



Research Journal of **Microbiology**

ISSN 1816-4935



Academic
Journals Inc.

www.academicjournals.com

Fermentation and Characterization of Apricot and Raisin Wine by *Saccharomyces cerevisiae* NCIM 3282

R.K. Bapat, S.B. Jadhav and J.S. Ghosh

Department of Microbiology, Shivaji University, Kolhapur 416004, MS, India

Abstract: Dried apricot or dried *Prunus armeniaca* as well as raisins or *Vitis vinifera* are very rich in nutrients. These contain vitamin A, lycopene, β -carotene, polyphenols like resveratrol like other antioxidants which quenches free radical damage to cells and tissues. β -carotene helps protect LDL cholesterol from oxidation thus prevent related heart disease. Dietary resveratrol modulate the metabolism of lipids, inhibit oxidation of low-density lipoproteins and aggregation of platelets. Keeping in mind the above mentioned advantages of these fruits, it was decided to provide the same in the form of wine, which is the primary aim of this study, where the small quantity of alcohol helps in absorption of these antioxidants, yet do not have the disadvantages like intake of high dietary fibers associated with the fruits. The wine from such fruit was prepared by using yeast *Saccharomyces cerevisiae* NCIM 3282, after 28 days of incubation there were 6% and 4 % alcohol, respectively. Further analysis was also carried out on HPLC and GCMS revealed that the wines were free from harmful substances like amylalcohol but had the antioxidants present in the fruits.

Key words: Dry fruits, resveratrol, antioxidants, alcoholic-fermentation, polyphenols

INTRODUCTION

Fruit wines are fermented alcoholic beverages made from a variety of ingredients (other than grapes) and having a variety of flavors. Fruit wines are usually referred to by their main ingredient fruit e.g., plum wine, apple wine, elderberry wine, peach wine, etc.

Fruit wines have traditionally been popular with home winemakers and in areas with cool climates such as North America and Scandinavia. Most fruits and berries have the potential to produce wine. Few fruits other than grapes have balanced quantities of sugar, acid, tannin, nutritive salts for yeast feeding and water to naturally produce a stable, drinkable wine. However, if the amount of fermentable sugars is low then it needs to be supplemented by a process called chaptalization in order to have sufficient alcohol levels in the finished wine (Robinson, 2006). Sucrose is often added so that fruits having excessive levels of acids (usually citric or malic acid), can split this into fermentable fructose and glucose sugars. If the specific gravity of the initial solution is too high, indicating an excess of sugar, water or acidulated water may be added to adjust the specific gravity down to the winemaker's target range. Many fruit wines suffer from a lack of natural yeast nutrients needed to promote or maintain fermentation (Robinson, 2006). Winemakers can counter this with the addition of nitrogen, phosphorus and potassium available commercially as yeast nutrient. Like many conventional white wines, fruit wines often do not improve with bottle age and are usually meant to be consumed within a year of bottling (Robinson, 2006).

Corresponding Author: Dr. Jai S. Ghosh, Department of Microbiology, Shivaji University, Kolhapur 416004, MS, India Tel: +91 9850515620

Two fruits used in this investigation are: dried apricots, i.e., dried *Prunus armeniaca*, syn. *Armeniaca vulgaris* Lam. and raisins, i.e., dried *Vitis vinifera*.

Apricot is very rich in beta-carotene which helps protect LDL cholesterol from oxidation, thus prevent heart disease (Bjelakovic *et al.*, 2007). These also contain vitamin A which is a powerful antioxidant, quenches free radical damage to cells and tissues especially the cornea of the eye (Cho *et al.*, 2004). The high iron content makes it an excellent food for anemia sufferers. The small but essential amount of copper in the fruit makes the iron available to the body.

Raisin contains eight times more sugar than grapes. The sugar consists of glucose and fructose. Therefore, raisins are an excellent food in all cases of debility and wasting diseases. These are also rich in antioxidants especially the dark purple variety as these have large quantities of anthocyanins which are antioxidants. These antioxidants, along with vitamin A, can reverse effect of free radicals, or oxidative stress, which often lead to macular degeneration (Cho *et al.*, 2004). Another important phytochemical found in raisins is resveratrol. Protection of the genome through antioxidant actions may be a general function of resveratrol (Shankar *et al.*, 2007) and by inhibiting gene expression associated with heart and skeletal muscle aging, it prevents age-related heart failure (Mancuso *et al.*, 2007).

Although, adoption of wine consumption is not recommended by some health authorities, a significant volume of research indicates moderate consumption, such as one glass of wine a day for women and two for men, may confer health benefits (Mukamal *et al.*, 2008). Evidence shows that wine polyphenols like resveratrol, lycopene, β -carotene provide physiological benefit whereas alcohol itself may have protective effects on the cardiovascular system (Sato *et al.*, 2002).

