamshibhai Jethabhai Somaiya College of Arts, Commerce and Science Kopargaon, Maharashtra, India

Abstract
The present study shows the Production of red wine at a laboratory scale by using four types of grapes including *Shiraz, Merlot, Tempranillo, and Cabernet sauvignon* respectively. Fermentation activity was carried out by using *Saccharomyces cerevisiae*. Due to the high glucose concentration, pulp containing organic acid and polyphenols the Vitis vinifera are commonly preferred for the red wine preparation. This variety of grapes makes quality wine. Traditionally wine is used as an alcoholic drink originated by fermenting fruit juice with the reaction of yeast. For this experiment, fresh grapes were harvested from Sinnar region. Shiraz variety was used for red wine production. The grapes were taken manually, for cold-soaking the grapes were kept at a low temperature of 5-6°C for 3 days. After the cold-soaking procedure, the grapes were crushed by hand, and juice was collected into the sterile flask. The fermentation process was carried out with *Saccharomyces cerevisiae* culture for 21 days at 30°C temperature in an incubator. After fermenting wine was filtered in sterile glass bottles. A study of Biochemical parameters was recorded. For microbial screening, samples were collected after 5 days on to the specific media by serial dilution method. Using specific media the microflora was grown and identified by the staining method. Yeast (*Saccharomyces cerevisiae*) was identified microorganism. Biochemical analysis of red wine including reducing sugar, pH, Brix %, Reducing sugar, TSS, Titrable acidity, Volatile acidity, Vitamins, and ethanol content was observed. A comparative study of Shiraz wine with physicochemical parameters was done with standard wine. Fore types of selected grapes are studied for wine production. Throughout the study, the sugar content was decreased. During the fermentation process in biochemical analysis, increase intitrable acidity was found and decrease in pH was observed. After 21 days of fermentation grape wine was prepared.

Keywords: *Saccharomyces cerevisiae*, grapes, fermentation, alcohol content. grape wine

Introduction
Wine made from grapes is the most casual fruit juice along with alcohol. The winemaking process has the most attention in the research area due to the commercialization of its industrial products. *Vitisvinifera* is a common grape for wine which is member of the *Vitis*species, belonging to the southwestern Asia, Mediterranean region, Europe, and, Morocco, Iran, Germany, and Portugal [5]. There are only a few verities of *Vitisvinifera* grapes available worldwide wild with commercial significance in winemaking. It consists of many phenolic compounds such as Anthocyanins are present in the skin of *V.vinifera* berries which gives color, another is hydroxycinnamic acids which are present in the pulp of berries, and also the proanthocyanidins condensed tannins are present in seeds. In the Wood and Skin, Stilbenoids can be detected [7]. In the grapes Isoprenoid monoterpenes are present, geraniol, tocotrienol, nerol, acyclic linalool, monocyclic alpha-terpineol, citronellal, and, frequently occurring as glycosides [6]. The ripening of grapes is accumulated by carotenoids. Volatile fragments and C13-norisoprenoids are produced by oxidation of the carotenoids. It has some strong odoriferous compounds which include damascenone having exotic fruits aroma, beta-inonol having fruits and flowers aroma, beta-ionone having viola aroma, and beta-damascone having rose aroma. An Alkaloid identified in grapes is Melatonin. There is a high content of unsaturated fatty acids in grapes seeds, which helps in lowering LDL cholesterol and total cholesterol level present in the blood [15]. The most important components necessary to make wine is present in grapes which are sugar and yeast which are used as inoculum having the bloom which appears like white powder. Yeast plays a significant role to convert sugar present in the grapes into carbon dioxide and alcohol through the process of fermentation. The production of wine from grapes involves the basic steps that are, Cold soaking, crushing, fermentation, filtration, clarification, and bottling. The subsequent processing practices using grapes as a raw material give a distinctive flavor to the wine. A volatile compound like terpenes contributed by grapes gives a specific fruity character to the final product. Some of the non-volatile compounds such as malic acid and tartaric acid are also contributed by grapes to improve the flavor of wine and the astringency and bitterness are due to tannins. The skin of grapes is the most important constituent in red wine because the tannins are present in the