MATERIALS AND METHODS

The experiments were carried out during the period July 2009 to March 2010. It was carried out in the Department of Microbiology, Shivaji University, Kolhapur, India.

Microorganism and Growth

Saccharomyces cerevisiae NCIM 3282 was grown in medium containing glucose 12%, peptone 3%, NaCl 9% and incubated at 25°C for 24 to 48 h. The culture was then adapted to grow at 12°C. The organism was maintained on a media containing glucose 0.5%, peptone 0.5%, yeast extract 0.3% with pH is 6.8 to 7.

Growth Curve

The organisms was grown in above medium at 25°C and the growth of microorganism was monitored at regular time interval by recording change in absorbance at 530 nm as well as by doing plate counts. Specific growth rate (F) was calculated using the expression:

$$\ln x_t = \ln x_0 + \mu t$$

Preparation of Wine

The dried apricots which were golden yellow in color and dark purple raisins were obtained from the market. These were separately crushed into medium containing ammonium sulfate 0.3%, K_2HPO_4 0.1%, KCl 0.05%. This mash was first filtered through a coarse filter like cheese cloth and then centrifuged at 5000x g for 20 min. The supernatant had a final sugar

concentration of 10.5% (w/v) and was sterilized at 110°C for 20 min. Adapted growth of *Saccharomyces cerevisiae* NCIM 3282 was inoculated in the flasks. The flasks were incubated for 21 days at 8°C and then at 25°C for 7 days.

Characterization of Wine

The fermented medium at the end of 28 days was centrifuged at 5000x g for 20 min to remove all yeasts. The clear supernatant which is now termed as cell free wine was used for checking titrable acidity like tartaric and acetic acid, alcohol content, amyl alcohol content (a component of fusel ol) and residual sugar.

Titration Acidity

The cell free wine was titrated against 0.1 N NaOH solution.

Tartaric acid content was determined by the expression:

$$\text{Tartaric acid (\%)} = \frac{\text{Volume of alkali} \times \text{Normality of alkali} \times 7.5}{\text{Weight of sample}}$$

Acetic acid content was determined by the expression:

$$\text{Acetic acid (\%)} = \frac{\text{Volume of alkali} \times \text{Normality of alkali} \times 6.0}{\text{Weight of sample}}$$

Alcohol (%)

The cell free wine was distilled at 78°C. The ethyl alcohol content (%) was estimated by potassium dichromate method (Knox and Pask, 1950).

Determination of Reducing Sugar

Reducing sugar concentration was determined before the start of fermentation and at the end of fermentation, i.e., after 28 days. The estimation was carried out by dinitrosalicylic acid method (Miller, 1959).

Antioxidant Properties

Antioxidant activity of the cell free wine samples were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Ko *et al.*, 1998). A control sample with no added wine sample was also analyzed and expressed as radical scavenging activity (RSA%):

$$\text{RSA (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where, A is absorbance at 517 nm.

Chemical Characterization of the 2 Wine Samples

In this investigation, the volatiles compounds were not considered and only those compounds which are non-volatiles were checked. The wine samples were rapidly dried at 25°C and dissolved in methanol. This was then used for GCMS analysis.

The wine samples were also analyzed for the presence of amyl alcohol by HPLC using C-18 column and for this purpose pure amyl alcohol was used as primary reference standard.

Statistical Analysis

All experiments were done in triplicate and in case of doubt were repeated 5 times till consistent results. The standard deviation wherever applicable, was 0.2 or less.

RESULTS AND DISCUSSION

It can be shown from Table 1, that the growth of the yeast; *Saccharomyces cerevisiae* NCIM 3282 was unaffected by the fruits. It showed the same pattern of growth as can be seen from the specific growth rate (μ) (Fig. 1), the rate of biomass formation and biomass formed remained the same. There was a slight change in the alcohol content like it was 6% in the apricot wine and 4% in the raisin wine. However, the residual sugar content and the antioxidants present (Table 2) are the same. Table 3 shows that on carrying the sensory analysis the wines were of a superior grade from the consumer acceptance point of view.

The HPLC analysis (Fig. 2, 3) show that there was no amyl alcohol produced during the fermentation of extracts of both the dry fruits (apricots and that of the raisin).