skin [16]. As compared to other species *Saccharomyces cerevisiae* can tolerate higher concentrations of ethanol [36] which is up to 14 % v/v and more than that, whereas other species can-not be able to tolerate alcohol concentrations of more than 5 % or 8%. Grapes are having multipurpose applications such as the preparation of wine, eating, and jam making [34]. The use of properly ripened grapes is very important. Colorless juice is obtained from red wine grapes. The significant red color is entirely present in the skin of grapes, due to which the juice is used to be kept in contact with the skin for a regulated time to get color to the wine. The making of red wine is followed by grapes crushing and fermenting the skin, the juice the seeds, and the pulp together for some days. From the solid material separation of the liquid content is followed by the use of a pneumatic press by the end of the sugar fermentation during winemaking [6]. Frequently available verities of grapes for red wine include Syrah**,** Merlot, Cabernet, Sauvignon, and Tempranillo. The most popular red wine in the world is obtained from the Merlot variety of grapes and the second most popular in America, the first is Cabernet Sauvignon. Cabernet Sauvignon is popular for its approachable style, and sensual and soft texture. It is prepared by using the grapes of redskin, which adapt to different climates for the production of food-friendly verities of wine at different cost points [8]. Grapes of redskin give red color to Merlot. The color of Merlot is usually faint in the Cabernet Sauvignon and darker than the Pinot Noir [17]. Grapes of Cabernet sauvignon variety are harvested from local regions; Dindori, Sholapur, Sinnar and Nashik, etc. Sula Cabernet Shiraz is India's highest-selling wine. It is a medium-boiled and smooth wine prepared from silky tannins, the aroma of black pepper, pulp notes, and ripened cherry. Drinking food-friendly wine is also the most preferred one [19]. Wine should be served in slightly chilled condition [26]. There is 13-14% alcohol content present in Cabernet Sauvignon wine. This wine hasa large production volume which is easily available and cost-effective [20]. Tempranillo variety is harvested from Niphad, Ozar, and Pimpalgaon Nashik districts. Tempranillo has thin skin and larger grapes as compared to Shiraz. The flavor is good and the texture is not oily or not thick [33].

Material and methods

Collection of Sample [21]

For wine production, fresh grapes were harvested from the Sinnar region. The grapes were taken manually fourvarietiesof grapes were used for red wine production. Pure culture of *Saccharomyces cerevisiae* Strain BRL97 was collected from the culture collection of SULA Vineyard, Nashik.

Fig 1: Variety of grapes shown *viz*., *Shiraz, Merlot, Tempranillo, Cabernet sauvignon* Respectively

Cold Soaking

Freshly harvested grapes of selected varieties were kept at 5-6 ºC for three days for the cold soaking process this will enhance the color and flavor of the wine this technique was adopted by several winemakers before fermentation [35].

Crushing

Before crushing grapes of selected varieties were washed with a solution of vinegar in distilled water to reduce surface microflora. Crushing was done by hand manually in a glass vessel aseptically. Crushing was carried out in a way to retain pills of grapes as it is. As gapes skin plays important role in the color and flavor development of wine. The crushed mixture was kept ina UV chamber for 15 min to remove microbial contamination. Then the mixture was analyzed for its pH and sugar content. After that mixture was transferred to a sterile glass vessel to carry out the fermentation process [34].

Fermentation

Before starting the fermentation process the mixture was kept at 4-5ºC for two days. This procedure will increase color intensity in the finished product. Then fermentation process was carried out using the cultures of (*Saccharomyces cerevisiae* strain BRL97). The process was carried out up to twenty-one days for each variety. While the fermentation process, selected parameters like pH and reducing sugar content were analyzed at regular five-day intervals [9].

Fig 2: Pure culture of *Saccharomyces cerevisiae* strain BRL97

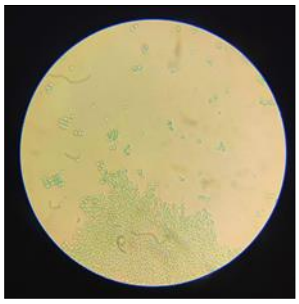

Fig 3: Staining of *Saccharomyces cerevisiae* strain BRL97

Fig 4: Fermentation broth for a variety of grapes *viz*., Merlot, Shiraz, Cabernet Sauvignon, Cabernet Sauvignon, and tempranillo

Filtration

After completion of the fermentation process, all flasks were filtered using sterile muslin cloths. The filtration process gives additional smooth texture to wine because it is very thick due to the higher content of tannin and some berry parts in the wine [11].

Bottling

Some amount of filtrate of each variety was filled in sterile bottles for the aging process the aging process will develop a unique test aroma and flavor further reaming part of the filtrate was kept for further analysis.

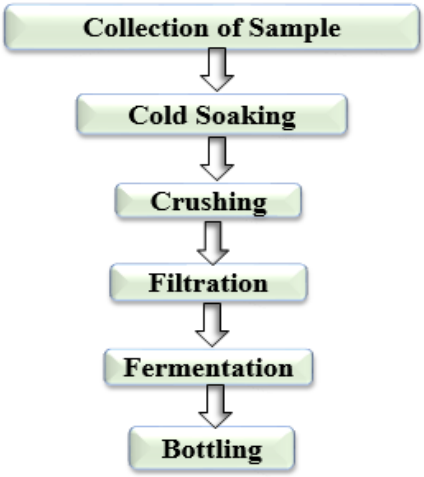

Fig 5: Steps are involved in winemaking procedure

Analysis of Physicochemical Parameters of grape wine for all four variety

Total Soluble Solids (TSS)

Total soluble solids in grape juice were examined with the help of a pocket refractometer. 2-3 drops of the sample were added tothe surface of the refractometer. Noted the readings onscreen. TSS was recorded [10].

Analysis of pH

pH measurement was done with the help of a pocket pH meter, by insertinga dry electrode slowly into the wine sample and noting the pH when the reading gets constant [12].

Analysis of Titratable Acidity

Wine sample of volume 10ml was mixed in 30 ml of distilled water. Adjusted the pH up to 8.25 by adding 0.1N NaOH drop by drop from the burette [9].

Titratable Acidity = Final Burette reading – Initial Burette reading × 0.75

Recovery to the extraction of Ethanol

A distillation setup was used to extract the ethanol from the grape wine sample. 500ml of wine samplewas added to a chamber and heatedat 78°C temperature. Water was introduced into the condenser,and a clean and dry flask was placed under the condenser. Collected distillate ethanol into the flask [9].

A qualitative examination of ethanol by Specific gravity Method [13]

Took clean, dry Specific Gravity bottle and weight of gravity bottle was measured. Water was filled to the rim level of the Specific Gravity bottle and weighed. The weight of the dry empty bottle was checked to find thewater volume. After that water was removed and derived ethanol from grape wine wasweighed by adding into the bottle. The weight of the dry bottle was subtracted to know the weightof the sample. Specific Gravity was estimated by the following derivation:

Specific gravity = Weight of Ethanol / Weight of water

Qualitative analysis of ethanol by Potassium Dichromate Method

1ml of ethanol was taken into the test tube. 5ml of Potassium Dichromate was mixed into the same. Thentest tube was put for 20 minutes at 60 ℃in a water bath. After cooling the mixer, absorbance was measured at 600nm. This method was used to make standard graph of ethanol for the grape wine sample. According to the standard graph, ethanol concentration was determined [22].

Analysis of Reducing Sugar

DNSA- method was used to measure reducing sugar. Ten tubes were taken and labeled (1to 10). D/W set as blank. Madeserial dilutions of glucose standards. Added 3ml of DNSA reagents in all tubes. Mixedit well. Put them into the hot water bath for 15 min. After these cooling tubes at room temperature using cold water, Absorbance was recorded at 540nm. With the help of a standard graph reducing sugar in grape wine was calculated [9].

Volatile Acidity

To determine volatile acidity distillation method was used. The distillate was collected and immediately added some drops of C20H14O4 in the flask and started titration with the 0.1N NaOH till the color changed into pink [34].

Volatile Acidity (g/l) = Titer volume × 0.3

Determination of Vitamin found in grape wine [32]

Presence of Vitamin B_1 (Thiamine) was determined by referring Pearson method. 1ml of sample mixed with 50 ml of distilled water and filtered. Reagent solution 2ml was mixed with filtrate of 2ml. Wait for 1 minute and 15ml of isobutyl alcoholis added and oscilated it vigorously for 2 min. The layer of isobutyl alcohol was dried, by moving a spatula tip of anhydrous sodium sulfate and again oscilated. The absorbance was recorded at 867nm. Isobutyl alcohol is set as a blank [32].

Vitamin B_2 (Riboflavin presence was recorded by referring method of Pearson. 1.5ml of filtrate sample mix with 6.5ml of distilled water and Reagent solution 2ml was added into the mixture. The absorbance was recorded at 525nm.

Vitamin B_6 (Pyridoxine) content was carried out with referring Pearson method. Sample of 1ml filtrate added in 2 ml of distilled water and 0.4ml of 50% sodium acetate were added. After this .5 %, sodium carbonate of 0.1 ml and diazotized reagent of 0.1 ml were added. Absorbance was recorded at 540nm with the help of a spectrophotometer.

Vitamin B_{12} (Cobalamine) content was determined by following the Pearson method. First, adjust the pH in range 9.5 to 10 by 10% NaOH in 2ml of sample filtrate. Then added 0.1g of sodium cyanide. After this Stand it

for 5h before adding 1g of sodium sulfate. After this again pH was adjusted in-between range of 11 and 11.5. Then added 2ml of benzyl alcohol and extracted before removing the aqueous layer. After this 3ml of chloroform and istilled water of 3ml were added to benzyl alcohol and shaken for 5min. The organic layer and aqueous layer were discarded prior to the addition of 1ml distilled water. 1ml solution of 10% sodium cyanide was added to another 5ml. pH was adjusted in between 5-6 by using a potassium dihydrogen phosphate solution of 12.5%. At 582 nm the reading were recorded [31].