It has been seen that grapes have so far been used to make wine readily and for most of the reasons, wine is consumed from the organoleptic qualities point of view. However, as

Table 1: Comparative summary of results of fermentation

| Parameters | Apricot | Raisin |
|---|---------------------|---------------------|
| μ (Specific growth rate) | 0.7265 | 0.7265 |
| p (Product-alcohol formed w/v) | 6% | 4% |
| q_p (Specific product formation rate) | 0.00605 | 0.00404 |
| pH (Final) | 6.5 | 6.5 |
| $y_{p/x}$ (Yield of product/biomass g) | 0.066 | 0.0443 |
| Initial sugar concentration (w/v) | 10.5% | 10.5% |
| X (Final biomass concentration-cells/100 mL) | 1200×10^6 | 1200×10^6 |
| dx/dt (Rate of biomass formation-cells h^{-1}) | 871.8×10^6 | 871.8×10^6 |
| Residual sugar (w/v) | 8.5% | 8.4% |
| Acidity | | |
| Acetate (%) | 0.3 | 0.375 |
| Tartarate (%) | 0.36 | 0.45 |
| Antioxidant activity | | |
| RSA (%) | 88.92 | 75.97 |

Table 2: GCMS analysis of cell free wines of apricot and raisins

| Compounds | Mol wt. |
|--------------------|---------|
| Resveratrol | 228 |
| Cyclopentasiloxane | 370 |
| Octadecane | 254 |
| Eicosane | 282 |

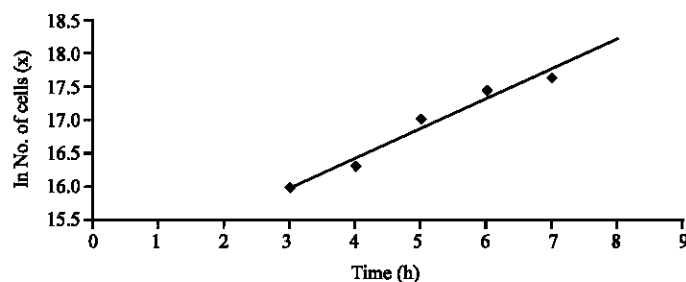


Fig. 1: Log phase growth pattern of *Saccharomyces cerevisiae* NCIM 3282. $R^2 = 0.9748$ and slope of the line (μ), i.e., specific growth rate = 0.7265

Table 3: Sensory characteristics of both the fruit wines

| Characters | Apricot wine | Raisin wine |
|------------|----------------|--------------------------|
| Color | Golden yellow | Reddish violet |
| Taste | Sweet | Sweet to slightly bitter |
| Aroma | Pleasant | Pleasant |
| Flavor | Apricot flavor | Raisin flavor |

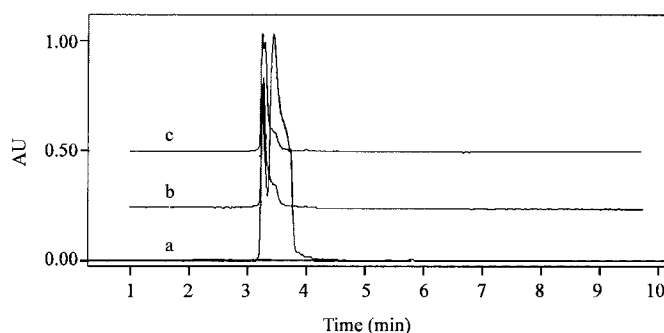


Fig. 2: HPLC results of cell free apricot wine (a) Pure amyl alcohol, after distillation (b) and (c) before distillation

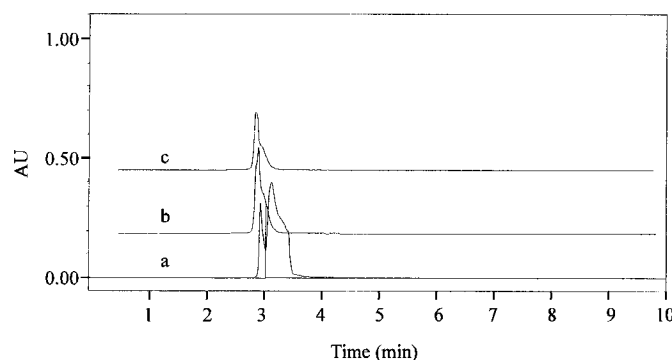


Fig. 3: HPLC results of cell free raisin wine (a) Pure amyl alcohol, (b) after distillation and (c) before distillation

time went by the medicinal properties of wine made its consumption as a reason for good health. Grapes were found to be rich in antioxidants like polyphenols especially the red grapes which beside the polyphenols also contained anthocyanins as antioxidants. However, the polyphenols like resveratrol drew maximum attention in the field of medicine as it was found to be a well know free-radical scavenger (Xia *et al.*, 2010; Hughes, 1975). Later on proanthocyanidins of grapes were found to prevent cancer (Nandakumar *et al.*, 2008). The only thing was that such substances could be taken from wines prepared from such berries like grapes. There were several views now on how much alcohol should be consumed by an individual and there were conflicting reports as to the good and bad effects of polyphenols (Arendt *et al.*, 2005; Brown *et al.*, 2009). In this study, an attempt has been made to produce wine from other fruits like dried apricots and dried grapes (raisins) to see if the same antioxidants could be found in these wines too.