To determine Vitamin C means Ascorbic acid content method of Pearson was used.1ml of sample was mixed with 20 ml of 0.4% of oxalic acid. Then 9ml of indophenol solution was added. Absorbance was recorded at 520 nm by using a spectrophotometer [32].

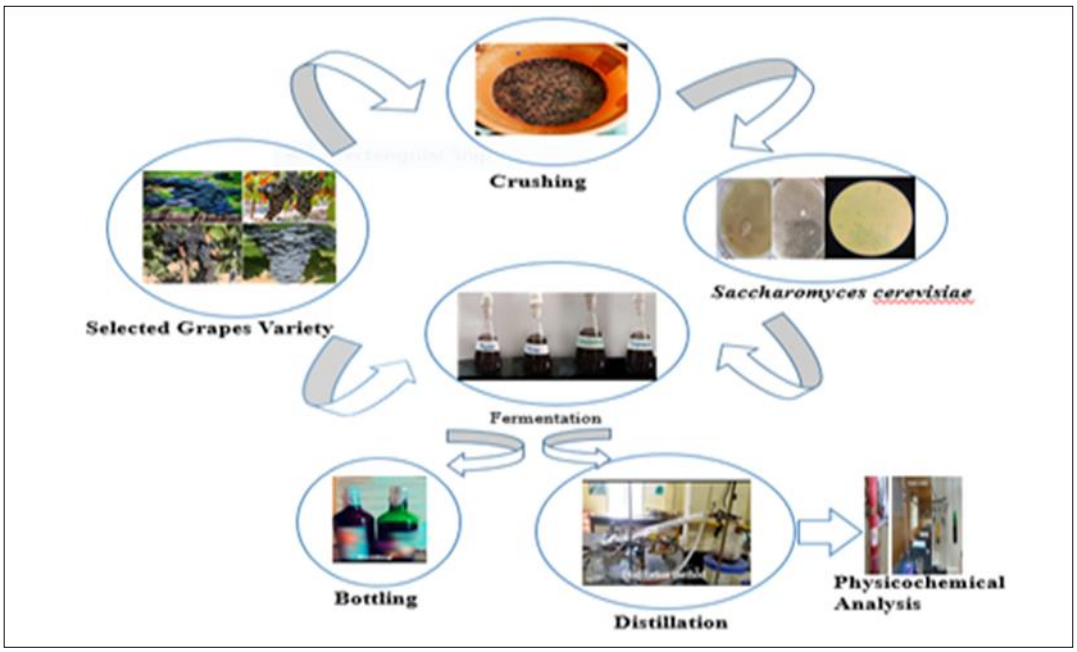

Fig 6: Diagrammatic representation of winemaking procedure

Result and Discussion

Table 1: Chemical analysis of finished wine produced from Sirahz, *Tempranillo, Merlot, and Cabernetsauvignon* respectively.

Chemical Analysis	Sirahz	*Tempranilla*	*Merlot*	*Cabernetsauvignon*
pH	3.66	3.87	3.91	3.79
TSS of grapes juice (°brix)	24°	26°	27°	26°
Vitamin B1	0.01	0.01	0.01	0.02
Vitamin B2	0	0	0	0.1
Vitamin B6	0.1	0	0.1	0.1
Vitamin B12	0	0	0	0
Vitamin C	0.002	0	0	0.001
Alcohol (%)	14.7%	5.9%	6.5%	7%
Titrable Acidity	6.67 gm/L	5.78 gm/L	5.77gm/L	5.68gm/L
Reducing Sugar	3.6 gm/L	5.9gm/L	5.8gm/L	5.1gm/L
Volatile Acidity	0.69 gm/L	0.79 gm/L	0.81gm/L	0.75gm/L

To check the quality of grape wine various test has been performed. pH, TSS, Volatile acidity, Reducing sugar, vitamins, and ethanol content. Throughout this experiment there are variations have been observed in physicochemical parameters for all four varieties. During the production of grape wine, sugar content decreases. Throughout the fermentation process in biochemical analysis, it was observed that the pH of the sample was reduced while the titrable acidity of the wine sample was enhanced. Shiraz showed a better result as compared to others with 14% ethanol.

Table 2: pH estimation during the fermentation process of wine from Sirahz, *Tempranillo, Merlot, and Cabernet sauvignon* respectively.

pH	Sirahz	*Tempranilla*	*Merlot*	*Cabernetsauvignon*
1st Day	5.87	5.99	5.91	5.89
5th Day	4.69	5.87	5.78	5.22

10th Day	4.22	4.78	5.11	4.25
15th Day	3.86	4.13	4.32	4.11
20th Day	3.66	3.87	3.91	3.79

During the fermentation process pH of all four variants of grape wine was decreased. In case the pH of wine is higher i.e. 4.0 or more then the wine will not stable concerning microorganisms. Low pH inactivate the growth of microorganisms.