It can be seen from the results that in spite of polyphenolic compounds present in dried apricots and raisins, the yeast could grow and produce sufficient alcohol. Since, these wines

can not be bottle-aged, harmful substances like amyl alcohol was not produced by the yeast. Further, incubation at 12°C also helped in preventing the production of these substances. The wines had sufficient amount of residual sugars which adds to their palatability, for those people who are not used to drinking alcoholic beverages (especially in country like ours where there are lot of reservations regarding such beverages). Finally, it must be remembered that these fruits also has lot of fibers. Therefore, those consumers who need such antioxidants and can not eat the fruits directly (digestive system being sensitive to fibers) can get all the benefits from the wine prepared from such sources.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Microbiology and Department of Biochemistry, Shivaji University, Kolhapur; for providing the necessary laboratory facilities to complete the study as a part of the Master's program in microbiology.

REFERENCES

- Arendt, B.M., S. Ellinger, K. Kelic, L. Geus and R. Fimmers *et al.*, 2005. Single and repeated moderate consumption of native or dealcoholised red wine show different effects on antioxidant parameters in blood and DNA strand breaks in peripheral leukocytes in healthy volunteers: A randomized controlled trial. *Nutr. J.*, 4: 33-33.
- Bjelakovic, G., D. Nikolova, L.L. Gluud, R.G. Simonetti and C. Gluud, 2007. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: Systematic review and meta-analysis. *J. Am. Med. Assoc.*, 297: 842-857.
- Brown, L., P.A. Knoon, D.K. Das, S. Das and A. Tosaki *et al.*, 2009. The biological responses to resveratrol and other polyphenols from alcoholic beverages. *Alcohol Clin. Exp. Res.*, 33: 1513-1523.
- Cho, E., J.M. Seddon B. Rosner, W.C. Willett and S.E. Hankinson, 2004. Prospective study of intake of fruits, vegetables, vitamins and carotenoids and risk of age-related maculopathy. *Arch. Ophthalmol.*, 122: 883-892.
- Hughes, R.E., 1975. James Lind and the cure of scurvy: An experimental approach. *Med. History*, 19: 342-351.
- Knox, K.D. and E.A. Pask, 1950. A modification of the dichromate method for the estimation of diethyl ether and ethyl alcohol. *Br. J. Anesthesia*, 22: 102-106.
- Ko, F.N., Z.J. Cheng, C.N. Lin and C.M. Teng, 1998. Scavenger and 354 antioxidant properties of prenylflavones isolated from *Artocarpus 355 heterophyllus*. *Free Radic. Biol. Med.*, 25: 160-168.
- Mancuso, C., T.E. Bates, D.A. Butterfield, S.C. Calafato and C. Cornelius *et al.*, 2007. Natural antioxidants in Alzheimers disease. *Expert Opin Investig Drugs*, 16: 1921-1931.
- Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, 31: 426-428.
- Mukamal, K.J., M. Kennedy, M. Cushman, L.H. Kuller and A.B. Newman *et al.*, 2008. Alcohol consumption and lower extremity arterial disease among older adults: The cardiovascular health study. *Am. J. Epidemiol.*, 167: 34-41.
- Nandakumar, V., T. Singh and S.K. Katiyar, 2008. Multitargeted prevention and therapy of cancer by proanthocyanidins. *Cancer Lett.*, 269: 378-387.

- Robinson, J., 2006. The Oxford Companion to Wine. 3rd Edn., Oxford University Press, London, ISBN-13: 978-0198609902, pp: 840.
- Sato, M., N. Maulik and D.K. Das, 2002. Cardioprotection with alcohol: Role of both alcohol and polyphenolic antioxidants. *Ann. N.Y. Acad. Sci.*, 957: 122-135.
- Shankar, S., G. Singh and R.K. Srivastava, 2007. Chemoprevention by resveratrol: Molecular mechanisms and therapeutic potential. *Frontiers Biosci.*, 12: 4839-4854.
- Xia, E.Q., G.F. Deng, Y.J. Guo and H.B. Li, 2010. Biological activities of polyphenols from grapes. *Int. J. Mol. Sci.*, 11: 622-646.