Table 3: Determination of Acidity during the fermentation process of wine for selected four varieties.

Time period in days	Shiraz	Marlot	Tempranilla	Cabernet sauvignon
1st Day	3.67	3.81	3.93	3.89
5th Day	4.31	4.22	4.26	4.23
10th Day	4.86	4.91	4.88	4.87
15th Day	5.38	5.35	5.11	5.28
20th Day	6.67	5.78	5.77	5.68

Acidity was determined by using the method of Titration. The present study shows that titrable acidity increases throughout the fermentation process.

Table 4: Comparative study of Shiraz wine and SULA Red wine

Chemical Analysis	Shiraz Red Wine	Sula Red Wine
pH	3.66	3.60
TSS of grapes juice (°brix)	24°	23°
Alcohol (%)	14.7%	13.0%
Titrable Acidity	6.67 gm/L	7.72 gm/L
Reducing Sugar	3.6gm/L	5 gm/L
Volatile Acidity	0.69 gm/L	0.60 gm/L

After a chemical analysis of the selected four wines, it was observed that Shiraz grape wine showeda better result. Therefore Shiraz grape wine was selected to compare with standard wine (Sula red wine). A comparative study shows that the quality of Shirazgrape wine produced on a laboratory scale is relatively close to the standard red wine of Sula.

Table 5: Comparative study of lab-scale wine at 30°C and room temperature

Chemical Analysis	Shiraz wine at 30C temperature in an incubator	Wine at room temperature
pH	3.66	5.11
TSS of grapes juice (°brix)	24°	26°
Alcohol (%)	14.7%	8.2%
Titrable Acidity	6.67 gm/L	5.22 gm/L
Reducing Sugar	3.6 gm/L	4.9 gm/L
Volatile Acidity	0.69 gm/L	0.73 gm/L

Throughout the process two jars were kept for fermentation one is in an incubator and another one is kept at room temperature. A wine that is produced in an incubator showsa better result as compared to wine prepared at room temperature. 5.4gm/L reducing sugar was observed at at room temperature while reducing sugar was observed at 3.6gm/L at 30^0C.

Calculations for Specific Gravity

Weight of Dry bottle(W1) = 20.49g
Weight of Bottle + Water (W2) = 47.95g
Weight of Bottle + Extracted Sample(W3) = 42.32g
∴Weight of Water = W2-W1
= 47.95- 20.49
= 27.46g
∴Weight of Sample = W3 - W1
= 42.32- 20.49
=21.83g
Specific Gravity = Weight of sample / Weight of Water
=21.83 / 27.46
= 0.79 g

Hence, the Specific Gravity was found to be 0.79 thai is similar to the specific gravity of Ethanol (0.79), therefore, the presence of Ethanol was observed.

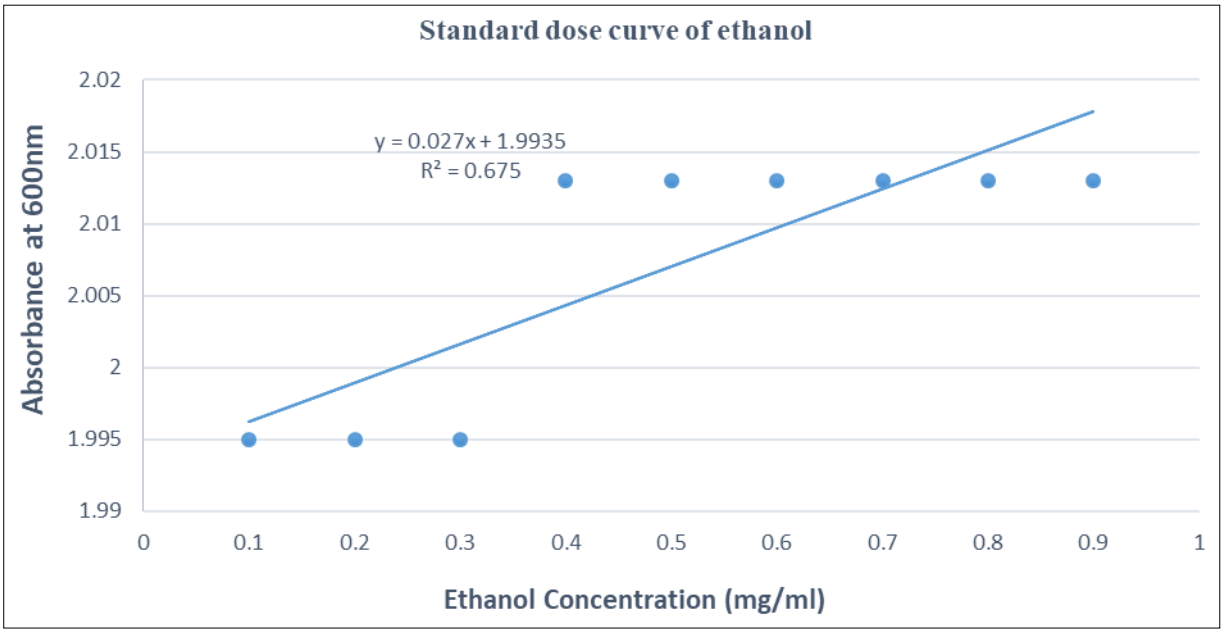

Fig 7: Standard Graph for ethanol estimation by Potassium Dichromate Method

From the method of Potassium Dichromate ethanol was estimated. The Sample absorbance was recorded at 600nm. After taking absorbance according to the standard graph ethanol was estimated from Shiraz Grape wine. Shiraz, *Tempranillo, Merlot, and Cabernet sauvignon* respectively.

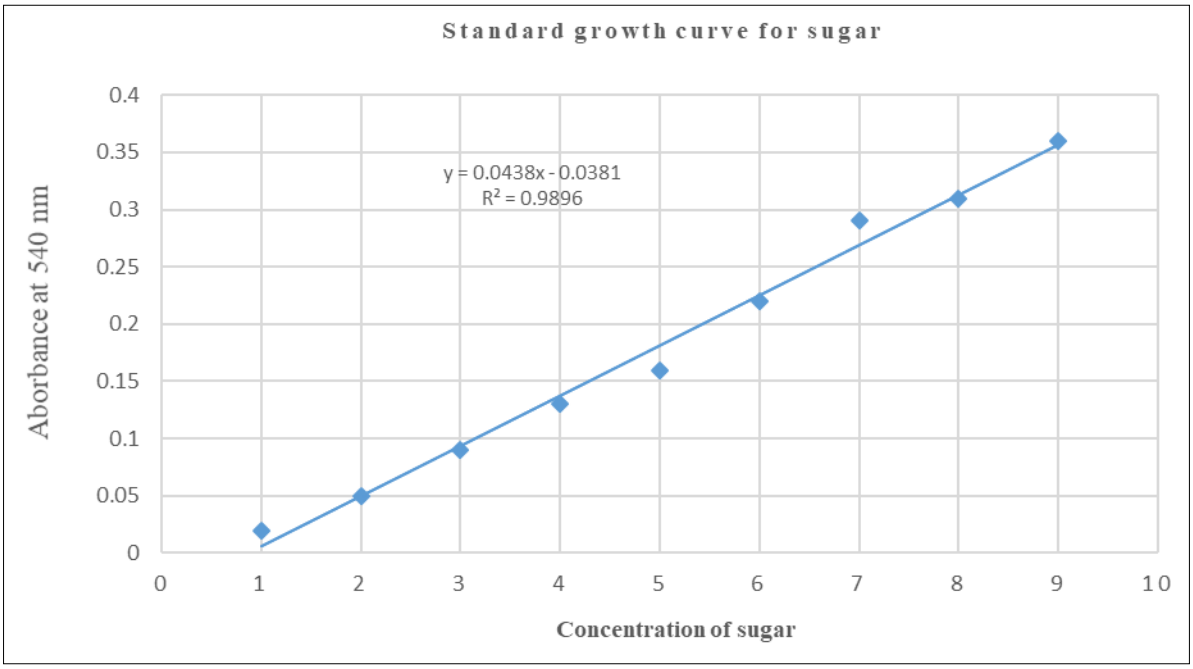

Fig 8: Standard dose curve for determination of reducing sugar

To determine reducing the sugar by the DNSA Method sample absorbance was recorded at 540nm. According to the standard graph reducing sugar was estimated from Sirahz, *Tempranillo, Merlot, and Cabernet sauvignon* respectively.

Conclusion

The study conclude that grape wine was successfully produced after fermentation process of 21 days from four Varieties of grapes including *Shiraz, Merlot, Tempranillo, and Cabernet sauvignon* respectively up to the laboratory level. *Shiraz*grape gave a better result as compared to the other three selected varieties. Therefore Shiraz grape wine was compared with the standard red wine. A comparative study shows that the quality of Shiraz grape wine produced on a laboratory scale is relatively close to the standard red wine of Sula.

Acknowledgment
Dr. B. S. Yadav, Principal, Karamshi Jethabhai Somaiya College of Arts, Commerce, and Science, Kopargaon, is grateful for his constructive remarks that helped to improve the quality of this Research Paper. The authors are also thankful to Mr. Aakash Pawar and Ms. Pradnya kadu for drafting the research article. The authors want to thank Sula Vineyards for their co-operation and help during the research work.

References

1. Iwasaki M, Murakami M, Ijiri Y, Shimizu M, Yamamoto J. Are all wines made from various grape varieties beneficial in the prevention of myocardial infarction and stroke? Future Sci OA,2020:7(2):FSO649. DOI: 10.2144/fsoa-2020-0098. PMID: 33437515; PMCID: PMC7787155.
2. Gao Y, Fangel JU, Willats WGT, Vivier MA, Moore JP. Differences in berry skin and pulp cell wall polysaccharides from ripe and overripe Shirazgrapes evaluated using glycan profiling reveals extensin-rich flesh. Food Chem,2021:363:130180. doi: 10.1016/j.foodchem.2021.130180. Epub 2021 May 24. PMID: 34157558.
3. Mattivi F, Zulian C, Nicolini G, Valenti L. Wine, biodiversity, technology, and antioxidants. Ann N Y Acad Sci,2002:957:37-56. doi: 10.1111/j.1749-6632.2002.tb02904.x. PMID: 12074960.
4. Jiménez-Cantizano A, Muñoz-Martín A, Amores-Arrocha A, Sancho-Galán P, Palacios V. Identification of Red Grapevine Cultivars (*Vitis vinifera* L.) Preserved in Ancient Vineyards in Axarquia (Andalusia, Spain). Plants (Basel),2020:9(11):1572. doi: 10.3390/plants9111572. PMID: 33203001; PMCID: PMC7697472.
5. Murphy CA. Grapes: origins and development. In Encyclopedia of global archaeology (pp. 3107-3111). Springer New York, 2014.
6. Imazio S, Labra M, Grassi F, Scienza A, Failla O. Chloroplast microsatellites to investigate the origin of grapevine. Genetic Resources and Crop Evolution,2006:53(5):1003-1011.
7. Grando S, Von Bothmer R, Ceccarelli S. Genetic diversity of barley: use of locally adapted germplasm to enhance yield and yield stability of barley in dry areas. Broadening the Genetic Base of Crop Production. CAB International. New York, 351-372.
8. https://www.slurrp.com/article/tracing-the-origin--the-history-of-wine-in-india-1628082207591
9. Goswami S, Ray S. Studies of process development for the fermentative production of wine from grape juice concentration. International Journal of food safety,2011:13:367-373.
10. Joshi V, Kumar V, Debnath MK, Pattanashetti S, Variath MT, Khadakabhavi S. Assessing quality of blended wine prepared from white and red varieties of Grape (Vitis vinifera L.). International Journal of Agricultural and Food Science,2014:5(1):1612.
11. Triwattana K. Protein differentiation of lactic acid bacteria between free-living cell and biofilm, 2018.
12. Guleria A. Production of grapes wine by the use of yeast, Saccharomyces. cervisiae. Global Journal for Research Analysis, 2014, 3(6).
13. Upendra RS, Pratima K, Priyanka S, Jagadish ML. Production of bioethanol from hitherto underutilized agro waste (Field beans/Green pea pods waste) incorporating zero waste generation technique, 2013.
14. Sneh-Lata, Mishra T, Kumari A. Wine Preparation from Different Fruit Substrates. International Research Journal of Biological Sciences,2014:3(7):61-64.
15. Shweta Parihar, Devender Sharma. A breif Overview On *Vitis vinefera*, Scolers Academic Journal Of Pharmacy, 2021. 10.36347/ saip.20121.V10i12.005.
16. Parker Mango, Capone Dimitra, Francis Ian, Herderich. 2017/02/21.,Aroma Precursors in Grapes and Wine: Flavor Release during Wine Production and Consumption.10.1021/acs.jafc.6b05255 Journal of agricultural and food
17. https://www.winemag.com/2018/11/26/everything-you-need-to-know-about-merlot/
18. https://www.masterclass.com/articles/learn-about-cabernet-sauvignon#what-does-cabernet-sauvignon-taste-like
19. Goldstein E. *Perfect pairings*. University of California Press, 2006.
20. Debenham S. Microsoft wine guide. *Visual Media*,1996:9(2):30-30.
21. Bajaj BK, Bajpai P. Selection and characteristics of yeast for desirable fermentation characteristics,2011:2:107-109.
22. Dias MO, Ensinas AV, Nebra SA, Maciel Filho R, Rossell CE, Maciel MRW. Production of bioethanol and other bio-based materials from sugarcane bagasse: integration to conventional bioethanol production process. Chemical engineering research and design,2009:87(9):1206-1216. https://doi.org/10.1016/j.cherd .2009.06.020.
23. El-Mekkawi SA, Abdo SM, Samhan FA, Ali GH. Optimization of some fermentation conditions for bioethanol production from microalgae using response surface method. Bulletin of the National Research Centre,2019:43(1):164. https://doi.org/10.1186/s42269- 019-0205-8.
24. Tiwari BK, Muthukumarappan K, O'Donnell CP, Cullen PJ. Modelling colour degradation of orange juice by ozone treatment using response surface methodology. Journal of Food Engineering,2008:88(4):553- 560. https://doi.org/10.1016/j.jfoode ng.2008.03.021
25. Priyadarsini P. Application of uv-c radiation for shelf life enhancement of sugarcane juice (Doctoral dissertation, CAET, OUAT).

26. Borji A, Borji F, Jourani A. A new method for the determination of sucrose concentration in a pure and impure system: spectrophotometric method. International journal of analytical chemistry, 2017. https://doi.org/10.1 155/2017/821412
27. Dziugan P, Balcerek M, Binczarski MJ, Kregiel D, Kucner M, Kunicka-Styczynska A *et al*. Ozonation as an effective way to stabilize new kinds of fermentation media used in biotechnological production of liquid fuel additives. Biotechnology for biofuels,2016:9(1):150. https://doi.org/10.1186/s13068- 016-0574-2
28. Manikantan MR, Pandiselvam R, Arumuganathan T, Indurani C, Varadharaju N. Low-density polyethylene based nanocomposite packaging films for the preservation of sugarcane juice. Journal of Food Science and Technology,2022:59:4:1629-1636.
29. Silva GPD, Araújo EFD, Silva DO, Guimarães WV. Ethanolic fermentation of sucrose, sugarcane juice and molasses by Escherichia coli strain KO11 and Klebsiella oxytoca strain P2. Brazilian Journal of Microbiology,2005:36(4):395-404. https://doi.org/10.1590/s1517- 83822005000400017.
30. Sobočan G, Glavič P. Optimization of ethanol fermentation process design. Applied thermal engineering,2000:20(6):529-543. https://doi.org/10.1016/s1359- 4311(99)00042-3.
31. Suleiman B, Abdulkareem SA, Afolabi EA, Musa U, Mohammed IA, Eyikanmi TA. Optimization of bioethanol production from nigerian sugarcane juice using factorial design. Advances in Energy Research,2016:4(1):069. https://doi.org/10.1351/pac19 6817020273.
32. Zabed H, Faruq G, Sahu JN, Azirun MS, Hashim R, *et al*. Bioethanol production from fermentable sugar juice. The Scientific World Journal, 2014.https://doi.org/10.11 55/2014/957102.
33. Mbaeyi-Nwaoha IE, Ajumobi CN. Production and microbial evaluation of table wine from tamarind (Tamarindus indica) and soursop (Annona muricata). Journal of Food Science and Technology,2015:52(1):105-116.
34. Reynolds AG. Viticultural and vineyard management practices and their effects on grape and wine quality. In *Managing wine quality*. Woodhead Publishing, 2022, 443-539.
35. Boulton R. Red wines. In *Fermented beverage production*. Springer, Boston, MA, 2003, 107-137.
36. Mane SS, Ghormade V, Tupe SG, Deshpande MV. Diversity of natural yeast flora of grapes and its significance in wine making. In *Yeast diversity in human welfare*. Springer, Singapore, 2017, 1-127.
37. Pawar Aakash & Kadam, Vijay & Joshi, Saish & Rashinkar, Abhishek & Jadhav, Bhagyashri & Patil, Jagruti & Journal, The. Isolation and Screening of Fungi from Sugarcane Bagasse Scientist The official Journal of Scientist R Academy,2022:1:155-162. 10.5281/zenodo.6378216
38. Microbial contaminant isolation from poultry feeds and remedy to overcome it", International Journal of Emerging Technologies and Innovative Research (www.jetir.org | UGC and issn Approved), ISSN:2349-5162,2022:9(4):b267-b272.
39. Chaudhari, Yogesh & Khairnar, Monali & Kashid, Shital & Pawar, Aakash & Kadu, Pradnya & Gakkhad, Pooja. Production of biofertilizers by using microbial isolates Scientist The official Journal of Scientist R Academy,2022:1:224-238. 10.5281/zenodo.6409149.
40. Pawar, Aakash & Chaudhari, Pradnya & Kadu, Rushikesh & Jadhav, Akshay & Darekar. Physico-Chemical Analysis of Soil and Screening for Biofertilizer Producing Microorganisms,2022:1:163-173. 10.5281/zenodo.6381323.
41. Pawar, Aakash, Jethabhai, Karamshibhai. Treatment and reclamation of wastewater, 2021, 6-7.
42. Tamrakar K, Lama A, Dhakal B, Adhikari L, Shrestha M, Amatya J. Qualitative analysis of wine prepared from banana and orange. Int J Food Sci Nutr. 2020;5(1):60-3.